



Procaine and salicylate-based ionic liquid: synthesis, in silico, in vitro, biophysical and biological studies

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Abstract

Herein, we explored the synergistic effect of ionic liquids as anticancer and antibacterial agents to avoid available combination therapies. We synthesized an API-based ionic liquid (IL) designated as [Pro-pip]SAL to meet our objectives. This compound features a cationic moiety derived from procaine and 1,3-benzodioxole, tagged with a salicylate salt as the anionic counterpart. Molecular docking studies showed that interaction is polar involving H-bonding and van der Waal forces. Spectroscopic studies were carried out to validate the results of computational studies for the binding interaction mechanism of [Pro-pip]SAL IL with bovine serum albumin (BSA). The UV–visible spectra showed an increase in absorbance intensity with bathochromic shift, and fluorescence spectra showed static quenching with bathochromic shift revealing that polar interaction played an important role in the interaction between Pro-pip]SAL IL with BSA. CD spectra showed no significant change in the secondary structure of BSA. The antibacterial study revealed that IL showed significant activity against *Staphylococcus aureus* (gram-positive) bacteria and no effect was observed on *E. coli* (gram-negative) bacteria. Cytotoxicity study on Vero cell line (non-cancerous cell line; kidney epithelium cells of African green monkey) showed IC₅₀ values 207.95 ± 1.25 μ M. Cytotoxicity analysis of [Pro-pip]SAL IL on A549 cell lines revealed antiproliferative properties with an IC₅₀ value of 54.55 ± 0.22 μ M. This study interpreted that Pro-pip]SAL IL could be used as an anticancer and antibacterial drug, warranting further exploration and development in these therapeutic areas.

Keywords A549 cell · Antibacterial · Biophysical study · BSA · Ionic liquid

Introduction

India's pharmaceutical sector is a vital contributor to its economy, accounting for approximately 1.72% of its GDP. With over 500 different active pharmaceutical ingredients (APIs) manufactured locally, it is the third-largest market globally, holding an impressive 8% share in the API industry.

Impressively, it contributes 57% of APIs to the prequalified list of the WHO, further cementing its position as a significant player in the pharmaceutical sector (English Annual Report 2020–21.pdf (pharmaceuticals.gov.in)). An API is a biologically active component of a medication that produces its intended therapeutic effect, which can be synthesized chemically or derived from natural sources such as plants or animals. They are the molecules that interact with specific targets in the body to produce the desired pharmacological activity [1–5]. Pharmaceutically active compounds in solid form present several challenges that can impact their effectiveness. Crystalline forms often exhibit low bioavailability due to poor solubility, while amorphous compounds may face issues related to handling and manufacturing, including polymorphic transitions and spontaneous crystallization. These factors can hinder the performance and stability of traditional API formulations [6–8]. To mitigate the limitations of conventional APIs, extensive research has focused on exploring alternative delivery mechanisms. These include

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strategies such as adsorption onto high surface area carriers, crystal engineering, micellar formulations, nano-suspensions, prodrug strategies, solid dispersions, and liquid salt formation. Notably, the conversion of APIs into liquid salts or the use of ionic liquid as an ionizing agent has demonstrated considerable potential in enhancing bioavailability and resolving challenges associated with polymorphic transformations [9–11].

A novel API-based ionic liquid salt formation approach is widely considered a viable solution to enhance their therapeutic efficacy. API-based ionic liquid refers to using ionic liquids in pharmaceutical applications, where the ionic liquid serves as API [12–14]. Ionic liquids (ILs) are a class of salts that exhibit liquid-like properties at or near ambient temperatures and are solely composed of ions. Ionic liquids have been investigated as potential drug candidates due to their unique properties, such as their ability to dissolve a wide range of compounds and low toxicity [15–19]. They have been explored for their potential in drug delivery, where they can be employed to enhance the solubility and bioavailability of poorly soluble drugs [20, 21]. In recent years, research has been focused on the development of ionic liquids based on APIs such as ibuprofenate [8], docusate [22], ampicillin [23], procaine [24, 25], lidocaine [26–29], dacarabazine [30, 31], indomethacin, mebendazole [32], salicylate [29, 33], levofloxacin [34], and many more. This captured the interest of researcher to develop more suitable drugs with manifold therapeutic effects.

In this research, an innovative active pharmaceutical ingredients-based ionic liquid drug is synthesized which will offer multiple-function and synergistic effects in one drug. This approach can minimize the use of unnecessary chemicals by reducing the number of excipients and other ingredients added to the drug to maintain its solid form for delivery. So, an API-based IL synthesized by combining 1,3-benzodioxole moiety (central moiety of many natural APIs having anticancer properties) [25, 28, 31, 35–39], procaine (local anesthetic, DNA methylation inhibitor) [24, 40–42], and salicylate salt (NSAID, nonsteroidal anti-inflammatory drug) [29, 31, 43]. The synthesized ionic liquid ([Pro-pip]SAL) was examined using NMR and IR spectroscopy to confirm structural integrity. Further, the interaction of [Pro-pip]SAL with BSA was investigated by computational studies (molecular docking, molecular dynamic simulation), and spectroscopic techniques (UV–visible, fluorescence, circular dichroism spectroscopy). Antibacterial studies of synthesized ionic liquid studied on *Staphylococcus aureus* (gram-positive) (MSSA 29213) and *E. coli* (gram-negative) bacteria strains and cytotoxicity assay studied on a non-cancerous cell line, Vero cell line, kidney epithelium cells of African green monkey.

Experimental

Materials

BSA (bovine serum albumin) was purchased from Merck and stored at 4 °C. Procaine, piperonyl alcohol, sodium salicylate, phosphorous tribromide, phosphate buffer tablets, analytical grade solvents, and reagents were procured from TCI and Thomas Baker.

All the spectroscopic studies were performed using stock solutions of BSA (1 and 5 μ M) prepared in phosphate buffer (10 mM, pH 7.4).

Synthesis of [Pro-pip]SAL

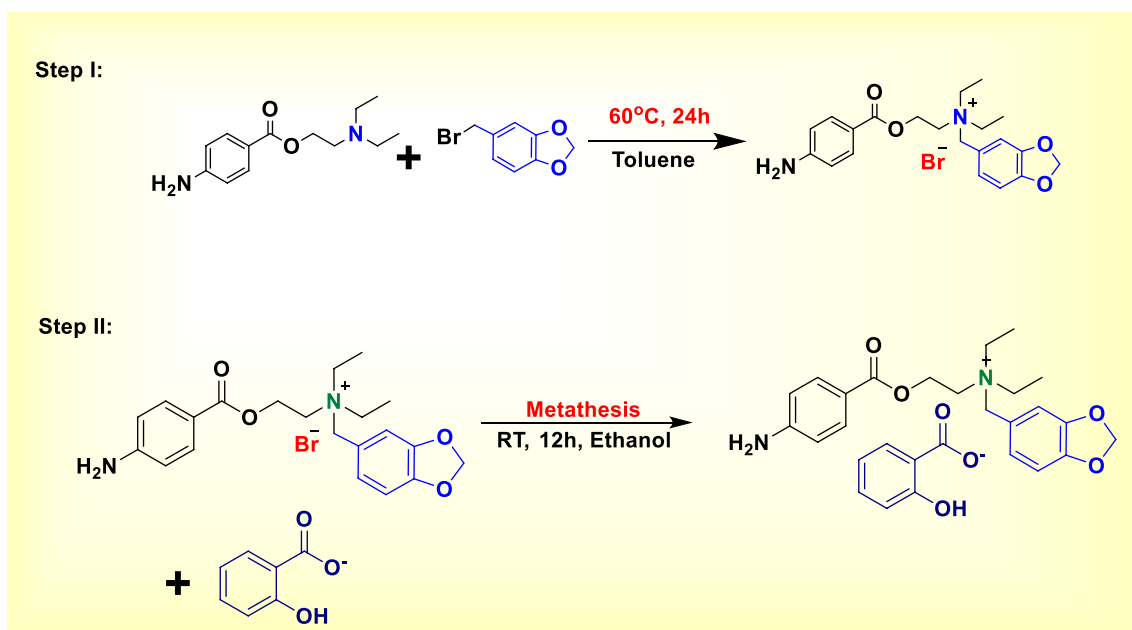
Piperonyl bromide was synthesized by reaction of equimolar ratio of piperonyl alcohol with PBr_3 in a round bottom flask containing dry diethyl ether. [Pro-pip]Br ionic liquid was synthesized using a synthetic route as per our previous research by doing a quaternization reaction of procaine with piperonyl bromide (detained synthesis procedure in SI). Further, [Pro-pip]SAL (2-((4-aminobenzoyloxy)-N-(benzo[d][1,3]dioxol-5-ylmethyl)-N,N-diethylethan-1-aminium 2-hydroxybenzoate) was synthesized by metathesis reaction of [Pro-pip]Br IL with salicylate salt in equimolar ratio in ethanol. After reaction completion, ethanol was evaporated followed by desolvation of residue in dichloromethane and filtration to remove byproduct. In the last step, dichloromethane evaporated to get the desired ionic liquid [25, 28] (Scheme 1).

Characterization

1H & ^{13}C NMR spectroscopy studies for [Pro-pip]SAL ionic liquid performed on Jeol JNM-ECZ 400S NMR spectrophotometer, FTIR studies performed on Bruker Alpha-II FTIR spectrophotometer and HRMS studies performed on Agilent G6530AA HRMS.

Characterization data

1H -NMR (400 MHz, DMSO- d_6) δ : 7.72–7.63 (m, 4H), 6.88–6.55 (m, 7H), 6.09 (s, 2H), 5.74 (s, 2H), 3.58 (s, 2H), 3.45–3.41 (m, 2H), 3.21–3.15 (m, 2H), 2.51–2.47 (m, 4H), 1.22 (td, $J=7.2, 2.7$ Hz, 6H); **^{13}C -NMR (100 MHz, DMSO- d_6) δ :** 8.6, 46.13, 49.5, 53.28, 54.95, 100.85, 108.1, 112.42, 112.65, 112.72, 116.12, 117.01, 120.3, 120.86, 127.27, 130.12, 131.41, 132.61, 147.34, 147.32, 148.84, 153.89, 162.14, 172.18; **FTIR (cm^{-1}):** 3311, 3208, 3055, 2983, 1701, 1599, 1448, 1443, 1263, 1173, 1101, 1038, 927, 845, 806, 731, 702; **HRMS m/z :** 371.1973. The ionic liquid SAL



Scheme 1. Synthesis route of [Pro-pip]SAL IL

IL was synthesized multiple times and thoroughly characterized using NMR, FTIR, and HRMS techniques. These analyses confirmed the high purity of the ionic liquid, and no impurities were detected in the sample (Figure S1–S2).

Methods

In silico study

Bovine serum albumin protein 3D crystal structure (PDB ID: 4f5s) was saved from PDB (<https://www.rcsb.org/>) with a resolution of 2.70 Å and prepared using Biovia Discovery Studio Visualizer [44–46]. In preparation for receptor protein water molecules, heteroatom was removed and polar hydrogens were added followed by the addition of Kollman charges for charge neutralization. The receptor files were saved in.pdb format. The three-dimensional structure of the protein was validated through the Ramachandran plot which was obtained from the PROCHECK server. 3D-structure of ligands (procaine and [Pro-pip]SAL) prepared in ChemDraw and energy optimization done in ChemDraw 3D using MM94 force field and saved as.mol2 format [47–49]. Molecular docking of BSA with ligands was performed using Hex 8.0.0 CUDA server using a fast Fourier algorithm [50–52]. The molecular docking results were further confirmed by using AutoDock Vina [53]. The grid box size is crucial for identifying the area involved in

active site docking. The grid box dimension in AutoDock was set to center ($x=34.888$, $y=23.991$, $z=98.766$) with a size of $40 \times 40 \times 40$ and with exhaustiveness of 8. Pharmacological and pharmacokinetic properties of ionic liquids studies using pkCSM online tool [54–57].

Spectroscopic methods

UV–visible and fluorescence spectroscopy

To understand the binding interaction of [Pro-pip]SAL with bovine serum albumin UV–visible absorption, spectra were recorded on Perkin Elmer Lambda 365(+) spectrophotometer at 298 K using quartz cuvettes of 1 cm path length in the 200–700 nm wavelength region. The fluorescence spectra were recorded on a Perkin Elmer FL 8500 fluorescence spectrometer. The absorption and emission spectra of BSA were recorded by varying concentrations of ionic liquid from 0 to 100 μM at pH 7.4. The instrument parameters were as follows: excitation wavelength of 280 nm, fluorescence emission wavelength range of 290–500 nm, slit width of 5 nm, excitation potential of 350 V, and scan rate of 240 nm/min.

It is important to remove the internal filter effect (IFE) as it may result in a decrease in the amount of light that reaches the center of the solution or the detector if there is strong absorbance of the quencher (ILs) near the excitation/emission wavelength region of the fluorophore (BSA). It can lead to a

situation where the emitted light may not be able to reach the detector fully. Equation (1) was used to correct the fluorescence data to nullify IFE.

$$F_{\text{corrected}} = F_{\text{recorded}} \times 10^{[(\lambda_{\text{ex}} + \lambda_{\text{em}})/2]}$$

F_{recorded} and $F_{\text{corrected}}$ = Recorded and corrected fluorescence intensity of BSA

λ_{ex} and λ_{em} = Absorbances at excitation and emission wavelengths

(1)

Circular dichroism spectroscopy

To get an insight into conformational changes in the 2° structure of BSA on interaction with [Pro-pip]SAL far-UV circular dichroism spectra were recorded. The CD titration was carried out by keeping the fixed concentration of BSA at 1 μM and varying the concentration of [Pro-pip]SAL from 0 to 3 μM using a cuvette of 1 cm path length at 298 K, pH 7.4 and under a nitrogen environment on JASCO-CD polarimeter (J-815). The CD spectra were observed in range (200–260 nm) with 100 nm/min scan speed and of 1 s response time.

Antibacterial activity study

Spot assay: The antimicrobial susceptibility assay evaluated the antimicrobial potential of synthesized [Pro-pip]SAL IL and procaine (control) in *Staphylococcus aureus* (gram-positive) (MSSA 29213) and *E. coli* (gram-negative) bacteria strains. Both bacteria strains were cultured overnight in Luria Bertani and muller Hinton broth and agar plates and spread in equal quantities on respective agar plates. Fifty-millimeter concentration of [Pro-pip]SAL and procaine each were added to respective wells in agar plates to assess their antimicrobial potential against tested strains. Ampicillin, an antibiotic drug, and dimethyl sulfoxide (DMSO) were used as positive control and solvent respectively for the compounds. Absolute DMSO was used as a control to normalize the effect of DMSO on the compound activity. The agar plates were incubated at 310 K and then the antimicrobial activity of [Pro-pip]SAL IL and procaine (control) was measured in the form of the zone of inhibition.

Culture of animal cell line

Vero cells (normal, kidney epithelium cells of African green monkey) were cultured in Dulbecco's Modified Eagle Medium accompanied with fetal bovine serum (10%) and penicillin–streptomycin with a continuous supply of 5% CO_2 at 310 K in a CO_2 incubator.

Vero cell cytotoxicity assay

Cell viability of Vero cell was inspected using (3-(4,5-dimethylthiazole-2-yl)–2,5-biphenyl tetrazolium bromide (MTT) assay. After the culture reached sufficient

confluency, cells were harvested with 0.25% trypsin–EDTA. Cell counting was done with a hemocytometer. In 96 well plates, 1×10^4 cells/well were seeded and incubated for 24 h in a CO_2 incubator for cell attachment and growth. After 24 h, [Pro-pip]SAL IL and procaine (control) with varying concentrations (starting from 1000 μM as the highest concentration) were introduced to the culture plates and incubated for 48 h. The viability of the treated cell culture with [Pro-pip]SAL IL and procaine (control) was assessed after 48 h of incubation. The wells were gently washed with 1X phosphate buffer saline (PBS) to remove the [Pro-pip]SAL IL and procaine (control) and 20 μl MTT salt solution (5 mg/mL in 1X PBS) and incubated at 310.15 K. After 3 h of incubation, DMSO was used to solubilize the formazan crystal and incubated for 30 min. The 96 well plates were then observed for absorbance in the Synergy H1 microplate reader at 570 nm (Figure S14). IC_{50} values were calculated for [Pro-pip]SAL IL and procaine (control).

Culture of human cell line

For the anti-cancer study, Dulbecco's Modified Eagle Medium was used to culture A549 cells (human lung adenocarcinoma cells). The medium was supplemented with fetal bovine serum (10%) and penicillin–streptomycin with a continuous supply of 5% CO_2 at 310.15 K.

A549 cell viability assay

Cell viability was investigated with an MTT assay. After the culture reached sufficient confluency, cells were harvested with 0.25% trypsin–EDTA. Cell counting was done with a hemocytometer and in 96 well plates 1×10^4 cells/well were seeded and incubated for 24 h in a CO_2 incubator for cell attachment and growth. After 24 h, test compounds ([Pro-pip]SAL IL and procaine) with varying concentrations (starting from 1000 μM as the highest concentration) were added to the culture plate and incubated for 48 h. The viability of the treated cell culture with test compounds was assessed after 48 h of incubation. The wells were gently washed with 1X phosphate buffer saline (PBS) to remove the test compounds and added MTT salt solution (5 mg/mL in 1X PBS) was then incubated at 310.15 K. After 3 h of incubation, DMSO was used to solubilize the formazan crystal and incubated for 30 min. The 96 well plates were then read for absorbance with a microplate reader at 492 nm. IC_{50} values were calculated for [Pro-pip]SAL IL and procaine.

Result and discussion

In silico study: molecular docking investigation

To get a comprehensive understanding of the interaction between a protein and a ligand, it is crucial to visualize

the most probable binding sites and the various types of interactions that occur. Molecular docking has emerged as the preeminent method to investigate these binding interactions. These cutting-edge techniques provide an in-depth understanding of the molecular-level interactions between the protein (fluorophore) and the ligand (quencher) and confirmation of experimental results. BSA (protein), [Pro-pip]SAL (ligand), and control prepared in Biovia Discovery Studio, and Ramachandran plot of BSA (4f5s) revealed that 91.9% residues were present in the most favored region

and respectively (Figure S3). Molecular docking was performed in the HEX 8.0.0 CUDA server (Figures S4–S8) and confirmed by AutoDock Vina with docking of cation and anion separately. Docking results revealed that the binding affinity of ionic liquid was better compared to the control molecule (procaine) (Table 1). Binding interactions of the protein with ligands were further analyzed in the Biovia Discovery Studio visualizer and active sites of protein bonded with ligands were shown in Fig. 1, Table 2 and with control molecule (procaine) shown in Figure S9–S12 (Supporting information).

Hydrogen bonds were observed in the visualization of the binding interaction between the cationic and anionic parts of ionic liquid with BSA (protein). These H-bonds were assumed as polar interactions between protein and ligand. Molecular properties and ADMET properties for

Table 1 Binding affinity of procaine(control) and [Pro-pip]SAL IL

Compounds	HEX (kcal/mol)	AutoDock Vina (kcal/mol)
Procaine (control)	−240.21	−6.7
[Pro-pip]SAL IL	−328.08	−8.0cat/−6.0an

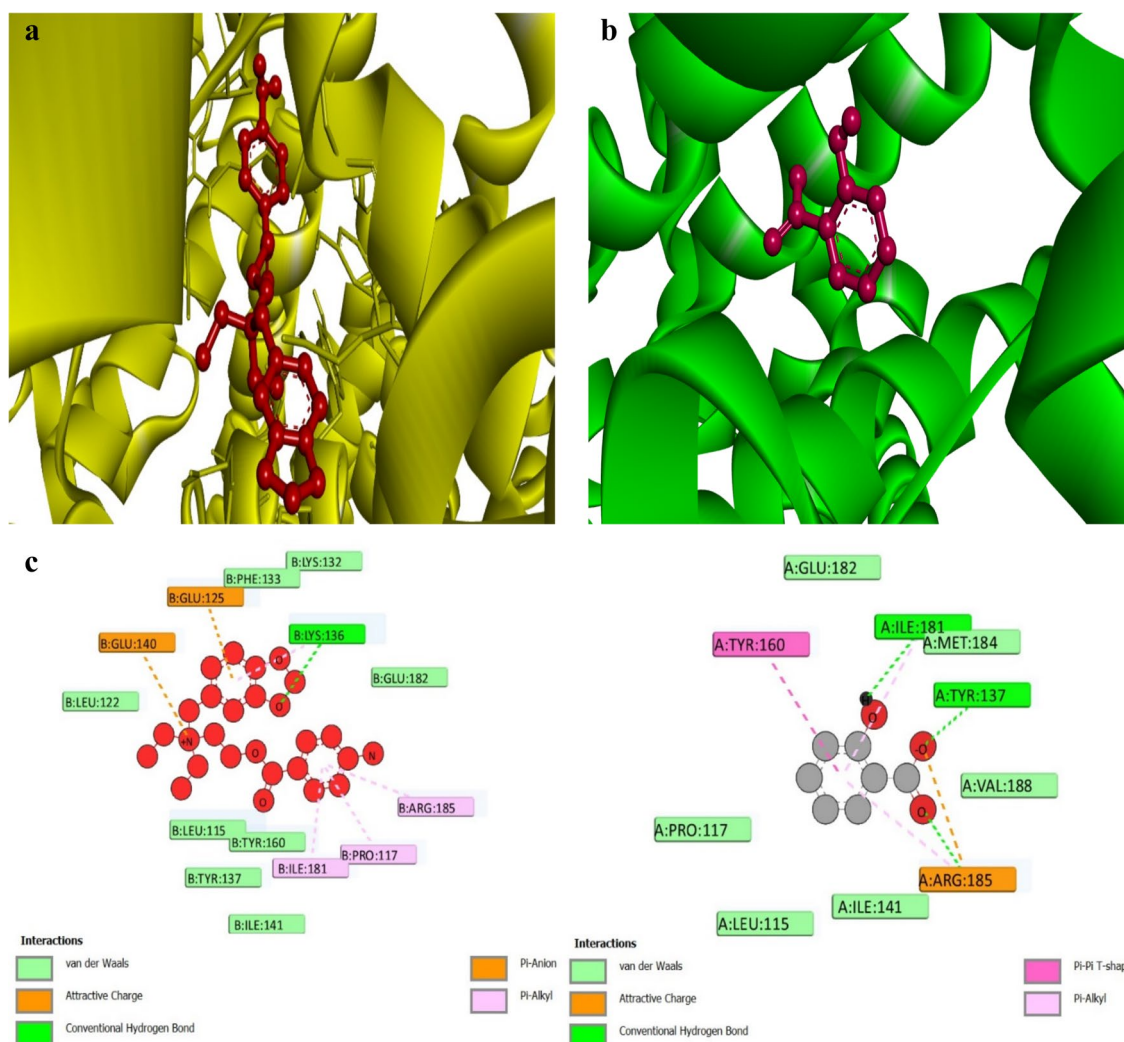
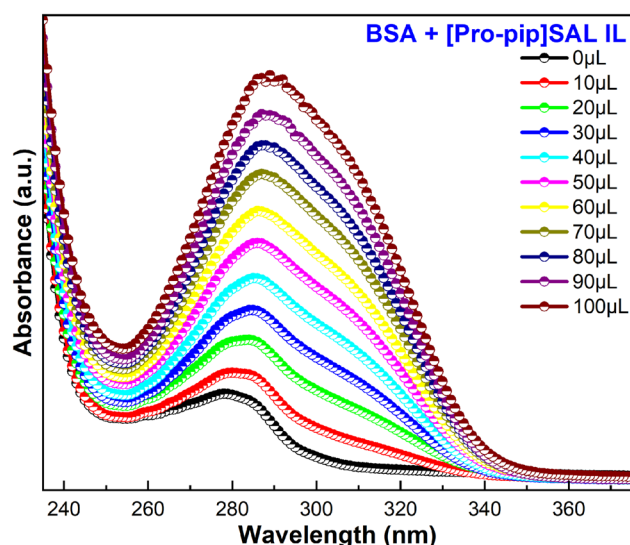


Fig. 1 **a** Interaction between BSA with cationic part of [pro-pip]SAL IL. **b** Interaction between BSA with anionic part of [pro-pip]SAL IL. **c** 2D-Interaction between amino acid residues of BSA with cationic and anionic part of [pro-pip]SAL IL

Table 2 Binding interaction of amino acid residues of BSA with [Pro-pip]SAL IL

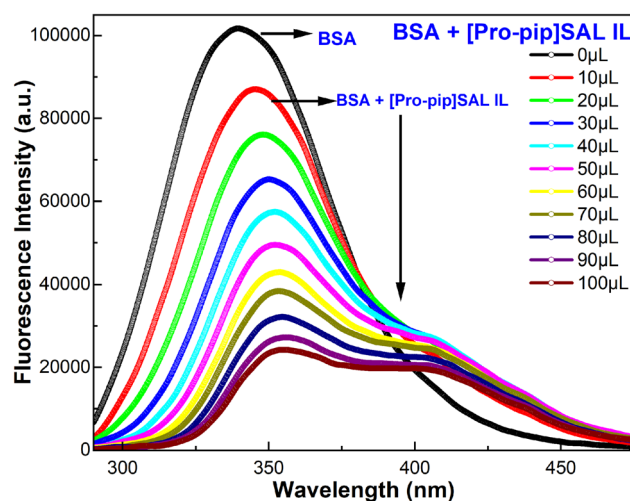
Binding interaction	Amino acid residues of BSA showing binding interaction with [Pro-pip]SAL IL
Van der Waals	PHE133, LYS132, LEU122, LEU115, TYR160, TYR137, ILE141, GLU182, PRO117, VAL188, MET184
Attractive charge	GLU125, ARG185
Conventional hydrogen bond	LYS136; ILE181, TYR137
Pi-anion	GLU140
Pi-alkyl	ILE181, PRO117, ARG185
Pi-pi T-shaped	TYR160

**Fig. 2** UV–visible absorption spectra of BSA with different molar concentrations of [Pro-pip]SAL at 298 K

[Pro-pip]SAL IL and control molecules are presented in Tables S1–S2.

UV–visible and fluorescence spectroscopy

UV–visible spectroscopy is one of the adequate procedures that is used to analyze the protein–ligand interaction and to explore changes in the structure of protein. BSA (bovine serum albumin) exhibited absorption peaks in two wavelength regions 200–240 nm and 260–300 nm corresponding to $n-\pi^*$ transition in polypeptide backbone framework and $\pi-\pi^*$ transition in aromatic amino acid residues: Trp, Tyr, Phe, respectively. UV–visible absorption study of BSA showed BSA exhibited a peak at 278 nm due to $\pi-\pi^*$ transition. To understand the interaction of [Pro-pip]SAL ionic liquid with BSA absorption spectra of BSA recorded with different molar concentrations of [Pro-pip]SAL ionic liquid. Due to the successive addition of IL to BSA, a change in absorbance intensity was observed suggesting perturbation in the secondary structure of protein. An increase in

**Fig. 3** Fluorescence emission spectra of BSA with different molar concentrations of [Pro-pip]SAL at 298 K

the concentration of [Pro-pip]SAL ionic liquid increases the absorption intensity of BSA along with the bathochromic shift of 11 nm (278 to 289 nm) (Fig. 2). A change in absorbance near 280 nm suggested a change in aromatic amino acid residues vicinity in the presence of ionic liquid.

The fluorescence intensity exhibited by a fluorophore is significantly influenced by the conditions in its immediate environment. As a result, fluorescence spectroscopy is an efficient method for exploring the molecular environment that surrounds chromophore molecules. By utilizing this technique, an insight was attained about the chemical and physical properties of fluorophore molecules and the interactions that occur between fluorophore (BSA) and ligand ([Pro-pip]SAL) and their surrounding environment. The fluorescence emission maximum for BSA was observed at 340 nm for Trp amino acid residues. With the increased concentration of [Pro-pip]SAL ionic liquids in BSA the emission intensity gradually decreased with a bathochromic shift of 15 nm (340 to 355 nm) (Fig. 3). Both UV–visible and fluorescence spectra studies disclosed that the surrounding microenvironment around the fluorophore (BSA) changed due to the binding of the drug ([Pro-pip]SAL)

with the active residue of BSA and the bathochromic shift showed that the polarity around the tryptophan amino acid residues had increased leading to increased hydrophilicity.

Fluorescence quenching mechanism

Fluorescence quenching is a well-known method that effectively reduces the fluorescence intensity of a sample. Through various molecular interactions with excited state fluorophores, such as collision quenching, energy transfer, ground-state complex formation, and molecular rearrangement. Fluorescence quenching is governed by two distinct mechanisms, namely dynamic and static quenching. In dynamic quenching mode, the fluorophore (protein) and quencher molecules (drug) collide resulting in the transfer

Table 3 Stern–Volmer plot parameters

Ionic liquid	K_{sv} (Lmol ⁻¹)	K_q (Lmol ⁻¹ s ⁻¹)	SD	R^2
[Pro-pip]SAL	3.14×10^4	3.14×10^{12}	1.06	0.95259

of energy from the excited state of the fluorophore to the quencher. On the other hand, static quenching involves the formation of a ground state complex between the fluorophore (protein) and quencher (drug). The ground state of complex is non-fluorescent and, therefore, reduces the overall fluorescence intensity. The quenching constant value obtained from calculations based on the Stern–Volmer plot was used to find out the mode of quenching. Using the Stern–Volmer equation (Eq. 2) K_{sv} and K_q were determined.

$$\frac{F_0}{F} = 1 + K_{sv}[Q] = 1 + kq\tau_0[Q]$$

F_0 and F = Fluorescence intensity in absence and presence of quencher respectively

K_{sv} = Stern volmer dynamic quenching constant

K_q = Biomolecular quenching constant

$[Q]$ and τ_0 = Free concentration of Drug and lifetime of protein respectively

(2)

For BSA value of τ_0 is $\sim 10^8$ s⁻¹ (lifetime of BSA in excited state). Stern–Volmer plot derived between F_0/F Vs $[Q]$ using Eq. 2, where slope gave a value of K_{sv} and K_q at 298 K (Fig. 4, Table 3). The value of K_q calculated, i.e., $K_q = 3.14 \times 10^{12}$ M⁻¹s⁻¹, from the graph was larger than the maximum diffusion rate of BSA ($\sim 2 \times 10^{10}$ M⁻¹s⁻¹) which confirmed the complex formation between BSA and [Pro-pip]SAL by static quenching mechanism.

Binding and thermodynamic parameters

To get an insight of complex formation in the ground state between fluorophore (BSA) and quencher ([Pro-pip]SAL), Eq. 3 was used and the number of binding sites was determined from the slope and binding constant determined from the intercept of the plot $\log((F_0-F)/F_0)$ Vs $\log[Q]$ (Fig. 5).

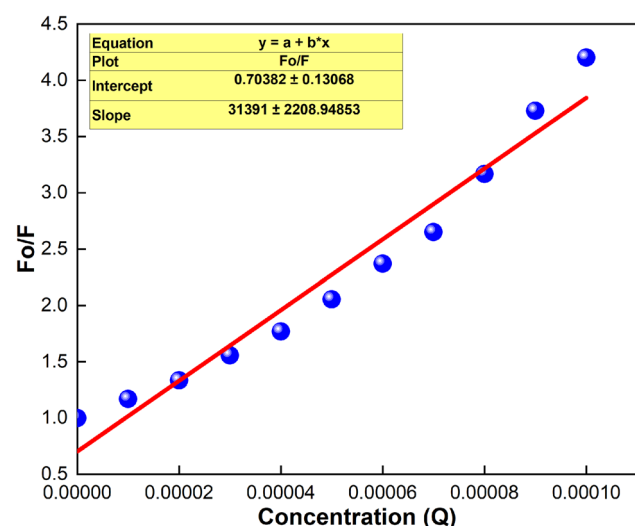


Fig. 4 Stern–Volmer plot for BSA Vs different molar concentrations of [Pro-pip]SAL at 298 K

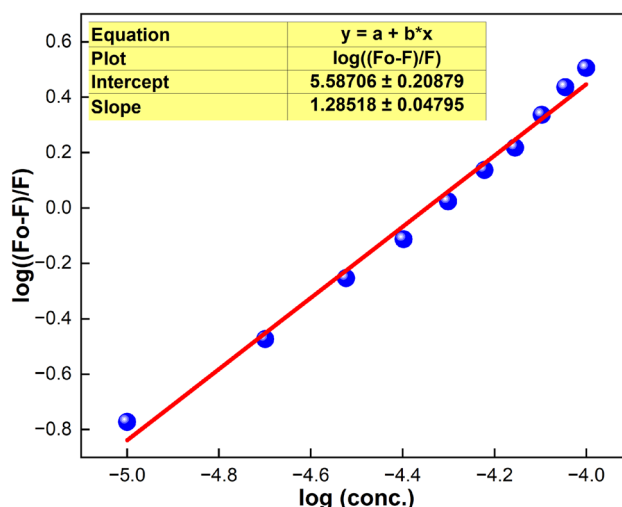


Fig. 5 Modified Stern–Volmer plot for BSA Vs different molar concentrations of [Pro-pip]SAL at 298 K

$$\log \frac{F_0 - F}{F_0} = \log Kb + n \log [Q]$$

Kb = Biomolecular binding constant (3)

n = number of binding sites

The number of binding sites was ~ 1 that means BSA and [Pro-pip]SAL bonded in a 1:1 ratio, and the K_b value was $3.86 \times 10^5 \text{ Lmol}^{-1}$ which lies in the range $10^5 - 10^8 \text{ Lmol}^{-1}$ suggesting that the binding strong interaction occurred in protein-drug complex (Table 4). Further spontaneity of complex formation was checked using Eq. 4 and ΔG° (Gibbs free energy) of protein-drug complex formation calculated. Negative Gibbs free energy value (-31.87 kJ/mol) employed that complex formation was spontaneous process.

$$\Delta G^\circ = -RT \ln Kb \quad (4)$$

Circular dichroism spectroscopy

CD spectroscopy is used for the quantitative study to learn about conformational changes that happened in the secondary structure of protein because of ligands. Here, CD spectra of BSA ($1 \mu\text{M}$) were recorded with different concentrations of [Pro-pip]SAL IL (Fig. 6). Two negative peaks were observed at 208 and 222 nm for $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition, respectively. Minor changes in spectra on addition of IL depicted slight changes in the microenvironment of BSA. When checked with BetSel online server (BeStSel-Protein Circular Dichroism Spectra Analysis (elte.hu)) no change was observed in the α -helical percentage and secondary structure of BSA when IL concentration was thrice the concentration of BSA. This depicted that the structure of BSA is stabilized and does not undergo structural changes in the presence of a high concentration of [Pro-pip]SAL IL.

Antibacterial activity

The antimicrobial effects of tested compounds [Pro-pip]SAL IL and procaine (control) were assessed against *Staphylococcus aureus* (gram-positive) (MSSA 29213) and *E. coli* (gram-negative) bacteria by spot analysis (Figure S13). From zone of inhibition (ZOI) interpretation as compared with ampicillin (control), it was observed that [Pro-pip]SAL IL showed strong antibacterial activity

Table 4 Modified Stern–Volmer plot parameters

Ionic liquid	K_b (Lmol^{-1})	n	SD	R^2	ΔG (KJ/mol)
[Pro-pip]SAL	3.86×10^5	1.28 ± 0.04	0.41141	0.98761	-31.8732

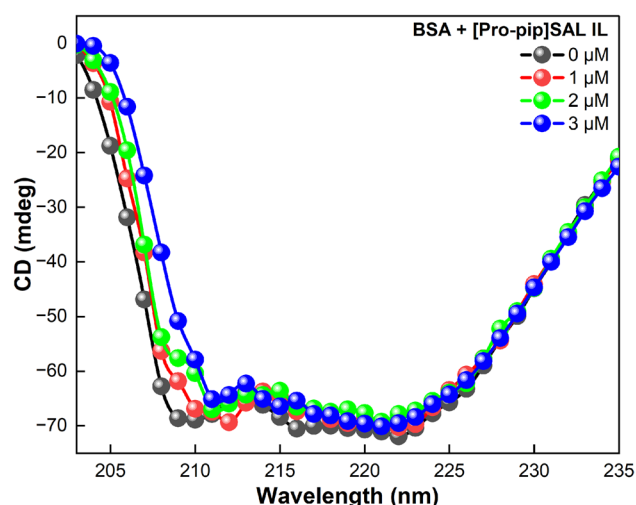


Fig. 6 CD spectra of BSA at different molar concentrations of [Pro-pip]SAL IL

against gram-positive bacteria at different concentrations. However, there was no effect on gram-negative bacteria and procaine (control) did not exhibit any antibacterial activity (Fig. 7).

Compound	Zone of inhibition
Ampicillin	22
[Pro-pip]SAL	16.5
Procaine	*

*No zone of inhibition observed

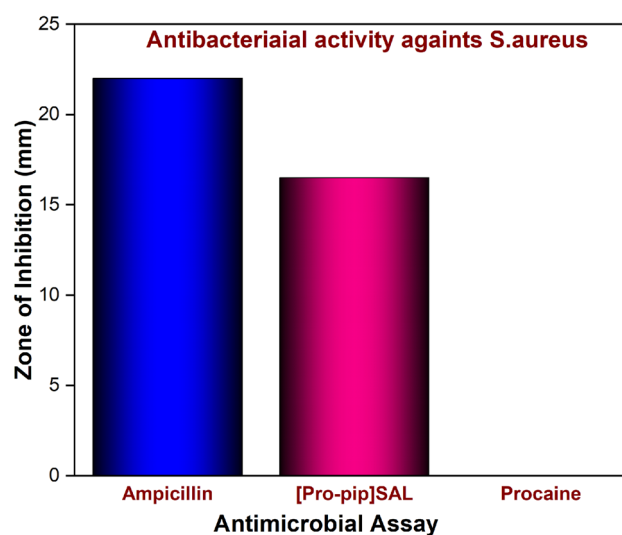


Fig. 7 Zone of inhibition study for [Pro-pip]SAL IL and procaine (control) in comparison with Ampicillin against *S. aureus*

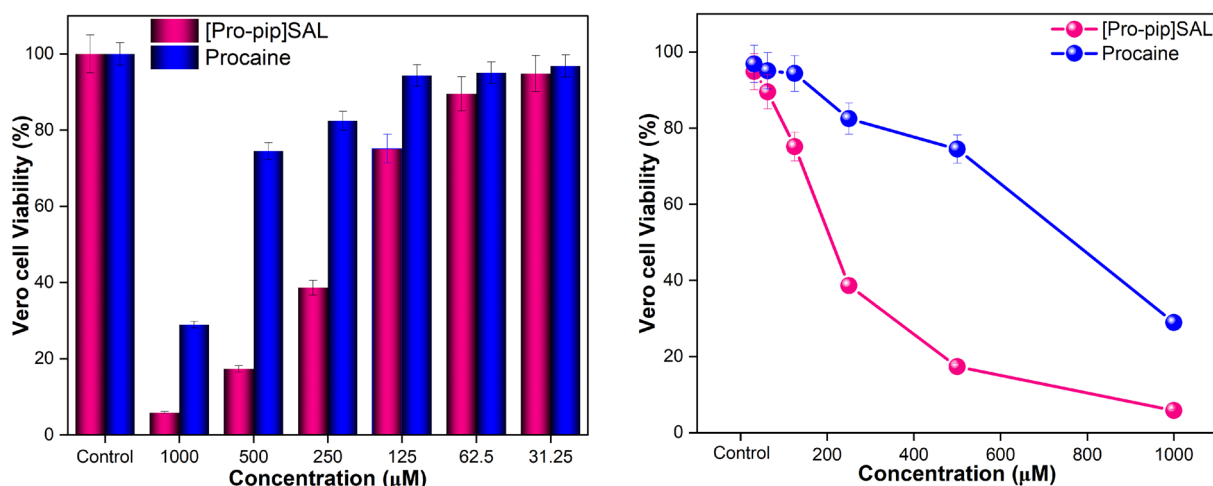


Fig. 8 Cytotoxicity analysis of Pro-pip]SAL IL and procaine (control) on Vero cell line

Vero cell cytotoxicity assay

The cytotoxicity evaluation against the Vero cell line for Pro-pip]SAL IL and procaine (control) was shown in Fig. 8. The starting experimental concentrations of all the test compounds were 1000 μM. [Pro-pip]SAL IL and procaine (control) possess cytotoxicity activity against kidney epithelium (Vero) cells. [Pro-pip]SAL IL and procaine (control) revealed the cytotoxic activity with IC_{50} values 207.95 ± 1.25 μM and 921.1 ± 0.2 μM, respectively. [Pro-pip]SAL IL showed lower IC_{50} values compared with procaine (control).

A549 cell cytotoxicity assay

The cytotoxicity evaluation against the A549 lung cancer cell line for Pro-pip]SAL IL and procaine (control) was

shown in Fig. 9. The starting experimental concentrations of all the test compounds were 1000 μM. [Pro-pip]SAL IL and procaine (control) showed anti-proliferative activity against the human lung adenocarcinoma cells. [Pro-pip]SAL IL displayed a better anticancer potential with IC_{50} values 54.55 ± 0.22 μM than procaine which exhibited IC_{50} 75.485 ± 0.205 μM respectively which is better than reported data by S. Sewariya et al. on cytotoxicity of noscapine based ionic liquid (consisting of 1,3-benzodioxole motif) [58]. In literature, procaine was studied for anticancer studies for lung cancer cell line [59, 60]. Thus, [Pro-pip]SAL IL consisting of 1,3-benzodioxole motif, procaine, and pharmaceutically active salicylate anion showed better antiproliferative activity than reported literature data and can be further used as therapeutic for cancer treatment.

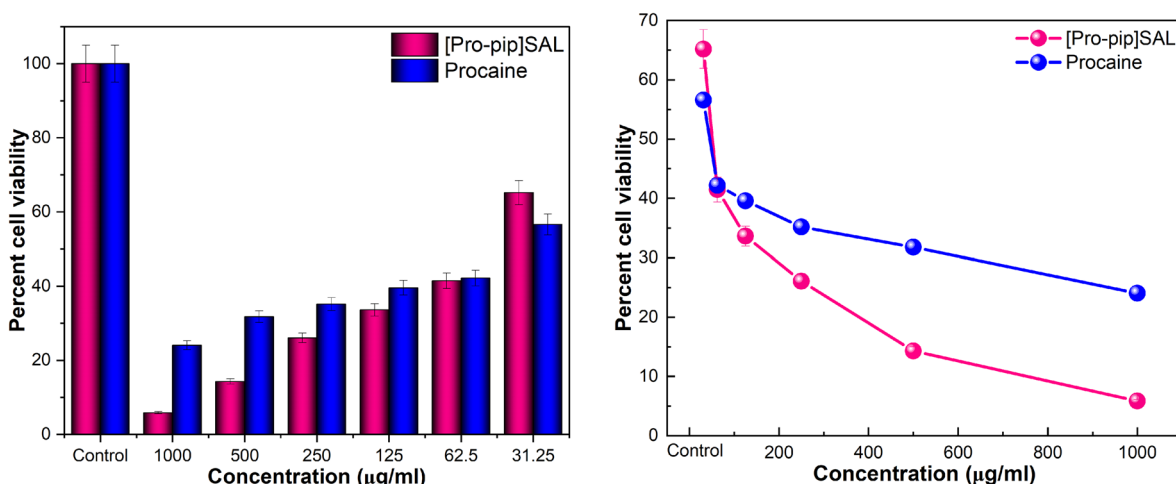


Fig. 9 Cytotoxicity analysis of Pro-pip]SAL IL and procaine (control) on A549 lung cancer cell line

Conclusion and futuristic aspects

In conclusion, the synthesized ionic liquid [Pro-pip]SAL exhibits promising potential in pharmaceutical development, particularly due to its dual antibacterial and anticancer activities. The molecular docking studies revealed that [Pro-pip]SAL interacts with bovine serum albumin (BSA) through polar interactions and hydrogen bonding, which was further confirmed by UV–visible and fluorescence spectroscopic analyses. These results suggest that [Pro-pip]SAL induces a polar environment around BSA, without significantly altering its secondary structure, as indicated by circular dichroism (CD) spectra. The antibacterial activity of [Pro-pip]SAL against *Staphylococcus aureus* (gram-positive) bacteria, with a zone of inhibition (ZOI) of 16.5 mm, demonstrates its potential for developing new antimicrobial agents while showing no effect on *Escherichia coli* (gram-negative) when compared with Ampicillin. Cytotoxicity study of [Pro-pip]SAL IL on Vero cell line showed IC_{50} values 207.95 ± 1.25 μ M. Additionally, its significant antiproliferative activity against A549 lung cancer cells, with an IC_{50} value of 54.55 ± 0.22 μ M, underscores its potential as an anticancer agent. The unique dual therapeutic properties of [Pro-pip]SAL make it a highly promising candidate for the development of innovative treatments aimed at combating both bacterial infections and cancer. Its remarkable ability to address these two serious health concerns suggests that it could play a significant role in creating effective medical therapies that target multiple aspects of disease simultaneously.

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Author contribution Sangeeta: Conceptualization, Formal analysis, Investigation, Methodology; Anjana Sarkar: Conceptualization, Formal analysis, Data curation, Validation, Supervision; Neetu Kumari: Biological Study, Methodology, Investigation; Mulaka Maruthi: Conceptualization of biological data; Ravi Tomar: Conceptualization, Formal analysis, Data curation, Validation, Visualization, Supervision.

Data availability Supplementary data include characterization data, ADMET study data, molecular docking with Hex.8.0, antibacterial study and Vero cell cytotoxicity study data.

Declarations

Competing interest The authors declare no competing interests.

References

1. Lazar MS (2008) Active pharmaceutical ingredients. In: Berry IR, Martin RP (eds) *The Pharmaceutical Regulatory Process*, 2nd ed. Informa healthcare, New York, pp 255–268. <https://doi.org/10.3109/9781420070439>
2. Tabassum R, Kawsar SMA, Alam A et al (2024) Synthesis, spectral characterization, biological, FMO, MEP, molecular docking, and molecular dynamics simulation studies of cytidine derivatives as antimicrobial and anticancer agents. *Chem Phys Impact* 9:100724. <https://doi.org/10.1016/j.chphi.2024.100724>
3. Munia NS, Alanazi MM, El Bakri Y et al (2023) Uridine derivatives: synthesis, biological evaluation, and in silico studies as antimicrobial and anticancer agents. *Med* 59:1–26. <https://doi.org/10.3390/medicina59061107>
4. Ferdous J, Abul F, Ali F et al (2024) FTIR, 1H -/ ^{13}C -NMR spectral characterization, antimicrobial, anticancer, antioxidant, anti-inflammatory, PASS, SAR, and in silico properties of methyl α -D-glucopyranoside derivatives. *Chem Phys Impact* 9:100753. <https://doi.org/10.1016/j.chphi.2024.100753>
5. Ahmmed F, Al-Mijalli SH, Abdallah EM et al (2023) Galactoside-based molecule enhanced antimicrobial activity through acyl moiety incorporation: synthesis and in silico exploration for therapeutic target. *Pharmaceuticals* 16:998. <https://doi.org/10.3390/ph16070998>
6. Bica K, Rodríguez H, Gurau G et al (2012) Pharmaceutically active ionic liquids with solids handling, enhanced thermal stability, and fast release. *Chem Commun* 48:5422–5424. <https://doi.org/10.1039/c2cc30959a>
7. Handa M, Almalki WH, Shukla R et al (2022) Active pharmaceutical ingredients (APIs) in ionic liquids: an effective approach for API physicochemical parameter optimization. *Drug Discov Today* 27:2415–2424. <https://doi.org/10.1016/j.drudis.2022.06.003>
8. Rapaic M, Panić J, Teofilović B et al (2022) Synthesis and evolution of physicochemical properties of new pharmaceutically active ionic liquids-tetracainium salicylate and tetracainium ibuprofenate. *RSC Adv* 12:26800–26807. <https://doi.org/10.1039/d2ra04711j>
9. Cojocaru OA, Shamshina JL, Rogers RD, Rogers RD (2013) Review / Preview : Prodrug ionic liquids combining the prodrug and ionic liquid strategies to active pharmaceutical ingredients. *Chim oggi-Chemistry Today* 31:24–28
10. Wu X, Zhu Q, Chen Z et al (2021) Ionic liquids as a useful tool for tailoring active pharmaceutical ingredients. *J Control Release* 338:268–283. <https://doi.org/10.1016/j.jconrel.2021.08.032>
11. Thakor KP, Lunagariya MV, Bhatt BS, Patel MN (2019) Bipyrazole-based palladium(II) complexes as DNA intercalator and artificial metallonuclease. *Monatshefte für Chemie-Chem Mon* 150:233–245. <https://doi.org/10.1007/s00706-018-2316-6>
12. Hough WL, Smiglak M, Rodríguez H et al (2007) The third evolution of ionic liquids: active pharmaceutical ingredients. *New J Chem* 31:1429–1436. <https://doi.org/10.1039/b706677p>
13. Egorova KS, Seitkalieva MM, Posvyatenko AV et al (2015) Cytotoxic activity of salicylic acid-containing drug models with ionic and covalent binding. *ACS Med Chem Lett* 6:1099–1104. <https://doi.org/10.1021/acsmedchemlett.5b00258>
14. Berton P, Di Bona KR, Yancey D et al (2017) Transdermal bioavailability in rats of lidocaine in the forms of ionic liquids, salts, and deep eutectic. *ACS Med Chem Lett* 8:498–503. <https://doi.org/10.1021/acsmedchemlett.6b00504>
15. Shamshina JL, Kelley SP, Gurau G, Rogers RD (2015) Develop ionic liquid drugs. *Nature* 528:188–189. <https://doi.org/10.1038/528188a>

16. Patel DD, Lee JM (2012) Applications of ionic liquids. *Chem Rec* 12:329–355. <https://doi.org/10.1002/tcr.201100036>
17. Welton T (2018) Ionic liquids: a brief history. *Biophys Rev* 10:691–706. <https://doi.org/10.1007/s12551-018-0419-2>
18. Yadav S, Baweja K, Kumar C et al (2024) A review: applications of ionic liquids in medicinal chemistry. *Chem Africa*. <https://doi.org/10.1007/s42250-024-00960-z>
19. Sangeeta SA, Tomar R (2024) Unveiling the potential: 1,3-benzodioxole-based ionic liquids as dynamic players against cancer. *J Mol Liq* 413:126002. <https://doi.org/10.1016/j.molliq.2024.126002>
20. Hough WL, Rogers RD (2007) Ionic liquids then and now: from solvents to materials to active pharmaceutical ingredients. *Bull Chem Soc Jpn* 80:2262–2269. <https://doi.org/10.1246/bcsj.80.2262>
21. Stoimenovski J, MacFarlane DR, Bica K, Rogers RD (2010) Crystalline vs. ionic liquid salt forms of active pharmaceutical ingredients: a position paper. *Pharm Res* 27:521–526. <https://doi.org/10.1007/s11095-009-0030-0>
22. Frizzo CP, Wust K, Tier AZ et al (2016) Novel ibuprofenate-and docusate-based ionic liquids: emergence of antimicrobial activity. *RSC Adv* 6:100476–100486. <https://doi.org/10.1039/c6ra22237d>
23. Ferraz R, Costa-Rodrigues J, Fernandes MH et al (2015) Antitumor activity of ionic liquids based on ampicillin. *ChemMedChem* 10:1480–1483. <https://doi.org/10.1002/cmdc.201500142>
24. Panić J, Tot A, Drid P et al (2021) Design and analysis of interactions in ionic liquids based on procaine and pharmaceutically active anions. *Eur J Pharm Sci* 166:105966. <https://doi.org/10.1016/j.ejps.2021.105966>
25. Sangeeta S, Kumari N et al (2023) Synthesis of 1, 3-benzodioxole-based procaine-tagged ionic liquids and investigation of its physicochemical and anti-cancer properties against. *J Mol Liq* 391:123325. <https://doi.org/10.1016/j.molliq.2023.123325>
26. Panić J, Tot A, Janković N et al (2020) Physicochemical and structural properties of lidocaine-based ionic liquids with anti-inflammatory anions. *RSC Adv* 10:14089–14098. <https://doi.org/10.1039/c9ra08815f>
27. Zhang Y, Cao Y, Meng X et al (2020) Enhancement of transdermal delivery of artemisinin using microemulsion vehicle based on ionic liquid and lidocaine ibuprofen. *Colloids Surfaces B Biointerfaces* 189:110886. <https://doi.org/10.1016/j.colsurfb.2020.110886>
28. Sangeeta S, Tomar R et al (2023) 1, 3-Benzodioxole tagged lidocaine based ionic liquids as anticancer drug : synthesis, characterization and in silico study. *Chem Sel* 8:1–9. <https://doi.org/10.1002/slct.202204535>
29. Vraneš M, Panić J, Gadžurić S et al (2021) The nature of ions organisation in aqueous solutions of ionic liquids based on local anaesthetic drugs and salicylic acid. *J Mol Liq* 338:116673. <https://doi.org/10.1016/j.molliq.2021.116673>
30. Alshitari W (2020) Investigating the binding measurements of human α -acid glycoprotein with chlorambucil and dacarbazine in the presence of imidazolium based-ionic liquid by affinity capillary electrophoresis. *Arab J Chem* 13:7445–7452. <https://doi.org/10.1016/j.arabjc.2020.08.020>
31. Kharb S, Yadav S, Singh A et al (2023) Molecular docking and physicochemical studies of 1,3-benzodioxole tagged dacarbazine derivatives as an anticancer agent. *Artif Cells, Nanomed, Biotechnol* 51:520–530. <https://doi.org/10.1080/21691401.2023.2253470>
32. Fernandez-Stefanuto V, Tojo E (2018) New active pharmaceutical ingredient-ionic liquids (API-ILs) derived from indomethacin and mebendazole. *MDPI Proc* 9:48. <https://doi.org/10.3390/ecsoc-22-05781>
33. Pernak J, Borucka N, Walkiewicz F et al (2011) Synthesis, toxicity, biodegradability and physicochemical properties of 4-benzyl-4-methylmorpholinium-based ionic liquids. *Green Chem* 13:2901–2910. <https://doi.org/10.1039/c1gc15468k>
34. AbrarSiddiquee M, Saraswat J, Ud din Parrray M et al (2023) Spectroscopic and DFT study of imidazolium based ionic liquids with broad spectrum antibacterial drug levofloxacin *Spectrochim Acta Part A Mol Biomol. Spectrosc* 285:121803. <https://doi.org/10.1016/j.saa.2022.121803>
35. Kumar N, Tomar R, Pandey A et al (2018) Preclinical evaluation and molecular docking of 1,3-benzodioxole propargyl ether derivatives as novel inhibitor for combating the histone deacetylase enzyme in cancer. *Artif Cells, Nanomed Biotechnol* 46:1288–1299. <https://doi.org/10.1080/21691401.2017.1369423>
36. Sehrawat H, Kumar N, Tomar R et al (2020) Synthesis and characterization of novel 1,3-benzodioxole tagged noscapine based ionic liquids with in silico and in vitro cytotoxicity analysis on HeLa cells. *J Mol Liq* 302:112525. <https://doi.org/10.1016/j.molliq.2020.112525>
37. Dutta Gupta S, Rao GB, Bommaka MK et al (2016) Eco-sustainable synthesis and biological evaluation of 2-phenyl 1,3-benzodioxole derivatives as anticancer, DNA binding and antibacterial agents. *Arab J Chem* 9:S1875–S1883. <https://doi.org/10.1016/j.arabjc.2014.08.004>
38. Yong C, Devine SM, Abel AC et al (2021) 1,3-Benzodioxole-modified noscapine analogues: synthesis, antiproliferative activity, and tubulin-bound structure. *Chem Med Chem* 16:2882. <https://doi.org/10.1002/cmdc.202100363>
39. Shilpa, Sonaxi, Sangeeta, et al (2023) Azomycin based ionic liquids as an anticancer agent: a computational approach. *Rasayan J Chem* 16:2353–2360. <https://doi.org/10.31788/RJC.2023.1648581>
40. Bezu L, Kepp O, Kroemer G (2022) Local anesthetics and immunotherapy: a novel combination to fight cancer. *Semin Immunopathol*. <https://doi.org/10.1007/s00281-022-00960-6>
41. Li C, Gao S, Li X et al (2018) Procaine inhibits the proliferation and migration of colon cancer cells through inactivation of the ERK/MAPK/FAK pathways by regulation of RhoA. *Oncol Res* 26:209–217. <https://doi.org/10.3727/096504017x14944585873622>
42. Song Z, Tan J (2022) Effects of anesthesia and anesthetic techniques on metastasis of lung cancers: a narrative review. *Cancer Manag Res* 14:189–204. <https://doi.org/10.2147/CMAR.S343772>
43. Sangeeta S, Kumari N et al (2024) Biophysical insight into the binding mechanism of 1, 3-benzodioxole-based lidocaine tagged ionic liquids with BSA : an in-silico, and spectroscopic studies. *J Mol Liq* 398:124284. <https://doi.org/10.1016/j.molliq.2024.124284>
44. Baroroh SS, Biotek MU, Muscifa ZS, Destiarani W et al (2023) Molecular interaction analysis and visualization of protein-ligand docking using Biovia Discovery Studio Visualizer. *Indones J Comput Biol* 2:22. <https://doi.org/10.24198/ijcb.v2i1.46322>
45. Islam M, Khan IM, Shakya S, Alam N (2023) Design, synthesis, characterizing and DFT calculations of a binary CT complex co-crystal of bioactive moieties in different polar solvents to investigate its pharmacological activity. *J Biomol Struct Dyn* 41:10813–10829. <https://doi.org/10.1080/07391102.2022.2158937>
46. Khan IM, Khan A, Shakya S et al (2023) Investigating the binding of organic charge transfer co-crystals to human serum albumin by experimental and theoretical methods: spectroscopy, molecular docking and DFT/TD-DFT studies. *New J Chem* 48:2527–2546. <https://doi.org/10.1039/d3nj05194c>
47. Mills N (2006) ChemDraw Ultra 10.0 CambridgeSoft, 100 CambridgePark Drive, Cambridge, MA 02140. www.cambridgesoft.com. Commercial Price: \$1910 for download, \$2150 for CD-ROM; Academic Price: \$710 for download, \$800 for CD-ROM.

- J Am Chem Soc 128:13649–13650. <https://doi.org/10.1021/ja0697875>
48. Cousins KR (2011) Computer review of ChemDraw ultra 12.0. J Am Chem Soc 133:8388. <https://doi.org/10.1021/ja204075s>
 49. Bruno IJ, Cole JC, Edgington PR et al (2002) New software for searching the Cambridge Structural Database and visualizing crystal structures. Acta Crystallogr Sect B Struct Sci 58:389–397. <https://doi.org/10.1107/S0108768102003324>
 50. Macindoe G, Mavridis L, Venkatraman V et al (2010) HexServer: An FFT-based protein docking server powered by graphics processors. Nucleic Acids Res 38:445–449. <https://doi.org/10.1093/nar/gkq311>
 51. Alamri AS, Alhomrani M, Alsanie WF et al (2022) Spectroscopic and molecular docking analysis of π -acceptor complexes with the drug barbitol. Appl Sci 12:1–16. <https://doi.org/10.3390/app121910130>
 52. Hasan AH, Shakya S, Hussain FHS et al (2023) Design, synthesis, anti-acetylcholinesterase evaluation and molecular modelling studies of novel coumarin-chalcone hybrids. J Biomol Struct Dyn 41:11450–11462. <https://doi.org/10.1080/07391102.2022.2162583>
 53. Rauf MA, Zubair S, Azhar A (2015) Ligand docking and binding site analysis with PyMOL and Autodock/Vina. Int J basic Appl Sci 4:168–177. <https://doi.org/10.1007/s10822-010-9352-6>
 54. Pires DEV, Blundell TL, Ascher DB (2015) pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem 58:4066–4072. <https://doi.org/10.1021/acs.jmedchem.5b00104>
 55. Mvondo JGM, Matondo A, Mawete DT et al (2021) In silico ADME/T properties of quinine derivatives using SwissADME and pkCSM web servers. Int J Trop Dis Heal 42:1–12. <https://doi.org/10.9734/ijtdh/2021/v42i1130492>
 56. Dabhi RA, Dhaduk MP, Savaliya NK et al (2024) Influence of pyridine entangled novel hybrid quinoxaline spirane on the fluorescence and absorption spectra of biomolecules: molecular docking, pharmacokinetic, and in-vitro biological investigations. J Fluoresc. <https://doi.org/10.1007/s10895-024-03975-4>
 57. Dabhi RA, Dhaduk MP, Bhatt VD, Bhatt BS (2023) Synthetic approach toward spiro quinoxaline- β -lactam based heterocyclic compounds: spectral characterization, SAR, pharmacokinetic and biomolecular interaction studies. J Biomol Struct Dyn 41:5382–5398. <https://doi.org/10.1080/07391102.2022.2086176>
 58. Sewariya S, Sehwat H, Mishra N et al (2023) Comparative assessment of 9-bromo noscapine ionic liquid and noscapine: synthesis, in-vitro studies plus computational & biophysical evaluation with human hemoglobin. Int J Biol Macromol 247:125791. <https://doi.org/10.1016/j.ijbiomac.2023.125791>
 59. Ma XW, Li Y, Han XC, Xin QZ (2016) The effect of low dosage of procaine on lung cancer cell proliferation. Eur Rev Med Pharmacol Sci 20:4791–4795
 60. Gao Z, Xu Z, Hung M-S et al (2009) Procaine and procainamide inhibit the Wnt canonical pathway by promoter demethylation of WIF-1 in lung cancer cells. Oncol Rep 22:1479–1484. https://doi.org/10.3892/or_00000590

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