Annapoorna Vadivelu. *et al.* / *International Journal of Pharmacy & Therapeutics*, 5(2), 2014, 90-99.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

MOLECULAR DOCKING, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW SCHIFF BASES OF 2, 3 DISUBSTITUTED QUINAZOLINONE DERIVATIVES

Annapoorna Vadivelu*, Saranya A, Gopal V

College of Pharmacy, Mother Therasa Post Graduate and Research Institute of Health Sciences, Gorimedu, Puducherry-06, India.

ABSTRACT

A series of Schiff bases of 2,3 hetero substituted quinazolinones were designed and Docking simulation was performed to position compounds into the CDK2 structure active site to determine the probable binding model. A series of Schiff bases of 2,3 disubstituted quinazolinones were synthesized by thermal condensation and cyclization of a amide, hetero aromatic acid and anthranilic acid at elevated temperatures followed by condensation with aromatic amines to give desired Schiff bases. The structures of the newly synthesized compounds were established on the basis of TLC, IR, ^{1}H-NMR and MASS data. Further the ADME properties and molecular properties of the synthesized compounds was studied using ACD/I Labs and OSIRS Based on the results of virtual screening compound AA1 and AA3 were screened for anti-oxidant and cytotoxicity in breast cancer cell lines using MTT assay. The compounds showed significant activity.

Key Words:- Quinazolinone, Chemotherapeutic activity, Antioxidants.

INTRODUCTION

Cyclin-dependent kinases (CDKs) are serine/threonine protein kinases whose activity depends on binding and activation by cyclin partners. These heterodimeric complexes can phosphor late various substrates involved in the control of transcription and cell-cycle progression in response to different stimuli. Quinazolone moiety, the lead compound chosen permits structural variation by modifying or incorporating various substituent's in the heterocyclic ring at second or third position. Structure activity relationship studies of quinazolinone ring system revealed in various literatures suggest position 2, 6 and 8 are very much important for structure activity studies and position 3 should be attached to different heterocyclic rings for better chemotherapeutic activity. Further Free radicals are a part of the natural signaling systems in the cell. Useful drugs have been found to be antioxidants but many antioxidants have not been found to be useful drugs (Gordon W Rewcastle 1966).

Corresponding Author:

Annapoorna Vadivelu
E-mail : spoorna@rediffmail.com

Experimental Part

The QSAR study was carried out for 64 substituted derivatives of 2,3 disubstituted quinazolinones.

MATERIALS AND METHODS

The synthesis is based on condensation reaction by varying the heating conditions suitably. The melting points of the compounds were determined by capillary tube method and are presented uncorrected (Laurent Gouilleux 1996).

IR spectra were recorded using KBr pellets in the range of 4000-500cm on a FTIR spectrometer shimadzu-model. ^{1}H NMR of the synthesized compounds were done using proton nmr (400 MHZ) spectra was recorded in CDCL3 in jeol GSX liquid state NMR spectrometer. Chemical shifts are reported in parts per million downfield with reference to internal standard (TMS)

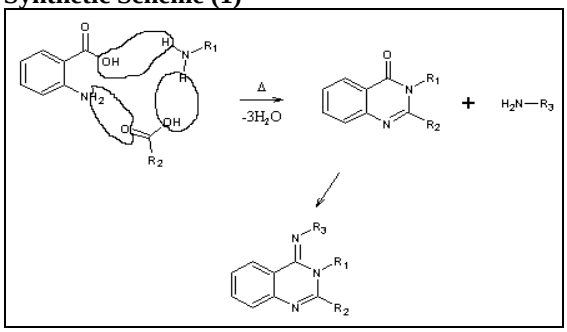
GENERAL PROCEDURE

Aliquot quantities of anthranilic acid, drug aromatic acid and amide were well mixed and pulverized. Fusions of all the reactants is taken into a borosilicate glass beaker and introduced into microwave. The temperature was maintained for few minutes.

The molten residue was boiled with water and filtered while hot. The compounds crystallized on cooling. Crystals so obtained were recrystallised and subjected to further purification (Ernest Hamel 1996).

The completion of reaction was checked by TLC using Precoated Aluminum sheets of Alugram.

Synthetic Scheme (1)

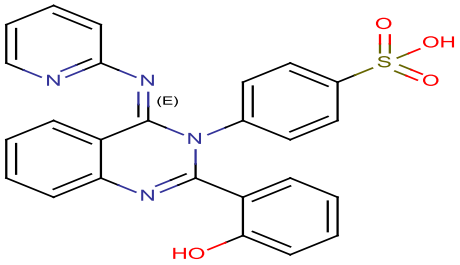


PHYSIOCHEMICAL AND SPECTRAL DATA OF THE SYTNHESISED COMPOUNDS

Compound Code : AA1

Compound name :4-[(4E)-2-(2-hydroxyphenyl)-4(pyridin-2-yl) imino]-3, 4-dihydro quinazolin-3-yl] benzene-1-sulfonic acid(AA1)

Chemical Structure:



Molecular formula : C25H18N4O4S

Molecular weight : 470

% yield : 75%

Melting Point : 169–173ºC

Rf value : 0.41

Log P Value : 2.6

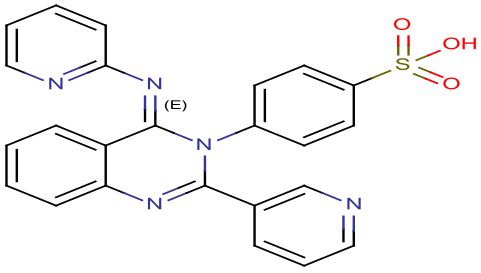
IR Data υ^{cm-1}_{KBr} : 1418(C-H), 1322(Ar-OH), 1558(C=C), 1653(C=N), 1154(C-N).

^{1}H NMR $\delta^{ppm}_{CDCl_3}$: 7-7.3 (m,5H, 4H);7.4 (m,4'H); 7.7 (d, 4'H) ; 9.5 (s, 1'H) 10.5(s, 1H), 11.5(s, 1H)

MS m/z : 470 m+ Ion Peak

Compound name :4-[(4E)-4-[(pyridin-2-yl)imino]-2-(pyridin-3-yl)-3,4-dihydroquinazolin- 3- yl] benzene-1-sulfonic acid2- Pyrazinyl- 4- quinazolone.(AA3)

Chemical Structure:



Molecular formula : C24H17N5O3S

Molecular weight : 455

% yield : 79

Melting Point : 171-175ºC

Rf value : 0.52

Log P Value : 1.8

IR Data υ^{cm-1}_{KBr} : 1420(C-H), 1247(Ar-OH), 1586(C=C), 1617(C=N), 1247(C-N).

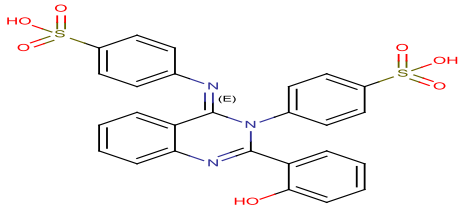
^{1}H NMR $\delta^{ppm}_{CDCl_3}$: 7.7 (m, 4'H) ; 8.15 (m, 4H); 9.5 (s, 1'H). (Fig-15)

MS m/z : 455 $(M)^{+}$ Ion Peak (Fig 16)

Compound code : AS1

Compound name: 4-[(4E)-2-(2-hydroxyphenyl)-4-[(4-sulfophenyl)imino]-3,4-dihydro quinazolin -3-yl] benzene-1-sulfonic acid.

Chemical Structure:



Molecular formula : C27H20N2O7S2
Molecular weight : 548
% yield : 70
Melting Point : 165-169ºC
Rf value : 0.47
Log P Value : 0.5

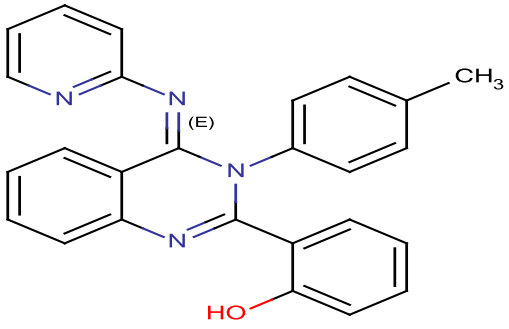
IR Data υ_{KBr}^{cm-1} : IR(KBr) : 1399(C-H), 1244(Ar-OH), 1599(C=C), 1648(C=N), 1244(C-N).

^{1}H NMR $\delta_{CDCl_3}^{ppm}$: 7.7 (d, 4'H); 8.15 (d, 4H) 9.5 (s, 2'H).

MS m/z : 548 $(M)^+$ Ion Peak

Compound code : BA1
Comound name: 2-[(4E)-3-(4-methylphenyl)-4-[(pyridin-2-yl)imino]-3,4-dihydro quinazolin-2-yl] phenol

Chemical Structure:



Molecular formula : C26H20N4O
Molecular weight : 404
% yield : 65
Melting Point : 182-186ºC
Rf value : 0.49
Log P Value : 6.1

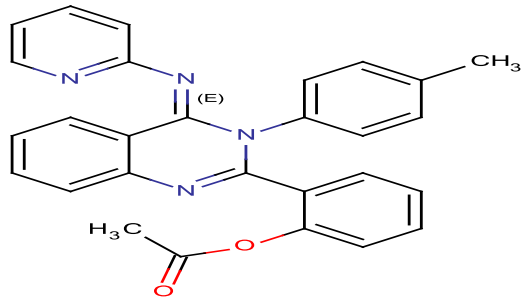
IR Data υ_{KBr}^{cm-1} : IR(KBr) : 1418(C-H), 1248(Ar-OH), 1585(C=C), 1670(C=N), 1248(C-N), 1485(CH3)

^{1}H NMR $\delta_{CDCl_3}^{ppm}$: 4(s CH3); 7-7.3 (m,6H, 7H, 8H), 9.5(s,OH);

MS m/z : 404$(M)^+$ Ion Peak (Fig 3)

Compound code : BA2
Compound name: 2-[(4E)-3-(4-methylphenyl)-4-[(pyridin-2-yl)imino]-3,4dihydroquinazolin- yl] phenyl acetate

Chemical Structure:



Molecular formula : C28H22N4O2
Molecular weight : 446
% yield : 69
Melting Point : 165-171ºC
Rf value : 0.55
Log P Value : 5.9

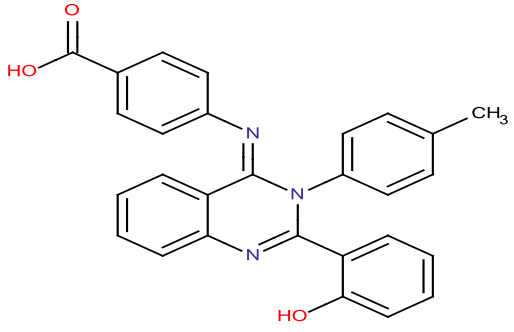
IR Data υ_{KBr}^{cm-1} : IR (KBr): 1402(C-H), 1670(C=O), 1538(C=C), 1666(C=N), 1245(C-N), 1485(CH3)

^{1}H NMR $\delta_{CDCl_3}^{ppm}$: 4 (s CH3); 7-7.3 (m,4H,);7.4 (m,4'H); 4.5 (s, 3'H).

MS m/z : 446$(M)^+$ Ion Peak

Compound code : BP1
Compound name: 4-{[(4E)-2-(2-hydroxyphenyl)-3-(4-methylphenyl)-3,4-dihydroquinazolin-4-ylidene]amino}benzoic acid

Chemical Structure:

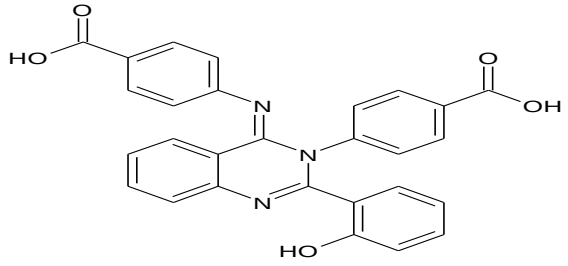


Molecular formula : C28H21N3O3
Molecular weight : 447
% yield : 70
Melting Point : 180-186ºC
Rf value : 0.62
Log P Value : 6.9

IR Data υ_{KBr}^{cm-1} : 1402(C-H), 1670(C=O), 1538(C=C), 1666(C=N), 1245(C-N), 1485(CH3), 1248(Ar-OH).
^{1}H NMR : 4(s CH3): 7.4 (m, 4'H); 7.7 (d, 4'H) ; 8.15 (d, 5H)10 (s, OH). 9.5 (s, OH).
MS m/z : 447(M)$^+$ Ion Peak

Compound code : CP1
Comound name: 4-[(4E)-4-[(4-carboxyphenyl)imino]-2-(2-hydroxyphenyl)- dihydro quinazolin-3-yl] benzoic acid

Chemical Structure:



Molecular formula : C28H19N3O5
Molecular weight : 477
% yield : 81
Melting Point : 195-201ºC
Rf value : 0.57
Log P Value : 6.4

IR Data υ_{KBr}^{cm-1} :1295(C-H), 3367(NH2), 1574(C=C), 1674(C=O),1624(C=N), 1245(C-N).

^{1}H NMR $\delta_{CDCl_3}^{ppm}$:10(s OH);7.4 (m,5'H); 7.7 (d, 4'H) ; 8.15 (d, 5H)11 (s, O'H).
MS m/z : 477 (M)$^+$ Ion Peak

The ADME, LD50 and molecular properties were alculated for the synthesized compounds using ACD/ILABS and OSIRS property explorer software.

INVITRO ANTI OXIDANT ACTIVITY

The synthesized AA1 and AA3 were screened for antioxidant activity using DPPH and Nitric oxide model. Free radical scavenging activity using 1,1-Diphenyl picryl hydrazyl model (DPPH)

DPPH is a stable free radical with a destructive ESR signal. Its reaction with antioxidants can be followed by the loss of the ESR signal or loss of absorbance at 517nm. The % inhibition of scavenging =

$$\frac{\text{(Absorbance control- Absorbance test)}}{\text{Absorbance control}} \times 100$$

Inhibition of Nitric oxide Radical Generation

At physiological pH aqueous solution of sodium nitroprusside generate nitric oxide (NO).
The % reduction in absorbance =

$$\frac{\text{(Absorbance control- Absorbance test)}}{\text{Absorbance control.}} \times 100$$

IN VITRO CYTOTOXIC EVALUATION

Human Breast cancer MCF-7 cell lines was obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.
In vitro assay for Cytotoxicity activity (MTT assay).
The Cytotoxicity of samples on MCF-7 cells was determined by the MTT assay (*Mosmann et al*.,1983).

% cell viability = A570 of treated cells / A570 of control cells × 100%.

Table 1. Energy minimization table

S. No	Compound Code	Binding Energy	No of hydrogen bonds	Site of hydrogen bonds
1	AA1	4.66	2	LYS 65
2	AA2	4.31	1	HIS 60
3	AA3	4.57	1	LYS 65
4	AA4	4.24	1	GLU 28
5	AP1	4.62	2	LYS 142, LYS 142
6	AP2	3.32	2	LYS 65, LYS 142
7	AP3	5.2	3	LYS 142, PHE 82
8	AP4	4.99	1	LYS 142
9	AS1	5.36	4	PHE 82, LYS 65,
10	AS2	5.85	2	LYS 65, HIS 121
11	AS3	6.54	1	LYS 65
12	AS4	6.3	2	LYS 65, ASN 59
13	AT1	4.54	0	-----
14	AT2	3.95	1	PHE 82

15	AT3	4.3	0	-----
16	AT4	4.75	0	-----
17	BA1	4.78	0	-----
18	BA2	4.47	1	LYS 65
19	BA3	4.56	0	-----
20	BA4	4.25	1	LYS 65
21	BP1	4.65	2	PHE 82, LYS 65
22	BP2	4.26	2	PHE 82, LYS 65
23	BP3	5.1	1	PHE 82
24	BP4	5.08	1	PHE 82
25	BS1	4.55	2	PHE 82, LYS 65
26	BS2	3.53	0	-----
27	BS3	5.27	1	PHE 82
28	BS4	5.46	1	PHE 82
29	BT1	5.38	0	-----
30	BT2	5.25	1	LYS 142
31	BT3	5.28	0	-----
32	BT4	5.55	0	-----
33	CA1	1100	0	-----
34	CA2	2.98	0	-----
35	CA3	4.83	2	LYS 65, LYS 142
36	CA4	5.03	2	LYS 65, LYS 142
37	CP1	4.43	0	-----
38	CP2	4.38	2	PHE 82, LYS 142
39	CP3	3.97	2	LYS 65, ASN 59
40	CP4	5.68	3	ASN 59, PHE 82 HIS 60
41	CS1	3.66	2	ARG 245, ARG 45
42	CS2	2.18	1	PHE 82
43	CS3	4.12	1	PHE 82
44	CS4	5.01	2	LYS 65, PH2 82
45	CT1	4.57	1	PHE 82
46	CT2	3.46	1	ARG42
47	CT3	5.16	1	LYS 65
48	CT4	2.98	0	-----
49	DA1	657.68	1	ASP 185
50	DA2	4.91	1	LYS 65
51	DA3	4.63	0	-----
52	DA4	4.55	0	-----
53	DP1	5.38	1	PHE 82
54	DP2	5.2	2	LYS 65, PHE 82
55	DP3	6	2	ARG 22
56	DP4	6.23	2	VAL 29, ARG 22
57	DS1	5.89	2	LYS 65, PHE 85
58	DS2	4.62	1	PHE 82
59	DS3	4.72	2	LYS 65, PH2 82
60	DS4	6.19	1	PH2 82
61	DT1	5.36	1	LYS 65
62	DT2	5.95	2	GLU 81, LYS 142
63	DT3	5.52	1	LYS 65
64	DT4	5.73	1	LYS 65

Table 2. Calculation Of ADME properties of the synthesized compounds

Comp code	Oral Bio	BBB, Brain/plasma Eq Rate	Vd	Genotoxicity
AA1	>30%	CNS inactive, -3.6	0.36 L/Kg	Nill
AA3	30%-70%	CNS inactive, -3.5	0.26 L/Kg	Nill
AS1	>30%	CNS inactive, -5.1	0.26 L/Kg	Nill
BA1	30%-70%	CNS active, -2.9	2.86 L/Kg	Nill
BA2	30%-70%	CNS active, -3	2.96 L/Kg	Nill
CP1	30%-70%	CNS inactive, -4.1	0.33 L/Kg	Nill

Table 3. Calculation of LD50 of the synthesized compounds

Comp code	Mouse (IP) mg/kg	Mouse (oral) mg/kg	Mouse (IV) mg/kg	Mouse (sc) mg/kg	Rat (IP) mg/kg	Rat (oral) mg/kg
AA1	690(B)	1500(NR)	130(B)	360(NR)	430(NR)	1300(NR)
AA3	290(B)	1300(B)	120(B)	670(NR)	340(NR)	650(NR)
AS1	700(B)	6200(B)	520(M)	1500(B)	730(B)	4100(NR)
BA1	240(B)	580(B)	33(B)	150(B)	190(NR)	440(NR)
BA2	330(M)	650(M)	47(B)	140(B)	220(NR)	420(NR)
CP1	800(B)	930(NR)	350(NR)	990(B)	260(NR)	1200(B)

(B)- Borderline (NR) Not Reliable (M) Moderate

Table 4. Calculation of molecular properties of the synthesized compounds

Comp code	Mutagenic	Reproductive effective	Tumerogenic	Irritant	clogP	Solubility	Drug likeness	Drug Score
AA1	Nill	Nill	Nill	Yes	1.87	-3.95	0.93	0.47
AA3	Nill	Nill	Nill	Yes	1.09	-3.45	1.49	0.54
AS1	Nill	Nill	Nill	Yes	0.06	-2.82	-1.19	0.32
BA1	Nill	Nill	Nill	Nill	4.59	-5.68	0.76	0.4
BA2	Nill	Nill	Nill	Nill	4.82	-6.28	1.24	0.35
CP1	Nill	Nill	Nill	Nill	3.91	-5.64	-1.05	0.29

Table 5. Effect of (AA1) and (AA3) on DPPH model

Concn. (µg/ml)	AA1	AA3
	% Inhibition	% Inhibition
5	35.4	26.1
10	39.9	32.3
15	41.6	42.0
20	42.8	46.5
25	58.5	61.1
30	71.4	69.3

Table 6. Effect of (AA1) and (AA3) on Nitric oxide model

Concn. (µg/ml)	AA1	AA3
	% Inhibition	% Inhibition
50	16.6	34.4
100	20.44	41.6
150	23.94	51.3
200	24.64	55.4
250	30.74	59.6

Table 7. Anti Cancer Activity of *Sample AA1* on MCF-7 Cell line

S.No	Conc. (µg/ml)	Dilutions	Absorbance(O.D)	Cell viability(%)
1	1000	Neat	0.03	6.6
2	500	1:1	0.10	22.2
3	250	1:2	0.15	33.3
4	125	1:4	0.24	53.3
5	62.5	1:8	0.32	71.1
6	31.2	1:16	0.36	80.0
7	15.6	1:32	0.41	91.1
8	Cell control	-	0.45	100

Table 7. Anti Cancer Activity of *Sample AA3* on MCF-7 Cell line

S.No	Conc. (µg/ml)	Dilutions	Absorbance(O.D)	Cell viability (%)
1	1000	Neat	0.07	15.5
2	500	1:1	0.12	26.6
3	250	1:2	0.18	40.0
4	125	1:4	0.23	51.1
5	62.5	1:8	0.30	66.6
6	31.2	1:16	0.34	75.5
7	15.6	1:32	0.39	86.6
8	Cell control	-	0.45	100

Fig 1. AP1

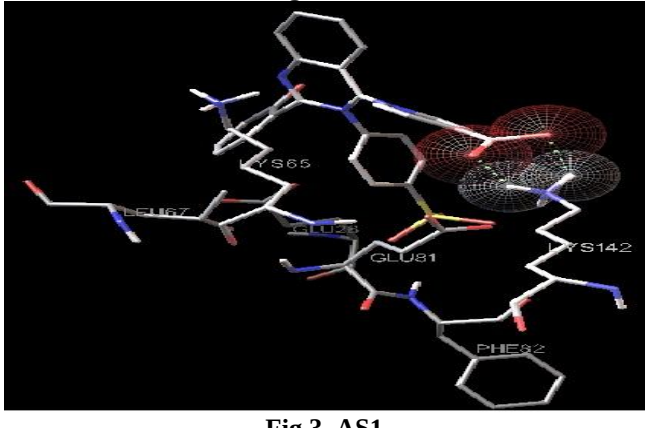


Fig 1. AP2

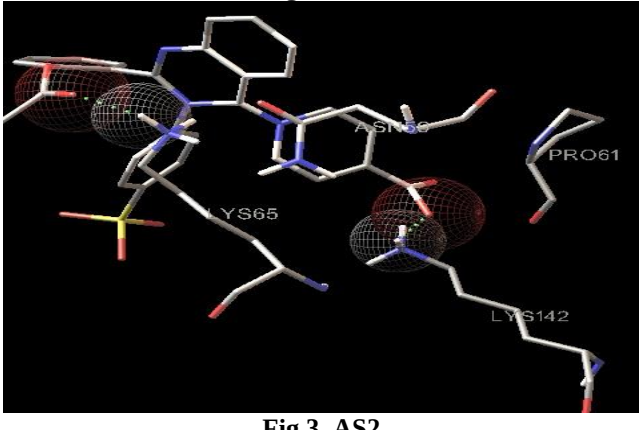


Fig 3. AS1

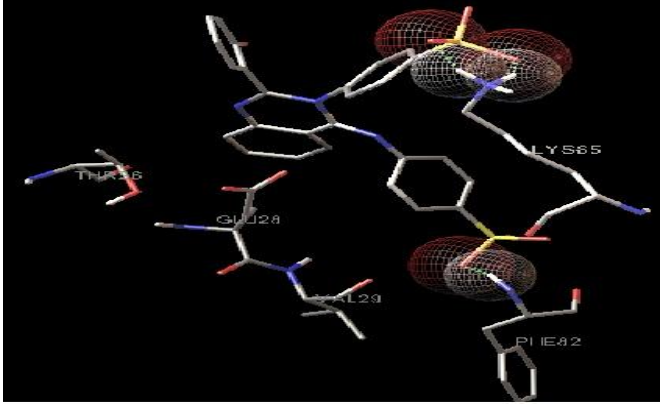


Fig 3. AS2

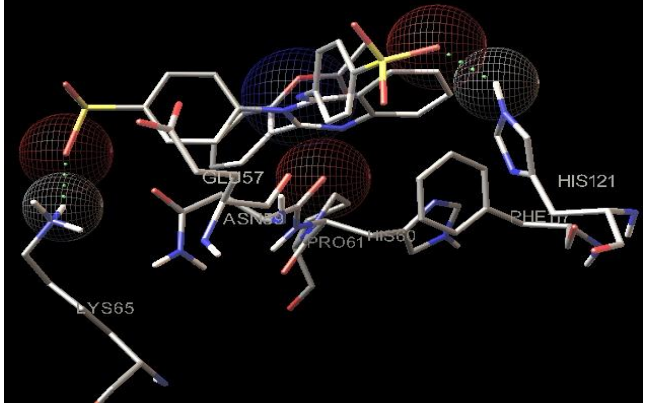


Fig 5. AP3

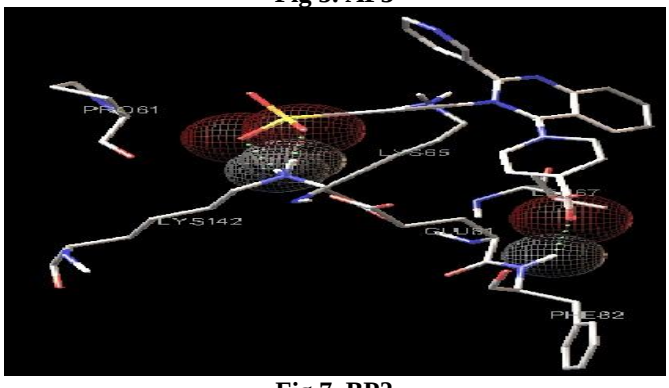


Fig 6. BP1

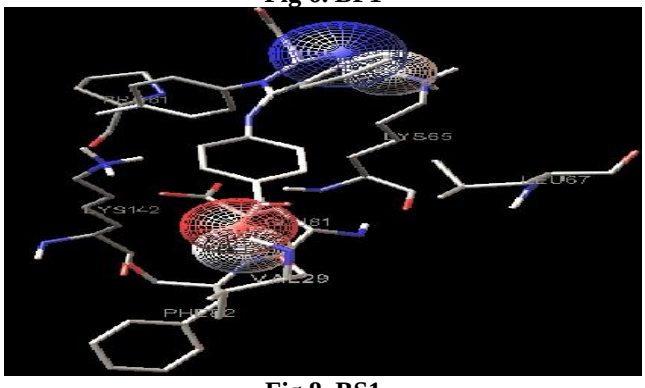


Fig 7. BP2

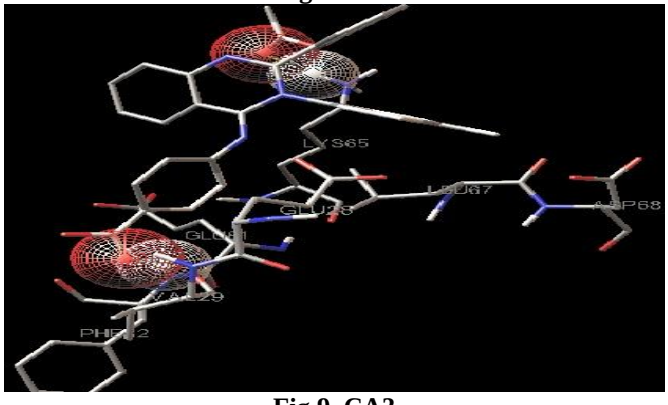


Fig 8. BS1

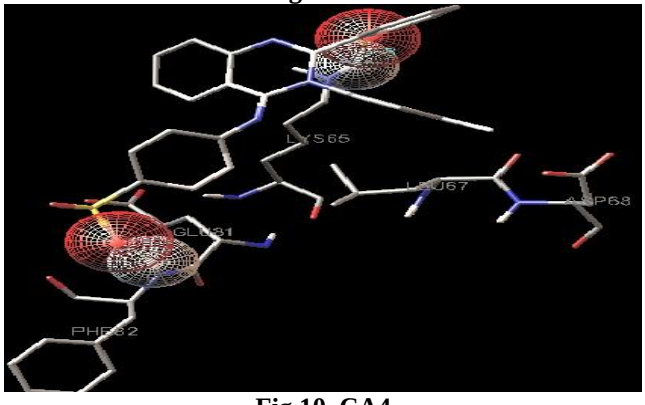


Fig 9. CA3

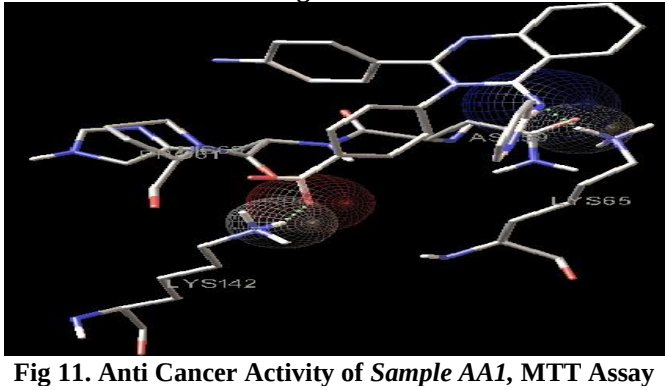


Fig 10. CA4

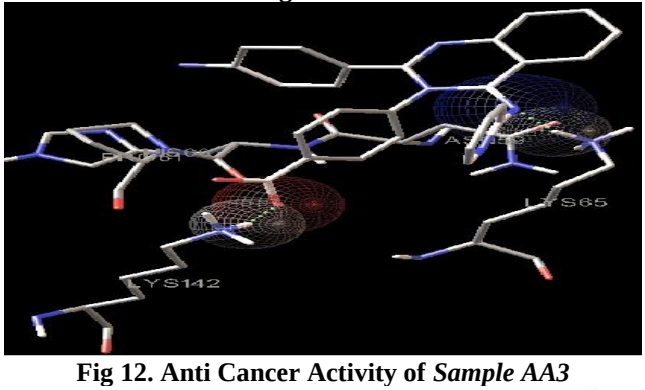


Fig 11. Anti Cancer Activity of *Sample AA1*, MTT Assay

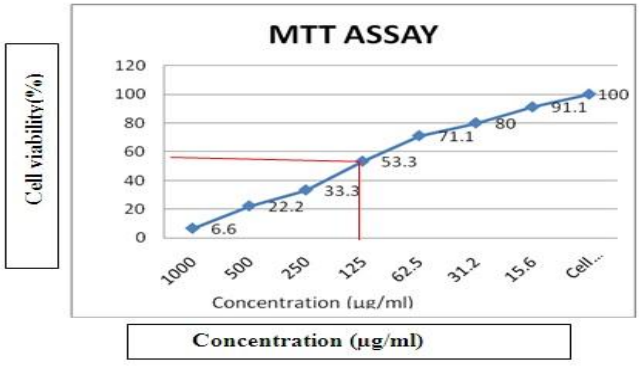


Fig 12. Anti Cancer Activity of *Sample AA3*

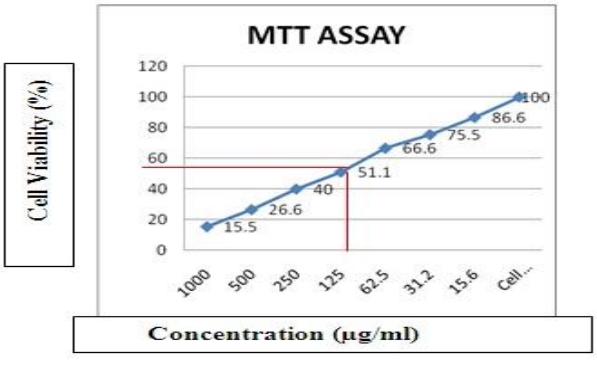


Fig 13. Anti Cancer Activity of *Sample AA1* on MCF-7 Cell line

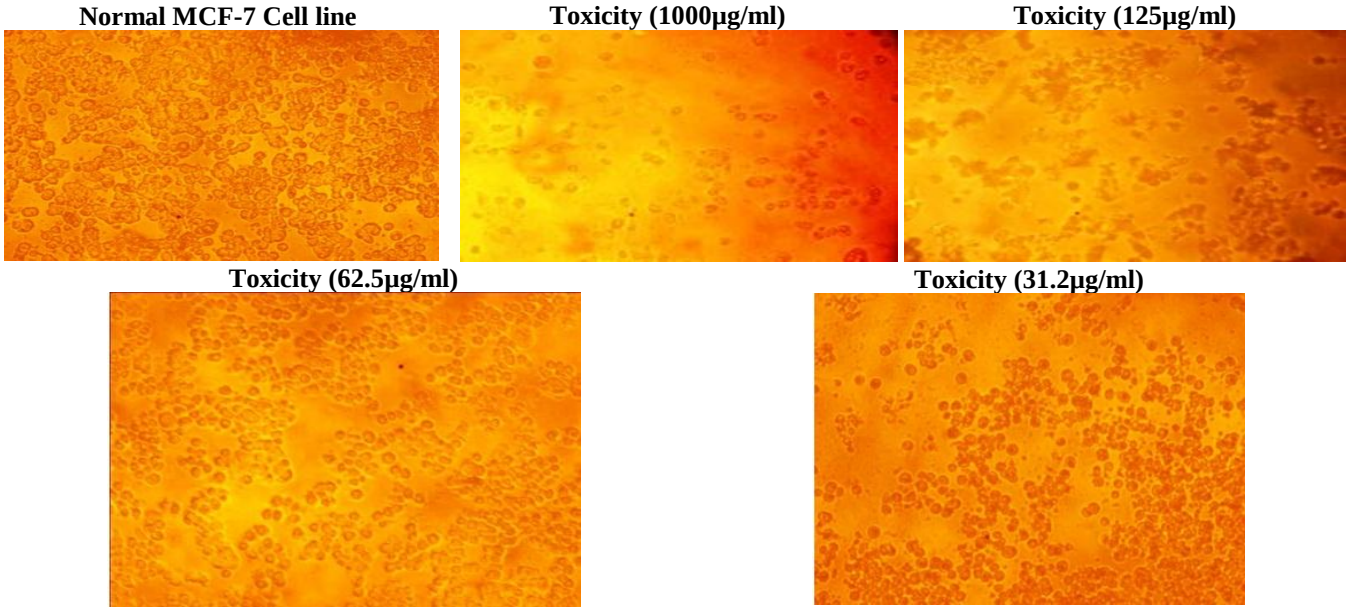
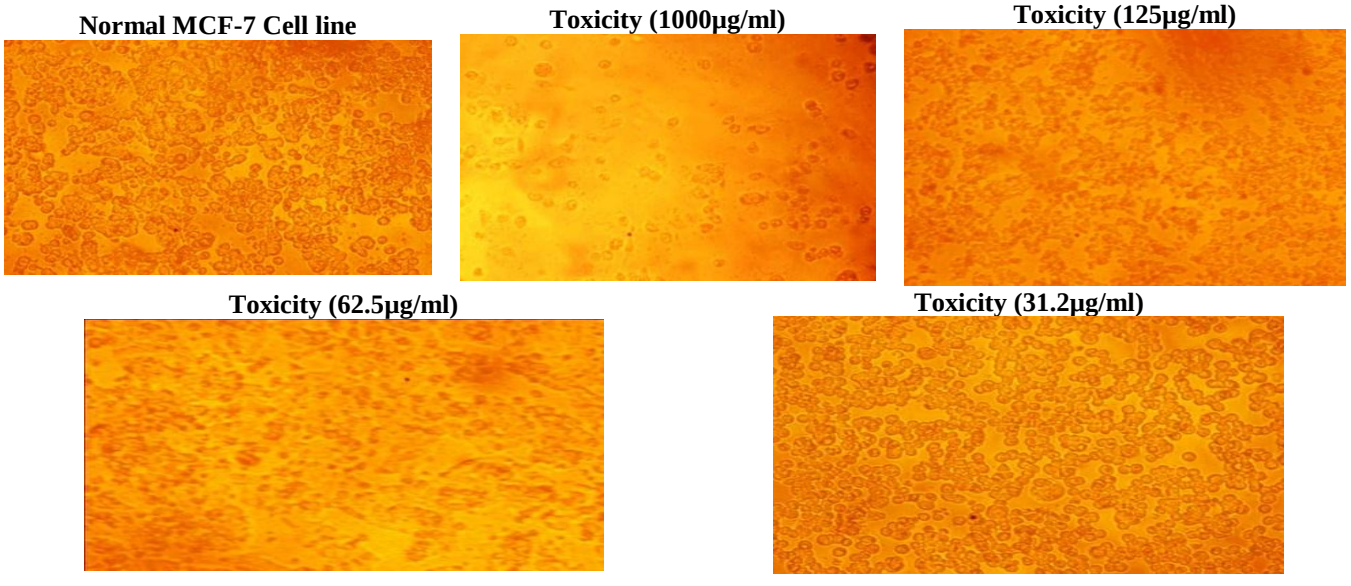


Fig 14. Anti Cancer Activity of *Sample AA3* on MCF-7 Cell line



DISCUSSION AND CONCLUSION

1. In the present thesis 64 schiff bases of 2.3 disubstituted quinazolinones were subjected to QSAR study and it was found out that all the compounds have good Cyclin dependant kinase 2 inhibitor activity.

2. From the results of the QSAR study it was found that AP1, Ap2, AS2, AS4, AP3, BS1, BP2, BP1, CP2,CP3, CS4, CP4, CA3,DS1, DP3,DP4 and DP2 were found to have a very good score and of them AP3, AP4, CP4 and DP2 were found to be very good CDK2 inhibitors.

3. Seven novel Schiff bases of 2,3 disubstituted quinazolinones compounds were synthesized by thermal condensation and cyclization of anthranalic acid, aromatic amine and aromatic acid . Followed by formation of schiff bases by condensation of the synthesized 2,3 disustituted quinazolinones with aromatic amines.

4. The Physiochemical parameters of the synthesized compounds were determined and Identification and characterisation of the compounds were done by Melting Point, IR. Spectra, ^{1}HNMR spectra and Mass spectra.
5. The ADME properties and Molecular properties were calculated for the synthesized compounds two compounds namely AA1 and AA3 were screened for in vitro-antioxidant by DPPh and Nitric oxide and In Vitro cytotoxicity was evaluated by MTT assay in Human Breast cancer cell lines. The concentration of the compounds AA1 and AA3 responsible for 50% death was found to be 125.5mcg/ml.
6. In this study, it was found that the free radical scavenging action of AA1 and AA3 were found to be 71 and 72 percentage in DPPH model, and 30.7 and 55.64 % in nitric oxide model respectively.
7. From the above results it can be concluded that the Schiff bases of different quinazolinones enhances anti cancer activity.
8. The future study is planned or in vivo studies of the synthesized compounds.

ACKNOWLEDGEMENT

Here by we acknowledge the Dean and Faculty of MTPG&RIHS for their constant encouragement throughout the research work.

REFERENCES

Anette Witt, Jan bergman. Tetrahedron, 56(37), 2001, 7245-7253.
Aysel Gursoy, Nilgun Karali. *European journal of medicinal chemistry*, 38(6), 2003, 633-643.
Francois-renealexandre, amaya berecibar, roger wriggles worth, thierry besson. *Tetrahedron*, 59(9), 2003, 1413-1419.
Ji-feng Liu, Christopher J. Wilson, galina beletsky, daniel yohannes, shi-chungng. *Bioorganic & medicinal chemistry letters*, 16(3), 2006, 686-690.
Raid J. Abdel-Jalil, Wolf Gang Voelter, and Muhammad Saeed. *Tetrahedron letters*, 45(17), 2004, 3475-3476.
Sheng-Li Cao, Yu-Ping Feng, Yu-Yang Jiang, Shi-Ying Liu, Guo-Yu Ding, Run-Tao Li. *Bioorganic & Medicinal Chemistry Letters*, 15(7), 2005, 1915-1917.