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Research Article

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Synthesis, characterization and evaluation of antioxidant activities of some new quinazolino-acetidinone derivatives

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ABSTRACT

Our aim was to develop novel quinazolino-acetidinone derivatives as potent antioxidant agents which could minimize or inhibit oxidative damage through blocking free radical formation or inhibiting chain reaction. In-silico design of novel analogues were carried out using ACD labs ChemSketch 12.0. Molinspiration software was used to analyse 'Lipinski Rule of Five' and drug likeness properties. Five derivatives which obeyed rule of five and having desired physico-chemical properties were synthesized by five step process. After the completion of reaction in each step, the compounds were isolated, recrystallised by using suitable solvents, purified by TLC and column chromatography. Analogues were characterized by FT-IR, H^1NMR, C^{13}NMR and Mass Spectroscopy. The Biological evaluation was done by DPPH radical scavenging activity method. The results were compared with effect of standard drug ascorbic acid. The results of present research work showed that novel quinazolino-acetidinone derivatives have comparable antioxidant effect with that of standard antioxidant drug ascorbic acid. This will lead to the development of promising lead compounds for treatment against tumors or other free radical-related diseases

Keywords: Quinazolino-acetidinone, antioxidant activity, DPPH, free radical

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INTRODUCTION

Many exogenous chemicals in food systems and endogenous metabolic process in human body produce highly reactive free radicals, particularly free radicals that derive oxygen. Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl and nitric oxide radicals, play an important role in oxidative stress related to pathogenesis of many important diseases [1-3]. The ROS are constantly generated in the human body and are involved in various physiologically important biological reactions. Under physiological conditions, there is a balance between the production of reactive oxygen and a biological system's ability to detoxify the reactive intermediates. Oxidative stress occurs when the generation of ROS in a system exceeds the system's ability to eliminate them [4-6]. Excessive generation of ROS induced by various stimuli leads to many pathophysiological abnormalities such as inflammation, atherosclerosis, stroke, genotoxicity, diabetes, dementia and cancer. Antioxidants act as a major defense against radical mediated toxicity by protecting the damage caused by free radicals and have significant role in the prevention and treatment of such complex diseases conditions. Therefore in recent years there has been increased importance in developing novel and efficient antioxidant agents [7-10].

Many naturally occurring compounds likes flavanoids, phenolic compounds etc are widely distributed in plants are reported to exhibit multiple biological effects such as anti-inflammatory, anti-oxidant, free radical scavenging abilities, ad anti-carcinogenic[11-12]. A number of heterocyclic nuclei exhibit wide range of pharmacological

activities and these structures form basis of many pharmaceutical, agrochemical and veterinary products. Of these Quinazolin-4[3H]-ones are important class of heterocycles that are of considerable interest because of the diverse range of biological activities like anti-microbial, anti-cancer, anti-convulsant, anti-tubercular, anti-inflammatory etc. The unique chemotherapeutic properties of β–lactam antibiotics continue to attract the attention of the chemical community [13-14]. One important application of the β-lactam moiety in synthesis involves the production of natural and non-natural α-amino acids. The simplest β-lactam is 2-azetidinone. The biological activity of β-lactam skeleton is believed to be associated with the chemical reactivity of the ring and on the substituents especially at nitrogen of 2-azetidinone ring [15]. The present research work aim to design novel chemical nuclei coupled with quinazoline and azetidinone moiety, characterize it and to evaluate their antioxidant potential.

EXPERIMENTAL SECTION

2.1. Materials and methods

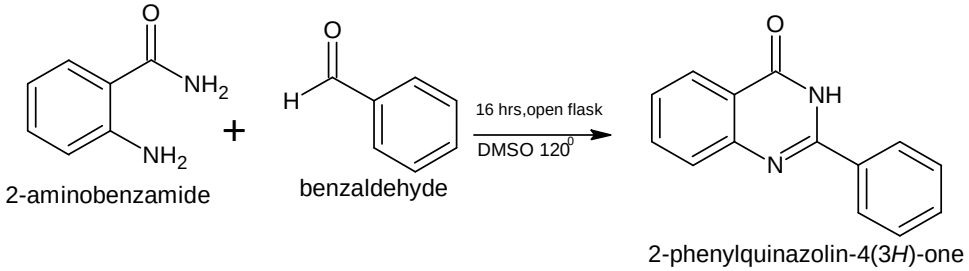
All the chemicals and reagents used in this research work were of analytical or synthetic grade from Sigma Aldrich, E-Merck (Germany) and S D Fine Chemicals (India). 1,1-diphenyl-2-picryl-2-hydrazine was purchased from Sigma-Aldrich, India, ascorbic acid and methanol were purchased from E-Merck. All the chemicals were dried and purified according to standard methods before use, wherever necessary. Software used for this study includes ACD Labs Chemsketch, Chemdraw Ultra 8.0, Molinspiration, and Osiris property explorer. All the reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. All the reaction courses and product mixtures were routinely monitored by aluminium coated TLC plates 60 F245 (E Merck) and visualized with UV light or iodine chamber. Melting point of synthetic compounds was determined on a Labindia MR-VIS visual melting point apparatus and is uncorrected. Absorbance values against wavelength were taken on a Systronic double beam UV-166 spectrophotometer. The FT-IR spectra were recorded using FT-IR (Agilent Cary 630 FT-IR spectrophotometer using KBr pellet. ^{1}H NMR spectra were recorded using NMR spectrophotomer (Bruker 400 ultra schield DPX 400) and chemical shifts are expressed as δ (ppm) using TMS as an internal standard in DMSO-*d6*. Mass spectra of the compounds were done with mass spectrometer (micromass-O-TOF-MS ES+).

2.2. *In-silico* drug design

In-silico methods used helped to identify and quantify the physico-chemical descriptors and to analyse whether any of these properties have significant effect on drug's biological activity. These methods could help in identifying drugs' possible targets and predict its activity using various bioinformatics tools. These methods can also used to analyze target structures for possible binding or active sites, generate candidate molecules, check for their drug likeness, and dock these molecules to improve binding characteristics. The Physico-chemical properties of the molecule were calculated by different software. The electronic, lipophilic and various steric parameters can be determined by ACD Labs Chemsketch. Drug likeness and analysis of Lipinski rule of five were carried out using Molinspiration software Maestro software. Thus, by comparing the structure of a new compound with structures of well-known biologically active substance it is possible to estimate if a new compound may have a particular effect.

2.3. Synthetic methods

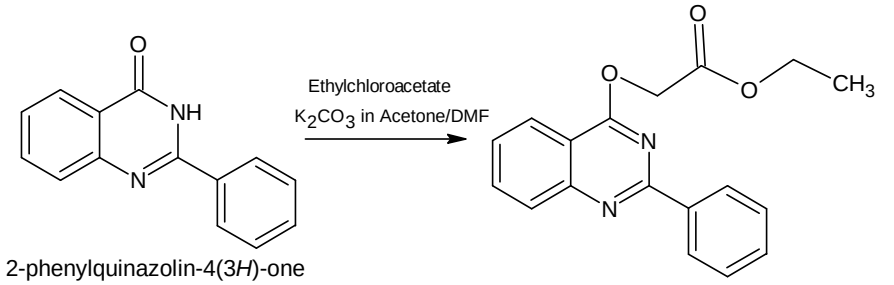
Step 1: Formation of 2-phenylquinazolin-4(3*H*)-one



2-aminobenzamide benzaldehyde 2-phenylquinazolin-4(3*H*)-one

To the solution of 2-aminobenzamide (0.01mole, 1.36g) and benzaldehyde (0.01 mole, 1.019 mL) in 10 mL Dimethyl sulfoxide, catalytic amount of acetic acid was added. The solution was heated in an open flask at 120^0c for 16 h. The progress of reaction was monitored using TLC 15% ethyl acetate in chloroform. After completion of reaction, the reaction mixture was cooled to room temperature and the product obtained was filtered washed with water and crystallized from absolute ethanol.

Step 2: Synthesis of ethyl [(2-phenylquinazolin-4-yl)oxy]acetate

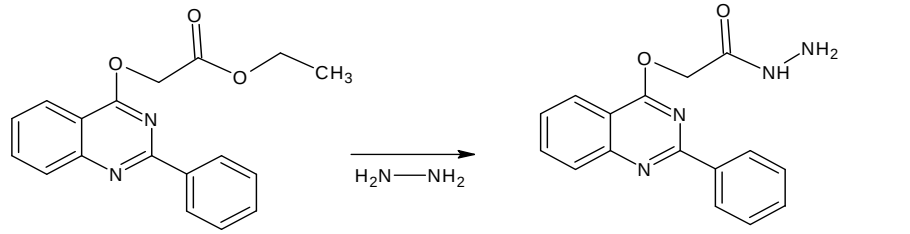


2-phenylquinazolin-4(3*H*)-one

ethyl [(2-phenylquinazolin-4-yl)oxy]acetate

In 500 mL Round bottom flask, take 15-20mL dry DMF. To this add 2-phenylquinazolin-4(3H)-one (0.01 mole, 2.22g), and ethylchloroacetate (0.01mole, 1.25mL) and anhydrous potassium carbonate (0.1 mole, 1.38g). The resultant mixture was stirred and refluxed for 9-10 hrs at 80°C. After completion of reaction, which was monitored by *in situ* TLC, the reaction mixture was filtered and poured into large amount of water. The solid separated was filtered and washed with water, the solid was dried and recrystallized from ethanol.

Step 3: Synthesis of 2-[(2-phenylquinazolin-4-yl)oxy]acetohydrazide

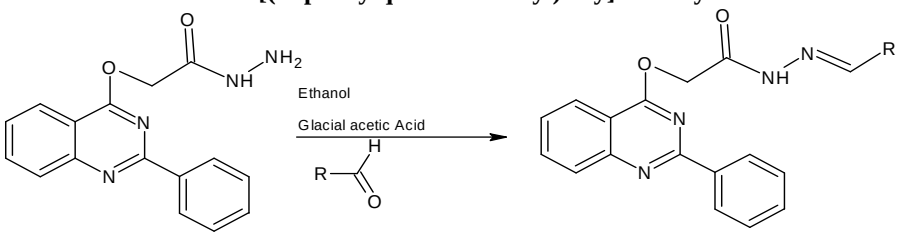


ethyl [(2-phenylquinazolin-4-yl)oxy]acetate

2-[(2-phenylquinazolin-4-yl)oxy]acetohydrazide

Ethyl [(2-phenylquinazolin-4-yl)oxy]acetate (0.05M) and hydrazine hydrate 99% (0.15M, 7.29 mL) was dissolved in sufficient quantity of ethanol (50 mL) to give clear solution and refluxed for 10 hrs at 100°C. The excess solvent was removed by distillation, allowed to cool, the solid mass that separated on cooling was washed with small amount of ice cooled ethanol, dried and recrystallized from ethanol.

Step 4: Synthesis of Schiff's bases of 2-[(2- phenylquinazolin -4-yl) oxy] acetohydrazide

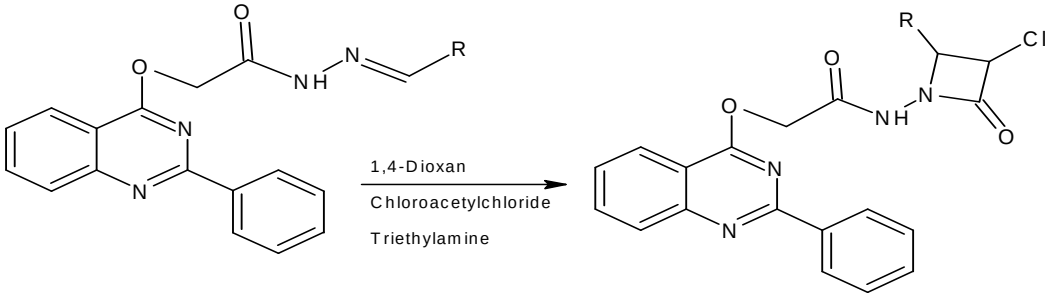


2-[(2-phenylquinazolin-4-yl)oxy]acetohydrazide

Q4(a-J)

To a solution of appropriate substituted benzaldehyde (1mmol, 3.5g) in ethanol (15mL), 2-[(2-phenylquinazolin-4-yl) oxy] acetohydrazide (1mmol, 3g) were added. Make pH around 4.5 by adding 2-3 drops of glacial acetic acid. The reaction was refluxed for 5-6 h and the course of reaction was monitored by TLC to its completion. The reaction mixture was cooled by keeping it in room temperature. A solid mass separated out, which was filtered and washed with water. The crude product was recrystallised from ethanol.

Step 5: Synthesis of substituted *N*-(3-chloro-2-oxoazetidin-1-yl)-2-[(2-phenylquinazolin-4-yl) oxy]acetamide



A mixture of Schiff's base [4a-4e] (0.01 mol) and triethylamine (5-6 drops) was dissolved in 1,4-dioxane (50mL), cooled and stirred. To this well-stirred cooled solution, chloroacetyl chloride (0.015mole, 1.68mL) was added drop wise within a period of 30 minutes. The reaction mixture was then stirred for an additional 3 hours at room temperature and refluxed for 7 hours. The reaction mixture was filtered to remove triethylamine hydrogen chloride and the resultant solution was concentrated, cooled and poured into ice-cold water with stirring. The solid thus obtained was recrystallized from acetone to yield desired 2-azetidinone derivatives (QAz3, QAz8, QAz13, QAz15, and QAz25)

2.4. Evaluation of anti-oxidant screening (DPPH radical scavenging activity)

2.4.1. Principle

The DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm and the solution has a deep purple colour. When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor and is reduced to 1,1-diphenyl-2-picryl hydrazine and the absorbance decreases. Radical to the DPPH-H results in decolourization of solution from deep purple to yellow with respect to the number of electrons captured. More the decolourization, more is the reducing ability and used as a measure of antioxidant ability.

2.4.2. Preparation of DPPH solution and drug solutions

DPPH solution (0.1mM) was prepared by dissolving 9.85 mg of DPPH in 250 ml ethanol. DPPH solution was protected from light influence by maintaining dark condition and covering by aluminium foil. Different concentrations of drug solutions were prepared by dissolving in methanol.

2.4.3. Procedure for antioxidant activity

3ml of DPPH solution was added to 1ml various concentration (10, 50 &100 µg/ml) of drug solutions or standard solution of ascorbic acid (10, 50 &100 µg/ml). The mixture was shaken and incubated in darkness at room temperature for 30 minutes and the absorbance was measured at 517 nm by using spectrophotometer. Ascorbic acid was used as standard. 3 ml DPPH reagent and 1 ml methanol was used as control. Methanol was used as blank. All experiments were done in triplicate and average was taken.

Scavenging activity of DPPH free radical in percentage was calculated by using the formula

Percentage inhibition = [OD of control-OD of test/OD of control] × 100

Where, OD is the optical density.

2.4.4. Evaluation of total antioxidant activity

The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate / Mo (V) complex at acidic pH. 0.3 ml of drug solution in methanol (100 µg/ml) was mixed with 3 ml of reagent solution, containing 1ml each of ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (0.6 M). The reaction solutions were incubated for 90 minutes at 90^0C and the absorbance of the green phosphomolydbenum complex was measured at 695 nm. Blank solution contained 3ml of reagent solution and 0.3 ml of methanol. Incubation was done under the same conditions as that of samples. Ascorbic acid, a water-soluble antioxidant, was used as standard and calibration curve was obtained using various concentrations of ascorbic acid. The antioxidant capacity was expressed as the equivalent of ascorbic acid.

RESULTS AND DISCUSSION

3.1. *In-silico* drug design

The *In-silico* molecular modeling studies of Quinazolino-acetidinone derivatives were carried out successfully with the aid of different software for selection of suitable drug candidates prior to wet lab synthesis. *In-silico* studies were performed on 30 analogues of Quinazolino-acetidinones by means of ACD Lab ChemSketch 12.0, ChemDraw 8.0, Molinspiration, PASS software. Among the 30 designed analogues, five analogues were found to obey Lipinski rule of five and their drug likeness were predicted by Molinspiration software. These analogues which are having desired physico-chemical properties and predicted antioxidant activity were chosen for wet lab synthesis **(Table 1,2,3,4).**

Table 1. Molecular descriptors for designed analogues generated by ACD Labs Chemsketch 12.0

Compound	Molecular Formula	Parachor ($cm^{3)}$	Molar Volume (cm^3)	Polarisability (10^{-24} cm^3)	Molar Refractivity (cm^3)
QAz3	$C_{25}H_{18}Cl_2N_4O_3$	973.6 ±6. 0	327.9 ±5.0	51.36 ± 0.5	129.55 ± 0.4
QAz8	$C_{26}H_{21}ClN_4O_3$	974.7 ±6.0	332.8 ±5.0	51.27±0.5	129.35 ±0.4
QAz13	$C_{25}H_{219}ClN_4O_4$	126.2 ± 0.4	314.0 ± 5.0	951.7 ± 6. 0	50.05 ± 0.5
QAz15	$C_{25}H_{19}ClN_4O_4$	126.2 ±0.4	314.0 ±5.0	951.7±6. 0	50.05 ± 0.5
QAz25	$C_{25}H_{18}ClFN_4O_3$	124.8 ± 0.4	321.6 ±5.0	943.8±6. 0	49.49 ± 0.5

Table 2. Analysis of Lipinski rule of five for selected quinazoline-acetidinone analogues

Compound	Log P	Mol. Wt.	No. of Hydrogen bond acceptors	No. of Hydrogen bond donors	No. of Rotatable bonds	Violations
QAz3	4.98	493.35	7	1	6	0
QAz8	4.75	472.93	7	1	6	0
QAz13	4.24	474.90	8	2	6	0
QAz15	3.82	474.90	8	2	6	0
QAz15	4.46	476.89	7	1	6	0

Table 3. Analysis of drug likeness score for selected derivatives

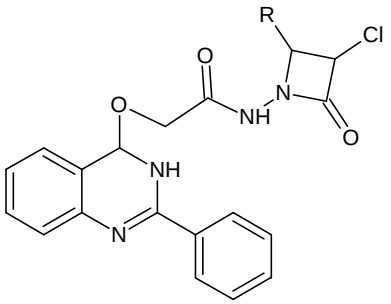
Compound	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear Receptor ligand	Protease Inhibitors	Enzyme Inhibitor
QAz3	-0.08	-0.35	-0.20	-0.50	-0.21	-0.08
QAz8	-0.11	-0.40	-0.22	-0.51	-0.23	-0.10
QAz13	-0.08	-0.34	-0.20	-0.42	-0.20	-0.04
QAz15	-0.04	-0.32	-0.16	-0.39	-0.18	-0.02
QAz25	-0.07	-0.36	-0.17	-0.47	-0.21	-0.07

Table 4. Prediction of Drug likeness of selected Quinazolino-acetidinone analogues using Osiris Property Explorer

Compound	C log P	Solubility	Molecular weight	Drug likeness	Drug score
QAz3	3.92	-7.4	464	3.77	0.18
QAz8	3.36	-7.01	444	1.06	0.17
QAz13	2.97	-6.37	446	2.09	0.21
QAz15	2.97	-6.37	446	2.77	0.22
QAz25	3.42	-6.98	448	2.07	0.17

3.2. Synthetic methods

The analogues which were designed by *in-silico* studies were selected for wet lab synthesis based on analysis of molecular descriptors, Lipinski rule of five, drug likeness property etc.



QAz

Figure 1. General structure of Quinazolino-acetidinone derivatives

The synthetic scheme involved was a five step reaction. After the isolation of product in each step the products were recrystallised and purified by TLC and column chromatography. The general structure of proposed analogues is shown in **Figure 1.** Five new derivatives were synthesized by conventional method (QAz3, QAz8, QAz13, QAz15, and QAz25). The percentage yield of the reaction, melting point, and R_f value of each compounds were calculated and shown in **Table 5.**

Table 5. Characterization data of synthesized acetidino-quinazoline derivatives

Code	R	Molecular Formula	Molecular Weight	Melting Point	Yield	R_f
QAz3	C_6H_5 p-Cl	$C_{25}H_{18}Cl_2N_4O_3$	493.35	130	68	0.61
QAz8	C_6H_5 p-CH_3	$C_{26}H_{21}ClN_4O_3$	472.93	120	75	0.59
QAz13	C_6H_5 o-OH	$C_{25}H_{19}ClN_4O_4$	474.9	145	69	0.6
QAz15	C_6H_5 p-OH	$C_{25}H_{19}ClN_4O_4$	474.9	142	68	0.63
QAz25	C_6H_3 p-F	$C_{25}H_{18}ClFN_4O_3$	476.89	136	63	0.66

3.3. Spectral characterization of Acetidino quinazoline derivatives

The newly synthesized novel Acetidino-quinazoline derivatives were further characterized by FT-IR, ^{1}HNMR, ^{13}CNMR and Mass spectral studies. The complete spectral analysis of prototype lead molecule QAz3 is shown in **Table 6.**

Table 6. Characteristic FT-IR, 1HNMR and Mass spectral analysis of synthesized analogues

Compound	IR (KBr υ cm^{-1})
Step 1	3,303 cm^{-1} (N-H str), 1,667 cm^{-1} (C=O), and 1,614 cm^{-1} (C=N)
Step 2	1,653 cm−1 (C=O), 1,609 cm−1 (C=N) and 1,152 cm−1 (C-O, ether),2851(CH aliphatic)
Step 3	3302-2922 (NH, NH2), 2852 (C-H alip.), 1653 (CO) carboxamide, 1511 (C=N), 1026 (C-O-C).
Step 4	3302.60 (N-H str.), 3062.51 (Ar C-H str.), 1657.82 (C=O str.), 1538.07 (C=N str.), 1566.09 (Ar C-C str.), 1292.23 (C-N str.), 890.91 (aliphatic C-H str. of N=CH-).
Step 5 **(QAz3)**	3302 (N-H str.), 3061.68 (Ar C-H str.), 1651.04 (C=O str.), 1613.96 (C=N str.), 1659.54(lactone), 1886.68cm^{-1} (-NCO, stretch.), 1148cm^{-1}(CH–Cl, stretch.), 860.32cm^{-1} (aromatic C=C)
^{1}HNMR QAz3	8.5(s,1H,NH), 7.06-8.01(m, 13H, aromatic ring), 7.409-7.430 (t, 1H, Ar-H), 6.244(s, 1H, Ar H), 5.417(s, 2H,CH_2), 2.50(s, CH_3),
Mass spectral Analysis	
QAz3	493.017 (Molecular ion peak), 102.345 (Base peak)

3.4. Evaluation of antioxidant activity of Quinazolino-acetidinone derivatives

The DPPH radical scavenging is considered as a good *in-vitro* model and is widely used to conveniently assess antioxidant efficacy. The percentage inhibitions of DPPH radical by different concentrations of quinazoline-acetidinone derivatives were compared with that of ascorbic acid. The novel derivatives showed moderate dose dependent inhibition of DPPH radical with an IC_{50} value of 11.01 µg/mL **(Figure 2).**

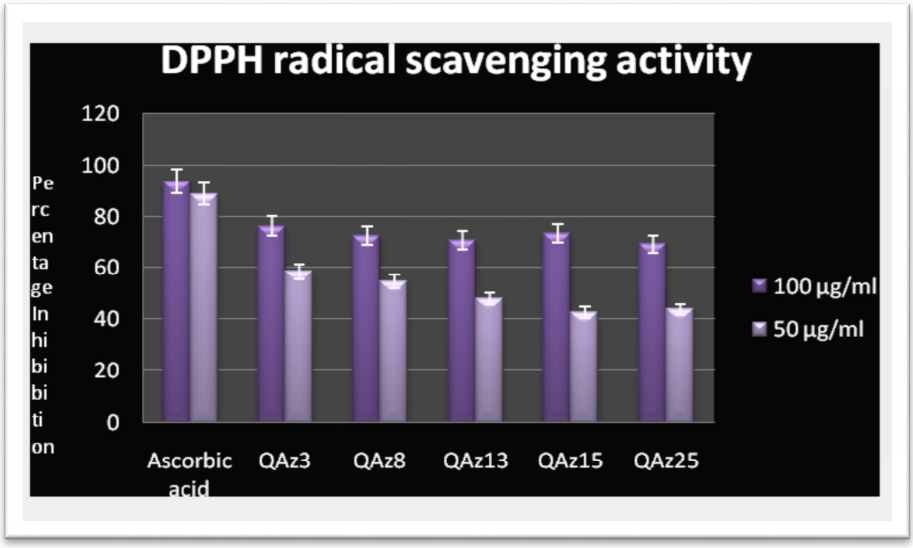


Figure 2. Comparison of DPPH radical scavenging activity of quinazolino-acetidinone derivatives with standard ascorbic acid

The total antioxidant activity of different concentrations of the ascorbic acid and in comparison with samples of quinazoline-acetidinone derivatives were shown in **Figure 3** and **Figure 4** respectively. Among these derivatives QAz15 and QAz25 showed comparatively good antioxidant activity.

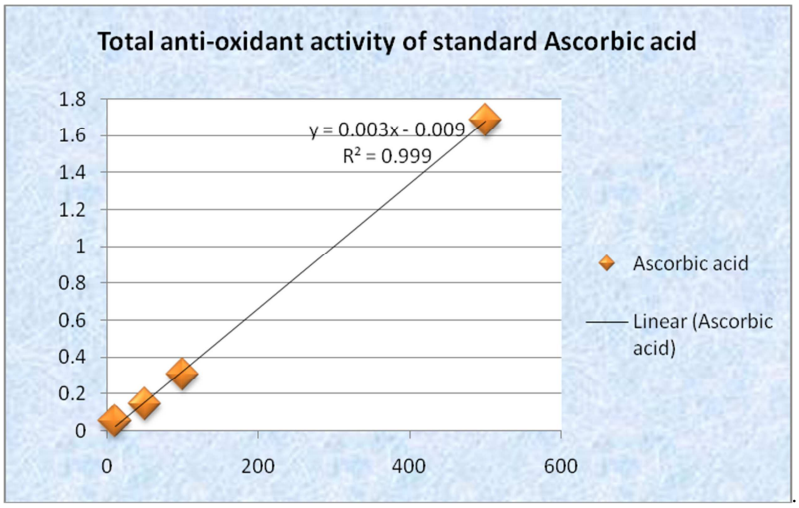


Figure 3. Total antioxidant activity of standard ascorbic acid

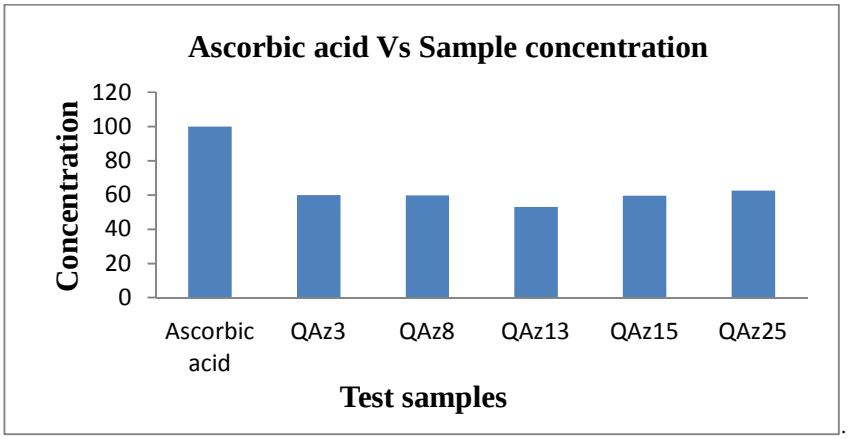


Figure 4. Comparative study of total antioxidant activity of quinazoline-acetidinone derivatives with standard ascorbic acid

CONCLUSION

The present work led to the development of novel antioxidant molecules containing Quinazolino-acetidinone pharmacophore. This research work was focused on the structure based drug design and development of novel quinazoline-acetidinone derivatives and their antioxidant evaluation. We have designed 30 new analogues and after *in-silico* molecular modeling and docking studies, selected five analogues for wet lab synthesis (QAz3, QAz8, QAz13, QAz15, QAz25). These derivatives were spectrally characterized by FT-IR, ^{1}HNMR, mass spectroscopy. The antioxidant evaluation of these derivatives was done by DPPH radical scavenging activity method. These compounds showed dose dependent antioxidant activity compared with that of standard ascorbic acid. From this study, this can be concluded that the synthesized Quinazolino-acetidinone derivatives can be lead candidates to be developed into useful antioxidant agents that could lead further research work on this potent nucleus. An extensive study is also warranted to determine additional physiochemical and biological parameters to have deeper insight into SAR and optimize the effectiveness of these lead molecules.

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