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Synthesis, Molecular Docking and Biological Evaluation of Some Quinazolinone Analogs

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ORIGINAL ARTICLE

Synthesis, Molecular Docking and Biological Evaluation of Some Quinazolinone Analogs

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ABSTRACT

Based on molecular docking studies eight molecules were selected for synthesis. These eight novel quinazolinone derivatives were synthesized and characterized by spectral analysis. These derivatives were screened for antioxidant activity, anticancer activity and toxicity studies. Molecular docking of the derivatives was performed using vLife MDS® 4.2 with Epidermal Growth Factor Receptor kinase domain (PDB ID: 1M17) as a target. Docked compounds were analyzed for molecular interactions. Analog **BSQ1** was found to have highest dock score (-76.56) and the binding interactions of all the other compounds were found to be favourable. Analog **BSQ1**exhibited mild antioxidant activity (IC_{50} 175 µg/ml), when compared with ascorbic acid (IC_{50}8.71 µg/ml). Analog **BSQ8** showed good anticancer activity on HCT-116 cell line (IC_{50} 11.6 µM) and **BSQ1**also showed good anticancer activity against MCF-7 cell line (IC_{50} 31.6 µM) which was compared to the positive control doxorubicin.

Keywords: Antioxidant, Anticancer, MTT assay, DPPH method, vLife MDS®

INTRODUCTION

Cancer is still a major health problem and feared disease in the world today. It is feared because it is difficult to cure. However since the past decade, a dramatic shift has occurred in cancer therapy. Despite serious side effects of existing anticancer drugs, the regular method of chemotherapy still remains the treatment of choice for many types of cancer, which include breast, colorectal, lung and pancreatic cancers as well as lymphoma, leukaemia and multiple myeloma[1]. Hence it is a major challenge to design new drugs that will be more selective for cancer cells with lesser side effects. Since the last fifty years more than hundreds of natural and synthetic chemical entities have been tested for anticancer activity, out of which only about 25 are in wide use today. This indicates how difficult this problem is[2].

Literature review has revealed that Quinazolinone derivatives are widely used in pharmaceutical industry, medicine and in agriculture due to their enormous biological activity. The quinazolinone moiety is versatile nitrogen containing heterocyclic compound and backbone for nearly 150 naturally occurring alkaloids and drugs. Derivatives of Quinazolinon-4(3H)-ones are of considerable interest because of the diverse range of their biological and pharmacological activities like antibacterial[3], anti-HIV[4], anti-tubercular[5], anticancer[6], anti-inflammatory[7], antiviral[8], antifungal[9], analgesic[10], antihistaminic[11], antiulcer[12], anticonvulsant[13], CNS depressant[14], antioxidant[15], anti-malarial[16], antihypertensive[17]etc. This work is a continuation of our research on Quinazolinon-4(3H)-ones. In our present communication, we describe the synthesis, docking and the evaluation of antioxidant activity by DPPH method and anticancer activity by MTT assay of someQuinazolinon-4(3H)-ones derivatives. As the derivatives were selected based on the best docking scores and their novelty, we were able to choose very few compounds for synthesis. Only eight derivatives were able to synthesise as they were not reported for anticancer activity. Antioxidant activity was carried out as there is growing evidence that reactive oxygen species are involved in the aetiology of fat-related neoplasms such as cancer of the breast and colorectum due to continued oxidative stress. The cell lines selected for the study are breast cancer cell lines, MCF-7 and Human colon epithelium cancer cell line, HCT-116.

MATERIALS AND METHODS

Materials

The solvents, reagents and chemicals used in the present work were purchased from Sigma Aldrich, E. Merck, Spectrochem, and S.D.Fine Chem., Hi-Media and used

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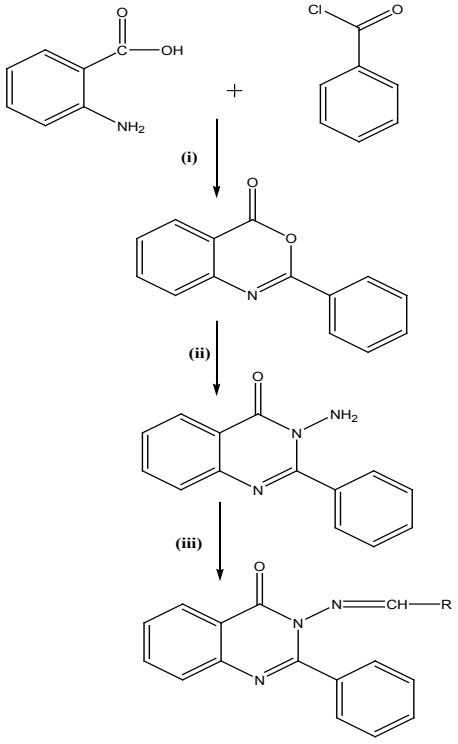
without further purification. TLC was used to check the purity of the synthesized compounds by using silica gel 60 F254 (E. Merck) aluminum plates. Melting points was determined using laboratory melting point apparatus (Toshniwal P. Ltd.) and were uncorrected. IR spectra of the synthesized compounds were recorded on FT-IRAffinity-1 (Shimadzu) IR Spectrometer. Mass spectra were recorded on GC-MS-QP5050*A* (Shimadzu). NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-*d*6 as the solvent.

Molecular Docking Studies

Molecular docking was done by GRIP batch docking method using vlife MDS® 4.2. The crystal structure of Epidermal Growth Factor Receptor kinase domain (PDB ID: 1M17) for anticancer docking studies was obtained from the protein data bank[18, 19].The parameter fixed for docking simulation was - number of placements: 50, rotation angle: 10º, ligand flexible, exhaustive method, scoring function: dock score. The ligand forming most stable drug-receptor complex was the one which was having minimum dock score. After docking simulation, the best docked conformer of each ligand was checked for its various interactions with receptor like hydrogen bonding, hydrophobic bonding and vander Waal's interaction. Dock score of the compounds are listed in Table 2.

Synthesis of quinazolinone derivatives

Scheme for the synthesis of Quinazolinone derivatives



Reagents and Conditions: (i) Pyridine, stir at 0-8^{0}C 1 hour, then at room temp 3-4 hour; (ii) Hydrazine hydrate (2 equiv), Pyridine, reflux (120-130^{0}C), 5 hours; (iii) Substituted aromatic aldehyde (1 equiv), ethanol, glacial acetic acid, reflux (80-90^{0}C), 10 hours.

Synthesis of 2-phenyl-3,1-benzoxazin-4(3*H*)-one[20]

Anthranilic acid (0.1mol) was solubilised in 25 ml of dry pyridine. Benzoyl chloride (0.1mol) was added dropwise with constant mixing and maintaining temperature of 8^{0}C for one hour. The reaction mixture was mixed for another 5 hours at room temperature. The reaction was watched over TLC. The solid obtained was poured into crushed ice and treated with sodium bicarbonate solution (20%) to remove the unreacted acid. Once the effervescence reduced, the reaction mixture was filtered and washed with water to remove inorganic materials and recrystallized from ethanol.

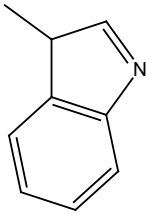
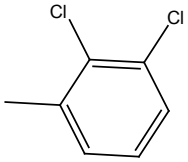
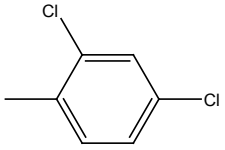
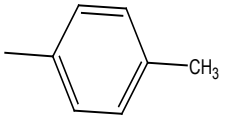
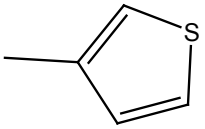
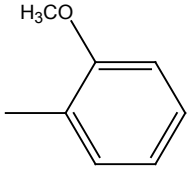
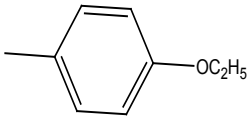
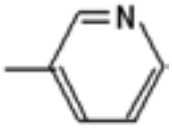
Synthesis of 3-Amino-2-phenyl-quinazolin-4(3H)-one

2-phenyl-3, 1-benzoxazin-4(3*H*)-one (0.05mol) was dissolved in anhydrous pyridine (30ml). To this, hydrazine hydrate 80% (0.1mol) was added drop wise in excess and refluxed for 8 hours at temperature of 120-130^{0}C under anhydrous reaction conditions . The reaction was watched over by TLC. When reaction was complete, the mixture was cooled to room temperature and poured into ice cold water containing dilute hydrochloric acid. The product was filtered off, rinsed repeatedly with water, dried and recrystallized from ethanol. Melting point was found to be 180-182^{0}C.

Synthesis of 3-(amino substituted arylidene)-2-phenyl-quinazolin-4(3H)-one

3-Amino-2-phenyl-quinazolin-4(3H)-one (0.01) was dissolved in ethanol. Ethanolic solution (20ml) of aromatic aldehyde (0.01mol) was added to the quinazolinone solution and the pH of the resulting solution was adjusted to 4.0-4.5 using glacial acetic acid. This mixture was refluxed for 10-12 hours at 110-120^{0}C. The reaction was monitored by TLC. When the reaction was complete, the mixture was cooled to room temperature and then poured into ice cold water. The separated solid was filtered, rinsed with water and recrystallized from ethanol.Yields and physical characteristics are listed in Table 1.

Table 1: Physical data of the quinazolinone derivatives

Compound	R	Mol. Formula	% Yield	Mt.pt (°C)	RfValue
BSQ1		$C_{23}H_{16}N_4O$	80.96	252-254	0.68
BSQ2		$C_{21}H_{13}Cl_2N_3O$	59.69	230-234	0.84
BSQ3		$C_{21}H_{13}Cl_2N_3O$	64.21	230-233	0.87
BSQ4		$C_{22}H_{17}N_3O$	65.59	206-209	0.83
BSQ5		$C_{19}H_{13}N_3OS$	60.95	226-228	0.9
BSQ6		$C_{22}H_{17}N_3O_2$	66.61	240-242	0.8
BSQ7		$C_{23}H_{19}N_3O_2$	84.80	218-222	0.82
BSQ8		$C_{20}H_{14}N_4O$	58.12	182-185	0.64

Spectral data of quinazolinone derivatives

3-[(3H-Indol-3-ylmethylene)-amino]-2-phenyl-3H-quinazolin-4-one (BSQ1): FTIR (KBr, cm^{-1}): 3253.91 (-NH), 3062.96 (C-H, Ar-H), 2924.09 (C-H, Ali-H), 1658.78 (C=O,), 1622.13 (C=N), 1519.91 (C=C); ^{1}H NMR (400MHz, DMSO-d6): δ = 7.193- 8.635(14H, Ar-H), 11.67(s, 1H, H–C=N), 11.83(1H, NH); GCMS (EI, m/z): 364(M^+).

3-[(2,3-Dichloro-benzylidene)-amino]-2-phenyl-3H-quinazolin-4-one (BSQ2): FTIR (KBr, cm^{-1}):3329.14 (-NH),590.22 (C-Cl), 3055.24 (C-H, Ar-H), 2956.87 (C-H, Ali-H), 1653.00 (C=O,), 1600.92 (C=N), 1525.69 (C=C);^{1}H NMR (400MHz, DMSO-d6): δ =7.288- 8.904(14H, Ar-H), 11.85(s, 1H, H–C=N) 11.83 (1H, NH); GCMS (EI, m/z): 393(M^+).

3-[(2,4-Dichloro-benzylidene)-amino]-2-phenyl-3H-quinazolin-4-one (BSQ3): FTIR (KBr, cm^{-1}):3217.27 (-NH), 586.88 (C-Cl), 3130.47 (C-H, Ar-H), 2983.88 (C-H, Ali-H), 1654.92 (C=O,), 1604.77 (C=N), 1531.48 (C=C);GCMS (EI, m/z): 393(M^+).

3-[(4-Methyl-benzylidene)-amino]-2-phenyl-3H-quinazolin-4-one (BSQ4): FTIR (KBr, cm^{-1}):3317.55 (-NH), 3062.96 (C-H, Ar-H), 2916.37 (C-H, Ali-H), 1661.07 (C=O,), 1602.86 (C=N), 1537.27 (C=C);GCMS (EI, m/z): 339(M^+).

2-Phenyl-3-[(thiophen-3-ylmethylene)-amino]-3H-quinazolin-4-one (BSQ5): FTIR (KBr, cm^{-1}): 3288.63 (-NH), 1178.51 (C-S), 3070.68 (C-H, Ar-H), 2881.65 (C-H, Ali-H), 1658.78 (C=O,), 1647.21 (C=N), 1591.27 (C=C);GCMS (EI, m/z): 331(M^+).

3-[(2-Methoxy-benzylidene)-amino]-2-phenyl-3H-quinazolin-4-one (BSQ6): IR (KBr, cm^{-1}):3233.48 (-NH), 1178.51 (C-S), 3070.68 (C-H, Ar-H), 2881.65 (C-H, Ali-H), 1658.78 (C=O,), 1647.21 (C=N), 1591.27 (C=C);GCMS (EI, m/z): 355(M^+).

3-[(4-Ethoxy-benzylidene)-amino]-2-phenyl-3H-quinazolin-4-one (BSQ7):IR (KBr, cm^{-1}):3062.96 (-NH), 2976.16 (C-H, Ar-H), 2893.22 (C-H, Ali-H), 1670.35 (C=O,), 1606.70 (C=N), 1554.63 (C=C);GCMS (EI, m/z): 369(M^+).

2-Phenyl-3-[(pyridin-3-ylmethylene)-amino]-3H-quinazolin-4-one (BSQ8): IR (KBr, cm^{-1}): 3057.17 (C-H, Ar-H), 2926.01 (C-H, Ali-H), 1645.28 (C=O), 1604.77 (C=N), 1581.63 (C=C),^{1}H NMR (400MHz, DMSO-d6): δ =7.228- 8.561(12H, Ar-H), 8.904 (s, 1H, H–C=N) 11.87 (1H, CNH); GCMS (EI, m/z):326.12(M^+).

BIOLOGICAL ACTIVITY

Antioxidant Activity

Antioxidant activity of the synthesized compounds was carried out by diphenylpicrylhydrazyl (DPPH) radical scavenging assay method[21]. This assay method involved the use of 96 well microtitre plate in which 100µl of test sample (the synthesised compounds) and standard solution (Ascorbic acid) was added to each well separately and in triplicates. Next, 100µl of DPPH solution was added to each well. Control wells were loaded with 100µl each of DMSO and DPPH. Sample blank and control blank were also performed. The plates were incubated at 37°C for 30 minutes without exposing to light and the absorbance of each solution was recorded with ELISA reader using 540 nm filters. The percentage scavenging of test samples at each concentration was calculated using the following formula:

[(Control absorbance – Test absorbance) / Control absorbance] X 100

The IC50 was calculated using the Microsoft excel.

Anticancer Activity

The cytotoxic effect of test samples on cancer cell lines (MCF-7 and HCT-116) was determined by MTT assay method[22]. In brief, exponentially growing cells (1 × 104 cells / well) were plated in 96 well micro titre plates and allowed to adhere for 24 hour prior to the addition of test samples. These samples were dissolved in 0.1% DMSO and then diluted with the medium. The cells were then exposed to different concentrations of the test samples for 24 hour. The cells in the control wells received medium containing the same volume of DMSO (0.1%). After the incubation, 100 µL of MTT reagent (1 mg/ml in PBS) was added, and cells were incubated for an additional 4 hour. The formazan produced by the viable cells was solubilized by addition of 100 µL DMSO. The suspension was placed on a microvibrator for 5 min, and the absorbance was recorded at 540 nm by the plate reader (ELx800; BioTek, Winooski, VT, USA). The experiment was performed in triplicate. Doxorubicin was used as positive control. The percentage cytotoxicity of the synthesized compounds was calculated using the following formula:

% cytotoxicity = [(Control absorbance – Blank absorbance) – (Test absorbance– Blank absorbance)/(Control absorbance– Blank absorbance)] × 100.

RESULTS

Compound **BSQ1** was found with highest dock score (-76.56) which indicated that it formed the most stable drug-receptor complex amongst all the eight synthesized compounds. The docking pose of **BSQ1** is shown in Figure 1which represents the hydrogen bonding with amino acid LYS721A, Vander Waal's interactions with few amino acid residues of the receptor and pi stacking interaction with amino acid PHE699A. Eight molecules with best dock score were selected for synthesis which were novel as anticancer activity was not explored for them. All eight compounds were synthesized by following established synthetic procedure and their structure was confirmed from spectral data. All the compounds were screened for antioxidant activity followed by anticancer activity. Cytotoxicity screening was carried out against Vero cell lines. Several concentrations ranging from 31.25-1000µg/ml of the synthesized compounds were tested for their antioxidant activity by using DPPH scavenging method. Ascorbic acid was used as the standard. The IC_{50} value of the compounds for antioxidant activity are shown in the Table 2. Among the tested compounds **BSQ1** and **BSQ6** showed antioxidant activity with IC_{50}

value of 175 and 835µg/ml respectively. The IC_{50} of all other compounds were above 1000µg/ml. These results showed that antioxidant activity of all eight synthesized compounds was mild when compared with IC_{50} of ascorbic acid (8.75µg/ml). The anticancer activity was done by MTT assay on MCF-7 and HCT-116 cell lines. Compound **BSQ1** showed good anticancer activity in MCF-7 cell line with IC_{50} 31.6µM/ml compared with IC_{50} of Doxorubicin (1.1µM/ml). Compound **BSQ8** showed good anticancer activity in HCT-116cell line with IC_{50} 11.6µM/ml compared with IC_{50} of Doxorubicin (0.6µM/ml). All the synthesised compounds showed IC_{50} value of more than 100µM/ml against VERO cell line indicating that the compounds are safe till 100µM/ml whereas the standard drug doxorubicin had IC_{50} value of 4.8µM/ml. Figure 2 and Figure 3 shows the graphical representation of IC_{50} for antioxidant activity of selected compounds and also the graphical representation ofIC_{50} for anticancer activity of all eight compounds against MCF-7 and HCT-116 cell lines.

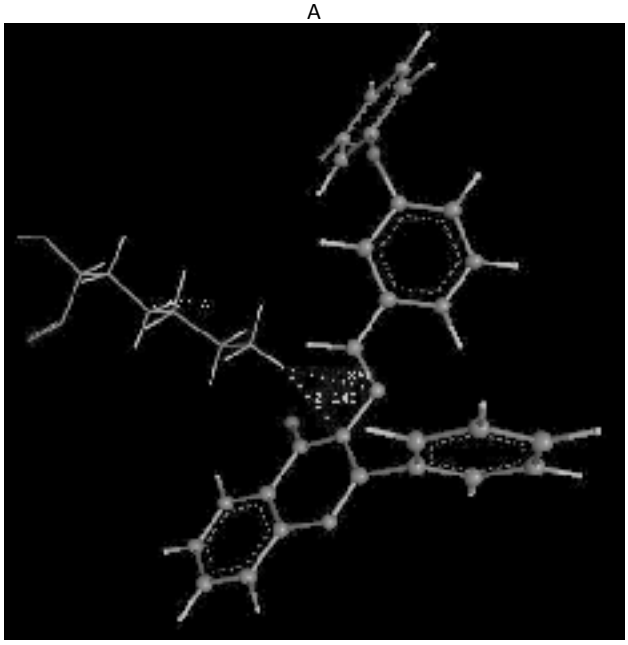


Figure 1: Docking poses of BSQ1 with the EGFR receptor pdb code 1M17.(A shows hydrogen bond interaction in green colour of the molecule with amino acid LYS721A , B shows vanderWalls interaction in pink colour with number of amino acid residues of the receptor and C shows pi stacking interaction in yellow colour with amino acid PHE699A of the receptor residue).

Table 2: Docking and Biological activity Results of Quinazolinone derivatives

Compound	Dock Score	Anti-oxidant IC_{50} (µg/ml)	MCF-7 IC_{50} (µM/ml)	HCT-116 IC_{50}(µM/ml)	VERO IC_{50}(µM/ml)
BSQ1	-76.56	175±0.96	31.6±0.86	26.9±1.00	>100±1.75
BSQ2	-58.11	>1000±1.05	84.9±1.18	23.9±0.83	>100±1.25
BSQ3	-73.22	>1000±2.71	>400±0.75	124.4±0.93	>100±1.35
BSQ4	-71.14	>1000±1.83	68.0±1.80	30.2±1.48	>100±1.18
BSQ5	-69.18	>1000±3.23	51.3±1.15	63.1±4.80	>100±1.78
BSQ6	-73.22	835±1.05	>400±1.98	27.0±2.60	>100±1.85
BSQ7	-74.68	>1000±4.17	>400±0.98	>400±1.90	>100±4.18
BSQ8	-67.16	>1000±2.10	201.7±0.80	11.6±0.55	>100±1.15
Ascorbic acid	_	8.75±0.78	_	_	_
Doxorubicin	_	_	1.1±2.40	0.6±3.35	4.8±2.65

Values are means of triplicate samples (n = 3). Data are presented as the mean ± SEM.

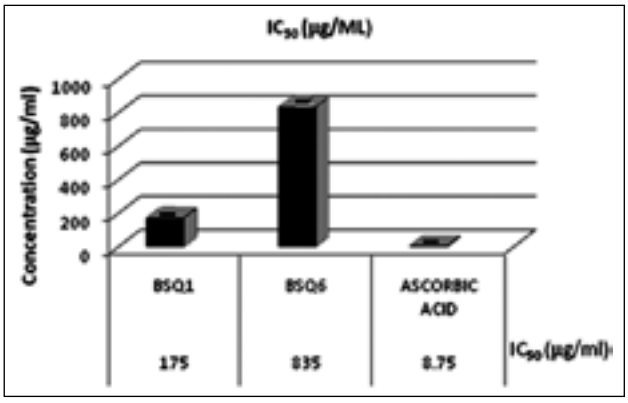


Figure 2: Graph of IC_{50} of selected compounds on antioxidant activity

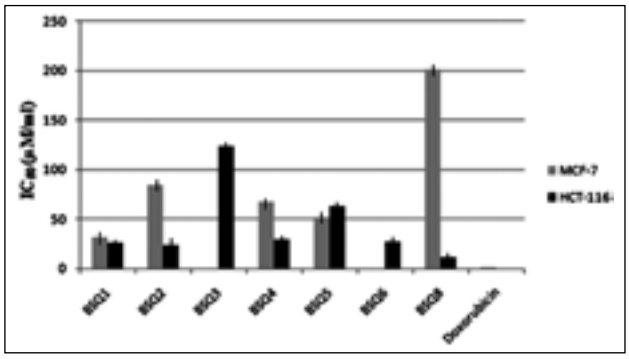


Figure3: Graph of IC_{50} of compounds onMCF-7 and HCT-116 cell lines

CONCLUSION

Based on the docking results, eight new quinazolinone derivatives were synthesized. As a preliminary study and as reactive oxygen species are involved in the aetiology of fat-related neoplasms such as cancer of the breast and colorectum due to continued oxidative stress all eightcompounds were screened for their antioxidant activity. Further all these compounds were tested for their anticancer activity using MCF-7, HCT-116 cell lines and cytotoxicity with VERO cell lines. The compounds **BSQ1, BSQ4, BSQ5** and **BSQ8** have shown highest dock score and have exhibited good anticancer activity against MCF-7 and HCT-116cancer cell lines indicating that substitution of lipophilic group at 3rd position has given good dock score as well as anticancer activity. These results revealed that these compounds can be evaluated further for their Epidermal Growth Factor Receptor Tyrosine Kinase (EGFR-TK) inhibitory activity.

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