Ind. J. Pharm. Edu. Res, 2023; 57(2):531-539.
https://www.ijper.org

Original Article

In vitro Antioxidant, Cytotoxicity Study on EAC Cell Line of Quinazolin-4(3H)-one Derivatives: Synthesis, Molecular Docking, *in silico* Drug Likeness

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ABSTRACT

Aim: The quinazolin-4(3)-one exist an important sort of therapeutic drug candidates which attain integer of biological actions. In this research intended on design, synthesis, and Drug likeness, screened their antioxidant by DPPH method, Cytotoxicity tumor cell line by EAC method. **Materials and Methods:** The existing amendment evaluated their antioxidant actions of quinazolin-4(3H)-one derivatives (1-8) using Assay of 2,2-diphenyl-1-picryl hydrazyl radical scavenging (DPPH) followed by *in vitro* cytotoxicity done with tryban blue exclusion technique by Ehrlich ascites carcinoma cells (QSL2 and QSD3). Molecular docking studies performed using PDB: 1M17, 2kw6 by PyRx virtual screening Autodock Vina and also software's like admit SAR, Pkcsm used for physiochemical studies and Pharmacokinetic prediction as well as structures of synthesized molecules inveterate with spectral scrutiny of IR, ^{1}H, ^{13}C NMR as well as Molecular mass. **Results:** Among (1-8) Ligands in order to be docked with the enzyme EGFR TK'sand CDK'S the substituted NO_2 and $N(CH_3)_2$ group with Quinazolin-4(3H)-one (QSL2 and QSD3) produced the typical effectual with in the middle of elevated requisite rate of -9.0 kcal/mol and -8.7kcal/mol. The ligands contains of Cl, OCH_3 gathering exibits best docked score of (QSL1–QSL4)8.6kcal/mol and 8.7kcal/mol and residual ligands all possess good docking scores more than-7.0kcal/mol against EGFR and CDK'S enzymes. Among all the selected compounds refusal violation exibits drug likeness properties. **Conclusion:** On the whole study showed to most of the compounds is appropriate action against antioxidant, tumor cell line of anticancer agents. Depending upon the docking scores and *in vitro* study, drug likeness properties of the composite be selected, also endorse *in vivo* study which may perhaps carry on beneficial enroute for broad better inhibitoryquinazolin-4(3H)-one derivatives.

Keywords: Quinazolin-4(3H)-one, Antioxidant activity, Cyto-toxicity, Molecular docking, DPPH Method, EAC method.

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Received: 21-09-2022;
Revised: 10-12-2022;
Accepted: 17-02-2023.

INTRODUCTION

The quinazolin-4(3H)-one compounds are of extensive medicinal magnitude as of their assorted biological tricks. It have been experiential which exibits like antiviral, anticancer, antibacterial, antifungal, antitubercular, anticoccidial,[1] anti-inflammatory and analgesics, antidepressant, anticonvulsant, antimalarial, antioxidant, antileishmanial,[2] neuroprotectivie,[3] obesity,[4] antihypertensive,[5] anti-H1-antihistaminic,[6] and antiprotozoal activities.[7] The quinazoline candidate is a central part in array of drugs for instance Gefitinib,[8] Nolatrexed,[9] CS 1101 (CAL 101),[10,11] Erlotinib,[12] Milciclib,[13,14] and Lapatinib[15] (Figure 1a-b). The Cytotoxicity on tumor cell lines actions of quinazolinone beside unusualtumor cell lines were reported by diverse probe groups.[16-18] In quinazolinone candidates are effective in EGFR lane of TK's inhibitors.[19-21]

The medical relevance of chemotherapy for malignancy healing is one of the valuable methods though to have its possess restriction owing towards conditions of the side effects among the progress of cancer cell conflict adjucent to these carcinogenic agents. Typically the proven supervision of elevated doses of antitumor drug campaign to beat battle leads to cruel toxicities.[22] Consequently new Cancer agents through high effectiveness also compact toxicity are immediately necessary to organize the troubles of tumor plus towards conquer the remedial conflicts are reported at the duration of metabolism along with respiration into individual remains and the free radicals as well as Reactive Oxygen Species (ROS) are formed so as to causes a integer of distressing possessions lying on soul well being.[23,24] More invention of ROS

DOI: 10.5530/ijper.57.2.65

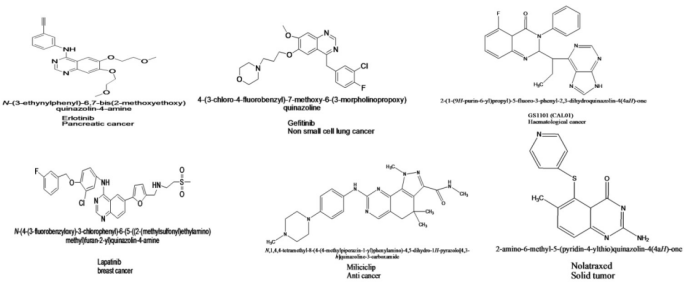


Figure 1: a. Examples of marketed drugs which contains quinazoline moiety and uses.

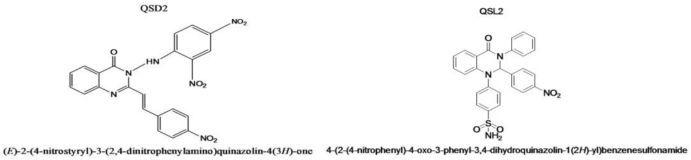


Figure 1: b. The tested derivatives of quinazolinone.

is accountable pro oxidative smash up to DNA towards leads various cancers.[25,26] The oxidative injure via free radicals plus ROS is infertile with the antioxidants[27] and take action through several behaviors, scavenging free radicals is one among them. Near decrease possessions of oxidation lying on person carcass followed by new with effective antioxidants are essential.[24] At this point we proposed towards study the bioactivities of a number of quinazolinones as antitumor, antioxidants in an intend headed for discover innovative synthetic source of drug candidate. molecular docking analysis be done near fit the anticipated quinazolinones (−8) hooked on the energetic position of human cyclin-dependent kinase 2 enzyme and also EGFR–TK's in sequence near revise the interface among requisite mold with *in vitro* antitumor, antioxidant evaluation.

MATERIALS AND METHODS[28,29]

General procedure designed for synthesis of Quinazolin 4(3H)-one

2 hydroxy benzoic acid (0.01mol mL), in aromatic amice (0.01 mol) were refluxed in 100mL RBF under heating at 30 min to produce 2-hydroxy phenyl salicylamide which is intermediate product. The 2-hydroxy phenyl salicylamide (0.01mL) intermediate was added to Aromatic aldehydes in THF. The reaction mixture was refluxed for 10-12 hr and left overnight to produce 2, 3 diphenyl 2, 3 Dihydro benzoxazine- 4-one followed by which is treated with (0.01mol) sulphonamide in 100mL of dry pyridine was refluxed for 6hr to form the solid product of Dihydro quinazoline 4 one derivatives after cooling into crushed ice. The reaction condition was maintained under TLC control and product was recrystallized from ethanol. Melting point of synthesized molecules was resolute via open capillaries method. The homogeneity, Purification of synthesized derivative was routinely monitored by TLC on siliga G plates, benzene: chloroform as mobile phase and visualization were done by iodine chamber. The infra red range of synthesized quinazolinones predicted at province 4000-400cm^{-1} via KBr discs scheduled JASCO 4100 FTIR, NMR ethereal revise be made throughout used DMSO like regents over JOEL FX90Q, Fourier renovate NMR spectrometer.

Molecular docking[30]

Docking studies were performed use the Autodock Vina utensil was retrieved from PyRx virtual screening Tools website and biovia Discovery studio visualizer from https://www.3dsbiovia.com/biovia-discovery. The Ligand to PDB configure change were done via Chem 3D pro 8.0, macromolecule of PDBset-up translation was carried out by using Swiss dock. The Protein data bank archive on crystal structures of human CDK's II with PDB ID:2KW6[31] as accessed, EGFR-TK's with PDB ID: 1M17[30] were obtained as of the RCSB website. Regulation plus optimization of macro molecule as well as ligand performed. Resolve the important amino acids into binding position were conceded out the presentation of the docking technique be screened through docked ligands keen on the arranged energetic position of relevant enzymes toward establish the Root mean square deviation scores. The docking process be conceded for each and every definite of compound at preferred dynamic location. Every docking candidate was organized value based on near its robust at the Ligand Binding Pocket (LBP) and also requisite interaction approach.

Biological activity

Anticancer activity[32,39]

The investigation of compounds were deliberate short term *in vitro* cytotoxicity by means of Ehrlich ascites carcinoma Cells (EAC). Lump cell aspirated as of peritoneal void of growth attitude micebe cleaned thrice either in PBS or usual sect procession. Cubicle feasibility be performed via tryban blue segregation technique. Viable cells deferment be extra to tubes containing a concentration range of the test candidates also the level be compose upto 1mL with Phosphate Buffered Cell line (PBS). Control tube enclosed no more than chamber deferment. To evaluate fusion be incubate in favor of 3hr at 37°C. Auxiliary cubicle deferral be assorted at 0.1mL in 1% try ban blue also reserved meant for 2-3 min in addition to laden scheduled a haemocytometer followed by deceased unit acquire at blue colour of trypan blue whereas survive groups no need to capture the dye. Integer marked with unstained compartment numbered independently.

$$\% \text{ Cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of live cells} + \text{No. of dead cells}} \times 100$$

Antioxidant assay[33]

The antioxidant bustle of the synthesized molecules was determine by the 2,2-diphenyl-1-picrylhydrazyl-DPPH free drastic scavenging assay.[33] New (.3mM: 3mg/25mL) methanol solvent of DPPH be equipped, from this 187 µg/mL as well as added to different test tubes containing methanol. To this solution, added different concentrations (10-100µg/mL) test samples in a maximum volume of 10uL. The reaction volume was making up to 1mL with methanol followed by final concentration DPPH was 0.5Mm. The tubes were then incubated in dark up to 20 min after starting the reaction, the absorbance was measured at 515nm. Ascorbic acid be use as an Indication standard (control). Te absorbance of DPPH radical devoid of antioxidant be calculated seeing that control moreover 95% methanol be worn like void. The entire determinations be functioned and averaged.

Percentage scavenging of the DPPH free radical be precise with the subsequent equation:

$$= \frac{(\text{Absorbance of control}) - (\text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

RESULTS

Molecular docking analysis

In sort to label the latent applicant pro supervision of malignant for that molecular docking performed followed by processed newly synthesized molecules of quinazolin-4-one derivatives under the binding pocket enzyme EGFR TK's also CDK's-II (PDB ID:1M17,2KW6). In addition variety of journalism says that quinazolin-4(3H)-one related moieties be instigate towards hamper the EGFR-TK's. In this epidermal growth factor receptor stand in crucial component into compartment extension, directive among solitary of the mainly momentous reflection intentional target of tyrosine kinase (TK's) inhibitors.[34] CDK-II might encompass a key responsibility into G2 segment of the unit succession. The implication of cycline dependent kinase-II (CDK-II) for cell phase evolution healing into contrary towards malignancy also additional hectic-proliferative.[35] In this study selected PDP ID: 1M17 where epidermal growth factor receptor towardsexplorethebindingaffinityofquinazolin-4-onederivatives, the entire synthesized compounds (QSL1-4,QSD1-3,QSD14) were docked in opposition to the target protein Cancer as well as ranked depends on their docked value. The reference compounds were used 5-fluoro Uracil and reference 2 was gefitinib with docked score value (-4.9kcal/mol,-7.4kcal/mol) against CDK'S Enzyme. Generally, Compounds exhibiting docked score of 7.0 or even less or more than that are thought concerning the better agent for restraint of the tumor activity.[36] A complete assessment could be done and enlisted in the Table 1 and the table exemplify the inventory of active compounds acquired later on docking

Figure 2: 3D crystal structures of the Macromolecule (a)EGFR -TK's (1M17) and (b)CDK's (2KW6).

studies. Those active as well as synthesized and docked molecules possess excellent docked value of more than 7.0 kcal/mol. Among 8(QSL1-4, QSD1-3, QSD14) compounds were chosen depends on the binding affinity among 1M17 and 2KW6 (Figure 2a-c) and also QSD1-3 and QSD14 against (PDB:1M17) docked score, drug likeness etc. followed by the results present in another paper[38] as well as from synthesized and proposed molecules of Quinazolin-4-one derivatives QSL2 having the Excellent docked value (-9.0kcal/mol) with cancer activity of Cyclic dependent kinase 2 (2KW6) and also QSD3 possess the best docked value of (-8.7kcal/mol) with Cancer of EGFR TK's (1M17).

Molecular Interaction Studies

Drug likeliness, Bio activity and ADMET evaluation

In drug development, ADME properties take part take in a vital task into victory otherwise stoppage of aspirant compounds. Reduced properties could edge the disclosure of the molecules to the target enzyme. Toxicity is one more especially imperative aspect which regularly overshadow of ADME actions. Lipinski's rule be functional into assess the bioavailability of the vocally treated drugs. The newly synthesized molecules were studied for the drug likeness of molecular properties as well as bioactivity by Molinspiration. Further the prediction of ADMET Properties was used admet SAR databases. All the newly designed compounds in order to veber's rule which they have rotatable bonds less than 10 as well TPSA not more than 140 and also its indicate that designed compounds may have good oral absorption.[37] Table 2 and 3 represents that all the new quinazolin-4-one of drug likeness properties. Human Intestinal Absorption value should be in the 0.9 and further which indicates good intestinal absorption. AMES toxicity evaluation engaged near find whether a drug be a carcinogenic or else so as all the synthesized compounds (1-8) which is influenced negative values, that is they are non-mutagenic as well as Non-carcinogenic and also designed compounds have exhibited lower LD_{50} (which is a measure to causes of 50% trial residents). And also was found to be somewhat higher range, could be measured to be safe as well as range of LD_{50} (listed in Table 3. The bioactivity value of the designed quinazolin-4-one derivatives) as G-protein coupled receptor ligand, ion channel modulator, nuclear receptor ligand a kinase inhibitor, protease inhibitor, also enzyme inhibitor were analyzed as well represented

in Table 4. A Moiety exhibited bioactivity value of more than 0.00 be mainly expected towards reveal significant biological bustle. Bioactivity range be additional to 0.0pro enzyme inhibition when compare to added mechanism.[36] Compounds had bioactivity value hit the 0.00 for enzyme inhibition, therefore which could be they can be measured to possess momentous natal motion with the respective system. The newly designed compounds given bioactivity score between -0.03 and -0.42. These results validate the basis at the back of designing and synthesized series as anticancer inhibitors.

Anticancer study

EGFR be within excess of articulated in numerous growth like lung, colon, breast, ovarian, brain, bladder, head and prostate cancer.[34] Further many core include be presented since persuasive CDK's enzyme inhibitors including pyrimidine, purines also quinazolines.[35] Our present exploration was based on motivated related study of anticancer belongs of the quinazolinone candidates (QSL2, QSD3) against Ehrlich ascites carcinoma cells using positive control. The information produced be worn to conspire a dose response arch of which concentration of assessment molecule essential to eradicate 50% of the cell populace (IC_{50}) (be dogged. The feasibility ethics also IC_{50} of quinazolinones QSL2, QSD3) against the Earlich's carcinoma cell lines are presented in Table 5, respectively. The results from Table 6 exposed that quinazolinones (QSL2, QSD3) be extra effective Cytotoxicity raised with increased concentration against Ehrlich ascites carcinoma cell lines with the respective (IC_{50} values with positive control dead cells).

Antioxidant activity

Here, in this study, Antioxidant bustle of synthesized quinazolinone derivatives (1-8) performed *in vitro* study with DPPH sweeping scavenging entitlement compare among ascorbic acid as a referred drug also outcome were presented at Figure 3 and Table 7 and also the results states that the tested compounds possess elevated (IC_{50} value than standard ascorbic acid).

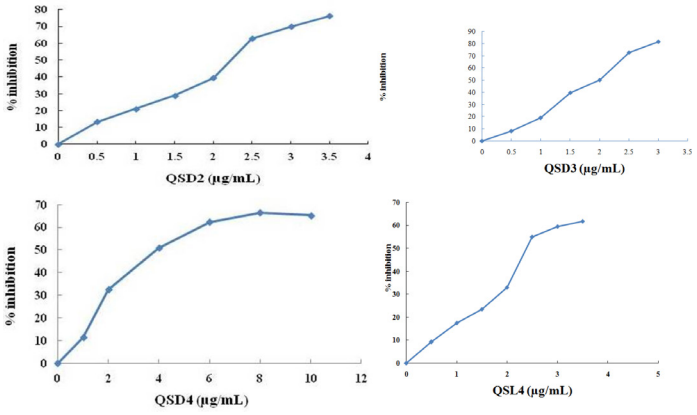


Figure 3: Some of compounds of slope curve on antioxidant activity by DPPH method.

H NMR, (δppm):7.24,(7.26)(Ar –(CH,C=C), 7.9(Ar-C=O (N), 4.0(Ar-C-NH),9.05(Ar-C-N-N),6.6(1-ethylene)7.2(-Cl), MS *m/z*: 463

^{13}C NMR (500 MHz, DMSO-d6, δppm): 164 (imine), 161.0(amide), 128.8(C-Cl), 133.5(CH—N=C –Ar, C=O), 138.2(ethylene), 142.5(Cphenyl ring), 161.2(Ar-C=O), 139.1 (Ar-C-N). 152.3(Ar-C-N), 158.7(Ar-C-C-O).

QSD-2: IR (KBr, v_{max}, cm^{-1}):3042(Ar-CH), 1616(C=O):1521.84 (C=N), 1530(C-N), 1319(C-NO_2)

H NMR, (δppm):7.4(CH, Ar), 7.9(Ar-C=O (N), 4.0(Ar-C-NH), 6.44 (Ar-N-C=O), 9.0(Ar-CH-N-N).8.0 (Ar-NO2) MS *m/z*: 474

^{13}C NMR (500 MHz, DMSO-d6, δppm): 151.7(imine), 160.9(O=C-NH), 138.7(ethylene), (Ar-NO_2), 142.5(Cphenyl ring), 161.2(Ar-C=O), 139.1 (Ar-C-N). 152.3(Ar-C-N), 158.7(Ar-C-C-O).

QSD-3:IR (KBr, v_{max}, cm^{-1}):3042(Ar-H) 1616(C=O):1456(C=N) 1530(C-N), 1031(CO-C), 1568 (-$N(CH_3)_2$)

H NMR, (δppm):7.4(CH, Ar), 7.9(Ar-C=O (N), 4.0(amine), 6.6(Ar-C-NH), 6.44(Ar-N-C=O), 2.85 (N $(CH_3)_2$). MS *m/z*: 472

^{13}C NMR (500 MHz, DMSO-d6, δppm): 164.1(imine), 161.0(amide), 147(C=O), 160.9(O=C-NH),138.7(ethylene), 122.4(2CH), 163,114.9-(C=N), 40.3 (N $(CH_3)_2$)), 142.5(Cphenyl ring),161.2(Ar-C=O), 139.1 (Ar-C-N). 152.3(Ar-C-N),158.7(Ar-C-C-O).

QSD-14: IR (KBr, v_{max}, cm^{-1}):3042(Ar-H) 1616(C=O):1456(C=N) 1530(C-N), 1031(CO-C), 2723 (-OCH_3)

H NMR, (δppm):7.4(CH, Ar), 7.9(Ar-C=O (N), 4.0(amine), 8.1(Ar-C-NH), 6.44 (Ar-N-C=O), 3.73 (O-CH_3. MS *m/z*:459

^{13}C NMR (500 MHz, DMSO-d6, δppm): 164(imine), 161(amide), 147(C=O), 147.7(C-CH_3), 160(O=C-NH), 138.7(ethylene), 163,114.9-(C=N), 55.9 (Aliphatic-OCH_3), 142.5(Cphenyl ring),161.2(Ar-C=O), 139.1 (Ar-C-N). 152.3(Ar-C-N),158.7(Ar-C-C-O).

QSL-1: IR (KBr, v_{max}, cm^{-1}):3042(Ar-H) 1616(C=O):1456.26(C=N) 1530(C-N), 1031.92(CO-C), 3017 (Ar-H),687(C-Cl).

H NMR, (δppm):7.4(CH, Ar), 7.9(Ar-C=O (N), 7.72(H N-S=O), 2.0(amine), 6.01 (methine), 6.71(Ar-CH (N-C-S=O), 7.15(C-Cl). MS *m/z*: 489

^{13}C NMR (500 MHz, DMSO-d6, δppm): 133.1(Ar-CH-N(C=O),77.6 (Aliphatic alpha, beta CH-N)), 161.2(amide), 132.3(Ar-CH-Cl), 129.2(Ar-CH-N-S(=O) 142.5(Cphenyl ring),161.2(Ar-C=O), 139.1 (Ar-C-N). 152.3(Ar-C-N),158.7(Ar-C-C-O).

QSL-2: IR (KBr, v_{max}, cm^{-1}):3042(Ar-H) 1616(C=O):1456.26(C=N) 1530(C-N), 1031.92(C-O-C),1320(Ar-H-C-NO_2)

Table 1: Interactions of EGFR (1M17) and CDK2 (2KW6) Macromolecules amino acid residues among synthetic compounds on receptor site.

Code	PDP ID	Binding affinity (kcal/mol)	Vander Waals	H. bond	Pi-alkyl	Pi-sigma
QSL1	1m17	-8.5	ALA A:678,743, LEU A:679,LYS A:828,GLN A:767,VAL A:718,ARG A:681.	ARG A:752	TRP A:707(pi –pi T Shaped)	ILE A:716,LEU A:754, VAL A:714.
	2kw6	-8.6	GLU B:276, 265,LEU B:291,HIS B:258,GLY A:294	ARG B:290	ARG B:293,ILE U:269,273,ALA B:268	GLU B:272 (pi anion), HIS B:297)pi-pi T shaped)
QSL2	1m17	-8.4	ALA A:678,743,LYS A:828,GLN A:767,VAL A:718,ARG A:681.	ARG A:752	TRP A:707(pi –pi T Shaped)	ILE A:716,LEU A:754
	2kw6	-9.0	ARG B:278,GLU B:272,GLY B:252,274,HIS B:253,255,ALA B:268,LYS A:92	GLU B:271	ILE A:96 and LYS A:275(pi-pi T shaped).	ARG A :99 (pi cation)
QSL3	1m17	-8.5	ALA A:678,743,LYS A:828,GLN A:767,VAL A:718,ARG A:681.	ARG A:752	TRP A:707(pi –pi T Shaped), VAL A:714	ILE A:716,LEU A:754,
	2kw6	-8.2	GLU B:276,GLY B:294,LEU B:291.	ARG B:290 GLU B:275	ARG B:293,LEU B:273(HIS B:297,ALA B:268,ILE B:269 – pi-pi T shaped)	GLU B:272 (pi anion),
QSL4	1m17	-8.3	PRO A:910,912,913,THR A:885,SER A:888,LYS A:889,ASP A:892,GLY A:893,ARG A:908,GLU A:907.	TRP A:881	TYR A:891 (pi-pi T shaped)ILE A:894	
	2kw6	-8.7	GLU B:272,GLY B:252,274,HIS B:253,ALA B:268,ARG B:278	GLU B:271,HIS B:255	ILE A:96,LYS B:275	ARG A:99 (pi cation)
QSD1	2kw6	-7.8	HIS B:253,255,ILE A:95,96,ILE B:277,LYS A:92,LYS B:275	ARG A:99,ARG B:278,GLU B:271	LYS B:275	GLY B:274(amide pi stacked)
QSD2	2kw6	-7.8	HIS B:253,255,ILE A:95,96,ILE B:277,LYS A:92,GLU B:272	ARG A:99,ARG B:278,GLU B:271,LYS B:275	-----	GLY B:274(amide pi stacked)
QSD3	2kw6	-7.5	HIS B:253,255,ILE A:96,ILE B:277,LYS A:92	ARG A:99,ARG B:278	LYS B:275 and GLU B:272,272 (pi cation, anion)	GLY B:274(amide pi stacked)
QSD14	2kw6	-7.7	HIS B:253,ILE A:95,96,ILE B:277,LYS A:92	ARG A:99,ARG B:278,GLU B:271	LYS B:275	GLY B:274(amide pi stacked)

H NMR, (δppm):7.4(CH, Ar), 7.9(Ar-C=O (N), 7.71(H N-S=O), 2.0(amine), 6.01 (methine), 6.71(Ar-CH (N-C-S=O), 8.07(Ar-CH-N-(=O)). MS *m/z*: 500

^{13}C NMR (500 MHz, DMSO-d6, δppm): 133.1(Ar-CH-N(C=O),77.6(Aliphatic alpha,beta CH-N)), 161.2(amide), 132.3(Ar-CH-Cl), 129.2(Ar-CH-N-S(=O) 142.5(Cphenyl ring),161.2(Ar-C=O), 139.1 (Ar-C-N).,146.4(Ar-CH-N=O). 152.3(Ar-C-N),158.7(Ar-C-C-O).

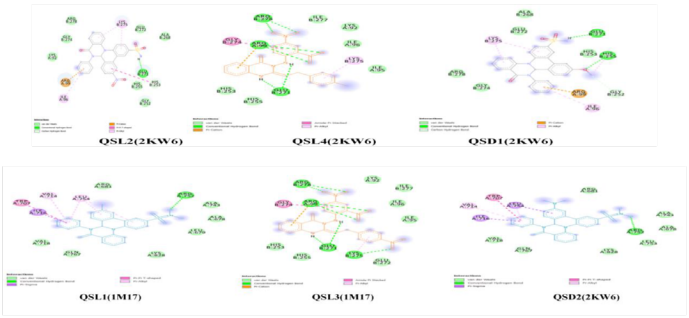
QSL-3: IR (KBr, v_{max}, cm^{-1}):3042(Ar-H) 1616(C=O):1456(C=N) 1530(C-N), 1031(C-O-C), 1560 (-N$(CH_3)_2$)

H NMR, (δppm):7.4(CH, Ar), 7.9(Ar-C=O (N), 7.71(H N-S=O), 2.0(amine), 6.01 (methine), 6.71(Ar-CH (N-C-S=O), 2.85 (N $(CH_3)_2$). MS *m/z*: 498

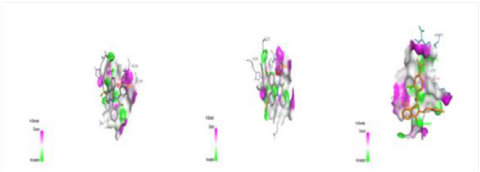
^{13}C NMR (500 MHz, DMSO-d6, δppm): 133.1(Ar-CH-N(C=O),77.6(Aliphatic alpha, beta CH-N)), 161.2(amide),

Table 2: Physicochemical possessions of the active molecules with the rules of drug-likeness.

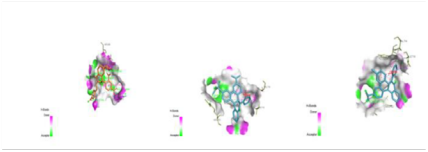
Ligands	MW	LogP	AlogP	HBA	HBD	TPSA	Nrb	No. of violation
QSL1	489.98	5.59	5.60	6	2	83.71	4	1
QSL2	500.54	4.88	4.86	9	2	129.54	5	1
QSL3	498.61	5.02	5.01	7	2	86.95	5	2
QSL4	485.56	4.97	4.96	7	2	92.95	5	1
REF1	2kw6	-4.9						
REF2	2kw6	-7.5						



3D Best binding poses of the molecular intention with CDK'S and EGFR TK'S Enzymes (6M2N, 6VW1)



QSL2(2KW6) QSL4(2KW6) QSD1(2KW6)



QSD2(2KW6) QSL3(1M17) QSL1(1M17)

Figure 4: b. Best 2D Structure of the molecular intention with CDK'S and EGFR TK'S (2KW6,1M17).

132.3(Ar-CH-Cl), 129.2(Ar-CH-N-S(=O) 142.5(Cphenyl ring),161.2(Ar-C=O), 139.1 (Ar-C-N)., 40.3 (N $(CH_3)_2$)), 152.3(Ar-C-N),158.7(Ar-C-C-O).

QSL-4: IR (KBr, v_{max}, cm^{-1}):3042(Ar-H) 1616(C=O):1456.26(C=N) 1530(C-N), 1031.92(CO-C), 3017 (Ar-H), 2723 (-OCH_3)

H NMR, (δppm):7.4(CH, Ar), 7.9(Ar-C=O (N), 7.72(H N-S=O), 2.0(amine), 6.01 (methine), 6.71(Ar-CH (N-C-S=O), 3.73 (O-methyl). MS *m/z*: 485

^{13}C NMR (500 MHz, DMSO-d6, δppm): 133.1(Ar-CH-N (C=O),77.6(Aliphatic alpha, beta CH-N)), 161.2(amide), 129.2(Ar-CH-N-S(=O) 142.5(Cphenyl ring),161.2(Ar-C=O), 139,55.9 (Aliphatic-O-CH_3),152.3(Ar-C-N),158.7(Ar-C-C-O).

DISCUSSION

The titled compounds were synthesized by 3-4 different reaction step followed by the formed product yield (75%-86%),which contains hetero moieties like substituted sulphonamide (Figure 4a), (QSL1-4) aromatic aldehydes derivatives of quinazolinones in the suitable reagents and catalyst as well as styryl moiety of phenyl hydrazine substitution quinazolinones (QSD1-3, QSD14) and the completion of reaction condition monitored by chromatography of TLC and melting point were performed via an open capillary tube method and structures of synthesized quinazolinone candidate were established by IR, NMR and MASS Spectrum.

From above study the docking poses was produced according towards docking parameters with their resultant binding pockets. Current Investigations subsist supposed cooperative for considerate the fastening relations above embattled protein. Molecular docking analysis of preferred quinazolin-4-one derivatives exist take away also additionally docked binding affinity of selected candidates consequential in the binding value of -7.1 kcal/mol and -9.0 kcal/mol as well as reference drug 1 and 2 which listed at Table 1 and Figure 2 and 5. Each and every one of the proposed molecules be set up towards effectively clutch behind the cancer inhibitors EGFR and CDK'S Enzyme as a outcome of entirely the capable site into intend Macromolecule. The result of docking exploration is exhibited en route for all individual of the docking moieties include lesser energy value (elevated binding energy value). In addition to assorted interface score of select Ed molecules (QSL1-4, QSD1-3 and QSD14) which showed at Table 1. Demonstrate the mostly outstanding less binding energy (more binding energy values) in favor of the docking molecule candidates. Alongside by (1-8) Ligands in order to be docked with the enzyme EGFR TK's and CDK'S, the substituted NO_2 and $N(CH_3)_2$ group with Quinazolinone of ligand (QSL2 and QSD3) produced the mainstream efficient at the center of high binding score of -9.0 kcal/mol and -8.7kcal/mol. Among, ligands of substituted Cl, OCH_3 assembly exibits greatest docked score of (QSL1–QSL4)8.6kcal/mol and 8.7kcal/mol and remaining Substituted ligands all had good docking scores more than-7.0kcal/mol against EGFR and CDK'S enzymes. Among all the selected ligands refusal violation exibits drug likeness. Extra

Table 3: ADMET Properties of newly synthesized compound.

Ligands	HIA	BBB	AMES Toxicity	Carcinogenicity	LD_{50} in rat(mol/kg)
QSL1	0.9761	0.9665	Non toxic	Non –carcinogenic	3.211
QSL2	0.9211	0.9729	Non toxic	Non-carcinogenic	2.979
QSL3	0.9692	0.9741	Non toxic	Non-carcinogenic	3.523
QSL4	0.9780	0.9707	Non toxic	Non-carcinogenic	3.108
QSD1	0.9258	0.9743	Non toxic	Non-carcinogenic	2.482
QSD2	0.9242	0.9732	Non toxic	Non-carcinogenic	2.902
QSD3	0.9177	0.9735	Non toxic	Non-carcinogenic	2.587
QSD4	0.9249	0.9704	Non toxic	Non-carcinogenic	2.482

Table 4: Bioactivity score of proposed molecule among standard.

C.Code	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear Receptor ligand	Protease inhibitor	Enzyme Inhibitor
QSL1	0.17	0.38	0.21	0.35	0.07	0.06
QSL2	0.27	0.42	0.29	0.38	0.13	0.10
QSL3	0.16	0.39	0.17	0.31	0.04	0.03
QSL4	0.20	0.43	0.22	0.34	0.08	0.07

Table 5: The inhibitory bustle of the screened molecules verses Ehrlich ascites carcinoma cells at various Concentrations.

Drug concentration(ug/mL)	% Cytotoxicity	
	QSD3	QSL2
10	3.64±0.7	4.82±0.42
20	4.97±1	5.92±0.34
50	6.55±1.2	7.54±0.89
100	12.4±1.4	11.3±1.91
200	20.3±.2	15.3±1.86

The statistics are articulated as (IC_{50} value ± Standard error)
Control Tube contains 5 dead cells and sample dissolved in DMSO.

Table 6: Physical characterization of newly synthesized Quinazolinone derivatives.

Compound code	Colour of the compound	Melting Point (°C)	R_f Value	%Yield
QSL1	Colourless	195	0.82	78
QSL2	Colourless	200	0.86	80
QSL3	Colourless	215	0.76	70
QSL4	Colourless	210	0.84	69
QSD1	Red solid	196	0.72	86
QSD2	Yellow solid	190	0.80	80
QSD3	Red solid	185	0.78	82
QSD14	Red solid	205	0.74	76

IR, ^1H, C^{13} NMR, MASS Spectral study of synthesized Compounds:
QSD1: IR (KBr, v_{max}, cm^{-1}):3431(Ar-H) 1720(C=O):1456.26(C-N) 1068.56(C-N-O), 686(-Cl)

Table 7: The *in vitro* antioxidant activity of synthesized quinazolinone derivatives (1-8) in DPPH Method.

Compound code	IC_{50} µg/mL
QSD1	4.89
QSD2	2.77
QSD3	2.33
QSD4(14)	5.73
QSL1	4.88
QSL2	2.97
QSL3	5.35
QSL4	2.67
Ascorbic acid (std)	1.86
Solvent	DMSO

with entire elected quinazolinones left in excess of the candidates exposed very good Human Intestinal Absorption (HIA), Blood Brain Barrier (B.B.B), among rejection carcinogenicity and AMES negative as well as good bioavailability. The docking ligand demonstrates electrostatic interaction, Hydrogen bond, and also Pi sigma and Pi alkyl interactions there into Table 1. Viewing to Ligands interactions bind reflective in the hub of active position of the quinazoli-4-one against action of anticancer enzymes.

Each and every one of the compounds (1-8) were evaluated for antioxidant activity and two molecules (QSL2.QSD3) done anticancer activity. Cytotoxicity screening was performed against Ehrlich ascites carcinoma cells. The concentrations ranging from 10 to 200 ug/mL of the proposed molecules are screened for their antioxidant evaluation by using DPPH Scavenging method. Ascorbic acid was used like reference (control) drug. The (IC_{50} score of) the candidates for their antioxidant properties are present in Table 2. Along with the tested molecules significant antioxidant activity of QSD3, QSL4, QSD2 and QSL2 with (IC_{50} value) of 2.33, 2.67, 2.77 and 2.97µg/mL correspondingly and also compounds possess antioxidant activity of QSL1, QSD1 with IC_{50} value of 4.88 and 4.89ug/mL respectively and also mild to moderate antioxidant activity of QSL3, QSD4 with (IC_{50} value of 5.35 and 5.73ug/mL) respectively. These consequences exposed to antioxidant activity of the entire (1-8) eight synthesized candidates while compared with (IC_{50} of ascorbic acid 1.89µg/mL). Further among synthesized compounds few (QSD3 and QSL2) were tested anti-cancer activity using EAC cell line as well as possess good cancerous activity against all the concentrations like percentage of cytotoxicity increased when concentrations increased. Furthermore indicating that substitution of sulphonamide at 1st position electro donating methoxy group better antioxidant activity compared to 2nd styryl moiety of phenyl hydrazine contains electron donating methoxy group ligands better antioxidant activity and also 2nd styryl moiety of phenyl hydrazine moiety of di methylamine better antioxidant action compared to sulphonamide at 1st position di methylamine derivatives as well as electron withdrawing Nitro group containing phenyl hydrazine and sulphonamide moieties better antioxidant activity. The present Anticancer study correlated with antioxidant, computational study followed by cytotoxicity in the results of better effective ligands of QSD3 and QSL2.

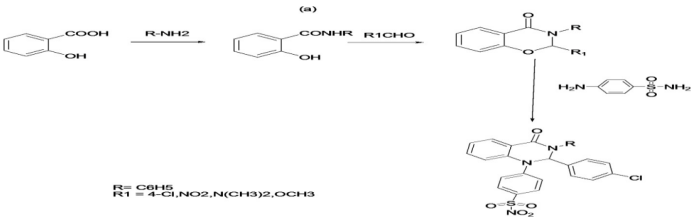


Figure 5: a. Proposed Scheme.

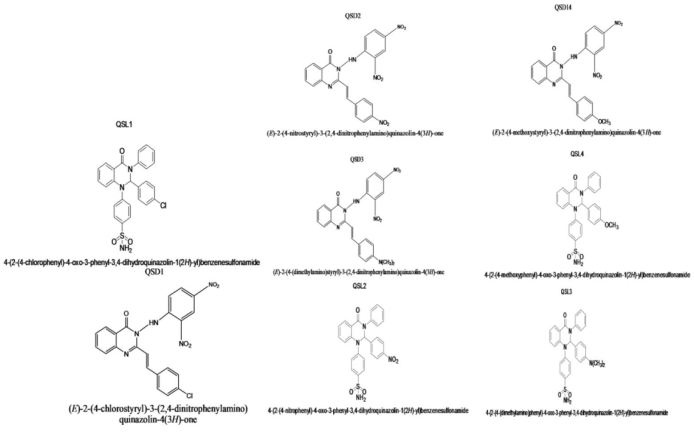


Figure 5: a. Proposed Scheme.

CONCLUSION

The outcome showed to synthesized quinazolinone compounds QSD3 as well as QSL2 exist potent activity against EAC cell defenses, among the evaluated compounds except few all have better antioxidant action and some have mild to moderate bustle compared with reference (control) ascorbic acid. The synthesized moieties of structures confirmed by IR, H^1 ^{13}C NMR and MASS spectral records. In the present study correlation among antioxidant and cytotoxicity study as well as *in silico* study data shows that the excellent docking score (-7.1kcal/mol to -9.0kcal/mol.) against PDB 1m17 and 2kw6 when compared to reference 1 and 2. And Depending upon the docking scores and *in vitro* study and drug likeness properties of the composite be elected also promote *in vivo* study. In addition this investigate suggest further study with intention of the chosen Compounds revealed substantial battle aligned with EGFR TKs enzyme and cyclic dependent kinase 2 enzyme which may perhaps survive constructiveen route for enlarge better inhibitory quinazolin-4-one drug molecules.

ACKNOWLEDGEMENT

The authors are very grateful to Principal also management of Karpagam College of Pharmacy for the agreed required facilities and infrastructure.

The authors are indebted and very much thankful to Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India, for the supervision for successful academic philosophy Research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EAC: Ehrlich ascites carcinoma; **EGFR:** Epidermal growth factor receptor; **TK's:** Tyrosine kinases; **CDK:** Cyclic dependent kinase; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **IR:** Infra-red; **NMR:** Nuclear magnetic resonance; **TLC:** Thin layer Chromatography; **DMSO:** Dimethyl Sulphoxide; **PDB:** Protein data bank; **PBS:** Phosphate buffered cell line suspension; **IC_{50}:** Half maximal inhibitory concentration; **GPCR:** G-Protein coupled receptor; **B.B.B:** Blood brain barrier; **TPSA:** Topical polar surface area.

SUMMARY

The current revision evaluated their antioxidant activities of quinazolinone using Assay of -1,1-iphenyl-2-picryl hydrazyl radical scavenging (DPPH). *In vitro* cytotoxicity performed using tryban blue exclusion method by Ehrlich ascites carcinoma cells. Docking studies performed using PDB 1M17(EGFR TK's),2kw6(CDK's) Docked by PyRx virtual screening Autodock Vina. The overall study showed that some of the compounds are suitable antioxidant. It might be fulfilled that most of the compounds showed momentous docking score as well as In silico ADME and toxicity profiles in order to facilitate into the threat and also among the emergence of drug resistance there by it is important to explore with develop more efficacious drugs QSD-3 and QSL-2 (Quinazolinone derivatives) is a potential candidate for managing cyto toxicity against EAC Cell line. They have no other toxicities as well as obey lipink;s rule of five suitable oral drug.

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Cite this article: Kavitha K, Srinivasan N, Suresh R, Mohan S. *In vitro* Antioxidant, Cytotoxicity Study on EAC Cell Line of Quinazolin-4(3H)-one Derivatives: Synthesis, Molecular Docking, *in silico* Drug Likeness. Indian J of Pharmaceutical Education and Research. 2023;57(2):531-9.