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AZOMYCIN BASED IONIC LIQUIDS AS AN ANTICANCER AGENT: A COMPUTATIONAL APPROACH

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ABSTRACT

The study aims to contribute to drug design and development by analyzing protein-drug interactions. It has been reported that 1,3-benzodioxole-based ionic liquid derivatives possess cytotoxic effects against human cancer cell lines. The derivatives are microtubule-targeted chemotherapeutic agents that can suppress microtubule dynamics leading to apoptosis and mitotic blocking. We have focused attention on neomycin-based Ionic Liquids. We report here the screening of a set of derivatives to identify suitable inhibitors against protein function based on binding affinity, and physicochemical and pharmacokinetic studies. The tubulin protein structure was evaluated and validated using various validation tools for proteins *viz*. VADAR, Verify3D. The molecular docking of 1,3-benzodioxole-based neomycin-tagged ionic liquids was accomplished with tubulin protein to illustrate the interactions of the most potent compound, followed by pharmacokinetic and pharmacological studies. 3-(benzo[*d*][1,3]dioxol-5-ylmethyl)-2-nitro-*1H*-imidazol-3-ium dimethyl phosphate was found to be a tubulin polymerization inhibitor revealing the binding energy -327.18 kcal/mol. 3-(benzo[*d*][1,3]dioxol-5-ylmethyl)-2-nitro-*1H*-imidazol-3-ium dimethyl phosphate has shown 69.78% intestinal absorption, -1.29 log BB, -2.73 logKp, maximum tolerated dose: 0.22 log mg/kg/day, LD50: 2.89 mol/kg. The results revealed that these compounds interact efficiently with the receptor and that compound 1 can be evaluated as a potential therapeutic candidate.

Keywords: Molecular Docking, Preclinical Studies, 1, 3-Benzodioxole, Anticancer Agent, 2-Nitroimidazole, azomycin.

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INTRODUCTION

Cancer is a chronic illness caused by the change of healthy cells into tumor cells, which can divide indefinitely, migrate, and invade healthy tissues. According to a recent study, there will likely be roughly 19-20 million new cases of cancer worldwide annually. The five most prevalent malignancies are skin, breast, lung, colorectal, and prostate cancers.[1,2] Subsequently the molecular regulation of cancer advancement in various tissues may vary, and the diversity of tumor creation in various tissues creates a problem for treatment. Due to the many molecular pathways underpinning it, the control of metastasis, anti-apoptosis, cell-cycle regulation, and proliferation of various cancer types makes cancer treatment more difficult. For decades, cancer patients had just a few numbers of treatment choices to select from, which included chemotherapy, radiation therapy, and surgical resection, which may be used separately or in combination.[3] Despite the development of these novel treatment protocols and tactics for a variety of cancer forms, the average overall survival rate for the most prevalent adult cancer subtypes remains low.[4] It has been noted that the creation and development of innovative anticancer medications is a very laborious and challenging process that necessitates significant financial investment in vitro and in vivo investigations and follow-up clinical trials. Ionic liquids and salts are a brand-new family of substances that have their special qualities. The ability to modify their physiochemical properties by varying the cation/anion combination, which is not achievable for molecular compounds, is their most appealing feature. Ionic liquid has become a novel and expansive area of scientific study as a result of the

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skillful exploration of this trait to identify its applications in a variety of sectors, including synthesis, materials, speciality chemicals, etc. There have been several thousand papers on ionic liquids as a result of the amazing rise over the past two decades.[5,6,15,16,7–14] Numerous antimitotic drugs, including steganacin, podophyllotoxin, and combretastatin A-2, have the 1,3-benzodioxole molecule which showed cytotoxicity against numerous human cancer cells (colon tumor cells & multidrug resistant nasopharyngeal tumor cells).[17,18] Pharmaceutical researchers can alter the physical characteristics of a possible drug candidate *in silico* and anticipate its physicochemical characteristics before synthesis with the help of molecular modeling software. With real advantages, scientists have been able to apply structure-based drug-design methods to the crystallographic data on the receptor. One may instantly understand the strength that computer-based technologies offer given how challenging it is to produce organic substances.[19] In need for the design & development of new anticancer drugs our research group is working on 1,3-benzodioxole motif-based ILs as a potential drug.[20–26] In this study, we have carried out the molecular docking of azomycin-based ionic liquid using Hex 8.0 and predicted ADME properties to determine a potential anticancer drug.

EXPERIMENTAL

Structural Evaluation of Protein

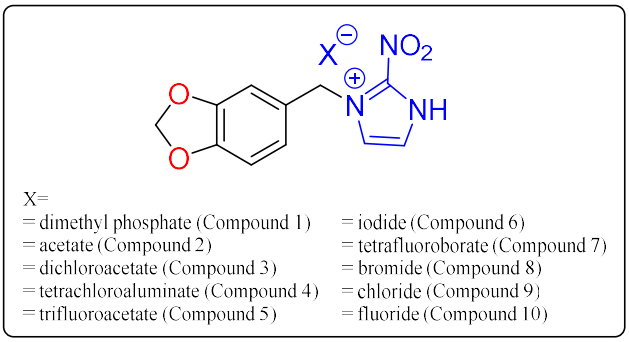
Tubulin protein crystal structure obtained from RCSB protein data bank (PDB ID: 1TUB). Vadar 1.8 server used to assess Ramachandran plot for stereochemical properties determination.[27] Other three-dimensional structural properties and protein quality were studied using the Verify3D server,[28] and ProSA web interface.[29]

Preparation of Protein

Before molecular docking, the protein was prepared using the Chimera tool. Undesirable ligands and H_2O molecules were removed and H atoms were added. After minimization of energy, the protein is saved in pdb file format.

Preparation of Ligand

ChemDraw Ultra software[30] was used to draw the derivatives of the 1,3-benzodioxole compound named Compounds 1-10 (Scheme-1). After energy minimization, the 3-D structures of these compounds were saved in PDB format using Chem-3D.



X=

= dimethyl phosphate (Compound 1)	= iodide (Compound 6)
= acetate (Compound 2)	= tetrafluoroborate (Compound 7)
= dichloroacetate (Compound 3)	= bromide (Compound 8)
= tetrachloroaluminate (Compound 4)	= chloride (Compound 9)
= trifluoroacetate (Compound 5)	= fluoride (Compound 10)

Scheme-1: Structures of the Designed Compounds

Molecular Docking Studies

Molecular Docking (MD) is one of the important approaches in structural molecular biology and automated drug synthesis. A protein with a known 3D structure structure is used in ligand-protein docking to predict the major mode(s) of ligand binding. We used HEX 8.0 to carry out molecular docking (http://hexserver.loria.fr/) which uses the Fast Fourier Transform (FFT), and it is two times quicker than traditional FFT docking techniques with the same resolution and scoring functions.[31] The server entails PDB format uploading for protein and ligand structures and produces up to 1000 docking predictions. The docked complex was analyzed for non-bond interactions through Discovery Studio Client.

Physicochemical and Pharmacokinetic Properties Analysis

pkCSM and SwissADME web servers are used for studying the physicochemical and pharmacokinetics properties of the compound.[32,33] The physicochemical properties studied include molecular weight / BM,

the bonds' number between rotating atoms (Torsion), the logarithm of the octanal/H_2O partition coefficient (log P), H-Bond Donor (HBD), H-Bond Acceptor (HBA) and Polar Surface Activity (PSA). The pharmacokinetic properties were monitored using the ADMET tool. The toxicity profile of biologically active compounds was obtained by using the pkCSM tool (online).

RESULTS AND DISCUSSION

Structural Evaluation of Protein

The 3D crystal structure of tubulin (PDB ID: 1TUB) (Fig.-1a) was validated by the Ramachandran plot. Ramachandran plot assessment of tubulin protein showed 80.5% amino acids lie in the favorable permissible region, 9.6% amino acids in the generously permissible region, and 9.7% amino acids lie in the disallowed region as shown in Fig.-1b.

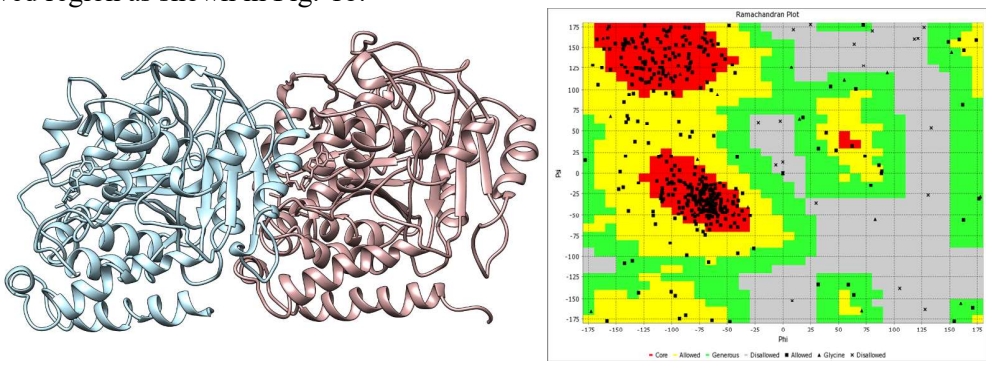


Fig.-1: (a) 3D- Structure of Tubulin Protein Showing Chains A and B in different Colors and (b) Ramachandran Plot Obtained from VADAR

The ProSA-web interface uses NMR and an X-ray plot based on existing structures to examine the tubulin structure. The Z-scores, which represent the inclusive quality of the model, are created and displayed in the results. With the aid of the X-ray crystallography technique (light blue color) and NMR spectroscopy (dark blue color), the Z-scores for all chains of proteins in the format of PDB are calculated in PROSA-we. The Z-score of tubulin protein models was in the range (denoted by the large black dot) as shown below in Fig.-2.

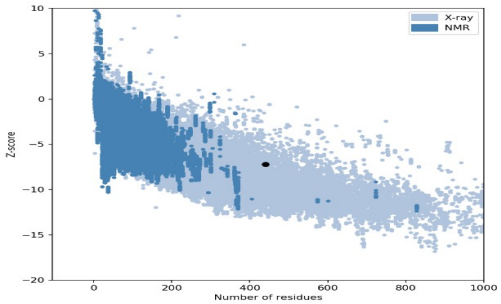


Fig.-2: Overall Model Quality Plot Obtained from ProSA Web Interface

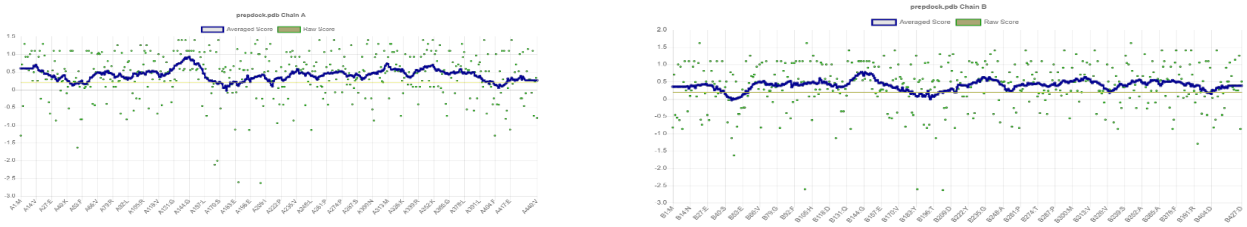


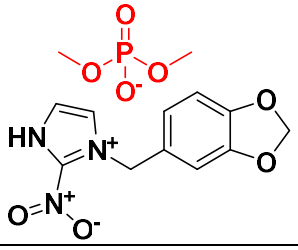
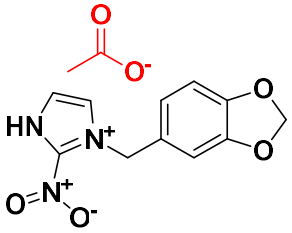
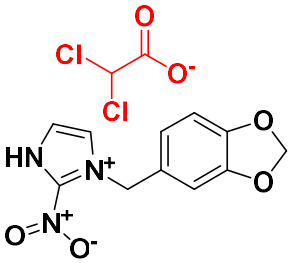
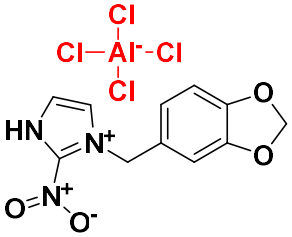
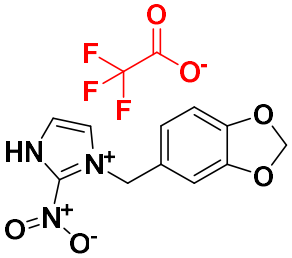
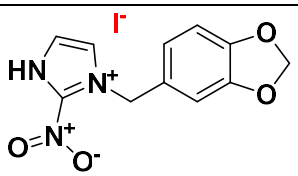
Fig.-3: Plot Obtained from Verify3D Web Server for Chain A and Chain B

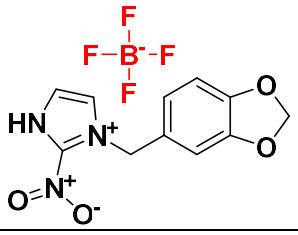
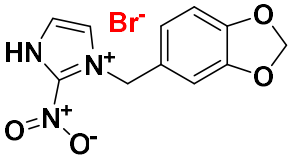
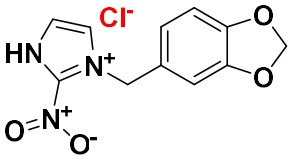
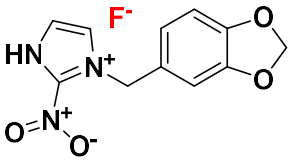
Verify3D analysis of tubulin protein shows that a number of the amino acid residues had a negative Z-score as shown in Fig.-3.

Molecular Docking Analysis

Molecular docking results of all compounds with tubulin protein by Hex 8.0.0 server revealed that compound 1 had the minimum binding energy (-327.18 kcal/mol) among these ten compounds as shown in Table-1.

Table-1: Binding Energy of Azomycin-Based Ionic Liquids with Tubulin Protein as Obtained from HEX Server Docking

Compound	Structure	B.E. (min) in kcal/mol
Compound 1		-327.18
Compound 2		-281.04
Compound 3		-280.74
Compound 4		-280.42
Compound 5		-274.44
Compound 6		-269.35

Compound 7		-264.85
Compound 8		-257.80
Compound 9		-251.92
Compound 10		-240.53

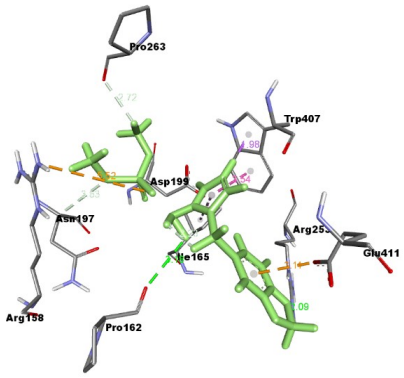


Fig.-4(a): Non-bond Interactions of Compound 1 with the Amino Acids of Tubulin Protein are Presented with Solid Dash Lines (Green Color for H-bonding, Orange Color for Electroctrostatic Interactions, and Violet Color for Hydrophobic Interactions along with Bond Distances)

Fig.-4 (b): Non-bonding interactions of Anionic Part (left side) and Cationic Part (right side) of Ionic Liquid with Amino Acid of Tubulin Protein are Presented in 2-dimensional Format

The protein-ligand interactions present in the docked complex were studied using Discovery Studio Visualiser. Compound 1 showed H-bond interactions with Arg253B, Pro162B, Asn197B, and Pro263B; hydrophobic interactions with Trp407B and Ile165B and electrostatic interaction with Arg158B (Fig.-4a and Fig.-4b).

Physicochemical and Pharmacokinetic Properties

The physicochemical and pharmacokinetic properties of the most potent compound studied using SwissADME and pkCSM web server are presented in Table-2 and Table-3.

Table-2: Physicochemical Properties of the Most Potent Compound (Compound 1) Observed using SwissADME Web Server

Physicochemical Properties	Value
Molecular weight	373.26 g/mol
Rotatable bonds (Numeric)	5
H-bond acceptor	8
H-bond donor	1
Topological Polar Surface Area	152.35 Å^2

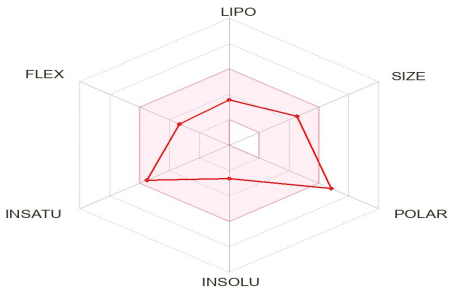


Fig.-5: Bioavailability Radar of Compound 1 Showing That the Pink-Colored Zone is Appropriate Physicochemical Space for Oral Bioavailability

Table-3: ADMET Properties of the Most Potent Compound (Compound 1) Observed in pkCSM Web Server

ADMET properties	Predicted Value
Adsorption	
Water solubility (log mol L^{-1})	-2.79
Human Intestinal Absorption (%)	74.72
Skin permeability (unit: log kp)	-2.73
p-glycoprotein substrate	Yes
Distribution	
Human VDss (logL kg^{-1})	-0.53
BBB permeability	-1.45
Excretion	
Total clearance rate (unit: log ml min^{-1} kg^{-1})	0.72
Renal OCT2 substrate	No
Toxicity	
Maximum tolerance dose (unit: log mg kg^{-1} day^{-1})	0.084
Acute oral rat toxicity (LD50)	2.55
Oral rate chronic toxicity	1.67
Skin Sensitization	No

Compound 1 obeys the Lipinski Rule (except N or O < 10) for drug-likeness; bioavailability score and synthetic accessibility were found to be 0.55 and 3.04 respectively. The ionic liquid is soluble in water

When orally administered, the compound is predicted to be well absorbed through the human small intestine. The predicted value has shown that the compound has relatively high skin permeability as logKp < -2.5. The P-glycoprotein (ATP-binding channel transporter) that serves as a biological barrier by releasing poisons from cells. The steady-state volume of distribution (VDss) is predicted low for this compound as log VDss < -0.15. The higher the volume of distribution is, the more drug (ILs) is distributed in tissue than blood plasma. The molecules with logBB less than -1 are poorly distributed to the brain. Compound 1 is the substrate for CYP3A4 and does not inhibit any isoform of cytochrome P450**.** Total clearance (hepatic clearance & renal clearance) is important for the determination of dose rates to attain steady-state concentrations. The maximum indorsed tolerated dose is predicted low for this compound. The oral rat chronic toxicity results are interpreted to know about the treatment lengths required and bioactive concentration.

CONCLUSION

We created a library of ILs based on 1,3-benzodioxole moiety to examine the binding potential to tubulin protein. Moreover, several protein validation methods were used to confirm its structural integrity followed by the pharmacological and pharmacokinetic studies of the designed ionic liquids. The results of the docking research showed that compound 1 successfully interacted with the tubulin protein and ADME studies indicated the compounds as effective anticancer agents.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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