Research in Pharmaceutical Sciences, May 2012; 7(2): 119-125
Received: Sep 2011
Accepted: Dec 2011

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Synthesis and cytotoxic evaluation of some new 4(3H)-quinazolinones on HeLa cell line

G.A. Khodarahmi, M. Shamshiri and F. Hassanzadeh*

Department of Medicinal Chemistry and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Science, Isfahan University of Medical Sciences, Isfahan, I.R.Iran.

Abstract

Quinazolinone backbone is present in a large number of bioactive substances. Since remarkable cytotoxic activity is associated with some 4(3H)-quinazolinones, in this study some 4(3H)-quinazolinone were synthesized and screened against HeLa cells. The synthesis was performed via reaction of anthranilic acid with dicarboxylic anhydrides to produce carboxylic acids derivatives. The products were heated in acetic anhydride to produce benzoxazinones. Finally, 4(3H)-quinazolinones were synthesized by reaction between benzoxazinones and primary amines. The assessment of the structure of the synthesized compounds was based on spectral data (FT-IR, Mass and ^{1}HNMR). Subsequently, cytotoxic activity of compounds **3**, **6**, **9** and **13** (individually and in combination with doxorubicin) was evaluated on HeLa cell line using MTT assay. The results indicated that the tested compounds did not show significant cytotoxicity alone and in combination with doxorubicin (1 and 20 µM).

Keywords: Quinazolinone; Synthesis; Cytotoxicity; HeLa cell

INTRODUCTION

Cancer is a life threatening disease with complex pathogenesis which threats human life greatly. Quinazolinone is a building block for several alkaloids isolated to date (1). 4(3H)-Quinazolinones are fused heterocycles (2) with diverse range of biological activities e.g.; cytotoxicity, antimicrobial, protein tyrosine kinase inhibitory and anti-inflammatory properties (1,3). Tricyclic quinazolinone derivative, "Deoxyvasinon" a natural alkaloid is well known as a cytotoxic agent (4,5).

Structure activity relationship studies indicated that substitutions at 2 and 3 positions of quinazolinone ring are highly associated with cytotoxic activity of these compounds. Therefore, altering the substitutions on these positions may result in enhancing cytotoxic activity of these compounds (6-8).

In the light of above considerations, we planed to synthesize a number of 2,3 disubstituted derivatives of quinazolinone. In addition, their possible cytotoxic activities were also assessed.

According to the literature, usually the first step in the production of quinazolinone ring is the reaction between anthranilic acid and an acyl chloride or acetic anhydride (8,9). The product in both reactions is an amide substituted with simple alkyl groups which upon ring closure gives the benzoxazinone. Finally, alkyl substituted quinazolinones are obtained from the reaction of benzoxazinone with different amines.

In the current study, anthranilic acid was reacted with cyclic dicarboxylic anhydrides (10). Application of cyclic dicarboxilic anhydrides like succinic anhydride instead of simple acyl chlorides or acetic anhydride offer a free carboxylic acid at the end of the amide side chain. The amide is changed to the corresponding benzoxazinone and reacted with different amines to give the final quinazolinone derivatives. The presence of an extra carboxylic moiety on the quinazolinone side chain may serve as a new site for further substitutions on quinazolinone side chain or possibly an extra ring closure to produce tricyclic quinazolinone derivatives.

***Corresponding author: F. Hassanzadeh, this paper is extracted from the Pharm.D thesis No. 388507
Tel. 0098 311 7922575, Fax. 0098 311 6680011
Email: Hassanzadeh@pharm.mui.ac.ir

The presence of a free carboxyl group on the quinazolinone structure could possibly alter the biological properties including cytotoxic activities.

Because it has been shown that different dicarboxylic acid derivatives possess considerable cytotoxic activities (11), all dicarboxylic acid intermediates produced in this work were also evaluated for their cell toxicities.

MATERIALS AND METHODS

Melting points were determined on an Electro thermal 9200 melting point apparatus. ^{1}HNMR spectra were recorded on a 400 MHz spectrometer Bruker (Germany); chemical shifts are expressed in ppm with reference to TMS. Mass spectral data were obtained on a Shimadzu LC/MS-2010 apparatus (Japan). Infrared (FT-IR) spectra were recorded on a WQF-510 FT-IR spectrometer (China). Thin layer chromatography was performed on Merck 20×20 cm pre-coated (0.25 mm) silica gel GF254 plates (Merck Co., Germany); compounds were detected with a 254-nm UV lamp (Perkin Elmer 550s, USA). Silica gel (60-320 mesh) was employed for routine column chromatography separations.

RPMI 1640, MTT and antibiotics (penicillin G/ streptomycin) were purchased from Sigma (England), fetal calf serum (FCS) and trypsin-EDTA were purchased from Gibco (Scotland). $NaHPO_4$, K_2HPO_4, NaCl, KCl, HCl and NaOH were purchased from Merck (Germany) and HeLa cell line was obtained from NCBI (Iran).

Chemistry

The general reaction scheme for the preparation of the target compounds is shown in Fig. 1.

*Synthesis of N-benzyl-3-(3-benzyl-4-oxo-3, 4-dihydroquinazolin-2-yl) propanamide **(5)***

A mixture of anthranilic acid **1** (5.4 g, 0.04 mol) and succinic anhydride **2** (4 g, 0.04 mol) was refluxed for 5 h in glacial acetic acid (GAA) (20 ml). The solvent was evaporated and the residue was recrystallized in acetone to yield 2-(3-carboxypropanamido) benzoic acid **3** as yellowish crystal. The resulting carboxylic acid **3** (2 g, 0.0084 mol) was heated at 130-140ºC in acetic anhydride (8 ml) for 1 h and then was concentrated using vacuum pump to afford 3-(4-oxo-4H-benzo[d][1,3]oxazin-2-yl) propanoic acid **4** as dark viscose oil. Benzoxazinone derivative **4** (1.84 g, 0.0084 mol) was refluxed with benzylamine (1.48 g, 0.011 mol) in chloroform (20 ml) for 3 h. The solvent was evaporated and resulting oily mass was dissolved in ethylene glycol (20 ml), NaOH (0.25 g) was added and the mixture was heated at 130-140ºC for 3 h. The mixture was kept over night at room temperature. The pH of resulting mixture was adjusted to 7-8 by addition of HCl 3%. The precipitate was filtered and washed with cold water and recrystallized in isopropyl alcohol to afford N-benzyl-3-(3-benzyl-4-oxo-3,4-dihydroquinazolin -2- yl) propanamide **5** as white crystals.

*Synthesis of 3-(4-oxo-3, 4-dihydroquinazolin-2-yl) propanoic acid **(6)***

3-(4-Oxo-4H-benzo[d][1,3]oxazin-2-yl) propanoic acid **4** (1.84 g, 0.0084 mol) which was already synthesized and formamide (1.133 g, 0.025 mol) were refluxed in absolute ethanol (25 ml). After 4 h, the hot suspension was filtered, the bluish semisolid compound obtained was characterized as 2,3-dihydropyrrolo [2, 1-b] quinazoline-1, 9-dione **7.** The filtrate was allowed to cool at room temperature. 3-(4-oxo-3, 4-dihydroquinazolin -2-yl) propanoic acid **6** was precipitated as white crystalline powder.

*Synthesis of N-benzyl-4-(3-benzyl-4-oxo-3, 4-dihydroquinazolin-2-yl) butanamide **(11)***

A mixture of anthranilic acid **1** (3.5 g, 0.026 mol) and glutaric anhydride **8** (4 g, 0.035 mol) was refluxed for 10 h in GAA (15 ml). The solvent was evaporated to afford semisolid product which was recrystallized in chloroform to yield 2-(4-carboxybutanamido) benzoic acid **9** as white crystals. The resulting carboxylic acid **9** (0.5 g, 0.002 mol) was heated at 130-140 ºC in acetic anhydride (2 ml) for 1 h. Solvent was evaporated to yield 4-(4-oxo-4H-benzo[d][1, 3] oxazin-2-yl) butanoic acid **10** as viscose oil. Benzoxazinone derivative **10** (0.47 g, 0.002 mol) was refluxed with benzylamine (0.98 g, 0.009 mol) in chloroform (5 ml) for 3 h. The solvent was evaporated and the resulting

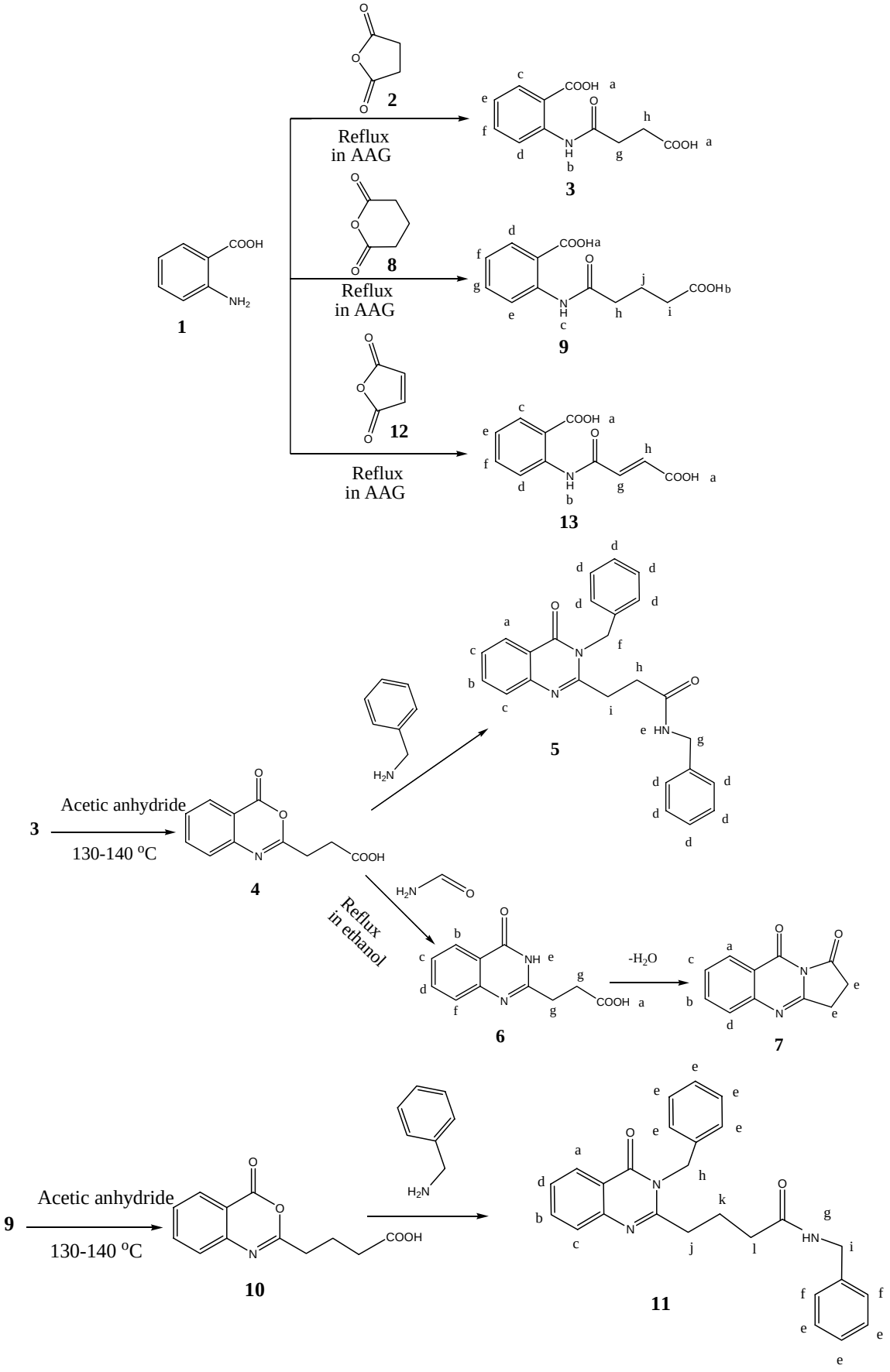

Fig. 1. General reaction scheme for the preparation of target compounds.

oil was dissolved in ethylene glycol (5 ml), NaOH (0.1 g) was then added and the mixture was heated at 130-140ºC for 3 h. The mixture was kept over night at room temperature. The pH of the resulting mixture was adjusted to 7-8 by addiition of HCl 3%. The resulting mixture was concentrated by rotary evaporator and fractionated on PTLC to give N- benzyl-4- (3-benzyl -4- oxo-3, 4-dihydroquinazolin-2-yl) butanamide **11** as white powder.

*Synthesis of 2-(3-carboxyacrylamido) benzoic acid **(13)***

In an attempt to produce N-benzyl-3-(3-benzyl -4- oxo -3 ,4- dihydroquinazolin -2 -yl) acrylamide a mixture of anthranilic acid **1** (5.4 g, 0.04 mol) and maleic anhydride **12** (3.92 g, 0.04 mol) was refluxed for 6 h in GAA (20 ml) to yield 2-(3-carboxyacrylamido) benzoic acid **13** as a pale yellow powder. Further reactions did not follow the usual procedure and desired compound was not isolated.

Biological activity

HeLa cell line was grown in RPMI 1640 medium (sterilized using 0.22 µM microbiological filters) supplemented with 10% FCS, 100 U/ml penicillin G, 100 mg/ml streptomycin and L-glutamine (12). The pH of the medium was then adjusted between 7.3 and 7.6 using HCl or NaOH and kept at 4ºC before use. Cells were cultured in an atmosphere of 5% CO_2 in humidified air at 37ºC (12). HeLa cell line was seeded in a 96-well plate at a cell density of 5×10^4cells/ml and incubated for 24 h. Then 20 µl of various concentrations (1-500 µM) of the synthesized compounds (3, 6, 9 and 13), DMSO 1% (as negative control) and doxorubicin 20 µM (as positive control) were added to the culture media and incubated for 48 h. In separate set of experiments, 22.5 µl of doxorubicin (1, 20 µM) and 22.5 µl of each compounds (100 µM) was added to each well and incubated at the same condition as mentioned above. Cytotoxicity was determined by rapid colorimetric assay using MTT. The cells were incubated at 37ºC for 3 h with MTT. Then the medium was removed and the resulting formazan crystals were dissolved in 180 µL of DMSO (13). Absorbance of each well was measured using a microplate reader at 540 nm. All results were compared with untreated control. The results were also compared with doxorubicin in the combination experiments. Each set of experiments was performed in triplicate within three consecutive days.

RESULTS

2-(3-Carboxypropanamido) benzoic acid (3)

$C_{11}H_{11}O_5N$; Yield 90%; m.p. 130 -132ºC; white powder; FT-IR (KBr, cm^{-1}): 3323 (N-H), 3150 (C-H Ar), 2939 (C-H Aliphatic), 2800-2200 (COOH), 1682 (C=O); ^{1}HNMR (400 MHz-DMSO): 11.8-13.6 (2H, s, Ha), 11.17 (1H, s, Hb), 8.47 (1H, d, j=8.4Hz, Hc), 7.97 (1H, d, j=8Hz, Hd), 7.57 (1H, t, j=7.2Hz, He), 7.13 (1H, t, j=7.6Hz, Hf), 2.6 (2H, t, j=6.4, Hg), 2.54 (2H, t, j=6, Hh); Ms (m/z, %): 236 (M-1, 100).

N- benzyl -3- (3- benzyl -4- oxo -3, 4-dihydroquinazolin-2-yl) propanamide (5)

$C_{25}H_{23}O_2N_3$; Yield 65%; m.p. 180-182ºC; white crystal; FT-IR (KBr, cm^{-1}): 3309(N-H), 3028(C-H Ar), 2954 & 2885 (C-H Aliphatic), 1682 & 1630 (C=O); ^{1}HNMR (400 MHz-CDCl3): 8.23 (1H, d, d, j=8Hz, j=1.2 Hz, Ha), 7.63(1H, d, t, j=8.4Hz, j=1.6Hz, Hb), 7.45-7.37 (2H, m, Hc), 7.27-7.12 (10H, m, Hd), 6.39 (1H, s, He), 5.39 (2H, s, Hf), 4.35 (2H, d, j=4.8Hz, Hg), 3(2H, t, j=5.6, Hh), 2. 7 (2H, t, j=5.6, Hi); Ms (m/z, %): 397 (M^+, 31).

3- (4- Oxo -3, 4-dihydroquinazolin -2- yl) propanoic acid (6)

$C_{11}H_{10}O_3N_2$; Yield 54%; m.p. 203-205ºC; white crystal; FT-IR (KBr, cm^{-1}): 3417 (N-H), 3064 (C-H Ar), 2935 (C-H Aliphatic), 1770 & 1693 (C=O); ^{1}HNMR (400 MHz-DMSO): 7.88 (1H, s, Ha), 7.7 (1H, d, j=7.6Hz, Hb), 7.58 (1H, t, j=7.4Hz, Hc), 7.5 (1H, t, j=7.4Hz, Hd), 7.28 (1H, s, He), 7.25 (1H, d, j=7.6, Hf), 2.85-2.65 (4H, m, Hg); Ms (m/z, %): 218 (M^+, 100).

2, 3-Dihydropyrrolo [2, 1-b] quinazoline-1, 9-dione (7)

$C_{11}H_8O_2N_2$; FT-IR (KBr, cm^{-1}): 2922 & 2852(C-H Aliphatic), 1803 & 1711 (C=O); ^{1}HNMR(400 MHz-DMSO): 8.14 (1H, d,

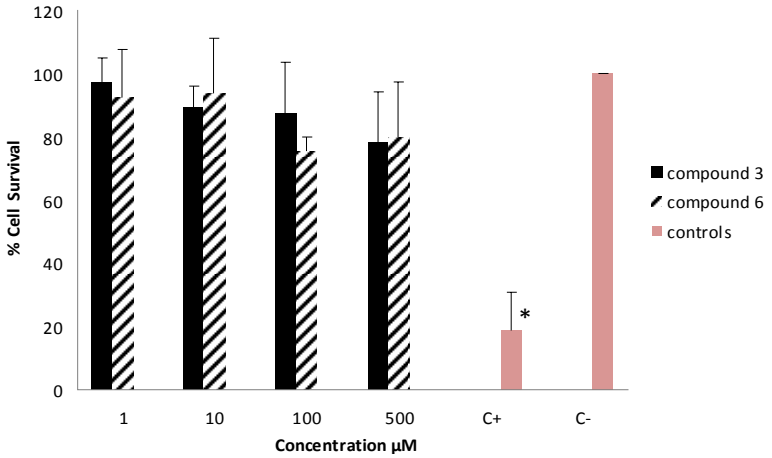

Fig. 2. Cytotoxic effects of compounds **3**, **6** on HeLa cells. Following exposure to different concentrations of compounds **3**, **6**, cell viability was assessed using MTT method. Data are presented as mean ± SD; * P<0.05 (compared to negative control); n=9; C+ =Positive control (Doxorubicin 20 µM); C- =Negative control (DMSO 1%).

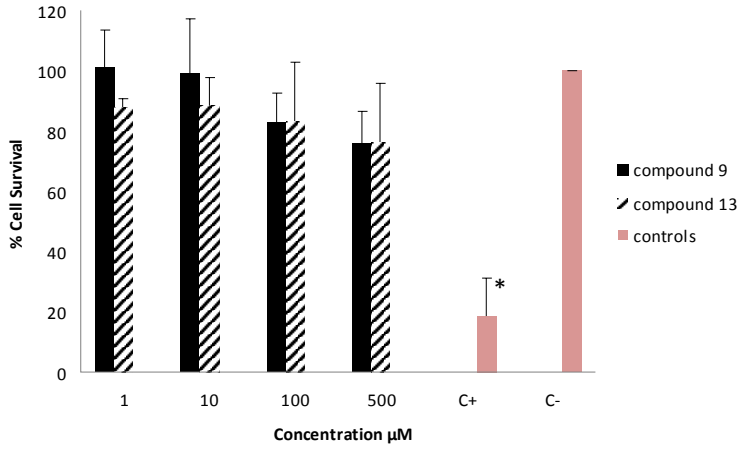

Fig. 3. Cytotoxic effects of compounds **9**, **13** on HeLa cells. Following exposure to different concentrations of compounds **9**, **13**, cell viability was assessed using the MTT method. Data are presented as mean ± SD; * P<0.05 (compared to negative control); n=9, C+ =Positive control (Doxorubicin 20 µM); C- =Negative control (DMSO 1%).

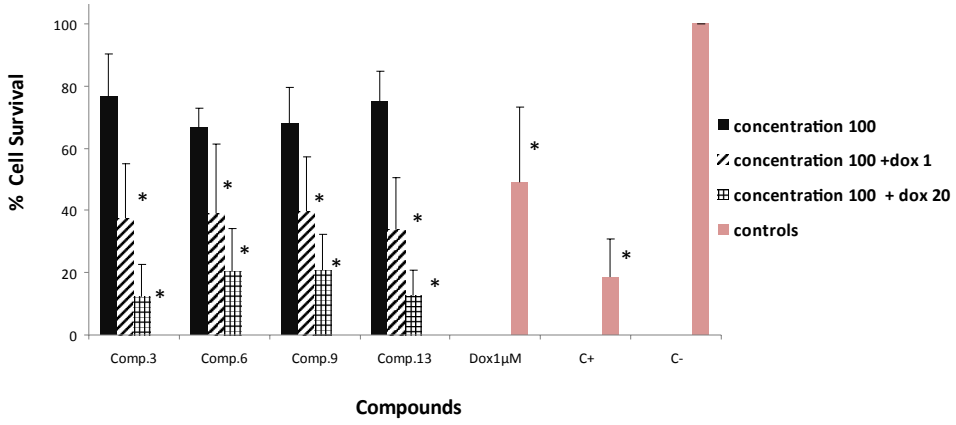

Fig. 4. Cytotoxic effects of compounds **3**, **6**, **9** and **13** individually and in combination with doxorubicin on HeLa cells. Following exposure to different concentrations of compounds **3**, **6**, **9** and **13** and doxorubicin, cell viability was assessed using the MTT method. Data are presented as mean ± SD; * P<0.05 (compared to negative control); n=9, Dox1µM = Doxorubicin 1 µM; C+ =Positive control (Doxorubicin 20 µM), C- =Negative control (DMSO 1%).

j=6.8 Hz, Ha), 7.88 (1H, t, j=6.8Hz, Hb), 7.75-7.45 (1H, t, j=6.8Hz, Hc), 7.46 (1H, d, j=7.6Hz, Hd), 2.9-2.7 (4H, m, He); Ms (m/z, %): 201 (M+1, 36)

2-(4-Carboxybutanamido) benzoic acid (9)

$C_{12}H_{13}O_5N$;Yield 60%; m.p. 120-122ºC; white powder; FT-IR (KBr, cm^{-1}): 3321 (N-H), 3118 (C-H Ar), 2960 (C-H Aliphatic), 2700-2400 (COOH), 1703 & 1676 (C=O); ^{1}HNMR (400 MHz-DMSO): 13.2-13.8 (1H, s, Ha), 12-12.5 (1H, s, Hb), 11.1 (1H, s, Hc), 8.46 (1H, d, j=8.4. Hz, Hd), 7.96 (1H, d, j=8Hz, He), 7.57 (1H, t, j=7.2Hz, Hf), 7.13 (1H, t, j=7.6Hz, Hg), 2.42 (2H, t, j=7.2Hz, Hh), 2.29 (2H, t, j=7.6Hz, Hi), 1.82 (2H, m, Hj); Ms (m/z, %): 251 (M^+, 20)

N- benzyl -4- (3- benzyl -4- oxo -3, 4- dihydroquinazolin-2-yl) butanamide (11)

$C_{26}H_{25}O_2N_3$;Yield 5%; m.p. 138-139°C; white powder; FT-IR (KBr, cm^{-1}): 3288(N-H), 3064 & 3030 (C-H Ar), 2951 & 2868 (C-H Aliphatic), 1680 & 1635 (C=O); ^{1}HNMR (400 MHz-CDCl3): 8.2 (1H, d, d, j=1.2Hz, j=9.2Hz, Ha), 7.64 (1H, d, t, j=1.6Hz, j=8.4Hz, Hb), 7.46 (1H, d, j=8Hz, Hc), 7.4 (1H, t, j=7.6Hz, Hd), 7.27-7.17 (8H, m, He), 7.11 (2H, d, j=6.8Hz, Hf), 6.13 (1H, s, Hg), 5.38 (2H, s, Hh), 4.34 (2H, d, j=5.6Hz, Hi), 2.76 (2H, t, j=7.2Hz, Hj), 2.27 (2H, t, j=6.8Hz, Hk), 2.13-2.05 (2H, m, Hl); Ms (m/z, %): 411 (M^+, 76.5)

2-(3-Carboxyacrylamido) benzoic acid (13)

$C_{11}H_9O_5N$; Yield 85%; m.p. 172-173°C; cream powder; FH-IR (KBr, cm^{-1}): 3072 (C-H Ar), 2900-2600 (COOH), 1689 (C=O); ^{1}HNMR (400 MHz - DMSO): 12.5-13.8 (2H, s, Ha), 11.31 (1H, s, Hb), 8.465 (1H, d, j=8Hz, Hc), 7.99 (1H, d, j=6.8Hz, Hd), 7.6 (1H, t, j=7.2Hz, He), 7.2 (1H, t, j=8Hz, Hf), 6.6 (1H, d, j=12Hz, Hg), 6.3 (1H, d, j=12Hz, Hh); Ms (m/z, %): 234 (M-1, 100) Cytotoxic evaluation of the prepared compounds (**3**, **6**, **9**, and **13**) on HeLa cell line revealed that these compounds at tested concentrations could not reduce the viability of cells significantly as shown in Figs. 2 and 3. Combinations of these compounds at a concentration of 100 µM with doxorubicin (1 and 20 µM) could not improve the cytotoxicity of doxorubicin either (Fig. 4).

DISCUSSION

Acylchlorides are the most reported substrates for the first nucleophilic attack (8, 9), carried out on anthranilic acid in the production of 2-substituted quinazolinone derivatives. Here cyclic dicarboxylic anhydrides (i.e. succinic anhydride) served as substrate to produce 2-substituted derivatives having a reactive carbonyl group attached to the end of the side chain (compounds **3**, **9** and **13**). The carboxylic derivatives produced easily was precipitated due to their higher polarity than the starting anthranilic acid.

In the next step benzoxazinone was produced in the presence of acetic anhydride, through dehydrative cyclization mechanism. The resulted lactones are very unstable (14,15) and ready for reaction with any nucleophiles available in the media including water and primary amines. Reactions of proper aliphatic amines with benzoxazinone resulted in the production of desired quinazolinones as the final compounds (compounds **5**, **6**, **7** and **11**).

Direct amidification of flunky carboxylic end group as well as the production of quinazolinone ring is also possible when nucleophile is used in excess quantity as seen in compounds **5** and **11**.

The results of cytotoxic evaluation of compounds **3**, **6**, **9** and **13** on HeLa cell line did not show significant cytotoxic activities even at 500 µM concentration. Combination of these compounds at a concentration of 100 µM with doxorubicin (1 and 20 µM) could not also improve the cytotoxicity of doxorubicin.

Despite ample amount of information related to the cytotoxic effects of similar quinazolinone derivatives, the lack of activity observed here could be attributed to the low solubility of the studied compounds in RPMI medium or the nature of the compounds. It should be noted that some of the compounds produced here were not soluble enough to be evaluated for cytotoxic activity with routine procedures (compounds **5**, **7** and **11**).

CONCLUSION

The chemical procedure applied here, successfully resulted in the synthesis of desired compounds and a number of side

products, which were elucidated through routine analytical procedures. Synthesized compounds did not show considerable cytotoxicity on HeLa cells at tested concentrations. Other biological evaluations including cytotoxicity on other cell lines and antimicrobial activities seems beneficial due to the structural similarity of synthesized compounds with known active cytotoxic substances.

ACKNOWLEDGMENT

This research was supported by the Research Council of Isfahan University of Medical Sciences.

REFERENCES

1. Mhaske BS, Argade NP. The chemistry of recently isolated naturally occurring quinazolinone alkaloids. Tet Lett. 2006;62:9787-9826.
2. Connolly DJ, Cusack D, O'Sullivan TP, Guiry PJ. Synthesis of quinazolinones and quinazolines. Tet Lett. 2005;61:10153-10202.
3. Liu JF, Wilson ChJ, Ye P, Sprague K, Sargent K, Si Y, *et al*. Privileged structure based quinazolinone natural product-templated libraries: identification of novel tubulin polymerization inhibitors. Bioorg Med Chem Lett. 2006;16:686-690.
4. Liu JF, Ye P, Sprague K, Sargent K, Yohannes D, Baldino CM, *et al*. Novel one-pot total syntheses of deoxyvasicinone, mackinazolinone, isaindigotone, and their derivatives promoted by microwave irradiation. Org Lett. 2005;7:3363-3366.
5. Lamchouri F, Toufik H, Bouzzine SM, Hamidi M, Bouachrine M. Experimental and computational study of biological activities of alkaloids isolated from Peganum harmala seeds. J. Mater. Environ. sci. 2010;1:343-352.
6. Raffa D, Edler MC, Diadon G, Maggio B. Synthesis, cytotoxicity and inhibitory effect on tubulin polymerization of a new 3-hetrocyclo substituted 2-styryl quinazolinones. Eur J Med Chem. 2004;39:299-304.
7. Selvam P, Babu K, Padamraj R, persoons L, Clercq E. Synthesis, antiviral and cytotoxic activities of some novel 2-phenyl-3-disubstituted quinazolin-4(3H)-ones. Afr J Pharm Pharmacol. 2008; 2:110-115.
8. Abbas SE, Abdel Gawad NM, Georgey HH, Abdullah JH. New quinazolinone derivatives: synthesis, anti-inflammatory and antitumor activities. Chem Tech. 2010;2:1560-1578.
9. Chandrika PM, Yakiah T, Narsaiah B, Sridhar V, Venugopal G, Venkateshwara Rao J, *et al*. Synthesis leadingto novel 2, 4, 6-trisubstituted quinazolinone derivatives, their antibacterial and cytotoxic activity against THP-1, HL-60 and A375 cell lines.Indian J Chem. 2009;48:840-847.
10. Majeed Khan A, Anjum Sh, Choudhary MI, Atta-ur-Rahman. Synthesis of bioactive succinylanthranilic acid ester and its analogues. World J Chem. 2006;1:30-32.
11. Hall IH, Izydore RA, Warren AE, Barnes CR. Cytotoxicity and mode of action of aliphatic dicarboxylic acids in L1210 lymphocytic leukemia cells. Anticancer Res. 1999;19:205-211.
12. Jantova S, Cipak L, Slamenova D, Horvath V, Rauko P. Induction of cytotoxicity and ssDNA breaks by 9-bromo-5morpholinotetrazolo[1,5-c] quinazoline in tumor cells cultured in vitro. Toxicol in Vitro. 2003;17:457-463.
13. Kamal A, Bharathi EV, Ramaiah MJ, Dastagiri D, Reddy JS, Viswanath A, *et al*. Quinazolinone linked pyrrolo[2,1-c][1,4]benzodiazepine (PBD) conjugates: Design, synthesis and biological evaluation as potential anticancer agents. Bioorg Med Chem Lett. 2010;18:526-542.
14. Madkour HMF. Reactivity of 4H-3, 1-benzoxazin-4-ones towards nitrogen and carbon nucleophilic reagents: applications to the synthesis of new heterocycles. Arkivoc J. 2004;i:36-54.
15. Nagar AA, Rathi LG, Chugh N, Pise VJ, Bendale A. Microwave assisted one pot synthesis of 2, 3-di-substituted Quinazolin-4-(3h)-ones and their potential biological activity. Der Pharma Chemica. 2010;2:37-43.