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SYNTHESIS AND *IN VITRO* CYTOTOXIC EVALUATION OF NEW QUINAZOLINONE-BASED CONJUGATES

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Abstract. Cancer is currently a global concern because of the mortality increase in recent decades. Although there has been a lot of advancement in cancer chemotherapy so far, the successful treatment of cancer remains a significant challenge in the future because of the drug resistance and adverse side effects of the chemotherapy. Therefore, the search for new compounds as more effective and reliable anticancer agents that overcome these limitations is urgent. In this research, a series of new quinazolione derivatives containing a conjugate system **13 a-l** was synthesized via a three step-procedure. The first step is the condensation of 5-hydroxyanthranilic acid (**10**) at 160 ^{o}C for 2 h to afford the intermediate **11** in high yield. This intermediate was then reacted with *n*-butylamine in acetic acid at 160 ^{o}C for 14 h to give **12** in 92 %. Finally, the reaction of **12** with different aldehydes in acetic acid at 140 ^{o}C for 14 h furnished new conjugates **13a-l** in 62 - 81 %. The bioassay results showed that several compounds displayed cytotoxic activity against two cell lines including HepG-2 and SKLu-1, among that **13h** exhibited the strongest cytotoxic activity against SKLu-1 with IC_{50} value of 5.05 µg/mL.

Keywords: cancer, conjugate, cytotoxicity, quinazolinone.

Classification numbers: 1.2.4.

1. INTRODUCTION

Nowadays, cancer is a global health problem. The disease is also a leading cause of death worldwide, accounting for an estimated 9.6 million deaths in 2018. It is predicted that the number of new cancer cases could increase by 50 % to 15 million new cases each year by 2020 [1]. Among cancer cases, lung and breast cancers are the most common cancers with an estimated 2.1 million diagnosed in 2018 [2]. Therefore, the cancer treatment is becoming one of the priority goals of health systems in both developed and developing countries. However, the drug resistance and side effects are barriers to effective treatment [3]. In order to overcome these problems, the development of effective and reliable anticancer agents is urgent.

Heterocyclic chemistry has been receiving a great deal of scientists' attention. In particular, approximately more than 70 % of all pharmaceuticals and agrochemical contains at least one

heterocyclic ring [4]. 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 [5 - 7]. Quinazolinone was first synthesized by P. Gries in 1869, followed by the discovery of new bioactive compounds containing the quinazolinone skeleton. Since then, 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 (Figure 1) [8 - 10].

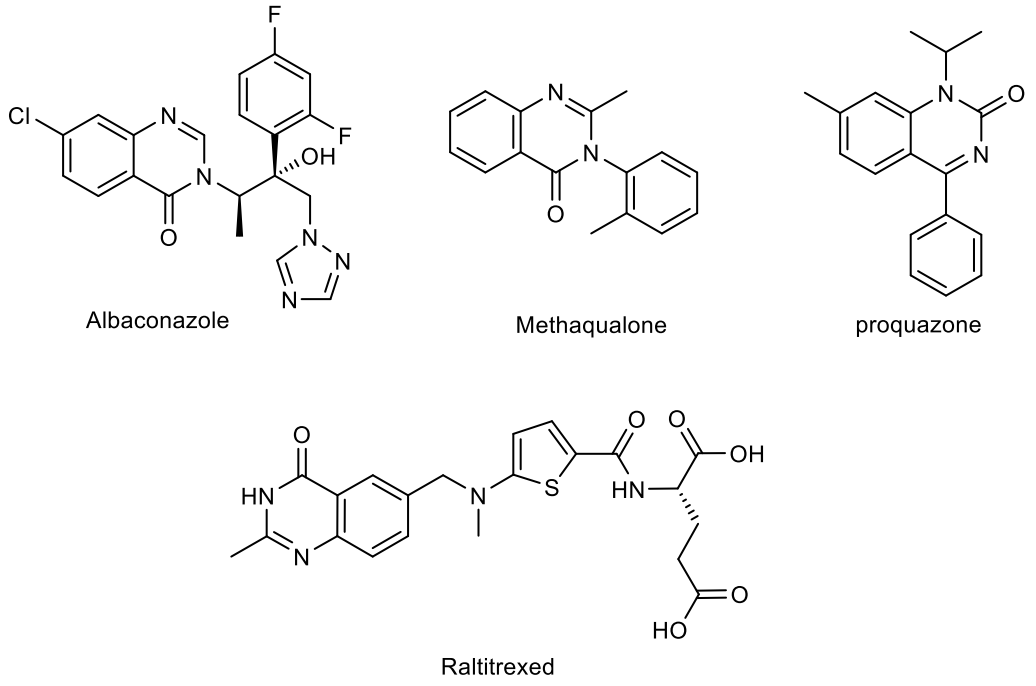


Figure 1. Several quinazolinone-based drugs.

Recently, several quinazolinone-based conjugates have been synthesized and displayed potent activity against methicillin-resistant (MRSA) strains, low clearance, oral bioavailability and shows efficacy in a mouse neutropenic thigh infection model. The structure−activity relationship study showed the importance of the conjugate structure in the molecules [11]. Being intrigued by this observation, in this report, we present a synthesis of new quinazolinone-based conjugates and evaluate cytotoxic activity against several cancer cell lines.

2. MATERIALS AND METHODS

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). IR spectra were recorded on FT-IR IMPACT-410 using KBr discs. Nuclear magnetic resonance spectra (1H and 13C NMR) were recorded using tetramethylsilane (TMS) as an internal standard on a Bruker 500

MHz spectrometer with $CDCl_3$, CD_3OD and 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.

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. 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 ^oC 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 by 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 OD_s were measured at 510 nm using an Elisa reader. The IC_{50} values were then calculated using Probits method. Ellipticin (sigma) was used as a positive control and the values reported for the compounds are presented as average of three determinations.

*Synthesis of 6-hydroxy-2methyl-4H-benzo[d][1,3] ozazin-4-one (**11**)*

A mixture of 5-hydroxy anthranilic acid (**10**) (5.0 g, 32.67 mmol) in acetic anhydride (15 ml) was refluxed at 150 ^oC for 2 h. The mixture was then poured in ice-water. The resulting precipitate was filtered, washed with distilled water and dried in vacuum to afford **11** (5.03 g, 87 %) which was used for next step.

*Synthesis of 3-butyl-6-hydroxy-2-methylquinazolin-4(3H)-one (**12**)*

A mixture of **11** (1.0 g, 5.64 mmol) and *n*-butylamine (1.235 g, 16.92 mmol, 3 eq) in acetic acid (10 mL) was refluxed at 120 ^oC for 14 h. The reaction was monitored by TLC (*n*- hexane : ethyl acetate = 1 : 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 residues which was subjected to column chromatography on silica gel using *n*-hexane/ethyl acetate as eluting systems to give desired **12** as a bright yellow solid (1.191 g, 91 %). Mp 140 - 141 ^oC. R_f = 0.57 (*n*-hexane : ethyl acetate = 1 : 1). ^1H NMR (500 MHz, $CDCl_3$, δ (ppm)): 7.85 (d, *J* = 3.0 Hz), 7.54 (d, *J* = 9.0 Hz), 7.31 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H), 7.63 (brs, 1H), 4.09 (t, *J* = 3.0 Hz, 2H), 2.64 (s, 3H), 1.74 - 1.70 (m, 2H), 1.50-1.46 (m, 2H), 1.0 (t, *J* = 2.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 162.2, 155.2, 152.4, 141.4, 128.3, 124.2, 121.2, 110.1, 44.7, 30.7, 22.8, 20.3, 13.7.

*General procedure for the synthesis of **13 a-l***

A mixture of **12** (1.0 g, 4.34 mmol) in acetic acid (10 mL) and benzaldehyde (2.5 eq) was refluxed at 140 ^{o}C for 16 h. The reaction was monitored by TLC (*n*-hexane : ethyl acetate = 1 : 1). The reaction mixture was then cooled to ambient temperature. Resulting crystal was filtered, washed with distilled water to get the crude product which was recrystallized in *n*-hexane : ethyl acetate to afford compound **13a-l**.

(E)-3-Butyl-6-hydroxy-2-styrylquinazolin-4(3H)-one (13a)

Bright yellow solid (847 mg, 61 %). Mp 175 - 176 ^{o}C; R_f = 0.55 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.82 (d, *J* = 15.5 Hz, 1H), 7.69 (d, *J* = 7.0 Hz, 2H), 7.62 (d, *J* = 9.0 Hz, 1H), 7.52 (d, *J* = 3.0 Hz, 1H), 7.46-7.43 (m, 2H), 7.40 (d, *J* = 7.0 Hz, 1H), 7.32 (d, *J* = 2.5 Hz, 1H), 7.30 (d, *J* = 15.5 Hz, 1H), 4.30 (t, *J* = 7.5 Hz, 2H, CH_2), 1.78 - 1.75 (m, 2H, CH_2), 1.51 - 1.46 (m, 2H, CH_2), 1.02 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 163.5, 157.9, 151.5, 142.3, 141.4, 137.0, 130.7, 130.0, 129.6, 128.8, 125.5, 122.4, 120.3, 110.3, 44.4, 32.2, 21.1, 14.1. ESI-MS *m/z*: 321.2 [M+H]+.

(E)-3-Butyl-6-hydroxy-2-(2-methoxystyryl) quinazolin-4(3H)-one (13b)

Bright yellow solid (832 mg, 65 %). Mp 206 - 207 ^{o}C. R_f = 0.57 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, DMSO-*d6*, δ (ppm)): 10.09 (s, 1H, OH), 7.81 (d, *J* = 15.0 Hz, 1H), 7.79 (overlap, 2H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.42 (d, *J* = 3.0 Hz, 1H), 7.39 (d, *J* = 15.0 Hz, 1H), 7.28 (dd, *J* = 3.0 Hz, 8.5 Hz, 1H), 4.27 (t, *J* = 7.5 Hz, 2H, CH_2), 3.38 (s, 3H, OCH_3), 1.62 - 1.59 (m, 2H, CH_2), 1.38 - 1.36 (m, 2H, CH_2), 0.91 (t, *J* = 7.5 Hz, 3H, CH3).^{13}C NMR (125 MHz, DMSO-*d6*, δ (ppm)): 160.8, 158.8, 157.4, 156.1, 148.8, 140.4, 137.4, 134.4, 133.8, 129.5, 128.8, 128.8, 124.0, 121.0, 120.6, 109.1, 55.2, 42.1, 30.9, 19.4, 13.6. ESI-MS *m/z*: 351.3 $[M+H]^{+}$.

(E)-3-Butyl-6-hydroxy-2-(3-methoxystyryl) quinazolin-4(3H)-one (13c)

Bright yellow solid (745 mg, 63 %). Mp 192 - 193 ^{o}C. R_f = 0.57 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.64 (d, *J* = 15.5 Hz, 1H), 7.59 (d, *J* = 9.0 Hz, 1H), 7.50 (d, *J* = 2.5 Hz, 1H), 7.35 - 7.28 (m, 2H), 7.25-7.22 (m, 2H), 7.19 (d, *J* = 15.5 Hz, 1H), 6.97 (d, *J* = 9.0 Hz, 1H), 4.27 (t, *J* = 7.5 Hz, 2H, CH_2), 3.85 (s, 3H, OCH_3), 1.76 - 1.73 (m, 2H, CH_2), 1.50 - 1.45 (m, 2H, CH_2), 1.02 (t, *J* = 7.5 Hz, 3H, CH_2). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 163.4, 161.6, 157.9, 151.4, 142.3, 141.4, 138.3, 131.0, 129.6, 125.5, 122.4, 121.2, 120.6, 116.4, 113.9, 110.3, 55.8, 44.4, 32.1, 21.0, 14.1; ESI-MS *m/z*: 351.2 $[M+H]^{+}$.

(E)-3-Butyl-6-hydroxy-2-(4-methoxystyryl) quinazolin-4(3H)-one (13d)

Bright yellow solid (787 mg, 66 %). Mp 215 - 216 ^{o}C. R_f = 0.57 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, DMSO-*d6*, δ (ppm)): 10.03 (s, 1H, OH), 7.81 (d, *J* = 15.0 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.42 (d, *J* = 3.0 Hz, 1H), 7.27 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H), 7.21 (d, *J* = 15.0 Hz, 1H), 7.0 (d, *J* = 8.5 Hz, 2H, CH_2), 4.26 (t, *J* = 7.5 Hz, 2H, CH_2), 3.80 (s, 3H, OCH_3), 1.65 - 1.60 (m, 2H, CH_2), 1.41 - 133 (m, 2H, CH_2), 0.92 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-*d6*, δ (ppm)): 160.84, 160.35, 155.78, 149.2, 140.5, 138.6, 129.4, 128.6, 123.9, 120.8, 117.1, 114.3, 109.0, 55.2, 41.9, 30.8, 19.5, 13.6; ESI-MS *m/z*: 351.4 $[M+H]^{+}$.

(E)-3-Butyl-6-hydroxy-2-(2-fluorostyryl) quinazolin-4(3H)-one (13e)

Bright yellow solid (1048 mg, 77 %). Mp 246 - 247 ^{o}C. R_f = 0.55 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.95 (d, *J* = 15.5 Hz, 1H), 7.78-7.75 (m, 1H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.53 (d, *J* = 2.0 Hz, 1H), 7.44 (d, *J* = 15.5 Hz, 1H), 7.33 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H), 7.29-7.26 (m, 2H), 7.23-7.19 (m, 1H), 4.31 (t, *J* = 7.5 Hz, 2H, CH_2), 1.80 - 1.77 (m, 2H, CH_2), 1.52-1.48 (m, 2H, CH_2), 1.04 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 163.6, 161.6, 157.9, 151.1, 142.4, 133.8, 132.2, 130.4, 129.9, 125.9, 125.4, 124.8, 123.4, 122.6, 117.1, 110.5, 44.4, 32.2, 21.0, 14.0; ESI-MS *m/z*: 339.7 $[M+H]^+$.

(E)-3-Butyl-6-hydroxy-2-(3-fluorostyryl) quinazolin-4(3H)-one (13f)

Bright yellow solid (771 mg, 72 %). Mp 245 - 246 ^{o}C. R_f =0.55 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.81 (d, *J* = 15.5 Hz, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.53-7.44 (m, 4H), 7.37 (d, *J* = 15.5 Hz, 1H), 7.33 (d, *J* = 9.0 Hz, 1H), 7.16-7.12 (m, 1H), 4.33 (t, *J* = 7.5 Hz, 2H, CH_2), 1.78 - 1.75 (m, 2H, CH_2), 1.51 - 1.47 (m, 2H, CH_2), 1.03 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 165.6, 163.4, 158.1, 151.1, 142.2, 140.0, 139.4, 131.8, 129.7, 125.5, 124.9, 122.5, 121.9, 117.3, 114.9, 110.3, 44.4, 32.2, 21.0, 14.1; ESI-MS *m/z*: 339.7 $[M+H]^+$.

(E)-3-Butyl-6-hydroxy-2-(4-fluorostyryl) quinazolin-4(3H)-one (13g)

Bright yellow solid (889 mg, 82 %). Mp 274 - 275 ^{o}C. R_f = 0.55 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.83 (d, *J* = 15.5 Hz, 1H), 7.77-7.74 (m, 2H), 7.64 (d, *J* = 9.0 Hz, 1H), 7.53 (d, *J* = 2.5 Hz, 1H), 7.34 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H), 7.29 (d, *J* = 15.5 Hz, 1H), 7.22 - 7.18 (m, 2H), 4.33 (t, *J* = 7.5 Hz, 2H, CH_2), 1.81-1.75 (m, 2H, CH_2), 1.52-1.48 (m, 2H, CH_2), 1.04 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 165.9, 163.5, 157.9, 151.5, 142.3, 140.1, 133.5, 130.9, 129.6, 125.5, 122.5, 120.3, 117.0, 110.3, 44.4, 32.2, 21.0, 14.1; ESI-MS *m/z*: 339.6 $[M+H]^+$.

(E)-3-Butyl-6-hydroxy-2-(2-nitrostyryl) quinazolin-4(3H)-one (13h)

Bright yellow solid (865 mg, 77 %). Mp 182 - 183 ^{o}C. R_f = 0.52 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm)): 8.15 (d, *J* = 15.0 Hz, 1H), 8.06-8.04 (dd, *J* = 1.0 Hz, 8.5 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.77 (t, *J* = 7.5 Hz, 1H), 7.64 (d, *J* = 9.0 Hz, 1H), 7.61 (d, *J* = 1.0 Hz, 1H), 7.53 (d, *J* = 3.0 Hz, 1H), 7.34 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H), 7.31 (d, *J* = 15.0 Hz, 1H), 4.32 (t, *J* = 8.0 Hz, 2H, CH_2), 1.80 - 1.74 (m, 2H, CH_2), 1.51-1.44 (m, 2H, CH_2), 1.01 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 163.4, 158.4, 150.4, 150.1, 142.1, 135.9, 134.6, 132.1, 131.1, 130.1, 130.0, 125.7, 125.6, 125.4, 122.7, 110.4, 44.5, 32.3, 21.0, 14.1; ESI-MS *m/z*: 366.2 $[M+H]^+$.

(E)-3-Butyl-6-hydroxy-2-(3-nitrostyryl) quinazolin-4(3H)-one (13i)

Bright yellow solid (876 mg, 71 %). Mp 263 - 264 ^{o}C. R_f = 0.52 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, DMSO-*d6*, δ (ppm)): 10.12 (s, 1H, OH), 8.60 (t, *J* = 1.5 Hz, 1H), 8.24 (d, *J* = 7.5 Hz, 1H), 8.20 (dd, *J* = 1.5 Hz, 8.0 Hz, 1H), 7.93 (d, *J* = 15.0 Hz, 1H), 7.12 (t, *J* = 8.0 Hz, 1H), 7.58 (d, *J* = 9.0 Hz, 1H), 7.56 (d, *J* = 15.0 Hz 1H), 7.43 (d, *J* = 3.0 Hz, 1H), 7.28 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H), 4.31 (t, *J* = 7.5 Hz, 2H, CH_2), 1.64 - 1.61 (m, 2H, CH_2), 1.39-1.34 (m, 2H, CH_2), 0.92 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-*d6*, δ (ppm)): 160.7, 156.3, 148.5, 148.4, 140.3, 137.3, 136.4, 133.9, 130.2, 128.9, 124.0, 123.5, 122.9, 122.1, 121.1, 109.1, 42.0, 30.9, 19.4, 13.6; ESI-MS *m/z*: 366.2 $[M+H]^+$.

(E)-3-Butyl-6-hydroxy-2-(4-chlorostyryl) quinazolin-4(3H)-one (13j)

Bright yellow solid (775 mg, 72 %). Mp 290 - 291 ^{o}C. R_f = 0.57 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.85 (d, *J* = 15.0 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 2H), 7.64 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 2.5 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 2H), 7.32 (d, *J* = 15.0 Hz, 1H), 4.33 (t, *J* = 7.5 Hz, 2H, CH_2), 1.82 - 1.76 (m, 2H, CH_2), 1.52 - 1.48 (m, 2H, CH_2), 1.03 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 163.3, 157.9, 151.1, 142.4, 139.7, 136.2, 135.9, 130.2, 129.9, 129.8, 125.4, 122.6, 121.5, 110.6, 44.3, 32.3, 21.0, 14.1; ESI-MS *m/z*: 355.2 $[M+H]^+$.

(E)-3-Butyl-6-hydroxy-2-(4-bromostyryl) quinazolin-4(3H)-one (13k)

Bright yellow solid (805 mg, 68 %). Mp 285 - 286 ^{o}C. R_f= 0.56 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR(500 MHz, CD_3OD, δ (ppm)): 7.85 (d, *J* = 15.0 Hz, 1H), 7.63 - 7.54 (m, 5H), 7.34 (d, *J* = 15.0 Hz, 1H), 7.34-7.31 (m, 2H), 4.33 (t, *J* = 7.0 Hz, 2H, CH_2), 1.80 - 1.75 (m, 2H, CH_2), 1.52 - 1.47 (m, 2H, CH_2), 1.03 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 163.3, 157.9, 151.0, 142.4, 139.7, 136.3, 133.2, 130.5, 130.0, 125.4, 124.5, 122.6, 121.6, 110.6, 44.3, 32.3, 21.0, 14.1; ESI-MS *m/z*: 399.1 $[M+H]^+$.

(E)-3-Butyl-6-hydroxy-2-(4-methylstyryl) quinazolin-4(3H)-one (13l)

Bright yellow solid (833 mg, 82 %). Mp 25 - 256 ^{o}C. R_f = 0.58 (*n*-hexane : ethyl acetate = 7 : 3). ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.91 (d, *J* = 9.0 Hz, 2H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 7.0 Hz, 1H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.26 - 7.22 (m, 2H), 7.06 (d, *J* = 15.5 Hz, 1H), 4.26 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 1.82 - 1.80 (m, 2H, CH_2), 1.52-150 (m, 2H, CH_2), 1.03 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ (ppm)): 162.4, 154.4, 149.9, 142.2, 140.4, 139.9, 133.2, 129.7, 129.2, 127.7, 124.3, 121.4, 118.1, 110.6, 43.7, 31.3, 21.4, 20.3, 13.7; ESI-MS *m/z*: 335.3 $[M+H]^+$.

3. RESULTS AND DISCUSSION

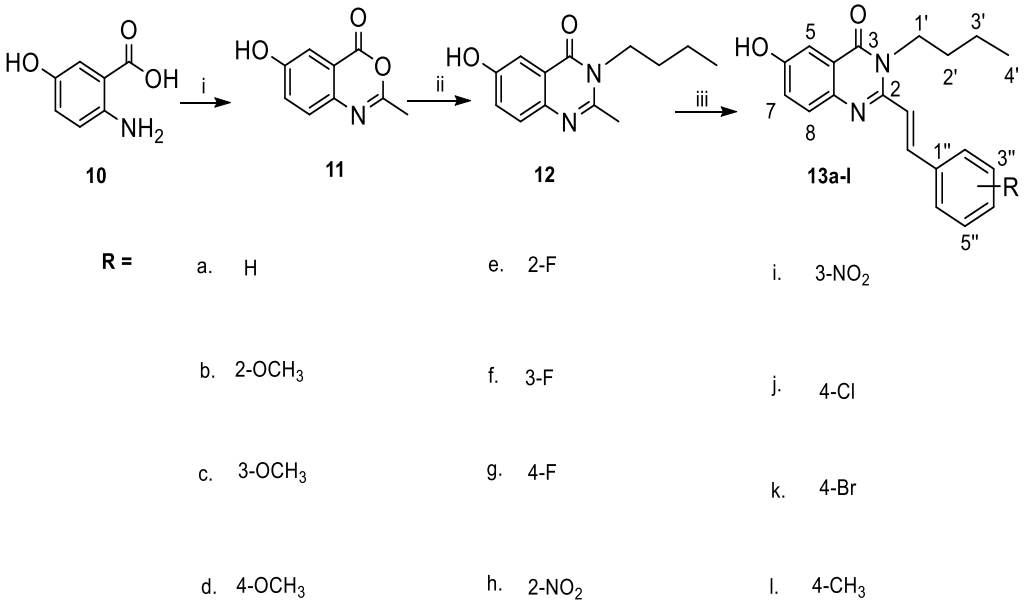
3.1 Chemistry

A series of new quinazolione derivatives **13a-l** containing a conjugated system was synthesized in good yields via a three-step procedure (Scheme 1). 6-Hydroxyanthranilic acid (**10**) was first condensed with the excess of acetic anhydride at 160 ^{o}C for 2 h to afford benzoxazinone **11** in 87 % yield. The purification of compound **11** was obtained by pouring the reaction mixture into the ice-water. The resulting precipitate was filtered, washed with distilled water, and dried in vacuum. Compound **11** was next reacted with *n*-butylamine in acetic acid at 120 ^{o}C for 14 h to give the intermediate **12** in 92 % yield. Finally, the reaction of **12** with different aldehydes in acetic acid at 140 ^{o}C for 16 h to furnish novel conjugates **13a-l** in 61 - 82 % yields. The structure of compounds were characterized by ^{1}H NMR, ^{13}C NMR, MS. Due to the structural similarity of target compounds, compound **13h** was used as an example to elucidate the structure.

The ^{1}H NMR spectrum of the compound **13h** indicated the presence of 18 protons, and it is easy to observe the resonance of two protons *β* and *α* of the conjugated system. The signal at the lowest field is attributed to proton *β* at δ_H 8.15 ppm and the other at δ_H 7.31 ppm with a coupling constant (*J* = 15.0 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 observed in which the proton H-5 resonates as a doublet at 7.53 ppm (J = 3.0 Hz) resulting from long coupling with H-7. The proton H-8 resonates as a doublet at δ_H 7.64 (J = 9.0 Hz) due to near coupling with H-7. The proton H-7 was observed as a doublet of doublet at δ_H 7.34 (d, J = 3.0 Hz, 9.0 Hz) due to coupling with H-8 and H-7. Four protons of aromatic ring were also observed as doublets and triplets ranging δ_H 8.06-7.61 ppm. In the upfield, resonance signals of the butyl chain are observed in which CH_2 connected to quinazolinone resonates as a triplet (J = 8.0 Hz) at δ_H 4.32 ppm, and two CH_2 resonate as multiples at δ_H 1.77 and 1.48 ppm. The strong triplet signal (J = 7.0 Hz) is attributed to CH_3.

The ^{13}C NMR spectrum showed the presence of 16 aromatic carbons and 4 carbon of the butyl chain, in which characteristic resonance signals at δ_C 163.4, 158.4 and 150.4 ppm are attributed to C-4, C-2 and C-6, respectively, and C-2″ resonates at δ_C 150.1 ppm. In addition, the resonance signals of butyl chain are observed at δ_C 44.5, 32.3, 21.0, 14.1 ppm, respectively.



Scheme 1. Reagents and conditions: (i) $(CH_3CO)_2O$, 160 ^oC, 2 h; (ii) acetic acid, *n*-butylamine, 120 ^oC, 14 h, 92 %; (iii) acetic acid, aldehydes, 140 ^oC, 16 h, 61 - 82 %.

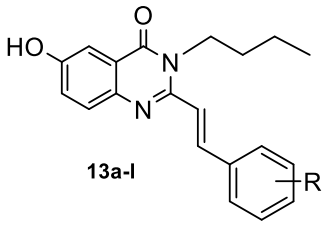
3.3 Bioassay

All target compounds **13a-l** 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.

As can be seen in the Table 1 that 5 compounds **13b-c**, 13f, **13h**, and **13k** displayed cytotoxic activity on the two human cancer cell lines tested with IC_{50} values ranging from 70.17 to 5.05 µg/mL. It was observed that these compounds showed better cytotoxic activity against SKLu-1 than HepG-2 and no compounds comparable to ellipticin in terms of cytotoxicity. Compounds **13b**, **13c** containing electron donating substituents (-OCH_3) in the *ortho* and *meta* positions increases cytotoxic activity on both tested cell lines while in *para* position deactivates (**13d**), and that electron donating groups tend to favor cytotoxic activity over electron

withdrawing groups except the compound **13h**. Compound **13f** containing a withdrawing group (-F) in the *meta* position of the conjugate moiety displayed better cytotoxic activity against SKLu-1 (IC_{50} = 37.10 µg/mL) than HepG-2 (IC_{50} = 70.17 µg/mL). It was found that compound **13g** and **13j** containing strong electron withdrawing group (-F, -Cl) in *para* position showed no cytotoxic activity against both tested cell lines while compound **13k** containing an electron withdrawing group (-Br) at the same position showed cytotoxic activity with IC_{50} values of around 50 µg/mL. Among synthesized compounds, **13h** exhibited the strongest cytotoxic effect against SKLu-1 with IC_{50} value of 5.05 µg/mL.

Table 1. *In vitro* cytotoxic activity of conjugates **13a-l**.



13a-l

No	Compounds	R	IC_{50} (µg/mL)	
			HepG-2	**SKLu-1**
1	**13a**	H	> 100	> 100
2	**13b**	2-OCH_3	8.94 ± 0.97	9.44 ± 0.37
3	**13c**	3-OCH_3	13.98 ± 2.02	10.98 ± 1.08
4	**13d**	4-OCH_3	>100	>100
5	**13e**	2-F	> 100	>100
6	**13f**	3-F	70.17 ± 2.48	37.10 ± 3.18
7	**13g**	4-F	> 100	> 100
8	**13h**	2-NO_2	15.07 ± 0.21	5.05 ± 1.19
9	**13i**	3-NO_2	> 100	> 100
10	**13j**	4-Cl	> 100	> 100
11	**13k**	4-Br	50.23 ± 5.27	51.03 ± 4.07
12	**13l**	4-CH_3	> 100	> 100
	Ellipticine		**0.40**	**0.43**

[a]Concentration (µg/mL) 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.

4. CONCLUSIONS

A series of new quinazolinone-based conjugates **13a-l** have been synthesized and 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 **13h** exhibited the strongest cytotoxic activity against SKLu-1 with IC_{50} value of 5.05 µg/mL.

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CRediT authorship contribution statement: Nguyen Thi Thanh, Nguyen Huong Linh carried out the main experimental work. Ho Duc Cuong collected and analysed experimental data. Ta Hong Duc participated in sampling of the compounds. Tran Khac Vu designed and supervised the study and wrote the manuscript.

Declaration of competing interest: The authors have declared no conflict of interest

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