Synthesis and Biological Evaluation of New Quinazolinone Derivatives

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Abstract

The paper presents a simple and efficient synthesis of a series of new quinazolinone derivatives 8a-h. First, the reaction of 5-hydroxyanthranilic acid (6) with acetic anhydride at 160–180ºC for 2 h gave the intermediate 7 in high yield. This intermediate was then reacted with amines in acetic acid at 180 ºC for 14 h afforded new quinazolinone derivatives 8a-h in 77–92%. Synthesized compounds were structurally confirmed using spectroscopic methods: ^{1}H, $^{13}CNMR$ and mass spectrum. The bioassay result using three cancer cell lines including SKLU-1 (lung cancer), MCF-7 (breast cancer) and HepG-2 (liver cancer) showed that only compound 8h exhibited significant cytotoxic effect against cancer cell lines tested with IC_{50} values of 23.09, 27.75 and 30.19 µg/ mL, respectively

Keywords: Quinazolinone, cytotoxic, cancer

1. Introduction

There is absolutely no doubt that cancer continues to be a major health problem in developing as well as underdeveloped countries. Although the extensive research and rapid progress in cancer chemotherapy has been made over the past several decades, the cancer burden remains substantial with more than 1.6 million newly diagnosed cases and 600,000 deaths estimated to occur in 2017 [1, 2] in the United States. The main reasons for this could be the drug resistance and adverse side effects of the chemotherapy [3]. In order to develop more effective and reliable anticancer agents that overcome these limitations, the search for novel antitumor agents is now urgent.

For the past few years, there has been an increasing interest in the development and pharmacology of heteroaromatic organic compounds [4-6]. Noticeably, among these structures, quinazolinone constitutes an important class of pharmacophores in medicinal chemistry because of their potential in H bonding and π–π stacking interactions with aromatic amino acid residues of receptors [7] and is considered to be the basic framework of biologically active compounds that exist in a number of drug molecules and biologically active compounds. Indeed, several quinazolinone derivatives (1-5) have been reported to exhibit various types of pharmacological activities, including anticancer [8], antioxidant [9], antiviral [10], anticonvulsant [11], anti-inflammatory [12], antitubercular [13], anti-HIV [14], and so on. Furthermore, quinazolinone and their derivatives have been found to display several benefits over the agents that are clinically used [15] and closely connected to the anti-cancer therapies [16, 17]. Some quinazolinone derivatives were proved substantial in treating human leukemia than the conventional agents and showed the significant effect of quinazolinones derivatives against breast cancer cell lines [18-21].

2. Experiments

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). Nuclear magnetic resonance spectra (1H and 13C NMR) were recorded using tetramethylsilane (TMS) as an internal standard on a Bruker 500 MHz spectrometer with CD3OD, 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). ESI-MS spectra were recorded on FTICR MS Varian. Reagents and solvents were purchased from Aldrich or Fluka Chemical Corp. (Milwaukee, WI, USA) or Merck unless noted otherwise. Solvents were distilled and dried before use.

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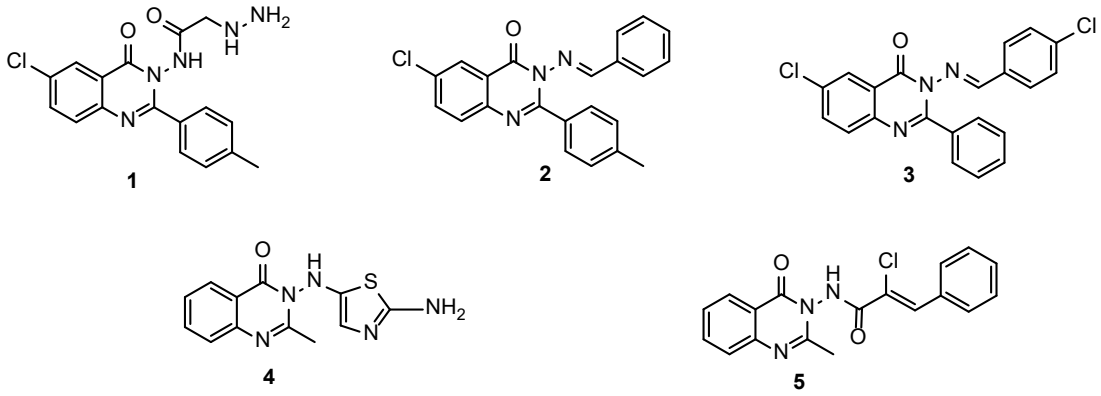


Fig. 1. Several reported quinazolinone derivatives as anticancer agents [8]

The in vitro cytotoxic evaluation was undertaken according to the described protocol. Briefly, the stock solution of the target compounds were prepared in dimethylsulfoxide (DMSO) at a concentration of 1 mg/mL, followed by dilution to obtain solution at concentration 100µg/mL which were serially diluted further for the bioassay on 96-well plates. The determination of IC_{50} was carried out using three cancer cell lines: Hep-G2, SK LU-1, and MCF-7 with ellipticine as a positive control. The IC_{50} values were determined from dose-dependent curve plotted from five different concentration regimens (0-100 µg/ml). At each regimen, mean of triplicate experiment was used for a point in the curve.

Synthesis of 6-hydroxy-2methyl-4H-benzo[d][1,3]oxazin-4-one (7)

A mixture of 5-hydroxy anthranilic acid (**6**) (5.0g, 32.67mmol) in acetic anhydride (15ml) was refluxed at 150°C for 2 h. The mixture was then poured in ice-water. The resulting precipitates were filtered, washed with distilled water and dried in vacuum to afford **7** (5.03 g, 87%); R_f = 0.54 (*n*-hexane: ethyl acetate = 7 : 3) which was used for next step [22].

General procedure for the synthesis of 8a-h

A mixture of **7** (1.0 g, 5.64 mmol) and primary amines (3 eq) in acetic acid (10 mL) was refluxed at 120°C 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 obtain the corresponding residues which was subjected to column chromatography on silica gel using *n*-hexane/ethyl acetate as eluting systems to give desired 8a-h.

3-Cyclopropyl-6-hydroxy-2-methylquinazolin-4(3H)-one (8a): Yellow solid; Yield: 88%; Mp: 243-244°C; R_f = 0.57 (*n*-hexane : ethyl acetate = 1 : 1); ^{1}H NMR (500 MHz, DMSO-d6, δ (ppm)): 7.87 (d, *J* = 3.0 Hz, 1H), 7.52-7.50 (d, *J* = 9.0 Hz, 1H), 7.29-7.27 (d, *J* = 3.0 Hz, 9.0 Hz, 1H), 2.96 (m, 1H), 2.71 (s, 3H, CH_3), 1.33 (m, 2H), 0.95 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d6, δ (ppm)): 163.44, 155.25, 153.75, 141.17, 128.18, 124.03, 121.78, 110.18, 27.79, 23.14, 10.40. ESI-MS m/z: 217.4 $[M+H]^+$.

6-Hydroxy-3-(2-methoxyphenyl)-2-methylquinazolin-4(3H)-one (8b): White solid; Yield: 88%; Mp: 156-157°C; R_f = 0.50 (*n*-hexane : ethyl acetate = 1 : 1); ^{1}H NMR (500 MHz, DMSO-d6, δ (ppm)): 10.31 (brs, 1H, OH), 7.52-7.48 (m, 2H), 7.38 (d, *J* = 2.5 Hz, 1H), 7.35 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H), 7.29 (dd, *J* = 2.5 Hz, 8.50 Hz, 1H), 7.25 (d, *J* = 8.50 Hz, 1H), 7.11 (t, *J* = 7.5 Hz, 1H), 3.76 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d6, δ (ppm)): 160.62, 155.83, 154.22, 151.28, 140.55, 130.58, 129.59, 128.22, 126.13, 123.89, 121.22, 120.95, 112.44, 109.13, 55.71, 22.72. ESI-MS m/z: 283.2 $[M+H]^+$.

6-Hydroxy-3-(3-methoxyphenyl)-2-methylquinazolin-4(3H)-one (8c): White solid; Yield: 92%; R_f = 0.49 (*n*-hexane: ethyl acetate = 1:1); ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.59 (d, *J* = 9.0 Hz, 1H), 7.52-7.49 (m, 2H), 7.35 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H), 7.13 (dd, *J* = 6.0 Hz, 8.5 Hz, 1H), 6.98 (t, *J* = 7.0 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 3.87 (s, 3H, OCH_3), 2.25 (s, 3H, CH_3). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 162.73, 162.47, 157.96, 153.47, 141.99, 140.18, 131.71, 128.90, 125.59, 122.68, 121.35, 116.31, 115.04, 110.57, 56.11, 23.50. ESI-MS m/z: 283.2 $[M+H]^+$.

6-Hydroxy-3-(3-methoxyphenyl)-2-methylquinazolin-4(3H)-one (8c): White solid; Yield: 92%; Rf = 0.49 (*n*-hexane: ethyl acetate=1:1); ^{1}H NMR (500 MHz, CD3OD, δ (ppm)): 7.59 (d, *J* = 9.0 Hz, 1H), 7.52-7.49 (m, 2H), 7.35 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H), 7.13 (dd, *J* = 6.0 Hz, 8.5 Hz, 1H), 6.98 (t, *J* = 7.0 Hz, 1H), 6.94 (d, *J* = 8.5 Hz,1H),

3.87 (s, 3H, OCH_3), 2.25 (s, 3H, CH_3). 13C NMR (125 MHz, CD3OD, δ (ppm)): 162.7, 162.5, 157.9, 153.5, 142.0, 140.2, 131.7, 128.9, 125.6, 122.7, 121.4, 116.3, 115.0, 110.6, 56.1, 23.5. ESI-MS m/z: 283.2 $[M+H]^+$.

6-Hydroxy-3-(4-methoxyphenyl)-2-methylquinazolin-4(3H)-one (8d): White solid (known compound) [22]; Yield: 79%; Mp: 263-264°C; R_f = 0.45 (*n*-hexane : ethyl acetate = 1 : 1); ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.58 (d, *J* = 9.0 Hz, 1H), 7.51 (d, *J* = 2.50 Hz, 1H), 7.35 (dd, *J* = 2.50 Hz, 9.0 Hz, 1H), 7.28 (d, *J* = 8.50 Hz, 2H), 7.14 (d, *J* = 8.50 Hz, 2H), 3.90 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 164.07, 161.73, 157.93, 154.08, 141.97, 131.57, 130.42, 128.86, 125.55, 122.67, 116.13, 110.58, 56.09, 23.74. ESI-MS m/z: 283.2 $[M+H]^+$.

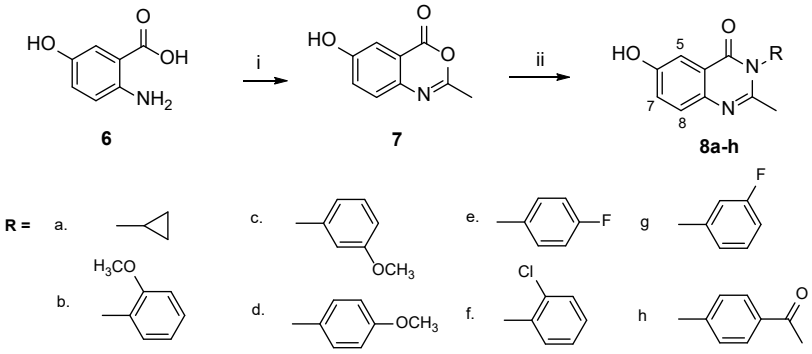
3-(4-Fluorophenyl)-6-hydroxy-2-methylquinazolin-4(3H)-one (8e): Bright yellow solid; 177-178°C; Yield: 82%; R_f = 0.51 (*n*-hexane : ethyl acetate = 1 : 1); ^{1}H NMR (500 MHz, DMSO-d6, δ (ppm)): 7.57 (d, *J* = 9.0 Hz, 1H, H-8), 7.43 (s, *J* = 3.0 Hz, 1H, H-5), 7.42-7.41 (dd, *J* = 3.0 Hz, 9.0 Hz, 2H), 7.36-7.32 (m, 3H), 4.83 (s, 2H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d6, δ (ppm)): 165.29, 163.84, 163.32, 157.99, 141.93, 135.24, 131.67, 128.95, 125.60, 122.59, 117.87, 117.68, 110.58, 23.74. ESI-MS m/z: 271.5 $[M+H]^+$.

3-(2-Chlorophenyl)-6-hydroxy-2-methylquinazolin-4(3H)-one (8f): White solid; Yield: 81%; Mp: 299-300 °C; R_f = 0.47 (*n*-hexane : ethyl acetate = 1 : 1); ^{1}H NMR (500 MHz, DMSO-d6, δ (ppm)): 7.73-7.71 (m, 1H), 7.61-7.57 (m, 3H), 7.55-7.52 (m, 2H), 7.38 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H), 2.16 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d6, δ (ppm)): 163.00, 158.15, 152.75, 141.97, 136.60, 133.49, 132.35, 131.71, 129.84, 129.13, 125.79, 122.47, 110.65, 22.98. ESI-MS m/z: 287.4 $[M+H]^+$.

3-(3-Fluorophenyl)-6-hydroxy-2-methylquinazolin-4(3H)-one (8g): White solid; Yield: 83%; R_f = 0.54 (*n*-hexane : ethyl acetate = 1:1); ^{1}H NMR (500 MHz, CD_3OD, δ (ppm)): 7.65-7.61 (m, 1H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.50 (d, *J* = 3.0 Hz, 1H), 7.35-7.32 (m, 2H), 7.28-7.25 (m, 1H), 7.24-7.22 (m, 1H), 3.25 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD, δ (ppm)): 165.6, 163.7, 158.0, 153.0, 141.9, 140.7, 132.5, 129.0, 125.7, 125.6, 122.6, 117.5, 117.1, 110.6, 23.6. ESI-MS m/z: 271.5 $[M+H]^+$.

3- (4-Acetylphenyl)-6-hydroxy-2-methylquinazolin-4(3H)-one (8h):

White solid; Yield: 77%; Mp: 247-248°C; R_f = 0.53 (*n*-hexane : ethyl acetate = 1 : 1); ^{1}H NMR (500 MHz, DMSO-d6, 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), 7.40 (d, *J* = 3.0 Hz, 1H), 7.30 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H), 2.65 (s, 3H, CH_3), 2.08 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d6, δ (ppm)): 197.34, 170.27, 160.97, 155.94, 150.20, 142.12, 140.50, 136.96, 129.35, 129.03, 128.31, 124.01, 121.21, 109.12, 26.83, 23.56. ESI-MS m/z: 295.6 $[M+H]^+$.



Scheme. 1. Reagents and conditions: (i) $(CH_3CO)_2O$, 160–180°C, 2 h; (ii) acetic acid, amines, 180°C, 14h, 77–92%.

3. Results and discussion

Novel quinazolinone derivatives 8a-h were synthesized as outlined in Scheme 1. 6-hydroxyanthranilic acid (6) was first condensed with the excess of acetic anhydride at 160°C for 2 h to afford the desired benzoxazinone 7 in 87% yield. The purification of compound 7 was simply carried out by pouring the reaction mixture into the ice-water. The resulting precipitates was filtered, washed with distilled water, and dried in vacuum. Compound 7 was next coupled with amines to give target compounds 8a–h in good to excellent yields. All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR. Due to the structural similarity of target compounds, compound 8a was used as an

example to elucidate the structure of synthesized compounds. In the ^{1}H NMR spectrum, the characteristic splitting pattern of 3 protons H-5, H-7 and H-8 as ABC system was easily observed. The proton H-5 of quinazolinone skeleton resonates at the lowest field as a doublet at δ 7.87 (*d*, *J* = 3.0 Hz), resulting from long coupling with H-7. At the lower field, the proton H-8 resonates as a doublet at δ 7.50 (*J* = 9.0 Hz) due to near coupling with H-7. The proton H-7 was observed as a doublet of doublet at δ 7.29 (*d*, *J* = 3.0 Hz, 9.0 Hz). The cyclopropyl side chain in the molecule was confirmed via the presence of 5 protons in which the proton connecting to tertiary carbon resonates at δ 2.96 ppm as a multiplet, and 4 other protons resonate at δ 1.33 and 0.95 ppm as multiplets. Finally, the strong single signal at δ 2.71 ppm was assigned to the only methyl group of quinazolinone skeleton. In the ^{13}C NMR spectrum, the carbonyl signal was observed at δ 163.44 ppm. The signal at δ 153.75 ppm was attributed to C=N group. Four aromatic carbons resonate at δ 110.18 -155.25 ppm. The methyl of quinazolinone resonates at 23.14 ppm, and three carbons of the cyclopropyl chain at 27.79 and 10.4 ppm.

Table 1. *In vitro* cytotoxic activity of quinazolinone derivatives **8a-h**

No	Compounds	R	IC_{50} (µg/mL)		
			SK-LU-1	**MCF-7**	**HepG2**
1	**8a**	Cycloropyl	>100	>100	>100
2	**8b**	2-Methoxyphenyl	>100	>100	>100
3	**8c**	3-Methoxyphenyl	>100	>100	>100
4	**8d**	4-Methoxyphenyl	>100	>100	>100
5	**8e**	4-Fluorophenyl	>100	>100	>100
6	**8f**	2-Chlorophenyl	>100	>100	>100
7	**8g**	4-Fluorophenyl	>100	>100	>100
8	**8h**	4-Acetophenyl	**23.09±2.07**	**27.75±1.94**	**30.19±0.02**
	Ellipticine		**0.43**	**0.43**	**0.40**

[a]Concentration (g/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: SKLU-1 (lung cancer), MCF-7 (breast cancer), HepG-2 (liver cancer).

All target compounds 8a-h were evaluated for their *in vitro* cytotoxicity. Three human cancer cell lines including SKLU-1 (lung cancer), MCF-7 (breast cancer), and HepG2 (liver cancer and were chosen for screening their inhibition effect using SRB method [23]. As shown in Table 1, most of the quiniazolinone derivatives were inactive against three cancer cell lines tested except compound 8h showing cytotoxic effect with IC_{50} values of 23.09, 27.75 and 30.19 µg/mL, respectively.

4. Conclusion

We have reported a series of new quinazolinone derivatives 8a-h. The structure of all synthesized compound has been confirmed based on ^{1}H and ^{13}C NMR and ESI-MS. Among synthesized compounds, compound 8h displayed cytotoxic effect against SKLU-1, MCF-7 and HepG2 with IC_{50} values of 23.09, 27.75 and 30.19 µg/mL, respectively, suggesting that it could be served as basics for further design of antitumor agents of this quinazolinone class.

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References

[1] Siegel RL, Miller KD, Jemal A. Cancer Statistics. CA Cancer J Clin., 67 (2017), 7- 30.

[2] Robert A. Smith; Kimberly S. Andrews; Durado Brooks; Stacey A. Fedewa; Deana Manassaram-Baptiste; Debbie Saslow; Otis W. Brawley; Richard C. Wender. Cancer Screening in the United States, 2018: A Review of Current American Cancer Society Guidelines and Current Issues in Cancer Screening. CA Cancer J Clin., 68 (2018), 297–316.

[3] Mellinghoff, I. K.; Sawyers, C. L. The emergence of resistance to targeted cancer therapeutics. Pharmacogenomics., 3 (2002), 603- 623.

[4] Welsch ME, Snyder SA, Stockwell BR. Privileged scaffolds for library design and drug discovery. Curr Opin Chem Biol., 14 (2010), 1–15.

[5] Asif M. Various chemical and biological activities of pyridazinone derivatives. Cent Eur J Exp Biol., 5 (2017), 1–19.

[6] Nikaljea AP, Bahetia K. Computer based drug design of various heterocyclic compounds having anticancer activity: a brief review. J Bioinform Genom Proteom., 2 (2017),1–13.

[7] Yadav MR, Naik PP, Gandhi HP, Chauhan BS, Giridhar R. Design and synthesis of 6,7-dimethoxyquinazoline analogs as multi-targeted ligands for α1- and aII-receptors antagonism. Bioorg Med Chem Lett., 23 (2013), 3959–3966.

[8] (a). Shetha, A. & Wijdan, I.A. Synthesis and characterization of new quinazoline–4(3H)-one Schiff bases. J Chem Pharm Res., 5 (2013), 42–45; (b). Amer M. Alanazi, Alaa A.-M. Abdel-Aziz, Ibrahim A. Al-Suwaidan, Sami G. Abdel-Hamide, Taghreed Z. Shawer, Adel S. El-Azab. Design, synthesis and biological evaluation of some novel substituted quinazolines as antitumor agents. Eur. J. Med. Chem., 79 (2014), 446-454; (c) Malleshappa N. Noolvi Harun M. Patel. Synthesis, method optimization, anticancer activity of 2,3,7-trisubstituted Quinazoline derivatives and targeting EGFR-tyrosine kinase by rational approach: 1st Cancer Update. Arab.J.Chem., 6 (2013), 35-48; (d). D. H. Fleita, R. M. Mohareb, O. K. Sakka. Antitumor and antileishmanial evaluation of novel heterocycles derived from quinazoline scaffold: a molecular modeling approach. Med. Chem. Res., 22 (2013), 2207-2221.

[9] Zaranappa et al. Synthesis and Antioxidant Activity of 3-Substituted Schiff bases of Quinazoline-2,4-diones. Int J Chem Tech Res., 4 (2012), 1527–1533.

[10] Krishnan, S.K. et al. Synthesis, antiviral and cytotoxic investigation of 2-phenyl-3-substituted quinazolin-4(3H)-ones. Eur Rev Med Pharm Sci., 15 (2011), 673–681.

[11] Patel, N.B. et al. Synthesis and microbial studies of (4-oxo-thiazolidinyl) sulfonamides bearing quinazolin-4(3h) ones. Acta Polo Pharm Drug Res., 67 (2010), 267–275.

[12] Saravanan, G., Pannerselvam, P. & Prakash, C.R. Synthesis, analgesic and anti-inflammatory screening of novel Schiff bases of 3-amino-2-methyl quinazolin 4-(3H)-one. Der Pharmacia Lett., 2 (2010), 216–226.

[13] Abid, O.H. & Ahmed, A.H. Synthesis and characterization of novel quinazoline derivatives via reaction of isatoic anhydride with schiff's base. Inter J Appl Nat Sci., 2 (2013), 11–20.

[14] Pati, B. & Banerjee, S. Quinazolines: an illustrated review. JAdv Pharm Edu Res., 3 (2013), 136–151.

[15] Katrin, S.N. Chemotherapy and Dietary Phytochemical Agents. Chem ther Res Prac., 3 (2012), 22–27.

[16] Manasa, A.K., Sidhaye, R.V., Radhika, G. & Nalini, C.N. Synthesis, antioxidant and anticancer activity of quinazoline derivatives. Current Pharma Research., 1 (2011), 101–105.

[17] Nerkar, B., Saxena, A., Ghone, S. & Thakeri, A.K. In Silico Screening, Synthesis and In Vitro Evaluation of Some Quinazolinone and Pyridine Derivatives as Dihydrofolate Reductase Inhibitors for Anticancer Activity. E-Journal of Chem., 6 (2009), 97–102.

[18] Danilov, A.V. Targeted therapy in chronic lymphocytic leukemia: past, present, and future. Clin Ther., 35 (2013), 1258–1270.

[19] Ahmed, M. F. & Youns, M. Synthesis and Biological Evaluation of a Novel Series of 6, 8-Dibromo-4 (3H) quinazolinone Derivatives as Anticancer Agents. Archiv der Pharmazie., 346 (2013), 610–617.

[20] Kumar, D. Design, synthesis, and cytotoxic evaluation of novel imidazolone fused quinazolinone derivatives. Arabian J. Chem. doi: 10.1016/j.arabjc.2014.07.001 (2014).

[21] Faraj, F.L. et al. Synthesis, Characterization, and Anticancer Activity of New Quinazoline Derivatives against MCF-7 Cells. Sci. Wld J. 212096; doi: 10.1155/2014/212096 (2014).

[22] Ahmed. K., E.V Bharathi., M. J Ramaiah., D. Dastagiri., J. S Reddy., A.Viswanath., FarheenSultana., S. N. C.V. L. Pushpavalli., ManikaPal-Bhadra., Hemant. K S., G. N Sastry., Aarti J., Subrata Sen., Surekha Zingde. Quinazolinone linked pyrrolo[2,1-c] [1,4] benzodiazepine (PBD) conjugates: Design, synthesis and biological evaluation as potential anticancer agents. Bioorg. Med. Chem., 18 (2010), 526-542.

[23] Scudiero, D. A., Shoemaker, R.H., Paull, K.D., Monks, A., Tierney, S., Nofziger, T.H., Currens, M. J., Seniff, D., Boyd, M. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res., 48 (1988) 4827–4833.