Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(8):1132-1138

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Anti-oxidant and anti-inflammatory activity of synthesized 3-substituted schiff bases of quinazoline 2, 4-diones

Deepali Gangrade* and Sayali Lad

Department of Pharmaceutical Chemistry, Vivekanand Education Society College of Pharmacy, Chembur (E), Mumbai, India

ABSTRACT

3-Substituted Schiff bases of Quinazolne-2,4-diones which belongs to the N-containing heterocyclic compounds. The aim of the present study is to study pharmacological study of 3-Substituted Schiff bases of Quinazoline2,4-diones. In the current course of work the novel Schiff bases of Quinazolne-2,4-diones have been synthesized and evaluated for Anti-oxidant and In vitro anti-inflammatory study. 3-Substituted Schiff bases of Quinazoline 2,4-diones have been evaluated for Antioxidant and anti-inflammatory activity. Antioxidant activity was evaluated by Ferric reducing antioxidant power (FRAP), 1,1-DPPH(2,2-diphenyl-1-picrylhydrazyl) And anti-inflammatory activity by membrane stabilization method. The newly synthesized compound F and I showed highest anti-oxidant activity .This compound was further evaluated for in-vitro anti-inflammatory activity by membrane stabilization method. The result of the present study thus demonstrates the anti-inflammatory and anti-oxidant activity of newly synthesized 3-Substituted Schiff bases of Quinazolne-2, 4-diones.

Keywords: Schiff base, Quinazoline 2,4-diones, Antioxidant, Anti-inflammatory, Ferric reducing antioxidant power (FRAP),1,1-DPPH, membrane stabilization method.

INTRODUCTION

Quinazoline derivatives, which belongs to the N-containing heterocyclic compounds, have caused universal concerns due to their widely and distinct biological activities [1]. Medicinal chemist synthesized a variety of quinazoline compounds with different biological activities by installing various groups to the quinazoline moiety using synthetic methods.

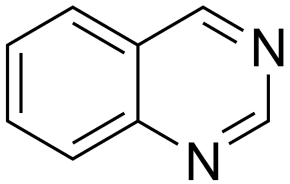

Figure.1: Quinazoline

In the current study, one such versatile, hetero aromatic system, Quinazoline2,4-diones , has been explored for its biological potential.

Another such pharmacologically privileged scaffold is the Schiff's Base [2]. A Schiff's base is a nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by C=N-R group [3] (also known as imine or azomethine group). Usually, a Schiff's base is a condensation product of an aldehyde or ketone with a primary amine.

Schiff's bases are an important class of compounds in organic chemistry and are useful in making carbon-nitrogen bond. The imine or azomethine group present in their structure is critical to their biological activity [4]. Schiff's bases are known to exhibit a broad range of biological activities including anti-oxidant, anti-bacterial, anti-fungal, anti-malarial, anti-inflammatory, anti-cancer, anti-viral and anti-pyretic properties [5]. Thus, Schiff's bases have gained considerable amount of attention of organic and medicinal scientists.

***IN VITRO* ANTIOXIDANT ACTIVITY:**

1)Ferric reducing antioxidant power (FRAP):

This method involves the reduction of Fe3+ to Fe2+ 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 Fe3+/ ferricyanide complex to the ferrous form, that is absorbed at 700 nm due to the formation of Perl's Prussain blue color of Fe4[Fe(CN)6]3. Increasing absorbance at 700nm indicated the increase in reductive ability [6].

2)DPPH scavenging activity:

Antioxidant activity of synthesized compounds was determined by DPPH Scavenging activity which is a spectrophotometric method [7-9]. It is non-enzymatic in vitro method for anti-oxidant activity. The principle of the DPPH Method is based on the reduction of DPPH in presence of a hydrogen donating antioxidant [10].

ANTI-INFLAMMATORY ACTIVITY:

Membrane stabilization method:

The anti-inflammatory activity was determined by membrane stabilization method. The membrane stabilization has been used as a method to study the in vitro anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the synthesized compounds may well stabilize lysosomal membranes [11]. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produces a various disorders [12]. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation.

EXPERIMENTAL SECTION

Materials for Antioxidant activity:

Ferric reducing antioxidant power (FRAP):

0.2 M phosphate buffer (pH 6.8) and 1% potassium ferricyanide, ferric chloride solution.

DPPH scavenging activity:

DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, methanolic DPPH Ascorbic acid.

Materials for Anti-inflammatory activity:

Membrane stabilization method:

Fresh whole human blood (10 ml), normal saline, Aspirin, test sample solution

Methods:

The newly synthesized 3-Substituted Schiff bases of Quinazoline2,4-diones in this research work are as follow in

Table-1: The newly synthesized 3-Substituted Schiff bases of Quinazoline2,4-diones in this research work

Compounds	Name of the compounds
1	3-[(3-Chlorophenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione
2	3-[(2-Cyanophenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione
3	3-[(2-Bromo phenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione
4	3-[(3,4-dimethoxy phenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione
5	3-[(2-Bromo phenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione
6	3-[(3,4-dimethoxy phenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione
7	3-((E)-3-phenylallylideneamino) quinazoline-2,3(1H,3H)-dione.
8	3-[(4-Bromophenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione
9	3-[(2-Flurophenyl)methylene] aminoquinazoline-2,4(*1H,3H*)-dione
10	3-[(2,3,4-Trihydroxy phenyl)methylene] aminoquinazoline-2,4(*1H,3H*) dione

***In vitro* antioxidant activity:**
Procedure
Ferric reducing antioxidant power (FRAP):
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 0.5M of 0.1% ferric chloride solution and absorbance was measured at 700 nm.
Increased absorbance of the reaction mixture indicated the increased reducing power.

DPPH scavenging activity
Procedure:
Preparation of DPPH Solution:
15mg of DPPH was dissolved in 10ml of methanol to prepare stock solution of 1.5mg/m; of DPPH in methanol. The resulting solution was covered with aluminum foil to protect from light.

Sample solution preparation:
The antioxidant activity of the synthesized compounds was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay .

The stock solutions were prepared by dissolving the compounds 1mg in 1mL of methanol [13]. 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.

Ascorbic acid was used as standard. A blank was prepared without adding standard or test compound.

Estimation of DPPH scavenging activity:
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 [14].

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

A= Absorbance of control
B= Absorbance of sample.

***In Vitro* anti-inflammatory activity**
Membrane Stabilization Test
Preparation of red blood cells (RBCs) suspension:
Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as10% v/v suspension with normal saline [15].

Heat induced hemolytic:
The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube [15]. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm [16].

The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula mentioned.

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

A= Absorbance of control B= Absorbance of sample.

RESULTS AND DISCUSSION

Antioxidant activity:

Ferric reducing antioxidant power (FRAP):

Reducing power of synthesized ten compounds at different concentration (50, 100, 150 and 200 µg/mL) was determined and absorbance values of those compounds are summarized in Table-2.

Results antioxidant activity of the novel compounds compared with the standards (Ascorbic Acid) is represented in Figure 2.

Table-2: Ferric ion reducing ability

	Reducing property at different concentrations in (µg/mL)			
Compound	50	100	150	200
A	0.75	0.83	1.21	1.34
B	0.025	0.67	0.79	1.01
C	1.12	1.34	1.65	1.85
D	0.28	0.47	0.78	0.9
E	0.67	0.92	1.12	1.43
F	1.32	1.78	2.01	2.23
G	0.65	0.8	0.46	0.65
H	0.76	0.83	0.67	1.07
I	1.27	1.07	1.54	1.23
J	0.56	0.46	0.76	0.52
Standard	0.78	0.65	1.25	1.55

All the ten synthesized compounds showed ferric ion reducing ability. This ability is in a dose dependant manner as increase in concentration of compounds there is significant increase in the absorbance at 700 nm. Compound F showed more reducing ability among all test compounds.

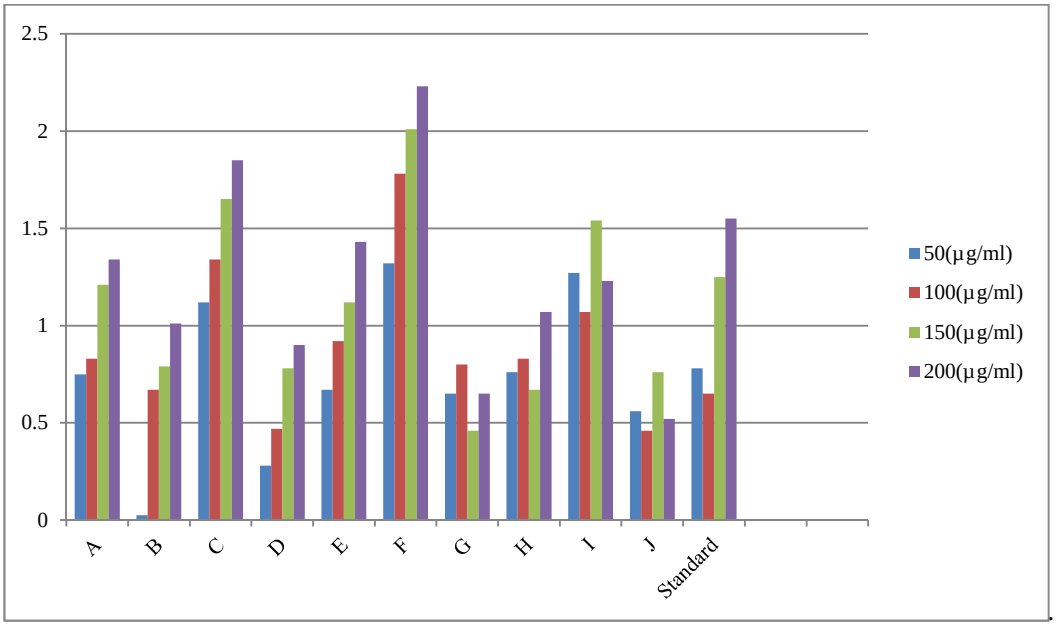

Figure 2: Reducing power method

DPPH scavenging activity

Percentage scavenging activity of the test samples are calculated as per standard procedure [16]. Majority of the synthesized compounds scavenge the DPPH radical by more than 50% and the values are summarized in Table 3. The percentage antioxidant activity of the novel compounds compared with the standard is represented in Figure 3.

Table-3: The percentage antioxidant activity

	% Scavenging activities at different concentrations (µg/ml)				
Compound	100	200	300	400	500
A	42	53	58	64	71
B	32	37	42	55	67
C	28	32	39	52	58
D	47	55	64	69	72
E	35	42	49	52	55
F	48	59	67	76	82
G	42	48	54	58	66
H	29	40	45	49	56
I	48	55	58	62	74
J	38	45	49	55	65
Standard	54	62	69	77	84

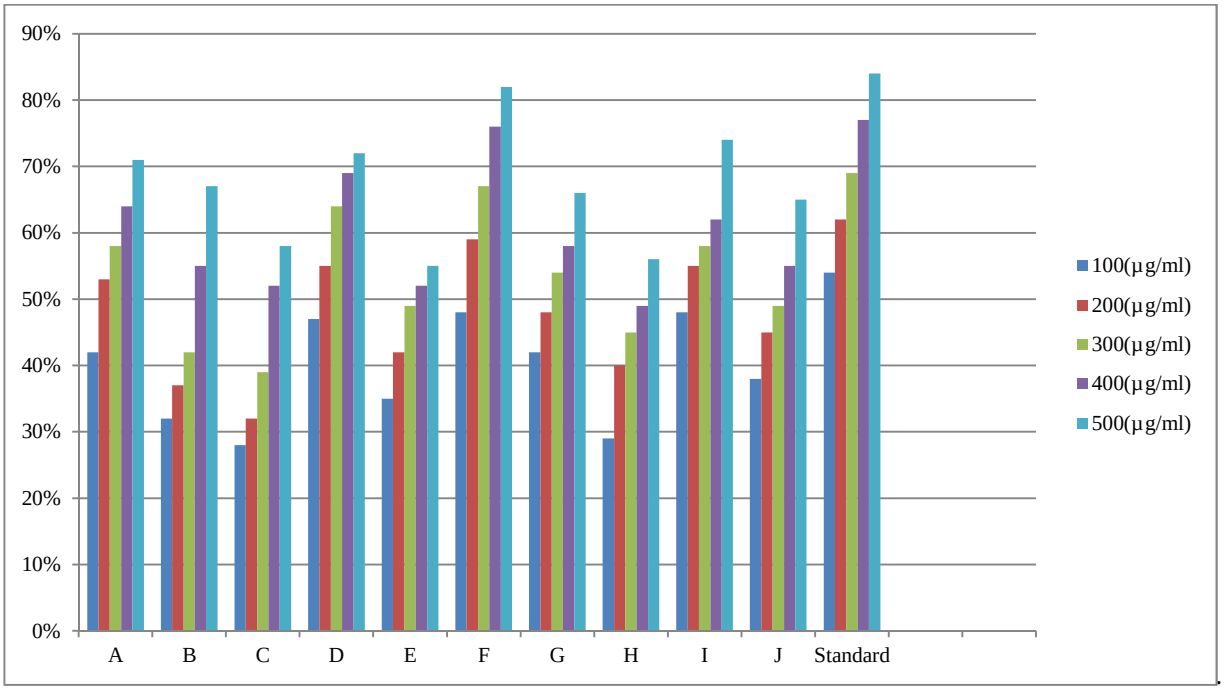

Figure 3: DPPH Free radical scavenging activity

The scavenging activity of synthesized compounds and ascorbic acid on the DPPH Radical was illustrated in figure 5.Synthesized compounds have significant effect on DPPH, it was increased with the increasing concentration from 100-500 µg/ml but the scavenging activity of the synthesized compounds was lower than that of the standard.

Fig 3 represents the percentage inhibition of ascorbic acid and synthesized compounds.

DPPH Radical is commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay.

In Vitro Anti-Inflammatory Activity:

The inhibition of hypo-tonicity induced HRBC membrane lysis i.e. stabilization of HRBC membrane was taken as a measure of anti-inflammatory study. The synthesized compounds F & I exhibited membrane stabilization effect by inhibiting hypotonic induced lysis of erythrocyte membrane. The percentage of membrane stabilization for synthesized compounds and standard were done at 10, 20, 40, 60, 80,100µg/ml. Compound F showed maximum inhibition of 70%.

Table 4: Effect of standard and synthesized compounds F & I on membrane stabilization

Concentration (µg/ml)	%inhibition +.SEM		
	Standard	F	I
10	45%	35%	25%
20	50%	42%	32%
40	56%	53%	42%
60	65%	64%	54%
80	72%	68%	60%
100	84%	70%	65%

All values are mean+- SEM (n=6).Two way ANOVA followed by Dunnett's test, where P<0.0001 When compared with standard and P<0.0001 when compared with control.

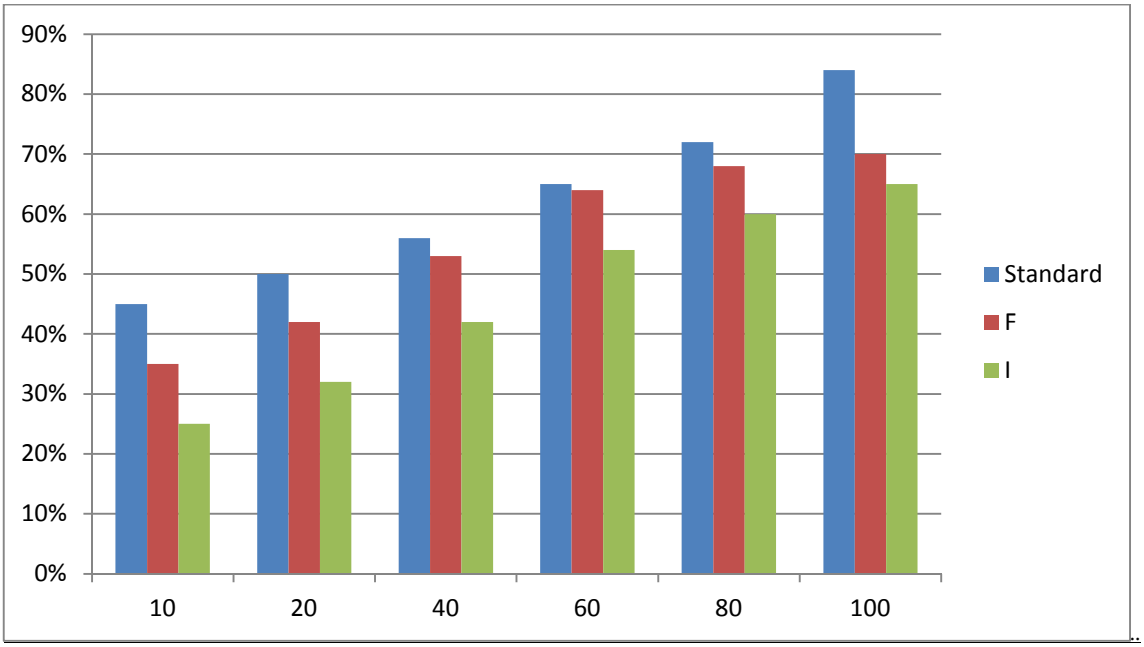

Figure 4: Multiple comparison of effect of test compounds F and I on membrane stabilization activity

CONCLUSION

In this research work, the description includes Pharmacological study of Schiff bases which have been synthesized using different aromatic aldehydes.

The series of Schiff bases of quinazoline2,4-diones have been synthesized successfully as per the designed scheme of synthesis.

All the compounds were screened for Antioxidant activity by Ferric reducing antioxidant power (FRAP) and DPPH free radical scavenging assay. These novel compounds proved to have improved the anti-oxidant property. Among all of the synthesized compounds F and I compounds showed highest anti-oxidant activity.

Anti-inflammatory activity by the membrane stabilization of the test compounds F, I and Standard are done. The results in demonstrate that compounds stabilized human blood cells membrane in dose dependent manner.

Results revealed that most of the compounds exhibit antioxidant and anti-inflammatory activity.

REFERENCES

[1] Eicher, T.; Hauptmann, S. *"The Chemistry of Heterocycles: Structure, Reactions, Syntheses and Applications"*, (**2003)**, 2nd Edition, Wiley-VCH.
[2] Block, B. H.; Beale, J. M. *"Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry"*, 11th Edition, Lipincott-Raven Publisher, New York, (**2004)**, 313-355.
[3] Dua, R.; Shrivastava, S.; Sonawne, S.; Shrivastava, S. K. *Advan. Biol. Res.*, (**2011)**, *3* (5), 12-144.
[4] Chavan, A. Greener and Rapid Access to some Bioactive Heterocycle. M.Pharm. Thesis, October (**2010**).

[5] Raju, G. N. & Nadendla, R. R. *Eur. J. Biomed. Pharm. Sci.* 2, 149–162 (2015).
[6] Shahwar, D., Shafiq, U. R., Naeem, A., Ullah, S., and Raza, M. A., *African J Biotech*, 2010, 9, 1086.
[7] S. Pratibha, D. Premansh, K. Ashok J Phar Res 8 (10):1355-1363 **(2014).**
[8] Antolovich, Prenzler, Patsalides, McDonald, & Robards, **(2002)**
[9] Progress, A. (2011). *European Review for Medical and Pharmacological Sciences*, 15(4), 376–378. http://doi.org/10.1016/j.bmcl.2010.12.025
[10] Pavlos, S., Charalampidis., Panos, V., and Spyros, K., angelos evangelous Blue Eros assay, A novel spectrophotometric method for the evaluation of the antioxidant capacity of various biological substances. Euro. J. Med.Chem. **2009**, 44, 4162-4168.
[11] S. R. Chatrasal and S. Shiwani *J Pharm ID* 907525:http://dx.doi.org/10.1155/2013/907525
[12] (**2013**).
[13] Raju, G. N., & Nadendla, R. R. (**2015**). *European Journal of Biomedical and Pharmaceutical Sciences*, 2(3), 149–162. http://doi.org/10.7897/2230-8407.04309.
[14] Y. Sawai and J. H. Moon, J. Agric. Food Chem., 2000, 48, 6247.
[15] R. Amarowicz, M. Naczk and F. Shahidi, J. Am. Oil Chem. Soc., **2000**, 77, 957.
[16] Kirtikar KR, Basu BD. Indian medicinal plants, vol. III. 2nd ed. Lalit Mohan Basu, Allahabad, India, **1933:**2390]2392.
[17] Puri VN, Kar K, Patnaik GK, Dhawan BN, Kulshreshtha BK, Rastogi RP. *Ind J Exp Biol* **1975**;13:369.