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Synthesis and Antioxidant Activity of 3-Substituted Schiff bases of Quinazoline-2,4-diones

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Abstract :A series of 3-substituted Schiff bases of quinazoline-2,4-dione have been synthesized from the reactions of quinazoline-2,4-dione (**2**) with substituted aromatic aldehydes. The structures of the newly synthesized compounds have been established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral studies. DPPH inhibition potential and FRAP (Ferric reducing antioxidant power) assay were carried out using *in vitro* models. All the compounds showed more than 50% inhibition potential against DPPH at IC_{50} values between 25-500 µg/mL and most of the compounds showed a dose dependant behavior in FRAP assay.
Key words: Isatoic anhydride, Quinazoline-2,4-dione, Schiff bases, Antioxidant activity.

Introduction

Reactive oxygen species (**ROS**) such as Singlet Oxygen ($^1O_2$), Superoxide anion ($O_2^{\cdot-}$) and hydroxyl (**$^{\cdot}$OH**) radical and hydrogen peroxide (H_2O_2) are often generated as the by products of biological reactions or from exogenous factors. These reactive species exert oxidative damaging effects by bombarding with living cells including DNA. They play an important roles in aging and in the pathogenesis of age related disorders such as cancer, hypertension, atherogenesis, Alzheimer's and Parkinson's diseases.

Quinazoline-2,4-diones exhibit biological activities like antioxidant[1-4], analgesic[5], anti-inflammatory[6], antibacterial[7], antitubercular[8], anticonvulsant[9], hypoglycemic[10] and anti HIV [11] agents.

With the objective of developing potential antioxidant agents, present work has been focused towards the synthesis of 3-substituted Schiff bases of quinazoline-2,4-diones using quinazoline-2,4-dione **2** with substituted aromatic aldehydes in presence of ethanol and screening for their antioxidant activity.

Materials and methods

Chemicals and instruments

All chemicals and solvents used were of analytical grade and purchased from Sigma Aldrich. The melting points were determined in open capillaries and are uncorrected. Purity of the compounds was checked by TLC having silica gel F28 as adsorbent using pet ether and ethyl acetate (8:2) as mobile phase. IR spectra were recorded in KBr on a Shimadzu FTIR 8400 spectrophotometer. ^{1}H NMR spectra were obtained on AMX-400 liquid state spectrometer at 440 MHz in $CDCl_3$. Mass spectrum were recorded on

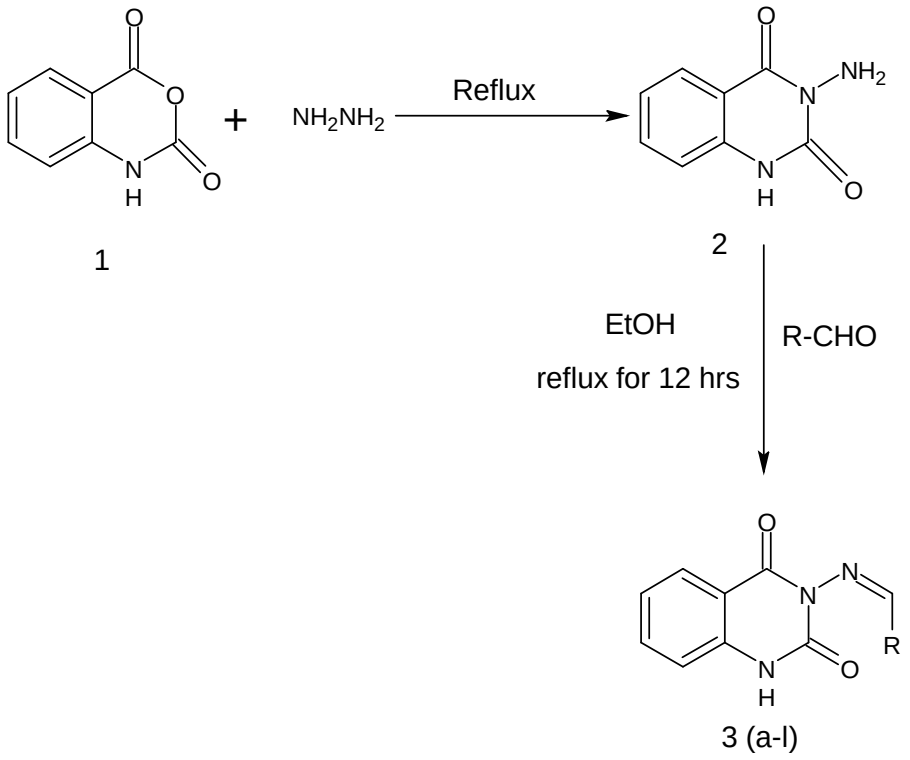
Shimadzu GCMS spectrometer 210. Elemental analysis (C, H, N) of these newly synthesized compounds were performed on a Carlo Erba-1108 elemental analyzer.

Synthesis of 3-amino quinazoline-2,4-dione (2)

An equimolar quantity of isatoic anhydride (11g, mol) and hydrazine hydrate (75ml) were taken in a 500ml round bottom flask and refluxed for 8hrs. The reaction mixture was cooled and poured into Petri plates to get white crystals, which was filtered washed with distilled water and recrystallized from aqueous ethanol.

General procedure for the synthesis of 3-substituted Schiff bases of quinazoline-2,4-diones 3(a-l)

Quinazoline-2,4-dione (0.01mol) was taken in 100ml round bottom flask and ethanol (30ml) was added to dissolve the compound. The substituted aromatic aldehydes (0.01mol) were added and refluxed for about 8hrs. The progress of the reaction was monitored by TLC, The excess of solvent was distilled off, the crystals formed on cooling were collected (Scheme 01).



Scheme 1. Synthesis of new Schiff bases of quinazoline-2,4-dione (3a-l)

Table 1. Newly synthesized Schiff base derivatives of quinazoline-2,4-dione

Compounds	R	Compounds	R
3a	4- $N(CH_3)_2C_6H_4$	3g	4-OH C_6H_4
3b	2-OCH_3, 4-OH C_6H_3	3h	2-Cl C_6H_4
3c	4-OCH_3 C_6H_4	3i	4-Cl C_6H_4
3d	2-OCH_3 C_6H_4	3j	3-NO_2 C_6H_4
3e	2-OH C_6H_4	3k	4-NO_2 C_6H_4
3f	3-OH C_6H_4	3l	4-F C_6H_4

Spectral analysis

3-[(4-Dimethylamino]phenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3a)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.77gm, 0.01mol), 4-N,N-dimethylaminobenzaldehyde (1.49gm, 0.01gm) :Yield 30%, m.p 110-115^{o}C IR (KBr,cm^{-1}):3345 (NH), 3069 (Ar-H), 1659 (C=O), 1524 (C=C of aromatic ring).^{1}H NMR ($CDCl_3$, δppm) 3.0 (s, 6H, N $(CH_3)_2$, 7.3-8.4(m, 8H, ArH), 9.4 (s,1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 309 Anal. Calcd for $C_{17}H_{16}N_4O_2$ C, 66.22; H, 5.23; N, 18.17. Found: C, 62.18; H, 5.19; N, 18.14.

3-[(4-Hydroxy-2-methoxyphenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3b)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.77gm, 0.01mol), 4-hydroxy-2-methoxybenzaldehyde (1.5gm, 0.01gm) :Yield 29%, m.p 178-180C^{o} IR (KBr, cm^{-1}):3560 (OH), 3343 (NH), 1659 (C=O),1527 (C=C of aromatic ring), ^{1}H NMR ($CDCl_3$, δppm) 3.5 (s, 1H, OCH_3) 5.7 (s, 1H, Ar-OH) 7.2-8.4 (m, 7H, Ar-H), 9.4 (s, 1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 312 Anal. Calcd for $C_{16}H_{13}N_3O_4$ C, 61.73; H, 4.21; N, 13.50. Found: C, 61.68; H, 4.17; N, 13.46.

3-[(4-Methoxyphenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3c)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.77gm, 0.01mol), 4-methoxybenzaldehyde (1.3gm, 0.01gm) :Yield 32%, m.p 210-215C^{o} IR (KBr, cm^{-1}):3343 (NH), 1659 (C=O), 1527 (C=C of aromatic ring).^{1}H NMR ($CDCl_3$, δppm) 3.5(s, 1H, OCH_3) 7.2-8.4(m, 8H, Ar-H), 9.4 (s, 1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 296 Anal. Calcd for $C_{16}H_{13}N_3O_3$ C, 65.08; H, 4.44; N, 14.23. Found: C, 65.03; H, 4.40; N, 14.19.

3-[(2-Methoxyphenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3d)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.77gm, 0.01mol), 2-methoxybenzaldehyde (1.3gm, 0.01gm) :Yield 42%, m.p 211-214C^{o} IR (KBr,cm^{-1}):3343 (NH), 1659 (C=O), 1527 (C=C of aromatic ring),. ^{1}H NMR ($CDCl_3$, δppm), 3.5 (s, 1H, OCH_3) 7.2-8.4 (m, 8H, Ar-H), 9.4 (s, 1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 296 Anal. Calcd for $C_{16}H_{13}N_3O_3$ C, 65.08; H, 4.44; N, 14.23. Found: C, 65.03; H, 4.40; N, 14.19.

3-[(2-Hydroxyphenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione(3e)

3-Amino-quinazoline-2, 4(*1H,3H*)-dione (1.77gm, 0.01mol), 2-Hydroxybenzaldehyde (1.2gm, 0.01gm):Yield 39%, m.p 205-210^{o}C IR (KBr, cm^{-1}):3560 (OH), 3343 (NH), 1659 (C=O), 1527 (C=C of aromatic ring),^{1}H NMR ($CDCl_3$, δppm) 5.7 (s, 1H, Ar-OH), 7.2-8.4 (m, 8H, Ar-H), 9.4 (s, 1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 282 Anal. Calcd for $C_{15}H_{11}N_3O_3$ C, 64.05; H, 3.94; N, 14.94. Found: C, 63.99; H, 3.89; N, 14.90.

3-[(3-Hydroxyphenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3f)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.77gm, 0.01mol), 3-hydroxybenzaldehyde (1.2gm, 0.01gm) :Yield 41%, m.p 168-172^{o}C IR KBr, cm^{-1}):3560 (OH), 3343 (NH), 1659 (C=O), 1527 (C=C of aromatic ring), ^{1}H NMR ($CDCl_3$, δppm) 5.7 (s, 1H, Ar-OH), 7.2-8.4 (m, 8H, Ar-H), 9.4 (s, 1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 282 Anal. Calcd for $C_{15}H_{11}N_3O_3$ C, 64.05; H, 3.94; N, 14.94. Found: C, 63.99; H, 3.89; N, 14.90.

3-[(4-Hydroxyphenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3g)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.77gm, 0.01mol), 4-hydroxybenzaldehyde (1.2gm, 0.01gm) :Yield 41%, m.p 210-214^{o}C IR (KBr, cm^{-1}):3560 (OH), 3343 (NH) 1659 (C=O), 1527 (C=C of aromatic ring). ^{1}H NMR ($CDCl_3$, δppm) 5.7 (s, 1H, Ar-OH), 7.2-8.4 (m, 8H, Ar-H), 9.4 (s, 1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 282 Anal. Calcd for $C_{15}H_{11}N_3O_3$ C, 64.05; H, 3.94; N, 14.94. Found: C, 63.99; H, 3.89; N, 14.90.

3-[(2-Chlorophenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3h)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.1gm, 0.01mol), 2-chlorobenzaldehyde (0.48gm, 0.01gm) :Yield 35%, m.p 170-172^{o}C IR (KBr, cm^{-1}):3300 (NH), 3030 (CH of heteroaromatic ring), 1679 (C=O), 1587, 1444 (C=C, C=N of aromatic ring), 670 (C-Cl). ^{1}H NMR ($CDCl_3$, δppm) 7.2-8.4 (m, 8H, ArH), 11.5 (s, 1H, N=CH). MS (m/z): 300 Anal. Calcd for $C_{15}H_{10}ClN_3O_2$ C, 60.11; H, 3.36; N, 14.02. Found: C, 60.07; H, 3.30; N, 13.98.

3-[(4-Chlorophenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3i)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.1gm, 0.01mol), 4-chlorobenzaldehyde (0.48gm, 0.01gm):Yield 45%, m.p 178-180^{o}C IR (KBr,cm^{-1}): 3300 (NH), 3030 (CH of heteroaromatic ring), 1587, 1444 (C=C, C=N of aromatic ring), 1679 (C=O), 670 (C-Cl).^{1}H NMR ($CDCl_3$, δppm) 7.2-8.4 (m, 8H, ArH), 11.5 (s, 1H, N=CH). MS (m/z): 300 Anal. Calcd for $C_{15}H_{10}ClN_3O_2$ C, 60.11; H, 3.36; N, 14.02. Found: C, 60.07; H, 3.30; N, 13.98.

3-[(3-Nitrophenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3j)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.77gm, 0.01mol), 3-nitrobenzaldehyde (0.35gm, 0.01gm)

:Yield 35%, m.p 178-180^{o}C IR (KBr, cm^{-1}):3071 (Ar-H), 3050 (NH),1679 (C=O), 1550 (C-NO_2), 1519, (C=C, of aromatic ring), ^{1}H NMR ($CDCl_3$, δppm) 7.2-8.3 (m, 8H, ArH), 9.4 (s, 1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 311 Anal. Calcd for $C_{15}H_{10}N_4O_4$ C, 58.07; H, 3.25; N, 18.06. Found: C, 58.02; H, 3.19; N, 18.02.

3-[(4-Nitrophenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3k)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.77gm, 0.01mol), 4-nitrobenzaldehyde (0.35gm, 0.01gm) :Yield 39%, m.p 176-178C^{o} IR (KBr, cm^{-1}):3071 (Ar-H), 3050 (NH), 1679 (C=O), 1550 (C-NO_2), 1519 (C=C, of aromatic ring), ^{1}H NMR ($CDCl_3$, δppm) 7.2-8.3 (m, 8H, ArH), 9.4 (s, 1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 311 Anal. Calcd for $C_{15}H_{10}N_4O_4$ C, 58.07; H, 3.25; N, 18.06. Found: C, 58.02; H, 3.19; N, 18.02.

3-[(4-Flurophenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione (3l)

3-Amino-quinazoline-2,4(*1H,3H*)-dione (1.77gm, 0.01mol), 4-flurobenzaldehyde (1.2gm, 0.01gm) :Yield 34%, m.p 175-178C^{o} IR (KBr,cm^{-1}): 3071 (Ar-H), 3050 (NH), 1679 (C=O), 1550 (C-F), 1519 (C=C, of aromatic ring).^{1}H NMR ($CDCl_3$, δppm) 7.2-8.5 (m, 8H, ArH), 9.4 (s, 1H, NH), 11.5 (s, 1H, N=CH). MS (m/z): 284 Anal. Calcd for $C_{15}H_{10}FN_3O_2$ C, 63.60; H, 3.56; N, 14.83. Found: C, 63.55; H, 3.50; N, 14.78.

Antioxidant activity

The antioxidant activity of the synthesized compounds was evaluated using the DPPH free radical scavenging assay [12]. The stock solutions were prepared by dissolving the compounds 1mg in 1mL of methanol. The test solutions (25, 50, 100, 250 and 500 µg/mL) were prepared by diluting the stock solution. 1 mL of test sample solution was added to 4 ml of methanolic DPPH (40mg/100mL of methanol). The mixture was incubated for 20 minutes at room temperature and the absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as standard. A blank was prepared without adding standard or test compound. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capacity to scavenge the DPPH radical was calculated using the following formulae and the results were tabulated in Table.1.

% of antioxidant activity = [A-B/A] X 100

A= Absorbance of control

B= Absorbance of sample.

Ferric reducing antioxidant power (FRAP)

This method involves the reduction of Fe^{3+} to Fe^{2+} in which the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each sample. In the presence of reducing behavior of the molecule, causes the conversion of Fe^{3+}/ ferricyanide complex to the ferrous form, that is absorbed at 700 nm due to the formation of Perl's Prussain blue color of $Fe_4[Fe(CN)_6]_3$. Increasing absorbance at 700nm indicated the increase in reductive ability [13].

Synthesized compounds in 1ml of methanol were mixed to the mixture of 2.5ml of 0.2 M phosphate buffer (pH 6.8) and 2.5ml of 1% potassium ferricyanide. The mixture was then incubated at 50^{o}C for 20 min. Trichloro acetic acid (2.5ml of 10%) was added to the mixture, which was centrifuged at 3000rpm for 10 min. By taking the upper layer of the solution the test compounds were prepared at different concentrations (50, 100, 150 and 200 µg/mL) and were mixed with 0.5mL of 0.1% ferric chloride solution and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power. Results are listed in Table.2.

Results and discussion

Antioxidant activity

Percentage scavenging activity of the test samples are calculated as per standard procedure. Majority of the synthesized compounds scavenge the DPPH radical by more than 50% and the values are summarized in Table 1. The percentage antioxidant activity of the novel compounds compared with the standard is represented in Figure 1.

Table 1. Antioxidant activity by DPPH radical scavenging method.

Sl. No	Compounds	% scavenging activity at different concentrations in (µg/mL)				
		25	50	100	250	500
1	3a	14.36	26.25	54.75	68.94	**79.13**
2	3b	13.36	17.94	28.37	39.13	56.47
3	3c	9.64	20.38	36.81	52.15	69.39
4	3d	08.31	12.52	21.83	34.54	50.42
5	3e	06.75	17.64	36.61	48.30	63.97
6	3f	08.53	21.75	35.12	52.50	68.58
7	3g	10.16	22.68	34.33	55.91	63.37
8	3h	19.56	34.71	66.72	87.39	**92.58**
9	3i	11.31	24.66	37.34	52.76	**73.63**
10	3j	10.43	25.70	37.84	54.99	**76.32**
11	3k	11.37	24.97	51.63	65.32	**85.63**
12	3l	09.28	20.31	33.18	55.86	**72.10**
13	Standard	10.42	19.11	34.85	61.53	**83.53**

Standard**:** Butylated hydroxytoluene (BHT)

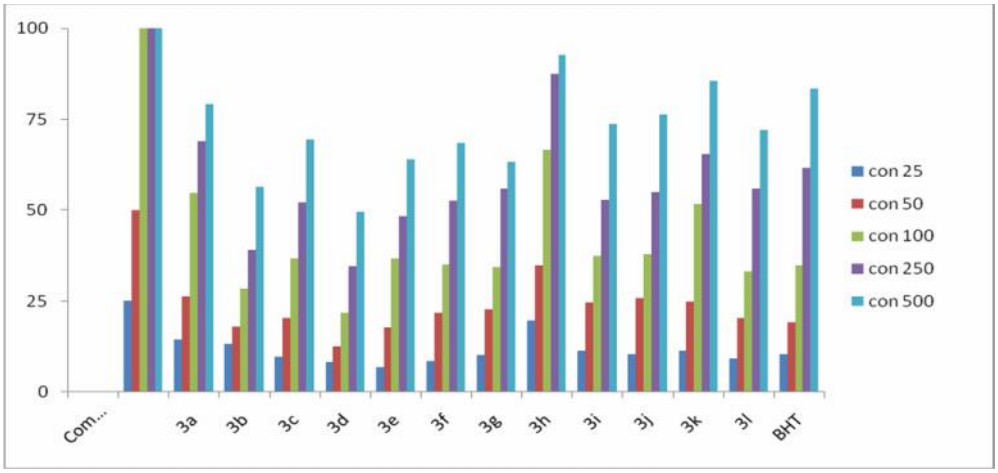


Figure 1. DPPH free radical scavenging assay

Compounds reacts with DPPH, which is a nitrogen centered radical with a characteristic absorption at 517 nm, and convert it to stable diamagnetic molecule 1,1-diphenyl-picryl hydrazine, due to its hydrogen donating ability at a very rapid rate. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons taken up. Such a change in the absorbance produced in this reaction has been widely applied to test the capacity of numerous molecules to act as free radical scavengers. The synthesized compounds scavenged DPPH radical significantly in a concentration dependent manner.

Compounds 3a, 3i, 3j, 3k and 3l showed more than 70% inhibition while compound 3h showed highest free radical scavenging property. Remaining all compounds exhibited moderate activity. Free radical scavenging property of the compounds due to the presence of electron withdrawing substituent like nitro, chloro and fluro groups. Moderate inhibition property displayed by the compounds 3b, 3c, 3d, 3e, 3f, 3g may be due to the presence of methoxy and hydroxyl groups.

Ferric reducing antioxidant power (FRAP)

Reducing power of test compounds at different concentration (50, 100, 150 and 200 µg/mL) was determined and absorbance values are summarized in Table 2. Results antioxidant activity of the novel compounds compared with the standards is represented in Figure 2.

Table.2. Reducing ability of synthesized compounds 3(a-l) at different concentration (µg/ml)

Reducing property at different concentrations in (µg/mL)				
Compounds	50	100	150	200
3a	0.855	1.11	1.365	1.512
3b	0.073	0.147	0.22	0.294
3c	0.572	0.844	1.216	1.488
3d	0.869	1.138	1.407	1.676
3e	0.071	0.143	0.214	0.286
3f	0.279	0.558	0.837	1.116
3g	1.329	2.659	3.988	5.318
3h	0.061	0.122	0.183	0.244
3i	1.461	1.723	1.984	1.246
3j	1.351	1.603	1.954	2.306
3k	1.095	1.361	1.875	2.372
3l	1.547	1.894	2.141	2.881
Standard	0.1878	0.3756	0.7512	1.1268

Standard is ascorbic acid

All the compounds showed ferric ion reducing ability in a dose dependant manner as we observed that with increase in concentration of compounds there is significant increase in the absorbance at 700 nm. Compound 3g showed more reducing ability among all test compounds. Remaining all other compounds showed fairly good activity and found to be more potent than the standard.

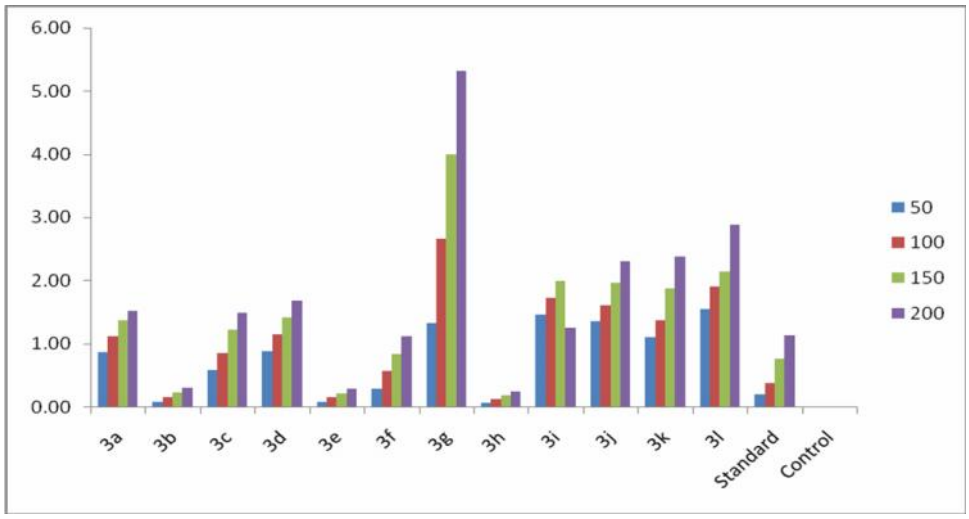


Figure 2. Reducing power method

Conclusion

Twelve new Schiff bases of quinazoline-2,4-dione were synthesized and structurally characterized by various spectral techniques. All the compounds were screened for Antioxidant activity by DPPH free radical scavenging assay and Ferric reducing antioxidant power method. Results revealed that most of the compounds are potent antioxidant agents.

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