COMPARATIVE IN-SILICO AND IN-VITRO STUDY OF BENZENE SULFONAMIDE AND ITS ANALOGUES WITH DOXORUBICIN AS AN ANTICANCER AGENT

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CANCER

• The division of normal cells is precisely controlled. New cells are only formed for growth or to replace dead ones.

• Cancerous cells divide repeatedly out of control, even though it is not required, they crowd out other normal cells and function abnormally.

• Destroy the correct functioning of major organs.
WHAT CAUSES CANCER?

• Cancer arises from the mutation of a normal gene.

• Mutated genes that cause cancer are called oncogenes.

• It is thought that several mutations need to occur to give rise to cancer.

• Cells that are old or not functioning properly normally self destruct and are replaced by new cells.

• However, cancerous cells do not self destruct and continue to divide rapidly producing millions of new cancerous cells.
CANCER TREATMENT

- Radiotherapy
- Chemotherapy
- Surgery
TYPES OF DRUGS USED

• **Antimetabolites**: Interfere with the formation of key bio-molecules within the cell including nucleotides (the building blocks of DNA). Ultimately interfere with DNA replication and therefore cell division.

• **Genotoxic Drugs**: Drugs that damage DNA. These agents interfere with DNA replication, and cell division.

• **Spindle Inhibitors**: Prevent proper cell division by interfering with the cytoskeletal components that enable one cell to divide into two.
Genotoxic drugs are chemotherapy agents that affect nucleic acids and alter their function.

These drugs may directly bind to DNA or they may indirectly lead to DNA damage by affecting enzymes involved in DNA replication.

Rapidly dividing cells are particularly sensitive to genotoxic agents because they are actively synthesizing new DNA.

If enough damage is done to the DNA of a cell it will often undergo apoptosis, the equivalent of cellular suicide.
GENOTOXIC CHEMOTHERAPY TREATMENTS

• **Alkylating agents:** The first class of chemotherapy agents used. These drugs modify the bases of DNA, interfering with DNA replication and transcription.

• **DNA binders (Intercalating, groove and surface binding agents):** These drugs lock themselves into the spaces between the nucleotides in the DNA double helix. They interfere with transcription, replication and induce mutations.

• **Enzyme inhibitors:** These drugs inhibit key enzymes, such as topoisomerase (involved in DNA replication inducing DNA damage).
IMPORTANT DRUGS USED IN CHEMOTHERAPY

• Daunorubicin
• Doxorubicin
• Epirubicin
• Idarubicin
• Mitoxantrone
• Cis-platin etc.
STRUCTURE OF ANTICANCER DRUG DOXORUBICIN

(7S, 9S)-7-[(2R, 4S, 5S, 6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6, 9, 11-trihydroxy 9-(2- hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5, 12-dione
MECHANISM OF ACTION OF DOXORUBICIN AND ITS SIDE EFFECTS

- Doxorubicin is an anthracycline anticancer drug used against various cancers such as non-Hodgkin’s lymphoma, breast cancer, lung cancer, ovary cancer, endometrium cancer, etc.

- Kills cancer cells by intercalating DNA, interfering topoisomerase II and initiation of DNA double strands breaks.

- Major side effects are hair loss, heart damage, liver dysfunction, diarrhea, vomiting & nausea. Main cause of concern.
SULFONAMIDES

- Sulfonamide a class of antibiotics in multiple clinical uses since 1930’s. First sulfonamide compound synthesized was effective antibacterial agent.

- Sulfonamide possesses many biological activities such as antibacterial, anticancer, antifungal, carbonic anhydrase inhibitor, diuretic and anti thyroid etc.

- Sulfonamide developed from prontosil dye and all are synthetic derivatives of sulfanilamide.

- Sulfonamide is similar in shape to p-aminobenzoic acid (PABA).
- These compounds bind to the active site and block PABA which synthesize folic acid for cell growth.

- Some of synthesized sulfonamide compounds are effective anticancer agents in comparison with doxorubicin.

- Sulfonamides carrying -SO₂NH₂- which lowers the polarity of sulfonamide and alkyl group increases the hydrophobicity and crosses cell wall more easily.

![Chemical structures of Sulfanilamide and PABA](image-url)
The chemical modification of this part of the molecule increases activity and modifies some pharmacological properties.

This part of the molecule cannot be modified chemically without loss of antibacterial activity.
STRUCTURE OF SYNTHESIZED BENZENE SULFONAMIDE ANALOGUES

(a) S
N
O
ClO
O O
S N
H
N
OO
O
S N
H
H
N
O
O
Cl
C2H5
C2H5
(b) S
N
O
CH3
S N
H
N
OO
O
C2H3
(c) S
O
N
H
S
N
O
O
O
CH3
S
N
H
H
N
O
O
O
CH3
S
N
H
H
N
O
O
O
(d) S
O
N
H
S
N
O
O
O
C2H5
C2H5
(e) S
O
N
H
S
N
O
O
O
(f) S
O
N
H
S
N
O
O
O

(a)
(b)
(c)
(d)
(e)
(f)
MATERIALS AND METHODS

- Growth inhibitory effect of benzene sulfonamides compounds measured by MTT assay on human breast cancer cell line.

- Cells are seeded in cell culture medium to obtain cell suspension which is seeded in tissue culture plate.

- The absorbance measured at 570 nm, IC$_{50}$ value of all the compounds as well as doxorubicin is calculated.
COMPARATIVE IC$_{50}$ VALUE OF DOXORUBICIN AND BENZENE SULFONAMIDES ANALOGUES ON HUMAN BREAST CANCER CELL LINES

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ value (µgm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>6.21</td>
</tr>
<tr>
<td>(b)</td>
<td>7.12</td>
</tr>
<tr>
<td>(c)</td>
<td>8.39</td>
</tr>
<tr>
<td>(d)</td>
<td>28.09</td>
</tr>
<tr>
<td>(e)</td>
<td>8.82</td>
</tr>
<tr>
<td>(f)</td>
<td>13.19</td>
</tr>
<tr>
<td>DOX</td>
<td>7.2</td>
</tr>
</tbody>
</table>
DOCKING STUDIES

METHODOLOGY FOR DOCKING STUDY

- Structure of doxorubicin and benzene sulfonamides are built using MOE software and optimized with MMF94x force field.

- Auto Dock Tools (ADT) is used for binding of doxorubicin and ligands with DNA duplex sequences.

- Best docked position is determined by comparing conformations.

- The free energy ($\Delta G$), inhibitory constant ($K_i$) and hydrogen bonding interactions of the complexes is calculated.
COMPARATIVE FREE ENERGY (ΔG) AND INHIBITORY CONSTANT (Kᵢ) OBTAINED FROM DOCKING STUDY OF DOXORUBICIN, COMPOUND (a) AND (b) WITH POLY(dA-dT).POLY(dA-dT) AND POLY(dG-dC).POLY(dG-dC).

<table>
<thead>
<tr>
<th>Complex</th>
<th>ΔG  (kcal mol⁻¹)</th>
<th>Kᵢ  (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOX-Poly(dA- dT).Poly(dA-dT)</td>
<td>-5.91</td>
<td>46.54</td>
</tr>
<tr>
<td>DOX-Poly(dG- dC).Poly(dG-dC)</td>
<td>-4.43</td>
<td>570.16</td>
</tr>
<tr>
<td>(a)-Poly(dA- dT).Poly(dA-dT)</td>
<td>-4.65</td>
<td>388.63</td>
</tr>
<tr>
<td>(a)- Poly(dG- dC).Poly(dG-dC)</td>
<td>-8.0</td>
<td>1.36</td>
</tr>
<tr>
<td>(b)-Poly(dA- dT).Poly(dA-dT)</td>
<td>-7.86</td>
<td>1.73</td>
</tr>
<tr>
<td>(b)- Poly(dG- dC).Poly(dG-dC)</td>
<td>-7.6</td>
<td>2.67</td>
</tr>
</tbody>
</table>
COMPARATIVE HYDROGEN BONDING INTERACTIONS OF POLY(dG-dC).POLY(dG-dC) AND POLY(dA-dT).POLY(dA-dT) WITH DOXORUBICIN AND BENZENE SULFONAMIDES ANALOGUES

<table>
<thead>
<tr>
<th>Complex</th>
<th>Hydrogen bonding</th>
<th></th>
</tr>
</thead>
</table>
| Compound (a) + Poly(dG-dC).Poly(dG-dC) | G11:H22-N  
G11:H22-O |          |
| Compound (a) + Poly(dA-dT).Poly(dA-dT) | T10:H3-N   
T10:HO3-O   
T10:H3-O |          |
| Doxorubicin + Poly(dG-dC).Poly(dG-dC) | G11:H21-DOX  
G11:H21-DOX  
C10:HO3*-DOX | C10:HO3 is additional interaction. |
| Doxorubicin + Poly(dA-dT).Poly(dA-dT) | T10:H3-DOX  
T10:HO3-DOX |          |
DOCKED IMAGES OF DOXORUBICIN AND COMPOUND (a) WITH POLY(dG-dC).POLY(dG-dC) AND POLY(dA-dT).POLY(dA-dT) SEQUENCES.

DOX + Poly(dG-dC).Poly(dG-dC)  
DOX + Poly(dG-dC).Poly(dG-dC)

Compound (a) + Poly(dG-dC).Poly(dG-dC)  
Compound (a) + Poly(dA-dT).Poly(dA-dT)
COMPARATIVE OF DOCKING AND CYTOTOXIC STUDIES

- $\text{IC}_{50}$ value of compound (a) and (b) are better than anticancer drug doxorubicin and other sulfonamide analogues on human breast cancer cell lines.

- Free energy and inhibitory constant of compound (a) and (b) are also better than doxorubicin.

- Both docking and cytotoxic study shows the better anticancer activity of compound (a) and (b) over doxorubicin and other sulfonamide analogues.
CONCLUSION

- Compound (a) shows best anticancer activity.

- Cytotoxic and docking study both support each other.

- Hydrogen bonding interactions shows some common interactions between drug and benzene sulfonamides with DNA duplex sequences.

- Further we are in progress to study cell cycle analysis, anticancer activity on different cancer cell lines, DNA binding on these benzene sulfonamides compounds, in order to confirm the anticancer activity.
REFERENCES


9. P. Awasthi, S. Dogra, R. Barthwal, multispectroscopic methods reveal different modes of interaction of anticancer drug mitoxantrone with Poly(dG-dC).Poly(dG-dC) and Poly(dA-
THANK YOU