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Triethylamine-based ionic liquids as anticancer agent: Synthesis, characterization, *in silico* and DFT studies

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The present study has focused on investigating the potential of triethylamine-based compounds tagged with 1,3-benzodioxole motif ionic liquids as inhibitors of tubulin proteins and anti-cancer therapeutics. Molecular docking analysis has been used to determine their binding capabilities with molecular targets and their impact on anti-cancer drug efficacy. *In silico* studies have found that N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium iodide (Compound 1) has the highest binding affinity to the tubulin protein. DFT studies have also been performed and various parameters recorded for compound 1. So, compound 1 has been synthesized using *meta*-synthesis methods and characterized using NMR techniques. Based on these findings, compound 1 has proven its potential as an anticancer agent with high potential for further studies.

Keywords: Anticancer drug, 1,3-Benzodioxole, Molecular Docking, Ionic Liquids, Tubulin

Cancer remains a substantial public health challenge in the world, accounting for an overwhelming number of new cases and deaths. As per the most recent cancer statistics, 958,310 new cases and 609, 820 fatalities were reported in 2023[1]. These figures highlight the urgent need for continued research, prevention, and treatment efforts to address this critical health issue. Eminent researchers from various research groups all over the world has reported many treatments and drugs for the cancer in last four decades[2]. However, the pharmaceutical industry is currently fronting significant challenges in discovering effective and innovative drugs along with their corresponding therapies. Almost 50% of accessible drugs are administered in form of salts, which can lead to several disadvantages such as polymorphism of drugs[3,4]. To combat this, the use of ionic liquids (ILs) is a promising strategy in the pharmaceutical field. While the definition of ILs is based on a physical property, their advantageous biological properties combined with the fact that the drug can be in a liquid state act as a crucial feature. This property can help address the issues related to the solid forms of drugs and also aid in the production of sustainable drugs[5–9]. ILs have the potential to become a promising class of anti-tumor agents due to their ability to tune their properties. They can be designed as anti-cancer agents, and one of their significant advantages is the ability to adjust their toxicity while tailoring the pharmacological and physiochemical properties required for the anticipated therapeutic applications[10–17]. Recently, our research group reported various 1,3-benzodioxole-based ionic liquids as anticancer agents[10–14,16–19]. Herein, in the present study synthesis and the anti-tumor activity of triethylamine-based ionic liquids using *in-silico* studies are reported.

Experimental Section

Materials and Methods

Synthesis and Characterization of Triethylamine-based Ionic Liquids

5-(Bromomethyl)-1,3-benzodioxole was synthesized using reported method in literature[16] and characterized using NMR techniques. 5-(bromomethyl)-1,3-benzodioxole was further used for the synthesis of triethylamine-based Ionic Liquid and followed by the *meta*-synthesis for the desired ionic liquids using our previous reported method. These ionic liquids have been characterized using ^{1}H and ^{13}C NMR techniques.

Preparation of Protein

The targeted tubulin protein (1SA0) with a resolution of 3.00 from the protein data bank[21] and Discovery Studio[22]was used to optimize the protein

structure by removing unwanted ligands and water molecules, adding hydrogen atoms, and performing an energy minimization process. Only two essential chains out of four and defined the binding pocket using Discovery Studio (Fig. 1).

Preparation of Ligand

New derivatives based on 1,3-benzodioxole were created and characterized using advanced analytical techniques such as NMR spectroscopy. The aim was to explore the pharmacological properties of these synthesized compounds using bioinformatics software. The Chem Draw Ultra software[23,24] was used to depict the structures of 1,3-benzodioxole derivatives and saved in pdb format. The optimized structures of the compounds were docked with the tubulin protein, and compounds showing strong interactions were optimized for their potential therapeutic applications (Fig. 2).

Molecular Docking Studies

Molecular docking is a critical process used in *in-silico* modeling, and it has become an essential tool in drug design. It is highly effective at producing accurate protein-ligand complexes and offers valuable insights into potential interactions[25,26]. For this study, AutoDock Vina, a molecular modeling suite that was known for its improved sampling capabilities, accuracy, and time efficiency[25,27]. To begin the molecular modeling process, the tubulin protein structure 1SA0 was obtained and saved in the PDB format[28–32]. Its stereo-chemical properties were analyzed by the Ramachandran plot[21,33,34] using Rampage, a powerful tool for studying protein conformations and distinguishing between allowed and disallowed states. To prepare the 1SA0 structure, Pymol[27] and Discovery Studio visualizer were used and removed water molecules and heteroatoms, such as magnesium ions and ligands, from the protein. Followed by defining a specific binding site, the colchicine-binding site in this case, by identifying a residue pocket and documenting its attributes and carrying out protonation, and adding Gasteiger charges to the protein. Steps were followed to prepare the groundwork for conducting molecular docking analysis using AutoDock Vina. The prepared ligand database was docked using AutoDock tool, which provided insights into module interactions and various bonds involved in the binding of the docked compounds. The Discovery Studio tools and Pymol were utilized for further analysis of the docked complexes, identifying hydrophobic interactions and hydrogen bonds, and analyzing various other important factors of the target protein structure (Fig. 3).

DFT Studies

DFT analysis is a powerful computational approach used to investigate the properties of ionic liquids. It provides insights into various attributes including electronic structure, thermodynamic characteristics, and molecular dynamics simulations[22,35]. In this study, DFT calculations were conducted using Spartan 14 software with the B3LYP/6-31 G*(d.p.) basis set.

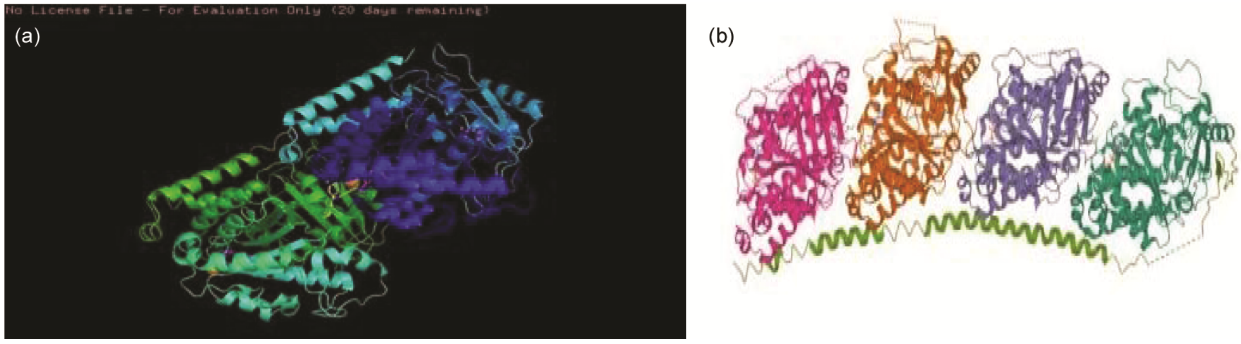


Fig. 1 — (A) -1SA0 Prepared protein -Chain A and chain B; (B) 3D- structure of Tubulin protein - 1SA0

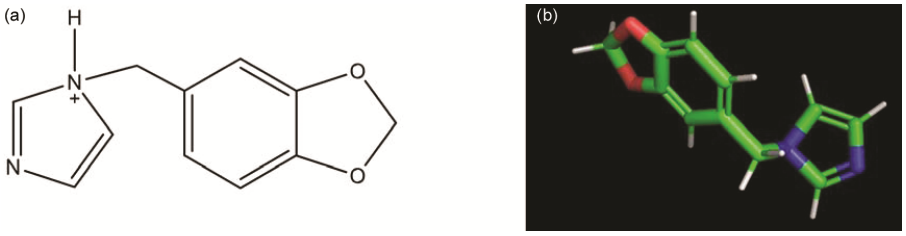


Fig. 2 — (A) - Prepared ligand – Compound 3; (B) - Prepared Ligand 3D – structure Compound 3

Results and Discussion

Molecular Docking

A recent study aimed to create a novel series of tubulin destabilizing agents inspired by 1,3-benzodioxole's exceptional properties in medicinal chemistry[12,36–42]. By examining the X-ray crystallographic structure of the tubulin complex with colchicine, the most favorable pose among ten generated poses was identified. The Compound 1 (iodide salt) demonstrated highly favorable binding energy in molecular docking experiments, suggesting its potential as a promising candidate for further exploration and development (Table 1 and Table 2).

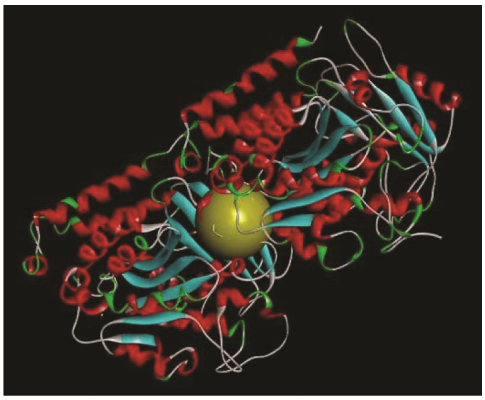


Fig. 3 — Prepared protein 1SA0 where yellow circle defines the colchicine binding pocket.

Table 1 — Binding energy of Ionic liquids with tubulin protein

S.No.	Compd	Binding Energy Kcal/ mol
1	N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium iodide	–6.2
2	N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium fluoride	–6.0
3	N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium bromide	–6.0
4	N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium chloride	–6.0
5	N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium fluoroacetate	–4.2
6	N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium hydroxide	–5.9
7	N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium phosphate	–6.0
8	N-(benzo[d][1,3]dioxol-5-ylmethyl)-N,N-diethylethanaminium salicylate	–5.8
9	N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium hydrogen carbonate	–3.4
10	N-(benzo[d][1,3] dioxol-5-ylmethyl)-N,N-diethylethanaminium acetate	–6.0

Table 2 — Interaction of ILs with tubulin protein

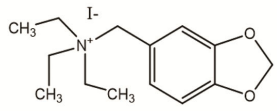
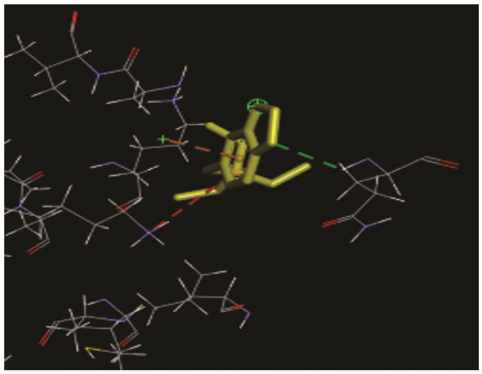
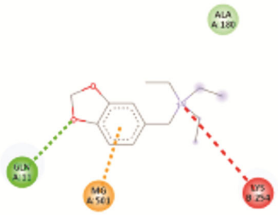
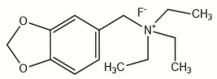
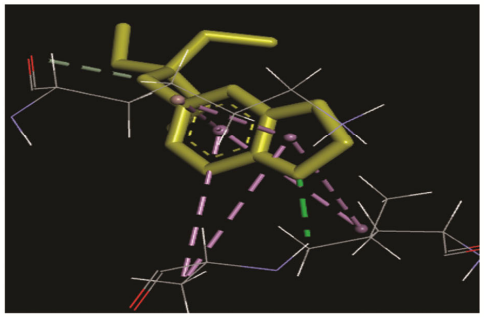
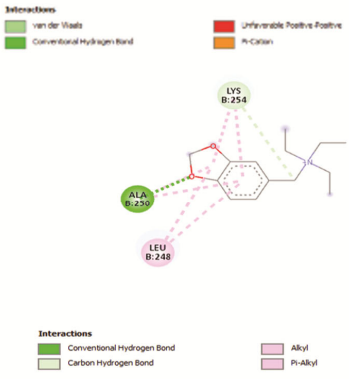
COMPOUND	3D - INTERACTIONS	2D - INTERACTIONS
		
 *N*-(benzo[*d*][1,3]dioxol-5-ylmethyl)-*N*,*N*-diethylethanaminium fluoride		

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Table 2 — Interaction of ILs with tubulin protein — (Contd.)

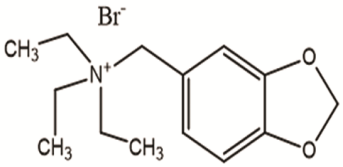
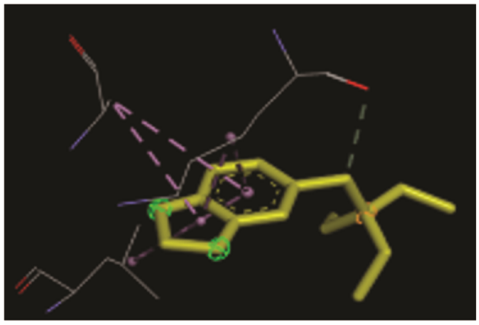
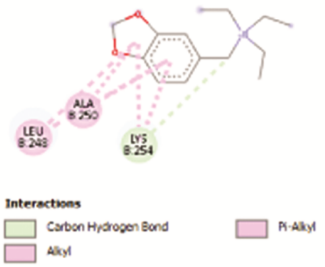
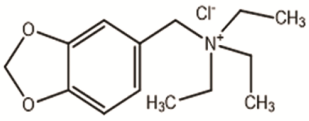
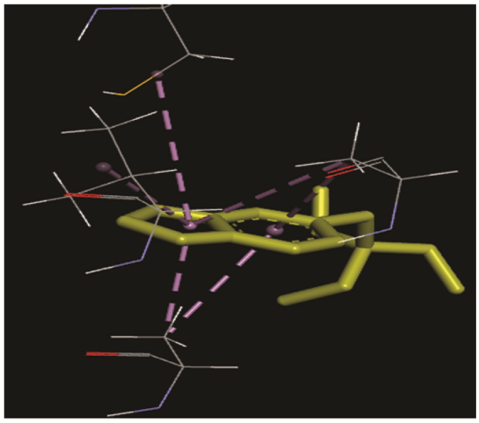
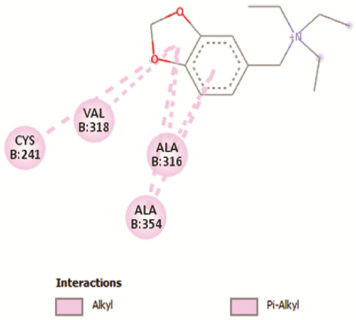
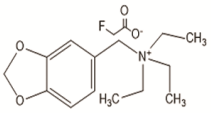
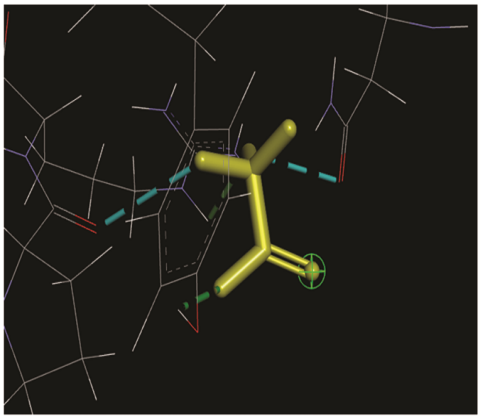
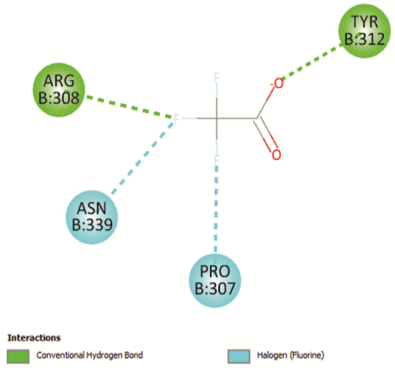
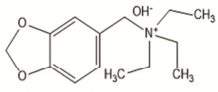
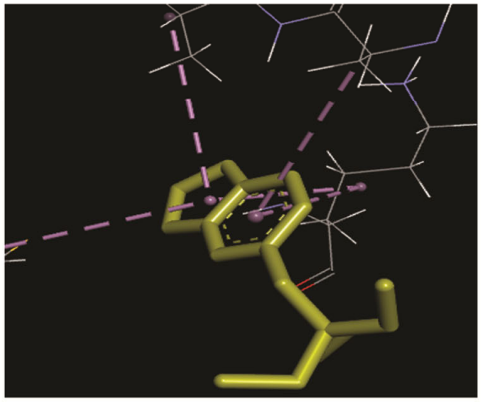
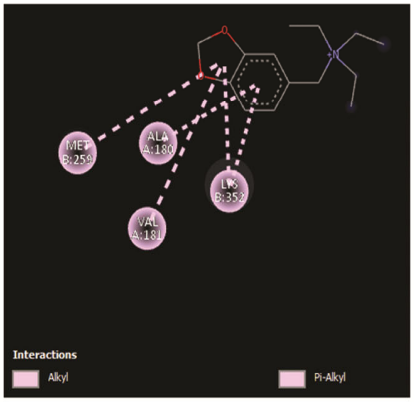
COMPOUND	3D - INTERACTIONS	2D - INTERACTIONS
		
 *N*-(benzo[*d*][1,3]dioxol-5-ylmethyl)-*N*, *N*-diethylethanaminium chloride		
 *N*-(benzo[*d*][1,3]dioxol-5-ylmethyl)-*N*,*N*-diethylethanaminium 2-fluoroacetate		
 *N*-(benzo[*d*][1,3]dioxol-5-ylmethyl)-*N*,*N*-diethylethanaminium hydroxide		

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Table 2 — Interaction of ILs with tubulin protein — (Contd.)

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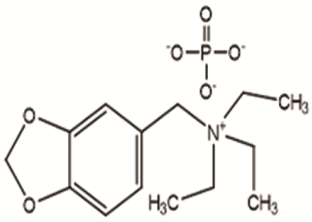
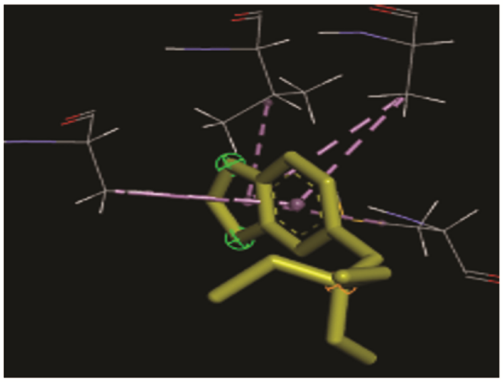
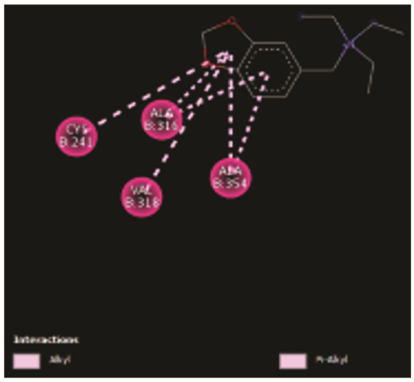
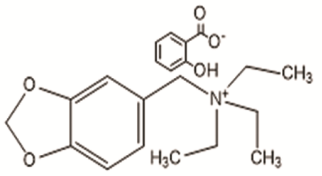
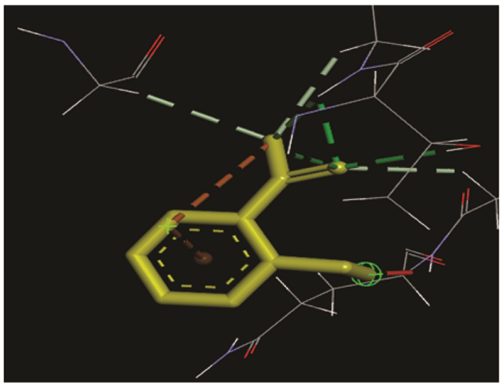
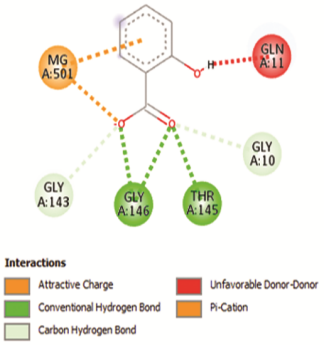
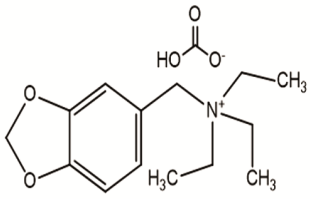
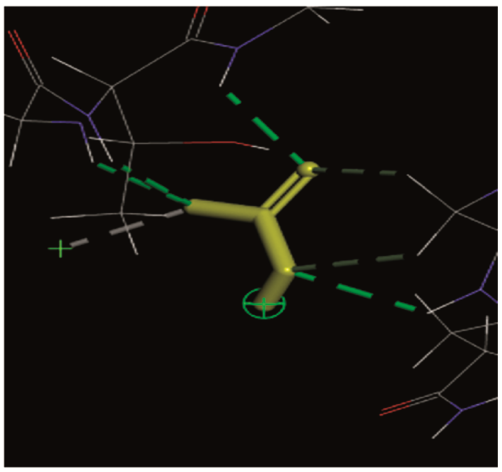
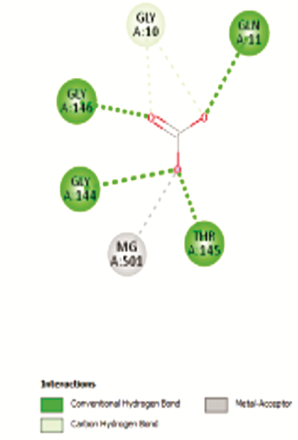
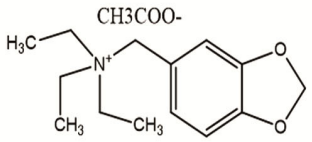
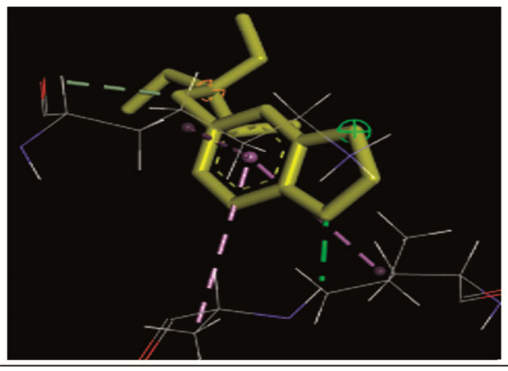
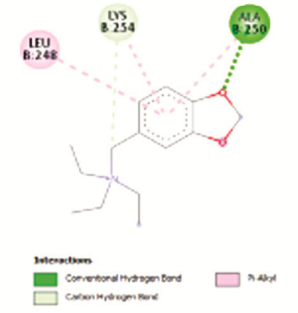
 *N*-(benzo[*d*][1,3]dioxol-5-ylmethyl)-*N*, *N*-diethylethanaminium phosphate		
 *N*-(benzo[*d*][1,3]dioxol-5-ylmethyl)-*N*, *N*-diethylethanaminium 2-hydroxybenzoate		
 *N*-(benzo[*d*][1,3]dioxol-5-ylmethyl)-*N*, *N*-diethylethanaminium hydrogen carbonate		
 *N*-(benzo[*d*][1,3]dioxol-5-ylmethyl)-*N*,*N*-diethylethanaminium acetate		

Chemistry

5-(Bromomethyl)-1,3-benzodioxole successfully synthesized and performed anion metathesis reactions to obtain the desired ionic liquids. Iodide salt was found to show best binding potential in molecular docking results and thus used to replace the bromide anion with the desired anion in synthesis. The resulting 1,3-benzodioxole-tagged tri-ethylamine-based ionic liquid obtained in excellent yield and characterized for purity through H^1-NMR, C^{13}-NMR as well as mass spectroscopy (Fig. 4).

Drug-Likeness Analysis

Drug-likeness rules include the criteria that evaluate the structural properties of compounds, allowing for a quick assessment of a molecule's potential as a drug. These rules are known for their effectiveness and efficiency. To calculate the drug-like properties of a molecule, researchers utilized DruLiTo and Swiss ADME server (Table 3 and Table 4)[43–52].

Pharmacological analysis and preclinical trials were conducted to investigate the pharmacological

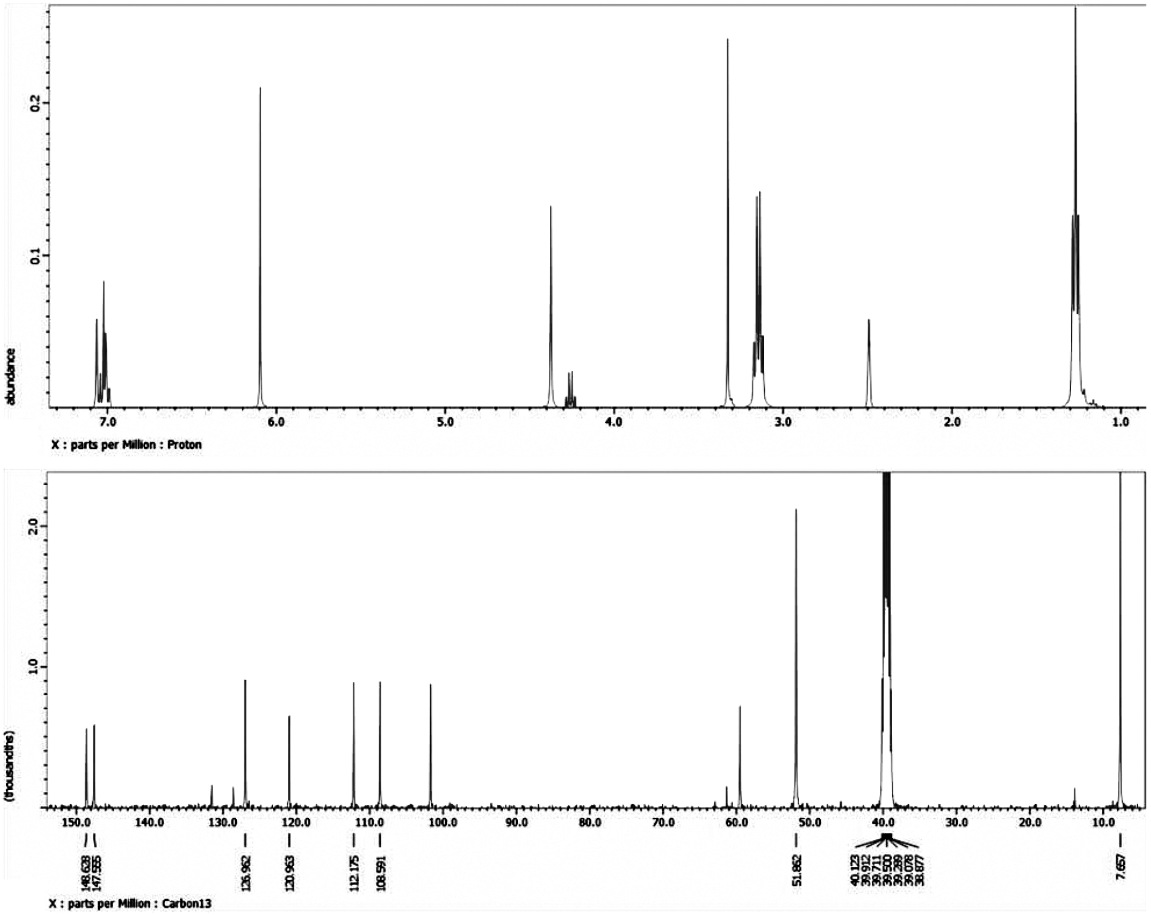


Fig. 4 — ^{1}H-NMR and ^{13}C-NMR of synthesized ionic liquid

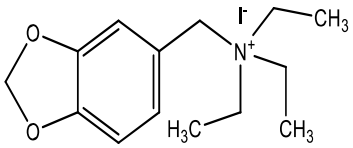
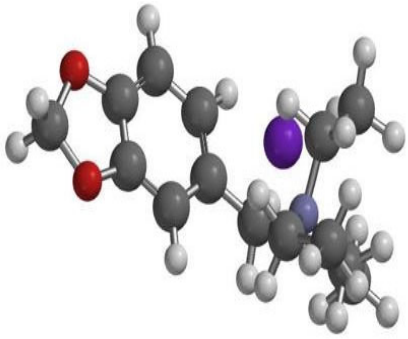
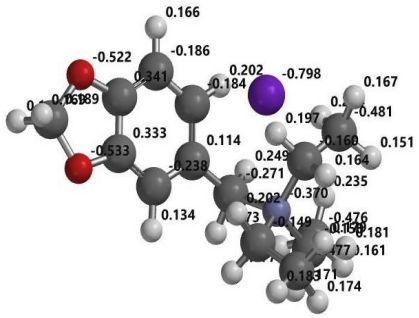
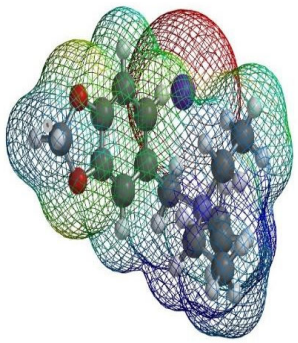
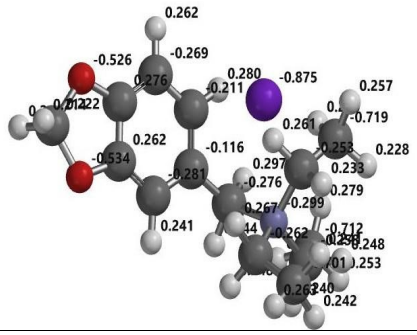
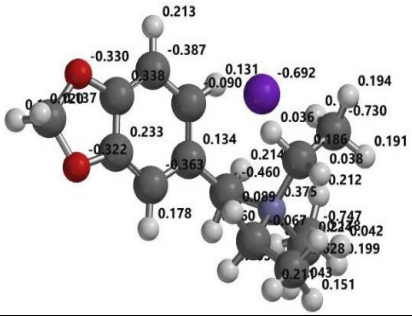
Table 3 — ADMET studies of ILs

Compd	Log kp cm/s	GI Absorption	BBBPermeability	*P*- gp substrate	CYP1A2inhibitor	CYP2C19inhibitor	CYP2C9inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Compound 1	–5.85	Low	No	No	No	No	No	No	No
Compound 2	–5.42	Low	No	Yes	No	No	No	No	No
Compound 3	–5.49	Low	No	Yes	No	No	No	No	No
Compound 4	–5.34	Low	No	Yes	No	No	No	No	No
Compound 5	–6.05	High	Yes	Yes	No	No	No	No	No
Compound 6	–6.13	High	yes	Yes	No	No	No	No	No
Compound 7	–7.75	High	No	Yes	No	No	No	No	No
Compound 8	–5.5	High	No	Yes	No	No	No	Yes	No
Compound 9	–6.16	High	No	Yes	No	No	No	No	No
Compound 10	–6.2	High	Yes	Yes	No	No	No	No	No

Table 4 — Pharmacokinetics study of ILs

S.No	Compd	log P	HBD	HBA	Lipinski's rule of 5 with zero violation	TPSA	MW
1.	Compound 1	3.057	0	2	yes	18.46	363.23
2	Compound 2	2.382	0	2	yes	18.46	255.33
3	Compound 3	2.857	0	2	yes	18.46	316.23
4	Compound 4	2.519	0	2	yes	18.46	271.78
5	Compound 5	2.145	0	0	yes	58.59	313.36
6	Compound 6		0		yes	41.52	253.34
7	Compound 7	–0.24	0	6	yes	114.52	331.3
8	Compound 8	2.005	0	5	yes	78.82	373.44
9	Compound 9	1.296	0	5	yes	78.82	297.35
10	Compound 10	1.446	0	4	yes	58.59	295.37

Table 5 — DFT study analysis of IL (with iodide anion)

Compd	Optimized Geometry	Mulliken Charges
		
MEP	**Nuclear Charges**	**Electrostatic Charges**
		

and biological properties of substances. The study included Lipinski's rule five, bioactivity assessment, and drug likeliness evaluation. Preclinical trials involved ADMET testing and recommendations for daily dosage analysis. The synthesized compounds do not violate the Lipinski rule of five, with high oral absorption values and moderately soluble in water. *P*-glycoprotein substrate properties analysis was studied, which may be exploited. The study paves the way for the development of a promising cancer treatment.

DFT analysis

Density Functional Theory (DFT) analysis of ionic liquids constitutes a potent computational approach employed for investigating the electronic structure, energetics, and diverse properties of these distinctive materials. DFT offers valuable insights into a range of attributes, including electronic structure, optical properties, thermodynamic characteristics, molecular dynamics simulations, pairing and aggregation, among others. In this study, DFT calculations were conducted by employing the B3LYP/6–31+G(d,p) basis set through Spartan 14 software. Electrostatic Potential (ESP) maps for isolated captions and anions were generated and Milliken charge analysis performed to determine atomic charge values. Additionally, nuclear charge values, electrostatic charges, and various geometric parameters such as bond lengths, valence angles, and selected dihedral angles were also obtained (Fig. 5). The results of the DFT analysis are presented in the Table 5.

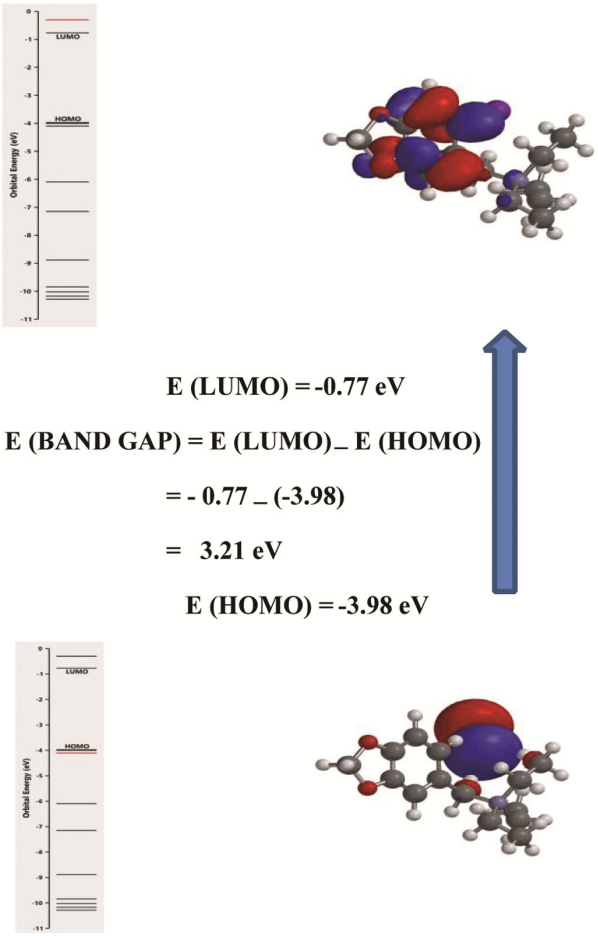


Fig. 5 — Frontier Orbital representation of N-(benzo[d][1,3] dioxol-5-ylmethyl) N,N- diethylethanaminium iodine

Conclusion

This research, centered on inhibiting tubulin, a pivotal protein in the cancer progression process, aspires to contribute to the creation of new and efficient chemical compounds. Studies have shown that ILs characteristics of structural diversity of ions and easy coupling motivated to biological applications *i.e.* better drug permeability and dissolution which leads to the designing of APIs and likewise in this present study, we have synthesized tri-ethylamine tagged 1,3-benzodioxole based ionic liquid that has been characterized by NMR spectroscopy and DFT analysis. Here, compound 1, has better binding affinities in comparison to other derivatives *i.e.* –6.2 kcal/mol. The findings have been quite positive as results of various *in-silico* studies like BBB permeability, follows Lipinski's rule of five that have been done with the Swiss ADMET online free server, DRULITO. Ultimately, this study paves the way for the discovery of innovative compounds that hold promise in the ongoing fight against cancer. In the course, of preclinical evaluations, we rigorously scrutinized various factors, including absorption, metabolism, excretion, toxicity, and synthetic feasibility, thereby ensuring their suitability for further development.

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