

ORIGINAL ARTICLE

Molecular Docking, ADME Studies, Synthesis, and Anti-oxidant Activity of Novel Quinazoline-4(3H)-one Derivatives

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Received date: October 20, 2022; **Accepted date:** November 21, 2022; **Published date:** December 31, 2022**Abstract**

Background: Free radicals are linked to numerous human diseases. Free radicals can be neutralised by antioxidants, thereby reducing their negative effects. We sought to learn more about the antioxidant and free radical scavenging abilities of quinazoline derivatives in this study.

Aim of the study: Drug discovery and development is a time-consuming, interdisciplinary and expensive process. Advances in computational procedures have empowered *in silico* routines, and specifically structure-based drug design technique, to accelerate new target choice for the improvement of lead compounds. Hence, the present work aimed to identify the potent quinazolinone compounds for synthesis.

Methodology: The synthesis was carried out from the reaction of anthranilic acid and primary aromatic amines with Vilsmeier reagent (DMF/POCl₃). Five derivatives which obeyed rule of five, having desired physio-chemical properties were synthesized (PDB code: 6DE4). The reaction occurred in few minutes under microwave irradiation providing good yields. The synthesized compounds were isolated, recrystallised by using suitable solvents, purified by Thin Layer Chromatography (TLC) and characterized by Fourier-transform infrared spectroscopy (FT-IR), Proton-Nuclear Magnetic Resonance (¹H NMR), and mass spectroscopy.

Results: All the synthesized compounds (3a, 4a, 8b, 9b, 10b) were evaluated for their anti-oxidant activities by 2,2-diphenyl-1-picrylhydrazyl (DPPH), Hydrogen peroxide (H₂O₂) assays. All of them showed significant anti-antioxidant activity, with 8b exhibiting the maximum activity compared to others.

Conclusion: On comparison with standard ascorbic acid, quinazolinone derivatives were found to possess effective *in vitro* antioxidant activity. These quinazoline analogues could be considered as useful templates for further development to obtain more potent antioxidant activity.

Keywords: One pot, Anthranilic acid, Docking studies, Anti-oxidant

Introduction

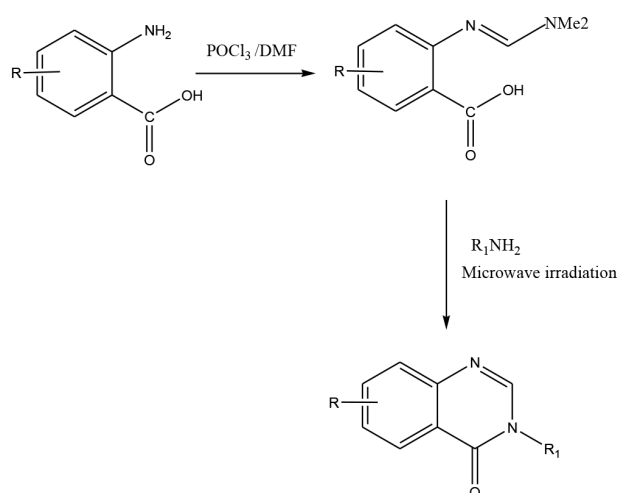
Quinazoline derivatives are classes of fused heterocyclic of extensive interest due to their varied biological activity including anti-inflammatory,^{1,2} antibacterial,³⁻⁸ antituberculosis,⁹ antimalarial,¹⁰ anti-HIV,¹¹ antiviral,¹² antiobesity,¹³ antipsychotic,¹⁴ antidiabetic,¹⁵ anticytotoxin,¹⁶ antispasmodic activities.¹⁷

The look for new molecules with antioxidant property is a popular area of research due to the fact that they could protect the human body from free radicals and retard the development of many continual diseases, including vascular diseases. Anti-oxidant activity is effective in the preventing complicated diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease

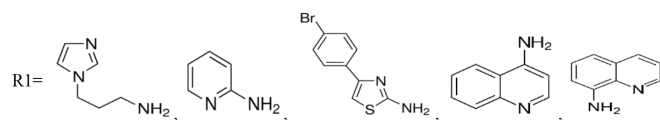
and cancer. Flavonoids and phenolic compounds are broadly available in plants which have been found to exert organic effects such as antioxidant, free radical scavenging abilities, anti-inflammatory and anticarcinogenic activities. It has attracted a great deal of interest in the research of herbal antioxidants. A wide variety of synthetic compounds such as quinazolinones have been explored for antioxidants abilities. This inspired our interest to synthesize a group of compounds containing quinazolin-4 (3H)-one derivatives associated with various primary hetero aromatic amines moiety and to evaluate their antioxidant potency. The antioxidant activity of synthesized compound turned into defined on the premise of general antioxidant interest with the aid of using scavenging activity of free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide radical scavenging activity.

Materials and Method

Design of compounds through *in silico* docking studies



R=(1a-5a) Anthranilic acid, (6b-10b) 5 Iodo Anthhranilic acid (11c-15c) 3 Iodo Anthranilic acid



Molecular docking

Before the docking analysis, ligands were prepared from the optimized compounds and saved in pdb file format using spartan, 14. The 3D compound of Dihydrofolate reductase (DHFR) tyrosine kinase was downloaded from the protein bank (with pdb ID:6DE4) (Table 1).

The enzyme was prepared with the help of discovery studio visualizer for the docking analysis. In the course of preparation, hydrogen was added and water molecule, heteroatoms and co-ligands were eliminated from the crystal compound saved in pdb file.

The docking of the ligands to the active site of DHFR was achieved with the help of pyrex software using Autodock vina. After successful docking protocol, reformation of the complexes (ligand-receptor) for further investigation was also achieved utilizing chimera software. Discovery studio visualizer and pyMOL were used to investigate the interactions of the complexes (Figure 1 to Figure 15).

Table 1: Docking and glide score of 6DE4

Sl. No	Compounds	6DE4	Interaction Of Amino Acids
1a		-7.1	Glu, Ile, Val, Phe, Leu
2a		-7.6	Glu, Ile, Phe
3a		-9.5	Phe, Ile, Ser, Leu
4a		-9.3	Ala, Ser
5a		-8.9	Ile, Val
6b		-7.4	Tyr, Ala, Phe
7b		-8.1	Tyr, Phe, Thr, Ile
8b		-9.2	Phe, Ala, Tyr
9b		-9.0	Ser, Ile, Val, Phe, Lys, Tyr
10b		-9.0	Ser, Ile, Leu
11c		-7.6	Ala, Tyr, Ile, Val, Phe, Leu
12c		-7.9	Glu, Ile, Ser, Thr, Tyr, Val
13c		-9.2	Ala, Lys, Thr
14c		-9.1	Asp, Tyr, Phe, Lys, Ile
15c		-8.9	Glu, Ile, Ser, Thr, Ala, Leu

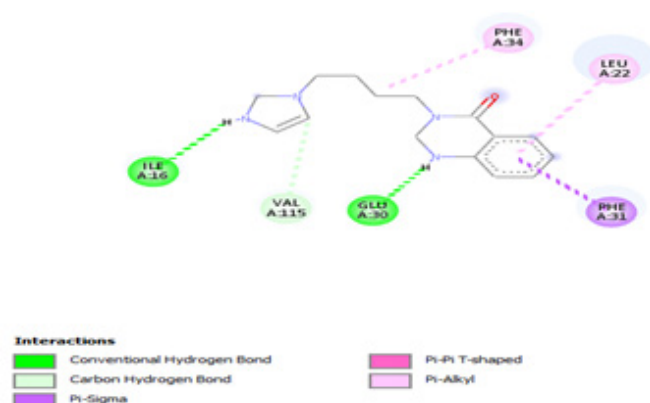


Figure 1: Compound 1a

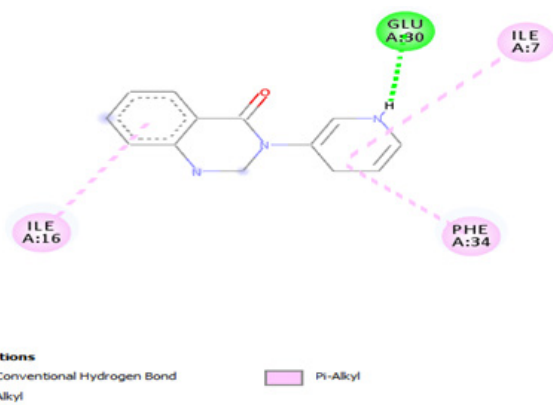


Figure 2: Compound 2a

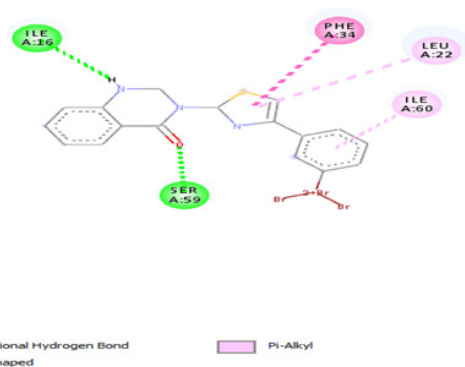


Figure 3: Compound 3a

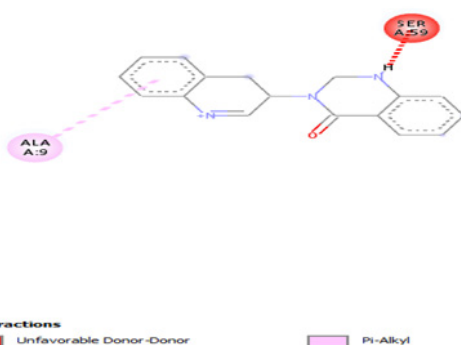


Figure 4: Compound 4a

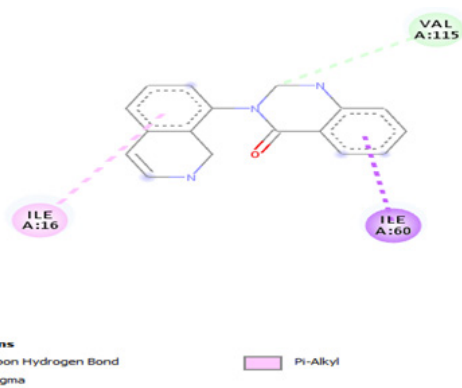


Figure 5: Compound 5a

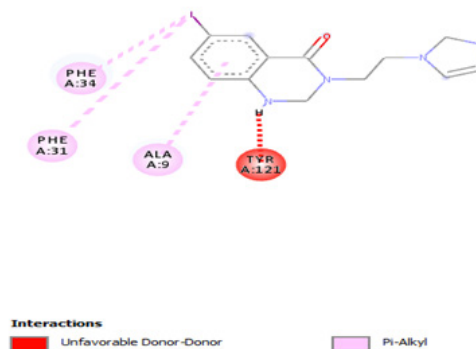


Figure 6: Compound 6b

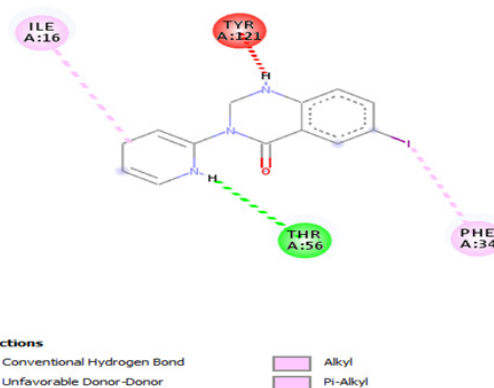


Figure 7: Compound 7b

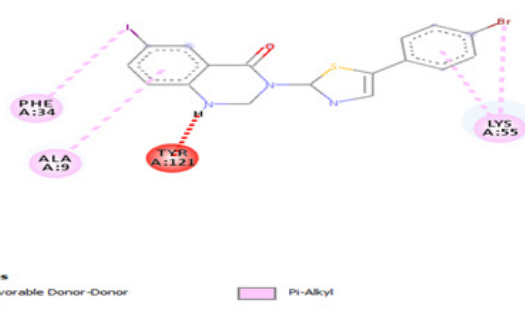


Figure 8: Compound 8b

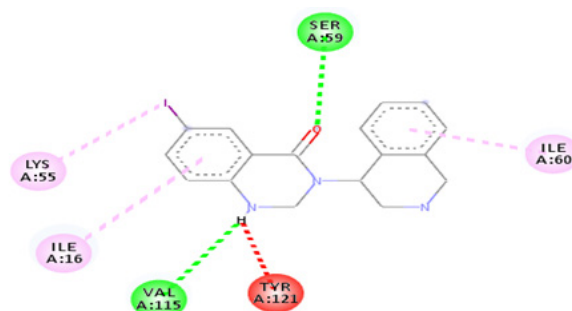


Figure 9: Compound 9b

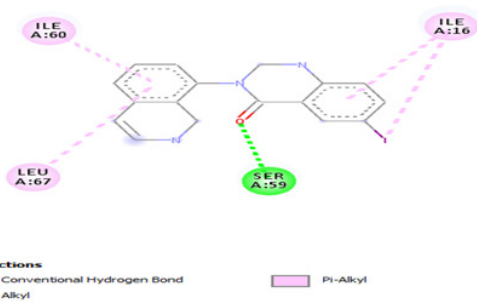


Figure 10: Compound 10b

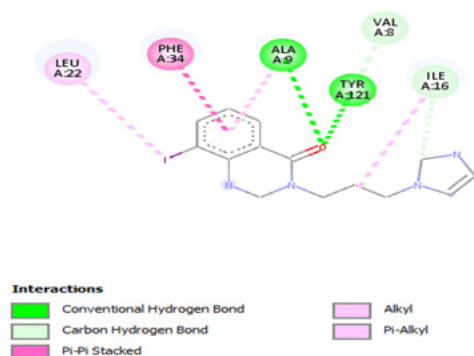


Figure 11: Compound 11c

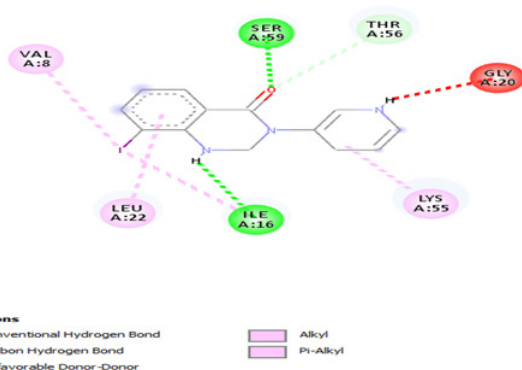


Figure 12: Compound 12c

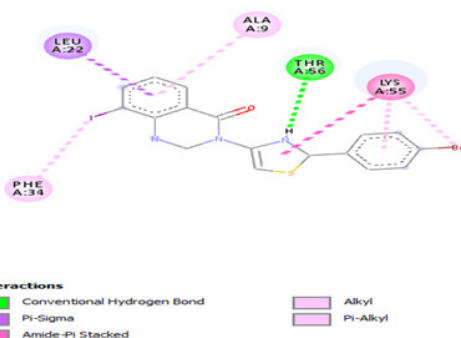


Figure 13: Compound 13c

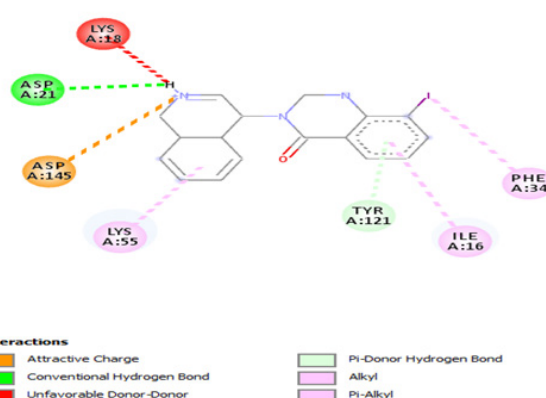


Figure 14: Compound 14c

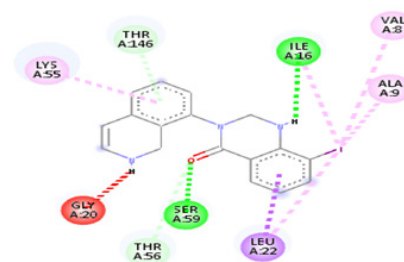
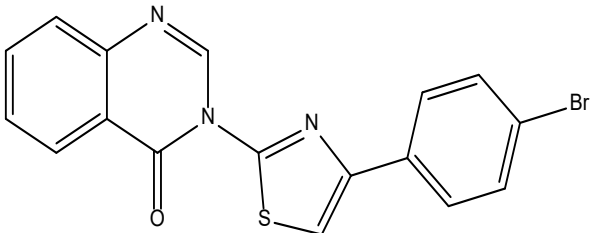
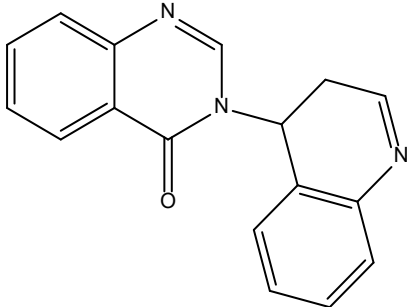
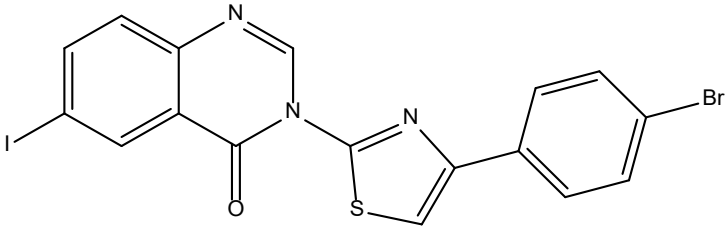
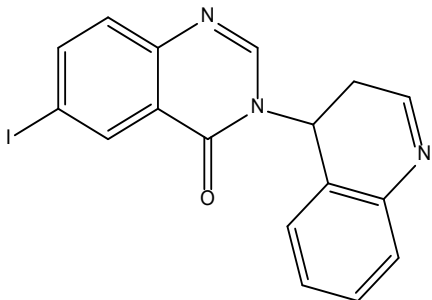
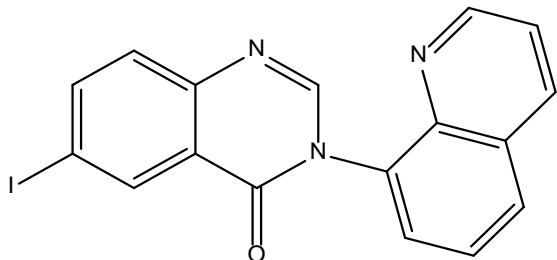


Figure 15: Compound 15c

Synthesis of designed compounds and their characterization

A series of 15 compounds were designed through *in silico* docking and high docking score (3a, 4a, 8b, 9b, 10b) synthesis by one pot synthesis of quinazolinone derivatives from the reaction of anthranilic acid and primary heterocyclic amines with Vilsmeier reagent (DMF/POCl₃) was carried out. The reaction occurred in few minutes under microwave irradiation providing excellent yields (Table 2). Various 4-(3H)-quinazolinones were synthesized by treating anthranilic acid and substituted anthranilic acid with Vilsmeier reagent (DMF/POCl₃) at 0° followed by the addition of primary heterocyclic amines. The reaction-mixture was supported on anhydrous sodium sulphate and exposed to microwave irradiation for 2-4 min, resulting in the formation of 3-substituted quinazolinones. The products were characterized by infrared spectroscopy (IR)), (Nuclear magnetic resonance (NMR) and mass spectra. All the synthesized compounds were screened for their *in vitro* antioxidant activity.

Table 2: Compounds and their characterization

Compounds	Structures	Reaction time (Min)	Yield (%)	M.P(°C)
3a	 <p>3-(4-(4-bromophenyl)thiazol-2-yl)quinazolin-4(3H)-one</p>	1.5	83	132
4a	 <p>3-(3,4-dihydroquinolin-4-yl)quinazolin-4(3H)-one</p>	1.7	81	178
8b	 <p>3-(4-(4-bromophenyl)thiazol-2-yl)-6-iodoquinazolin-4(3H)-one</p>	1.5	88	200
9b	 <p>3-(3,4-dihydroquinolin-4-yl)-6-iodoquinazolin-4(3H)-one</p>	1.7	84	198
10b	 <p>6-iodo-3-(quinolin-8-yl)quinazolin-4(3H)-one</p>	1.5	85	200

Characterized by IR, NMR, mass spectra

- 3a:** Brown crys-tals, ^1H NMR (CDCl_3 , 500 MHz): δ 7.39 (d, $J = 6.9\text{ Hz}$, 2H), 7.46 (t, $J = 7.6\text{ Hz}$, 1H), 7.50 (t, $J = 6.8\text{ Hz}$, 3H), 7.74 (t, $J = 7.5\text{ Hz}$, 1H), 7.78 (d, $J = 8.4\text{ Hz}$, 1H), 8.11 (s, 1H), 8.35 (d, $J = 7.6\text{ Hz}$, 1H); IR (KBr): 3056, 1668, 1591 cm^{-1} ; MS: m/z 223 ($\text{M}^+ + 1$). Anal. Calcd. for $\text{C}_{17}\text{H}_{10}\text{BrN}_3\text{OS}$: C, 53.13; H 2.61; Br. 20.78; N 10.94 O ,4.15 S,8.34 ,
- 4a:** Yellow crystals, ^1H NMR (CDCl_3 , 500 MHz): δ 7.43- 7.47 (m, 3H), 7.53 (t, $J = 6.9\text{ Hz}$, 1H), 7.59 (d, $J = 6.9\text{ Hz}$, 1H), 7.77 (d, $J = 8.4\text{ Hz}$, 1H), 7.79 (t, $J = 8.4\text{ Hz}$, 1H), 7.94 (s, 1H), 8.35 (d, $J = 7.65\text{ Hz}$, 1H); IR (KBr): 3061, 1677, 1603, 1082 cm^{-1} ; MS: m/z 257 ($\text{M}^+ + 1$), 259 ($\text{M}^+ + 3$). Anal. Calcd. for $\text{C}_{17}\text{H}_{13}\text{N}_3\text{O}$: C, 65.16; H, 4.75; N, 15.26. Found: C, 65.60; H, 3.56; N, 10.89%.
- 8b:** yellowish brown crystal, ^1H NMR (CDCl_3 , 500 MHz): δ 7.28 (d, $J = 9.2\text{ Hz}$, 2H), 7.49 (t, $J = 6.9\text{ Hz}$, 1H), 7.65 (d, $J = 8.3\text{ Hz}$, 2H), 7.74 (d, $J = 8.4\text{ Hz}$, 1H), 7.77 (t, $J = 6.9\text{ Hz}$, 1H), 8.06 (s, 1H), 8.32 (d, $J = 8.4\text{ Hz}$, 1H); IR (KBr): 3092, 1692, 1603, 1071 cm^{-1} ; MS: m/z 301 ($\text{M}^+ + 1$), 302 ($\text{M}^+ + 3$). Anal. Calcd. for $\text{C}_{17}\text{H}_9\text{BrIN}_3\text{OS}$: C, 55.84; H, 3.01; N, 9.30. Found: C, 55.92; H, 2.89; N, 9.31%.
- 9b:** Brown liquid, ^1H NMR (CDCl_3 , 500 MHz): δ 2.13 (s, 3H), 2.32 (s, 3H), 7.10 (q, $J = 7.62\text{ Hz}$, 2H), 7.18 (s, 1H), 7.51 (t, $J = 8.4\text{ Hz}$, 1H), 7.75 (m, 2H), 7.97 (s, 1H), 8.34 (d, $J = 8.4\text{ Hz}$, 1H); IR (neat): 1921, 1682, 1603 cm^{-1} ; MS: m/z 251 ($\text{M}^+ + 1$). Anal. Calcd. for $\text{C}_{17}\text{H}_{10}\text{IN}_3\text{O}$: C, 74.14; H, 5.64; N, 11.19. Found: C, 76.85; H, 5.63; N, 11.21%.
- 10b:** White crystals, ^1H NMR (CDCl_3 , 500 MHz): δ 3.83 (s, 3H), 7.01 (d, $J = 8.4\text{ Hz}$, 2H), 7.31 (d, $J = 9.1\text{ Hz}$, 2H), 7.50 (t, $J = 6.9\text{ Hz}$, 1H), 7.72 (d, $J = 7.65\text{ Hz}$, 1H), 7.77 (d, $J = 8.4\text{ Hz}$, 1H), 8.11 (s, 1H), 8.34 (d, $J = 8.4\text{ Hz}$, 1H); IR (KBr): 3044, 2981, 1682, 1609, 1261, 1032 cm^{-1} ; MS: m/z 252 ($\text{M}^+ + 1$). Anal. Calcd. for $\text{C}_{17}\text{H}_{10}\text{IN}_3\text{O}$: C, 71.42; H, 4.79; N, 11.10. Found: C, 71.35; H, 4.80; N, 11.08%

In vitro antioxidant activity

The synthesized compounds were screened for *in vitro* antioxidant activity.

The antioxidant capacity of quinazolinones was studied using different *in vitro* analytical methodologies such as DPPH scavenging, total reducing ability determination using Fe^{3+} , Fe^{2+} transformation method, and hydrogen

peroxide scavenging. These were used as the reference antioxidant radical scavenger compounds.

DPPH method**Preparation of control (DPPH) solution**

10 mg of DPPH was dissolved in 10 mL of methanol. From this stock solution, dilutions were made to obtain concentrations of 10 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$ and 250 $\mu\text{g/mL}$. The absorbance was recorded at 517 nm.

Preparation of standard solution (Ascorbic acid)

10 mg of ascorbic acid was dissolved in 10 mL of methanol. From this stock solution, dilutions were made to obtain concentrations of 10 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$ and 250 $\mu\text{g/mL}$ to which 1 mL of DPPH solution was added and volume was made up to 10 mL. The absorbance was recorded at 517 nm after a duration of 30 min.

Preparation of test or sample solutions

The test solutions were prepared in a similar manner as that of standard ascorbic acid and the absorbance was recorded at 517 nm after a duration of 30 min.

% inhibition was calculated by Scavenging (%) = $\frac{\text{AS blank (DPPH)} - \text{AS (Sample)}}{\text{AS control (DPPH)}} \times 100$. AS denotes absorbance (Figure 16).

Hydrogen peroxide radical scavenging method

Reaction mixture containing test samples /standard (Ascorbic acid) at different concentrations of 10 – 250 $\mu\text{g/mL}$ was added to 0.6 mL of hydrogen peroxide solution in phosphate buffer (pH 7.4). After incubating for 10 minutes at 37°C , the absorbance was measured at 230 nm. Appropriate blanks were taken. The experiment was carried out three times at 230 nm, and hydrogen peroxide absorbance in phosphate buffer was measured as control. Using below equation, the scavenging effect (%) was calculated. Hydroxyl radicals are created by hydrogen peroxide in cells. The test drug's ability to scavenge these free radicals was utilised to determine its antioxidant activity. The decrease in absorbance at 230 nm with increasing concentration of the test medication as shown in Figure 17 indicates the reduction of these radicals.

Scavenging (%) = $\frac{\text{Absorbance} - \text{Absorbance (Sample)}}{\text{Absorbance}} \times 100$

Results and Discussion

All the synthesized compounds (3a, 4a, 8b, 9b, 10b) were evaluated for their anti-oxidant activity by DPPH, H_2O_2

assays. All of them showed significant anti-antioxidant activity, with 8b showing the maximum activity compared to others. *In vitro* antioxidant activities of synthesized compounds were comparable to that of the standard drug used in the study.

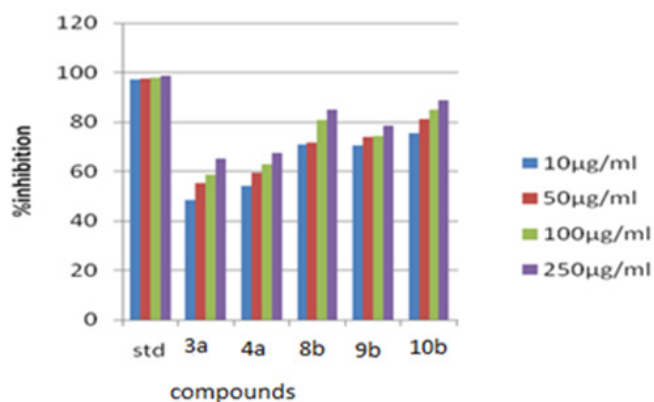


Figure 16: DPPH free radical scavenging activity

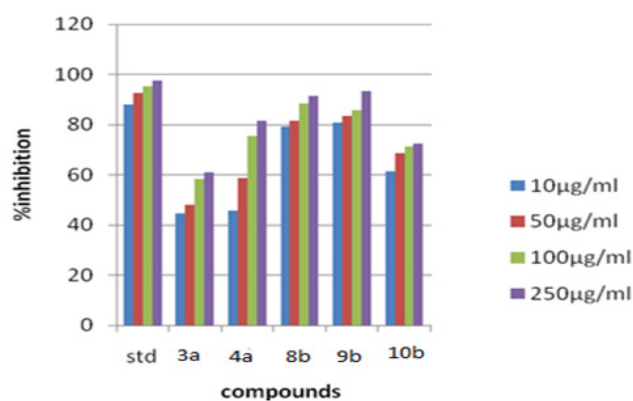


Figure 17: Hydrogen peroxide radical scavenging activity

Absorption, Distribution, Metabolism, and Excretion (ADME) studies

ADME properties and drug-likeness prediction of some of the selected anti-microbial agents among the data set was carried out using Swiss ADME, a free web tool used in evaluating ADME properties and drug-likeness of molecules (Table 3).

Table 3: ADME properties and drug-likeness of molecules

Molecules	MW	HBD	HBA	QPlog Po/W	QPlog BB	% Human Oral Absorption	Rule of Five
Acceptable range	130.0 - 725.0	0 - 6	2 - 20	-2.0 – 1.2	-3.0 – 1.2	> 80% is high	Maximum is 4
Compound 1a	253.305	0	4	-2.424	0.381	99.233	0
Compound 2a	223.235	0	4	-2.495	0.398	100	0
Compound 3a	384.258	0	5	-2.607	0.443	97.494	0
Compound 4a	281.359	0	4	-2.49	0.326	97.835	0
Compound 5a	273.295	0	4	-2.596	0.326	100	0
Compound 6b	280.12	0	4	-2.364	0.294	98.3	0
Compound 7b	256.234	0	4	-2.482	0.364	97.64	0
Compound 8b	510.154	0	4	-2.628	0.39	97.312	0
Compound 9b	407.255	0	4	-2.585	0.275	97.709	0
Compound 10b	399.191	0	4	-2.6	0.289	100	0
Compound 11c	294.164	0	4	-2.32	0.234	98.46	0
Compound 12c	289.126	0	5	-2.45	0.364	97.99	0
Compound 13c	510.154	0	5	-2.627	0.377	97.323	0
Compound 14c	407.255	0	4	-2.587	0.261	97.719	0
Compound 15c	399.191	0	4	-2.64	0.342	97.58	0

Conclusion

According to data obtained from the present study, quinazolinone derivatives were found to possess antioxidant activity by using *in vitro* assay including DPPH radical and hydrogen peroxide scavenging activities. We compared standard antioxidant compounds such as ascorbic acid, butylated hydroxytoluene (BHT) respectively. Based on the discussion above, these

quinazoline analogues could be considered as useful templates for further development to obtain more potent antioxidant activity. Quinazolinone derivatives could be very useful for virtual screening in the development of anticancer agents.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgement

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