Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 5, 2014

Original Article

IN SILICO DESIGN, SYNTHESIS AND PHARMACOLOGICAL SCREENING OF SOME QUINAZOLINONE METAL COMPLEXES AS DIHYDROFOLATE REDUCTASE INHIBITORS FOR ANTICANCER ACTIVITY: PART-II

MEGHA SAHU* AND AMIT G. NERKAR

Department of Pharmaceutical and Medicinal Chemistry, Sinhgad Technical education Society's Kashibai Navale college Of Pharmacy, Kondhwa (Bk) Pune, MS-411048, India.
Email: amit.nerkar@sinhgad.edu

Received: 31 Mar 2014 Revised and Accepted: 05 May 2014

ABSTRACT

Objective: The main objective of this research work was to *in silico* screening, synthesis, characterization of qunazolinone schiff's base and metal complexes for in vitro evaluation of anticancer activity as a DHFR Inhibitors.

Methods: This research study describes *in silico* screening of quinazolinone schiff base metal complexes by Vlife MDS 4.3 software, their synthesis and in vitro pharmacological screening. Metal complexes were used for electron transfer, binding and activation of oxygen and also for oxidation-reduction of substrate. Copper metal complexes hold unique position because of having high reduction potential. Protein environment may affect redox potential of transition metal based redox center. In case of selection of PDB, resolution factor and characterization methods were considered. Structure of the ligands were drawn by Marvinsketch and Chemdraw 12 software and to convert 2D molecules to Mol file followed by in controlled manner expanding their biological functionality. The prioritized metal complexes were synthesized further characterization of metal complex was done by AAS, ESR, IR, TLC and X-Ray diffraction. Hydrogen bonding, hydrophobicity and solvent assessibility of metal center exhibited to design metallocluster binding site for mutagenesis.

Results: Prioritized quinazolinone schiff base with comparable docking scores as compared with Methotrexate used as standard drug were synthesized. Prioritized molecules were characterized by using AAS, ESR, IR, TLC, XRD techniques and were found to comply with spectroscopic assignments. All molecules were evaluated for in vitro anticancer assay on ten different cell lines as per National Cancer Institute, Bethesda guidelines from ACTREC Center Navi Mumbai.

Conclusion: Prioritized compound showed promising activity on ten cell line at a concentration of ≤10 µg/ml, a requisite for compound to be active as per NCI guidelines.

Keywords: DHFR, Copper metal complexes, Hydrophobicity, Quinazolinone schiff's base.

INTRODUCTION

DHFR and DHFR Inhibitors

Dihydrofolate reductase (DHFR) is an enzyme of pivotal role in medicinal chemistry. DHFR catalyzes the reduction of folate or 7, 8-dihydrofolateto tetrahydrofolate and intimately couples with thymidylate synthase (TS). Overall, inhibition of hDHFR in cancerous cell affects essential step for nucleic acid synthesis and hence inhibit the growth as well as division of cancerous cells. DHFR inhibition disrupts synthesis of nucleic acid and effecting cell growth and proliferation. For this reason, DHFR is considered as good target for antitumor drugs. Inhibition of DHFR or TS activity in the absence of salvage leads to 'thymineless death.' [1–3] Compounds that inhibit DHFR exhibit an important role in clinical medicine. Methotrexate is used as in neoplastic diseases[4, 5], inflammatory bowel diseases [6], and rheumatoid arthritis[7], as well as in psoriasis[8, 9] and in asthma [10].

Metal complexes as anticancer agents

Many drug molecules are "organic" in nature, other elements in the periodic table, particularly metals, offer a much more diverse chemistry and have important therapeutic applications [11]. The use of metal-based compounds as therapeutic drugs dates back to over 5000 years. In modern days, the study of organometallic pharmaceuticals started with the pioneering work of Köpf and Köpf-Maier (in the late 1970's), who investigated the antitumor activity of early transition metal cyclopentadienyl complexes [12].

In case of inorganic anticancer agents are large variety of metal ions and ligand and many diverse designs tailored according to the specific receptor or biological target. So far, the major classes of metal-based anticancer drugs include platinum(II) and platinum(IV), palladium(II), gold(I) and gold(III), ruthenium(II) and ruthenium(III), bismuth(III), rhenium(I), and copper(II) compounds, as well as gallium(III) and tin(IV) derivatives, some of them have been reported to demonstrate higher in vitro anticancer activity than cisplatin [13]. Owing to the importance of quinazolinones, DHFR and metal complexes we chose them for research. In this research paper the quinazolinone metal complexes as human DHFR antagonists as anticancer agents leads have been investigated. The three leads were an outcome of *in silico* screening of quinazolinone metal complexes followed by their synthesis and characterization and *in vitro* anticancer screening on 10 Cell lines, for cell line anticancer cytotoxicity assay.

MATERIALS AND METHODS

In silico studies

The in silico ADME predictions were obtained from www.bmrd.org. Docking simulations were performed on Vlife MDS 4.3 Drug Design software on Windows OS. Marvin beans and Chem Draw Ultra 11.0 were used to draw the structures of the molecules and for conversion of 2-D structures into mol files.

Chemicals and materials

All chemicals were purchased from Sigma Aldrich, SD Fine, Spectrochem and Merck yields refer to purified products and are not optimized. Melting points were determined on VEEGO - VMP I melting point apparatus and are uncorrected. IR spectrums were recorded on SHIMADZU spectrophotometer. ^{1}H NMR were recorded at Diya labs Mumbai on 400 MHz Spectrophotometer facility, chemical shifts (δ) are reported in parts per million (ppm) with $CDCl_3$ and DMSO as solvent for NMR. TMS was used as internal standard for NMR. Splitting of signals is represented by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplates). Thin layer chromatography (TLC) was performed on Merk GF254 precoated

aluminium plate**.** AAS (Atomic Absorption Spectroscopy (AAS) analysis study was done on atomic absorption instrument model AA7000, in Department of Chemistry of University of Pune.) and ESR (Electron Spin Resonance Spectroscopy (ESR) analysis study was done on ESR instrument model JES - FA200 ESR Spectrometer with X band in Sophisticated Analytical Instrument Facility Department, IIT-Bombay, Powai, Mumbai. X-ray diffraction studies were performed at Diya labs Mumbai.

Experimental

I. *In silico* screening

1. ADME Predictions

In silico ADME parameters were obtained online from PreADMET software predicted by following parameters.[14]

a. Caco2 cell permeability

For prediction of Caco2 cell permeability in PreADMET, molecules were solvated *in silico* at pH 7.4. Caco2 cells are used to determine the apparent permeability values of compounds. The range of Caco 2 cell is 4-70 nm/sec.

b. MDCK cell permeability

MDCK cell means Madin-Darby Canine Kidney cell. MDCK cells are used to determine the apparent permeability values of compound. The range of MDCK is 25- 500 nm/sec.

c. Human Intestinal Absorption (HIA)

PreADMET can predict percent human intestinal absorption (% HIA). HIA data are the sum of bioavailability and absorption evaluated from ratio of excretion or cumulative excretion in urine, bile and faces. The range of HIA is 20- 70%.

d. Plasma Protein Binding (PPB)

Only the unbound drug is available for diffusion or transport across cell membranes and also for interaction with a pharmacological target.

As a result a degree of plasma protein binding of drug influences not only the drugs action but also its disposition and efficacy. The range of PPB is about 90%. *In silico* ADME prediction are shown in Table 1.

Table 1: ADME Parameters of synthesized compounds

Compound	HIA@	Caco2 cell permeability++	MDCK+++	PPB$
6a	97.37	47.7s5	0.24	95.39
6b	96.21	24.70	0.24	93.20
6c	99.40	21.34	0.25	93.84

@HIA = Human Intestinal Absorption. ++Caco 2 cell permeability = human colon adenocarcinoma and possess multiple drug transport pathways through the intestinal epithelium. +++MDCK = Madin-Darby canine kidney cell. $PPB = Plasma Protein Binding

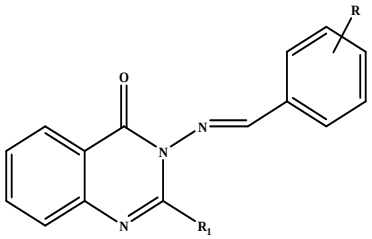
2. Docking study

The screening of molecule was done using Vlife MDS 4.3 software. Firstly selection of PDB, validation of protein, 2d to 3d conversation of molecule, force field minimization (MMFF) then docking of molecule by using Vlife 4.3. Conformers of the compound were generated by Monte Carlo method. All the Conformers were then energetically minimized up to the rms gradient of 0.001 and saved in separate folder. MMFF was used for optimizing molecule and geometry of molecule.

Library design and Ligand preparation

The Marvin Bean software was used to draw molecular structures of ligands and for the conversion of the 2D structure to 3D mol files.

Structures of ligands were designed shown from Series 1 **(6a-6j)** and Series 2 (**7a-7l)**. Library of 22 compounds was developed.



Series 1 of compounds

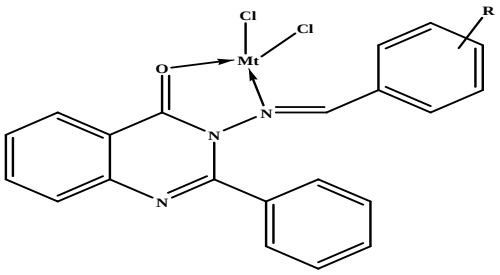
Table 2: It shows docking score of ligands performed on V-life sciences

Sr .No.	Code	R	R_1	Docking score
1	6a	4-OCH_3	CH_3	-59.32
2	6b	4-OH	CH_3	-46.04
3	6c	2-NO_2	CH_3	-71.81
4	6d	4-Cl	CH_3	-64.23
5	6e	2-OH	CH_3	-63.81
6	6f	4-OCH_3	C_6H_5	-89.45
7	6g	4-OH	C_6H_5	-59.48
8	6h	2-NO_2	C_6H_5	-63.20
9	6i	4-Cl	C_6H_5	-65.40
10	6j	2-OH	C_6H_5	-64.81
11	Methotrexate	-	-	-47.88

II. Synthesis

Synthesis of (quinazolinone schiff base) 3-(substituted benzylideneamino)-2-phenylquinazolin-4(3H)-one [15-17]: To a hot ethanolic solution of 2-amino 3-phenyl quinazolin 4-one 0.5g (0.002 mol) (1eq) and hot ethanolic solution of substituted benzaldehyde (1 eq) (0.26ml,0.002mol), 2-3 drop of H_2SO_4 was added. The reaction mixture was refluxed with stirring for 1 hr.

Solid precipitated out, was filtered under vacuum.



Series 2 of compound

Table 3: It shows docking score of ligands performed on V-life sciences

Sr .No.	Code	R	M*	Docking score
1	7a	4-OCH_3	Cu(II)	-45.61
2	7b	4-OH	Cu(II)	-62.99
3	7c	2-NO_2	Cu(II)	-58.67
4	7d	4-OCH_3	Ni(II)	-62.02
5	7e	4-OH	Ni(II)	-60.90
6	7f	2-NO_2	Ni(II)	-59.19
7	7g	4-OCH_3	Co(II)	-53.22
8	7h	4-OH	Co(II)	-48.90
9	7i	2-NO_2	Co(II)	-59.02
10	7j	4-OCH_3	Mn(II)	-52.46
11	7k	4-OH	Mn(II)	-57.07
12	7l	2-NO_2	Mn(II)	-52.74
13	Methotrexate	-	-	-47.88

M* = Metal

Synthesis of 3-(4-methoxybenzylideneamino)-2-phenylquinazolin-4(3H)-one (6a): To a hot ethanolic solution of 2-amino 3-phenyl quinazolin 4-one 0.5g(0.002 mol) (1 eq) and hot ethanolic solution of 4 methoxy benzaldehyde (1 eq) (0.26ml,0.002mol), 2-3 drop of H_2SO_4 were added.

The reaction mixture was refluxed with stirring for 1 hr. Solid precipitated was filtered under vacuum. **% Yield**: 65%, **Molecular Formula**: $C_{22}H_{17}O_2N_3$, **Molecular wt**: 355, **Rf**: 0.7 (Hexane:Ethyl acetate 50: 50), Melting Point:211-214 ^{0}C;**I.R. (KBr, cm $^{-1}$)**: 2921 (CH_2, Str.). 2854 (CH, Str.), 1764 (C=O Str), 1617 (C=N), 1600 (C-C, Str.), 1253 (C-O, Str.) **^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]**: 7.2-7.6(s, H, Ar-H); 3.9 (H, -CH_3); 7.95 (s, 1H, Ar-H); 8.3 (s, H,CH); 8.5 (d, H, Ar-H); 7.93 (m, Ar-H).

Synthesis of 3-(4-hydroxybenzylideneamino)-2-phenylquinazolin-4(3H)-one (6b): To a hot ethanolic solution of 2-amino 3-phenyl quinazolin 4-one 0.5g(0.002 mol) (1 eq) and hot ethanolic solution of 4 hydroxy benzaldehyde (1 eq) (0.2g 0.002 mol) and 2-3 drop of H_2SO_4 were added.

The reaction mixture was refluxed with stirring for 1 hr. Solid precipitated was filtered under vacuum. **% Yield**: 70%, **Molecular Formula**: $C_{21}H_{15}N_3O_2$, **Molecular wt**: 337, **Rf**: 0.6 (Hexane:Ethyl acetate 50: 50), Melting Point:251-254 ^{0}C;**I.R. (KBr, cm $^{-1}$)**: 3316 (OH, Str.). 1600 (C=C, Str.), 1650 (C=O Str), **^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]**: 6.8 (d, H, Ar-H); 7.2 (t, Ar-H); 7.85 (m, Ar-H); 7.95 (m, H, Ar-H); 8.3 (s, H, CH); 9.9 (s, OH).

Synthesis of 3-(2-nitrobenzylideneamino)-2-phenylquinazolin-4(3H)-one (6c): To a hot ethanolic solution of 2-amino 3-phenyl quinazolin 4-one 0.5g(0.002 mol) (1eq) and hot ethanolic solution of 2-nitro benzaldehyde (1 eq) (0.33g.0.002 mol) and 2-3 drop of H_2SO_4 were added.

The reaction mixture was refluxed with stirring for 1 hr. Solid precipitated was filtered under vacuum. **% Yield**: 80%, **Molecular Formula**: $C_{21}H_{24}N_4O_3$, **Molecular wt**: 370, **Rf**: 0.6 (Hexane:Ethyl acetate 50: 50), Melting Point:242-245 ^{0}C;**I.R. (KBr, cm $^{-1}$)**: 1671 (C=O Str), 1514 (NO_2), 1630 (C=C, Str.), **^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]**: 7.2 (t, H, Ar-H); 7.6-7.68 (m, H, Ar-H); 7.8 (m, H, Ar-H); 8.1 (m, H, Ar-H); 8.53 (d, H, Ar-H); 8.8 (s, C-H).

Synthesis of 3-(4-nitrobenzylideneamino)-2-phenylquinazolin-4(3H)-one (6d): To a hot ethanolic solution of 2-amino 3-phenyl quinazolin 4-one 0.5g(0.002 mol) (1eq) and hot ethanolic solution of 4-nitro benzaldehyde (1 eq) (0.33g.0.002 mol) and 2-3 drop of H_2SO_4 were added. The reaction mixture was refluxed with stirring for 1 hr. Solid precipitated was filtered under vacuum.

General synthetic procedure of quinazolinone schiff base metal complexes[21]: To a hot ethanolic solution of quinazolinone schiff base (6a =0.5g,0.00140 mol),(6b=0.1g 0.00029 mol), (6c =0.5g,0.00135 mol) (1eq) 0.5g hot ethanolic solution of metal salt (0.5eq) [($CuCl_2 2H_2O$) (6a=0.1g,0.0007 mol), (6b=0.02g,0.00014), (6c=0.11g,0.0006 mol/ ($NiCl_2 6H_2O$) (6c=0.03g,0.00014) was added respectively. The reaction mixture was refluxed with stirring for 2-3 hr. Solid was precipitated, washed with water and dried.

Synthesis of 3-(4-methoxybenzylideneamino)-2-phenylquinazolin-4(3H)-one Cu(II)complexes (7a): To a hot ethanolic solution of quinazolinone schiff base (6a =0.5g,0.00140 mol) (1eq) 0.5g hot ethanolic solution of metal salt (0.5eq) ($CuCl_2 2H_2O$) (0.1g,0.0007 mol) was added.

The reaction mixture was refluxed with stirring for 2-3 hr. Solid was precipitated, washed with water and dried. **% Yield:** 60%**, Molecular Formula:** $C_{22}H_{17}O_2N_3$, **Molecular wt:** 449**, Rf:** 0.5 (Hexane:Ethyl acetate 60: 40). **I.R. (KBr, cm $^{-1}$):** 1280 (C-O, Str.). 1640 (Ar., C=C, Str.), 3000 (CH_2 Str), **ESR (g value):** 2-2.09, **AAS (absorbance):** 0.055 or 70%.

Synthesis of 3-(4-hydroxybenzylideneamino)-2-phenylquinazolin-4(3H)-one Cu(II)complexes (7b): To a hot ethanolic solution of quinazolinone schiff base (6b=0.1g 0.00029 mol) (1eq) 0.5g hot ethanolic solution of metal salt (0.5eq) ($CuCl_2 2H_2O$) (0.02g,0.00014) was added. The reaction mixture was refluxed with stirring for 2-3 hr. Solid was precipitated, washed with water and dried.**% Yield:** 56%**, Molecular Formula:** $C_{21}H_{15}O_2N_3$, **Molecular wt:** 435**, Rf:** 0.6 (Hexane:Ethyl acetate 70: 30). **I.R. (KBr, cm $^{-1}$):** 3116 (OH, Str.). 1608 (Ar., C=C, Str.), 2927 (CH_2, Str), **ESR (g value):** 2-2.09, **AAS (absorbance):** 0.055 or 60%.

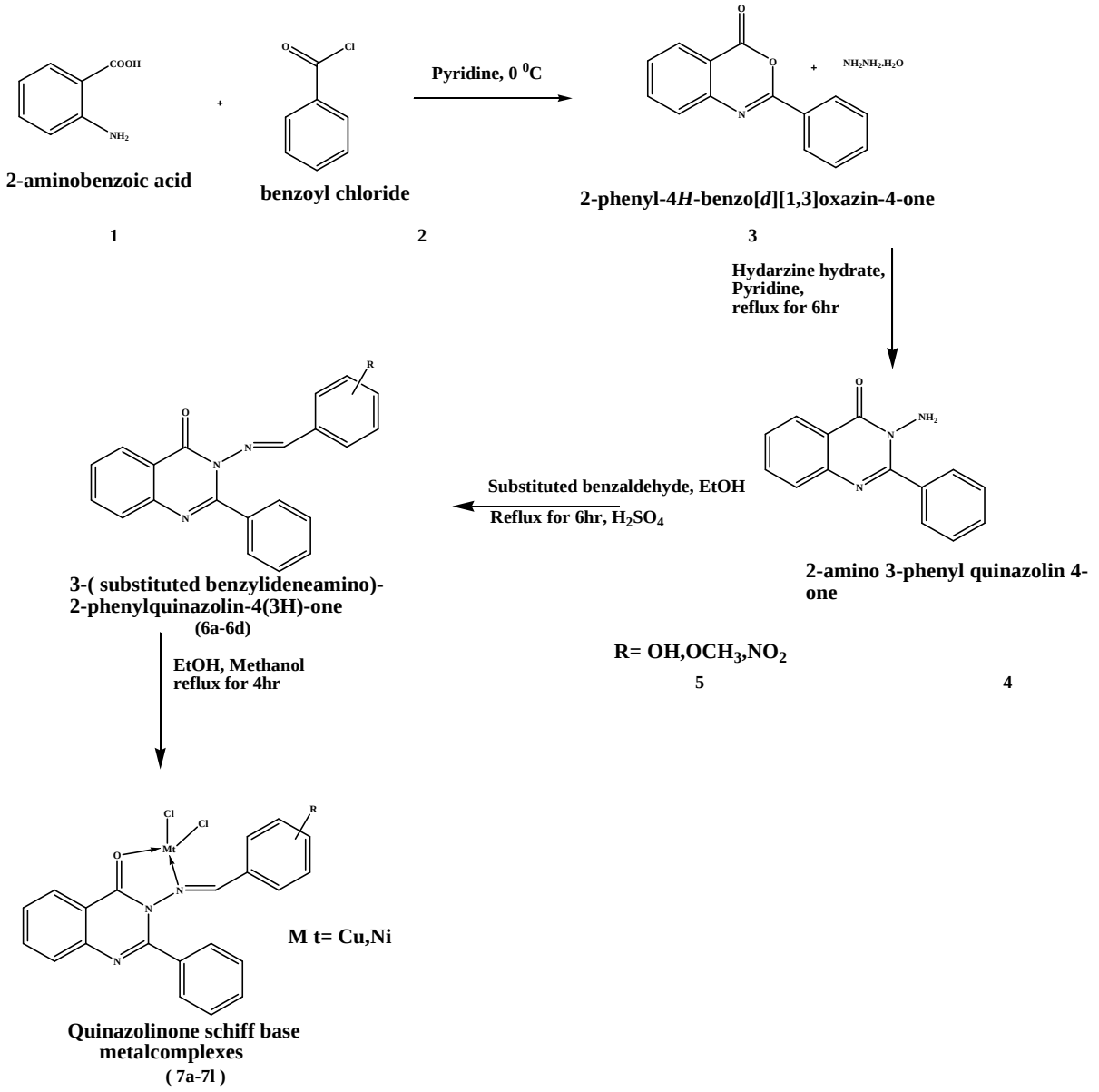


Fig. 1: General synthetic scheme

General synthetic Procedure of Schiff's base:

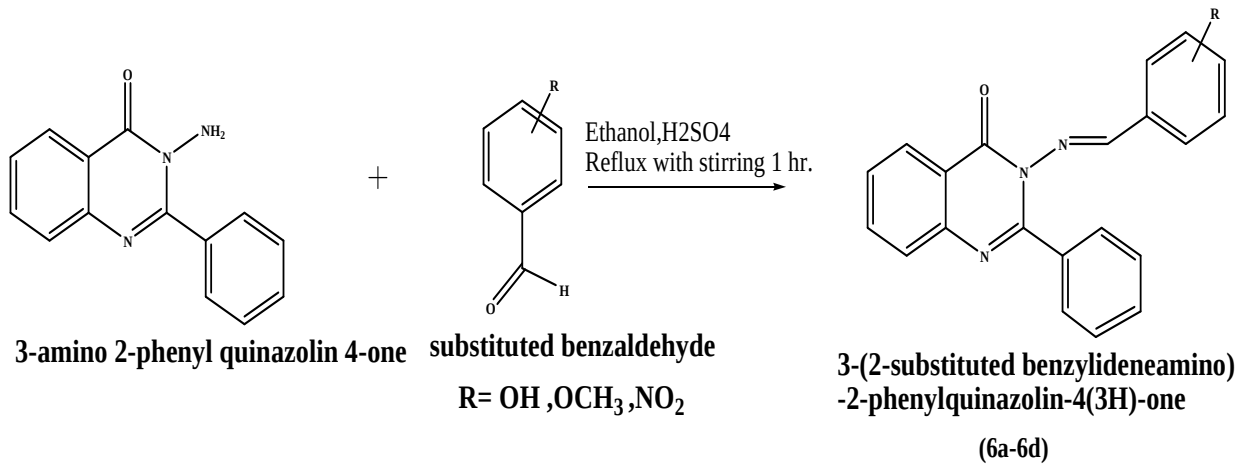
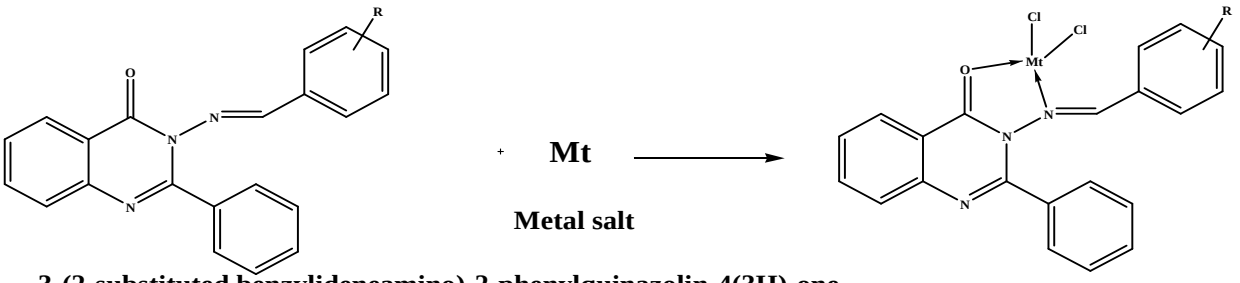


Fig. 2: General synthetic procedure of schiff's base

Synthesis of quinazolinone schiff's base metal complexes [18- 20]



3-(2-substituted benzylideneamino)-2-phenylquinazolin-4(3H)-one

Mt = $CuCl_2,2H_2O$
$NiCl_2,6H_2O$

Quinazolinone Schiff base metal complex

(7a-7l)

Fig. 3: Synthetic procedure of schiff's base metal complexes

Table 4: It shows In vitro Activity data of active compound (6a, 7a, 7b)

Sr. No.	Cell line	Compound code								
		6a			7a			7b		
		LC 50*	TGI+	GI 50&	LC 50*	TGI+	GI 50&	LC50*	TGI+	GI 50&
1	**A549**	>80	67.4	35.4	>80	>80	74.6	>80	>80	>80
2	**DU145**	>80	66.3	37.2	>80	>80	74.6	>80	>80	>80
3	**MCF7**	>80	47.3	12.5	>80	>80	74.6	>80	>80	>80
4	**SiHa**	>80	47.3	12.5	>80	>80	74.6	>80	>80	>80
5	**KB**	>80	47.3	12.5	56.8	28.1	<10	>80	>80	>80
6	**HCT15**	>80	>80	46.7	74.9	42.4	10	>80	>80	>80
7	**SK-OV-3**	69.9	49.7	29.5	39.8	<10	<10	>80	>80	>80
8	**K562**	>80	>80	>80	69.2	39.5	<10	>80	>80	>80
9	**HeLa**	>80	>80	51.3	48.8	25.6	<10	>80	>80	>80
10	**SK-MEL-2**	>80	>80	74.6	43.2	<10	<10	>80	>80	>80
Std.	**Methotrexate**	>80	>80	19.6	>80	75.9	14.1	>80	>80	>80
Drugs	**Adriamycin**	>80	37.6	<10	<10	41.3	<10	<10	41.3	<10

LC_{50}* - Concentration of drug causing 50% cell kill, GI_{50}& - Concentration of drug causing 50% inhibition of cell growth, TGI+ - Concentration of drug causing total inhibition of cell growth.

Table 5: Docking score and Interaction analysis of molecules on V-life sciences

Sr. No	Compound	Docking score	Hydrogen bond
1.	6a	-89.45	Ser 59
2.	6b	-59.48	Ser 59
3.	6c	-63.20	No
4.	7a	-62.99	Ser 59
5.	7b	-58.67	Ser 59,Tyr 121
6.	7c	-45.61	Ser 59
8.	Methotrexate	-47.88	Tyr 121,Ile 7, Thr 136,Thr146

Synthesis of 3-(2-nitrobenzylideneamino)-2-phenylquinazolin-4(3H)-one Cu(II)complexes (7c): To a hot ethanolic solution of quinazolinone schiff base (6c =0.5g,0.00135 mol) (1eq) 0.5g hot ethanolic solution of metal salt (0.5eq) ($CuCl_2 2H_2O$) (0.11g,0.0006 mol) was added. The reaction mixture was refluxed with stirring for 2-3 hr. Solid was precipitated out, washed with water and dried.

Synthesis of Synthesis of 3-(2-nitrobenzylideneamino)-2-phenylquinazolin-4(3H)-one Ni(II)complexes (7f): To a hot ethanolic solution of quinazolinone schiff base (6c =0.5g, 0.00135 mol) (1eq) 0.5g hot ethanolic solution of metal salt (0.5eq) ($NiCl_2 6H_2O$) (0.03g,0.00014) was added. The reaction mixture was refluxed with stirring for 2-3 hr. Solid was precipitated, washed with water and dried.

III. in vitro anticancer screening

***In vitro* cytotoxicity assay against human cancer cell lines:** The human cancer cell lines were procured from National Cancer Institute, Frederick, USA. Cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2mM glutamine, pH 7.4, supplemented with 10% fetal calf serum, 100 µg/ml streptomycin and 100 units/ml penicillin) in a carbon dioxide incubator (37 °C, 5% CO_2, 90% RH).

The cells at sub confluent stage were harvested from the flask by treatment with trypsin [0.05% in PBS (pH 7.4) containing 0.02% EDTA]. Cells with viability of more than 98% as determined by trypan blue exclusion were used for determination of cytotoxicity. The cell suspension of 1 x 10^5cells/ml was prepared in complete

growth medium. Stock solutions (2 x 10^{-2} M) of compounds were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 µg/ml of gentamycin to obtain working test solutions of required concentrations. Methotrexate and Adramycin were used as standard.

CONCLUSION

From the above result it was concluded that quinazolinone schiff's base metal complexes were found to be active as anticancer agents by *in silico* design. *In vitro* cytotoxicity of synthesized compounds against 10 Human Cancer Cell lines i.e. A549 (lungs), SK-OV-3 (ovary), HCT15 (colon), K562 (leukemia), HeLa (cervix), KB (Nesophayngea), MCF7 (breast) and DU145 (prostate) with standard Methotrexate used in the assay shows some comparable activity. Compound **7a** was found to be anticancer lead and can be consider as useful template to obtain more potent anticancer active lead. The DHFR in silico enzyme inhibition scores were obtained to consider the possibility of these agents acting through DHFR inhibition.

ACKNOWLEDGEMENT

This research work acknowledges Indian council for Medical Research (ICMR), a constituent of Ministry of Health and Family Welfare, New Delhi, autonomous for Medical Research in India for grant number 58/35/2011-BMS.

REFERENCES

1. Masur H, J. Infect Dis.1990; 161, 858-864.
2. Berman EM, Werbel LM, J. Med Chem. 1991; 34, 479–485.
3. Rot B, FedProc, 1986; 45, 2665–2772.
4. Jolivet JC, Curt KH, Clendninn GA, Chabner, N Engl J. Med. 1983; 309, 1094-1104.
5. Borst P, Quellette M, Annu. Rev. Microbiol., 1995, 49, 427–460.
6. Kozarek R, Patterson A, Gelfand DJ, Botoman MD, Ball VA, Wilske TJKR, Ann. Intern. Med. 1989; 110, 353–356.
7. Weinblatt M, Coblyn E, Fox JS, Fraser DA, Holdsworth P, Glass Trentham DEN, N Engl J. Med., 1985; 312, 818–822.
8. Weinstein GD, Frost P, Arch. Dermat. 1971; 103, 33–38.
9. Shakra Abu, Gladman M, Thorne DD, Long JC, Gough J, Faresell VTJ, Rheumat. 1995; 22, 241–245.
10. Majo VJ, Perumal PT, Tetrahedron Lett. 1996; 37, 5015.
11. Pegg AE, "Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy," Cancer Res., 1988; 48(4):759–774.
12. Köpf H, Köpf-Maier P, "Titanocene dichloride–the first metallocene with cancerostatic activity," Angewandte Chemie-Wiley online Libarary, 1979; 18(6):477–478.
13. Galanski M, Arion VB, Jakupec MA, Keppler BK, "Recent developments in the field of tumor-inhibiting metal complexes," Curr. Pharm. Des. 2003; 9(25):2078–2089.
14. http://preadmet.bmdrc.org/
15. Wheele AS, Oates WM, Bromination of Anthranilic Acid. J. Am. Chem Soc. 1910; 31,770-773.
16. Yaser A, El-Badry, Naglaa A, Anter Huda S, El-Sheshtawy, Synthesis and evaluation of new polysubstituted quinazoline derivatives as potential antimicrobial agents. Der Pharma Chemica, 2012; 4(3):1361-1370.
17. Nerkar AG, Saxena AK, Ghone SA, Thaker AK, Evaluation of Some Quinazolinone and Pyridine Derivatives as Dihydrofolate Reductase Inhibitors for Anticancer Activity, E –J. Chem., 2009; 6(S1):S97-S102
18. Kalagouda B, Gudasi etal., Transition metal complexs of potentioal anticancer quinazoline ligand, Transition Met. Chem. 2009; 34, 325-330
19. Shiva Prasad K, Shiva Kumar L, Chandan S, Vijaya B, Revanasiddappa HD, Synthesis, Characterization and DNA interaction studies of copper (II) complex of 4(3H) Quinazolinone derived Schiff base, Analele Universităţii din Bucuresti – Chimie (serie nouă), 2011; 07-13.
20. Kalagouda, B., Gudasi etal; Transition metal complexs of potentioal anticancer quinazoline ligand, Transition Met. Chem., 2009, 34,325-330.
21. Chandregowda V, Kush AK, Reddy CG, Synthesis and in vitro antitumor activities of novel 4-anilinoquinazoline derivatives. Eur. J. Med. Chem., 2009; 44(7):3046-3055.