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

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ABSTRACT

Objective: The main objective of this research work was to in silico screening, synthesis, characterization of quinazolinone 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. 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 accessibility of metal center exhibited to design metalcluster 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 folic acid or 7,8-dihydrofololate to 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 quinazolines, DHFR and metal complexes we chose them for research. In this research paper the quinazolino metal complexes as human DHFR antagonists as anticancer agents leads have been investigated. The three leads were an outcome of in silico screening of quinazolino 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 spectra were recorded on SHIMADUZU spectrophotometer. 'H NMR were recorded at Diya labs Mumbai on 400 MHz Spectrophotometer facility, chemical shifts (δ) are reported in parts per million (ppm) with CDCl₃, 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 (multiplets). 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 (Electronic 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**

1. **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 Caco2 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.

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**Table 1: ADME Parameters of synthesized compounds**

<table>
<thead>
<tr>
<th>Compound</th>
<th>HIA</th>
<th>Caco2 cell permeability</th>
<th>MDCK</th>
<th>PPB</th>
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<tbody>
<tr>
<td>6a</td>
<td>97.37</td>
<td>47.75s</td>
<td>0.24</td>
<td>95.39</td>
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<td>6c</td>
<td>99.40</td>
<td>21.34</td>
<td>0.25</td>
<td>93.84</td>
</tr>
</tbody>
</table>

@HIA = Human Intestinal Absorption. **Caco2 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

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**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.

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

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Code</th>
<th>R</th>
<th>R1</th>
<th>Docking score</th>
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<tbody>
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<td>1</td>
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<td>CH₃</td>
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<td>2</td>
<td>6b</td>
<td>4-OH</td>
<td>CH₃</td>
<td>-46.04</td>
</tr>
<tr>
<td>3</td>
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<td>4</td>
<td>6d</td>
<td>4-Cl</td>
<td>CH₃</td>
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<td>CH₃</td>
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<td>6f</td>
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<td>8</td>
<td>6h</td>
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<td>C₂H₅</td>
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<tr>
<td>11</td>
<td>Methotrexate</td>
<td>-</td>
<td>-</td>
<td>-47.88</td>
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</table>

**II. Synthesis**

Synthesis of (quinazolinone Schiff base) 3-{(substituted benzylideneamino)-2-phenylquinazolin-4(3H)-one [15-17]: To a hot ethanolic solution of substituted benzaldehyde (1 eq) (0.26ml,0.002mol). 2-3 drop of H₂SO₄ was added. The reaction mixture was refluxed with stirring for 1 hr. Solid precipitated out, was filtered under vacuum.

**Structures of ligands were designed shown from Series 1 (6a-6j) and Series 2 (7a-7l). Library of 22 compounds was developed.**
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 2-3 drop of 4(3H)-one Cu(II) complexes (7a): To a hot ethanolic solution of quinazolinone Schiff base (6b = 0.5g, 0.00140 mol) (1eq) 0.5g hot ethanolic solution of metal salt (0.05 eq) [(CuCl₂.2H₂O) (6a = 0.1g, 0.0007 mol), (6b = 0.02g,0.00014), (6c = 0.01g,0.0006 mol) (NiCl₂.6H₂O) (6c = 0.05g,0.00014) was added respectively. The reaction mixture was refluxed with stirring for 2-3 hr. Solid was precipitated, washed with water and dried. The reaction mixture was refluxed with stirring for 2-3 hr. Solid was precipitated, washed with water and dried. % Yield: 60%, Molecular Formula: C₁₇H₁₁N₄O₄, Molecular wt: 341, Rf: 0.5 (Hexane:Ethyl acetate 60: 40), I.R. (KBr, cm⁻¹): 3280 (C-O, Str.), 2927 (CH), 1514 (C-N), 1608 (C-C, Str.), 1156 (C-O, Str.), 1617 (C= N), 1598 (C=C, Str.). ESR (g value): 2.05. AAS (absorbance): 0.055 or 70%.

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) 0.5g hot ethanolic solution of metal salt (0.05 eq) [(CuCl₂.2H₂O) (6a = 0.1g, 0.0007 mol), (6b = 0.02g,0.00014), (6c = 0.01g,0.0006 mol) (NiCl₂.6H₂O) (6c = 0.05g,0.00014) was added respectively. The reaction mixture was refluxed with stirring for 2-3 hr. Solid was precipitated, washed with water and dried. % Yield: 60%, Molecular Formula: C₁₇H₁₁N₄O₄, Molecular wt: 341, Rf: 0.5 (Hexane:Ethyl acetate 60: 40), I.R. (KBr, cm⁻¹): 3280 (C-O, Str.), 2927 (CH), 1514 (C-N), 1608 (C-C, Str.), 1156 (C-O, Str.), 1617 (C= N), 1598 (C=C, Str.). ESR (g value): 2.05. AAS (absorbance): 0.055 or 70%.

% Yield: 60%, Molecular Formula: C₁₇H₁₁N₄O₄, Molecular wt: 341, Rf: 0.5 (Hexane:Ethyl acetate 60: 40), I.R. (KBr, cm⁻¹): 3280 (C-O, Str.), 2927 (CH), 1514 (C-N), 1608 (C-C, Str.), 1156 (C-O, Str.), 1617 (C= N), 1598 (C=C, Str.). ESR (g value): 2.05. AAS (absorbance): 0.055 or 70%.

% Yield: 60%, Molecular Formula: C₁₇H₁₁N₄O₄, Molecular wt: 341, Rf: 0.5 (Hexane:Ethyl acetate 60: 40), I.R. (KBr, cm⁻¹): 3280 (C-O, Str.), 2927 (CH), 1514 (C-N), 1608 (C-C, Str.), 1156 (C-O, Str.), 1617 (C= N), 1598 (C=C, Str.). ESR (g value): 2.05. AAS (absorbance): 0.055 or 70%.
2-aminobenzoic acid (1) + benzoyl chloride (2) → 2-phenyl-4H-benzo[d][1,3]oxazin-4-one (3)

**Figure 1:** General synthetic scheme

Pyridine, 0°C

Hydrazine hydrate, Pyridine, reflux for 6hr

Substituted benzaldehyde, EtOH

Reflux for 6hr, H₂SO₄

**Figure 2:** General synthetic procedure of Schiffs base

**General synthetic Procedure of Schiff’s base:**

3-amino 2-phenyl quinazolin-4-one + substituted benzaldehyde → 3-(2-substituted benzylideneamino) 2-phenyl quinazolin-4(3H)-one

**Figure 2:** General synthetic procedure of Schiff’s base

M = Cu,Ni

Quinazolinone schiff base metal complexes (7a-7l)
Synthesis of quinazolinone Schiff's base metal complexes [18-20]

![Chemical structure of quinazolinone Schiff's base metal complexes](image)

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

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

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<tr>
<th>Sr. No.</th>
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<th>Compound code</th>
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<th>TGI</th>
<th>GI 50 &amp;</th>
<th>LC 50*</th>
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<td>2</td>
<td>DU145</td>
<td>7a</td>
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<td>&gt;80</td>
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</table>

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**

<table>
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<th>Sr. No.</th>
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<th>Hydrogen bond</th>
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<td>4</td>
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<td>Methotrexate</td>
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</table>

**Synthesis of 3-(2-nitrobenzylideneamino)-2-phenylquinazolin-4(3H)-one Cu(II)complexes (7c):** To a hot ethanolic solution of quinazolinone Schiff base (0.05g,0.00135 mol) (1 eq) 0.5g hot ethanolic solution of metal salt (0.5eq) (CuCl₂2H₂O) (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 3-(2-nitrobenzylideneamino)-2-phenylquinazolin-4(3H)-one Ni(II)complexes (7f):** To a hot ethanolic solution of quinazolinone Schiff base (0.05g,0.00135 mol) (1 eq) 0.5g hot ethanolic solution of metal salt (0.5eq) (NiCl₂6H₂O) (0.03g,0.00014 mol) was added. The reaction mixture was refluxed with stirring for 2-3 hr. Solid was precipitated out, 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% CO₂, 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 10⁵ cells/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 Adriamycin 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
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REFERENCES