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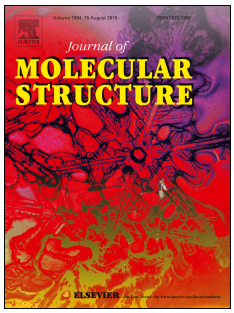

Synthesis, spectroscopic and molecular docking studies of imidazolium and pyridinium based ionic liquids with HSA as potential antimicrobial agents

Maria M. Trush, Ivan V. Semenyuta, Sergey I. Vdovenko, Sergiy P. Rogalsky, Evgeniya O. Lobko, Larisa O. Metelytsia

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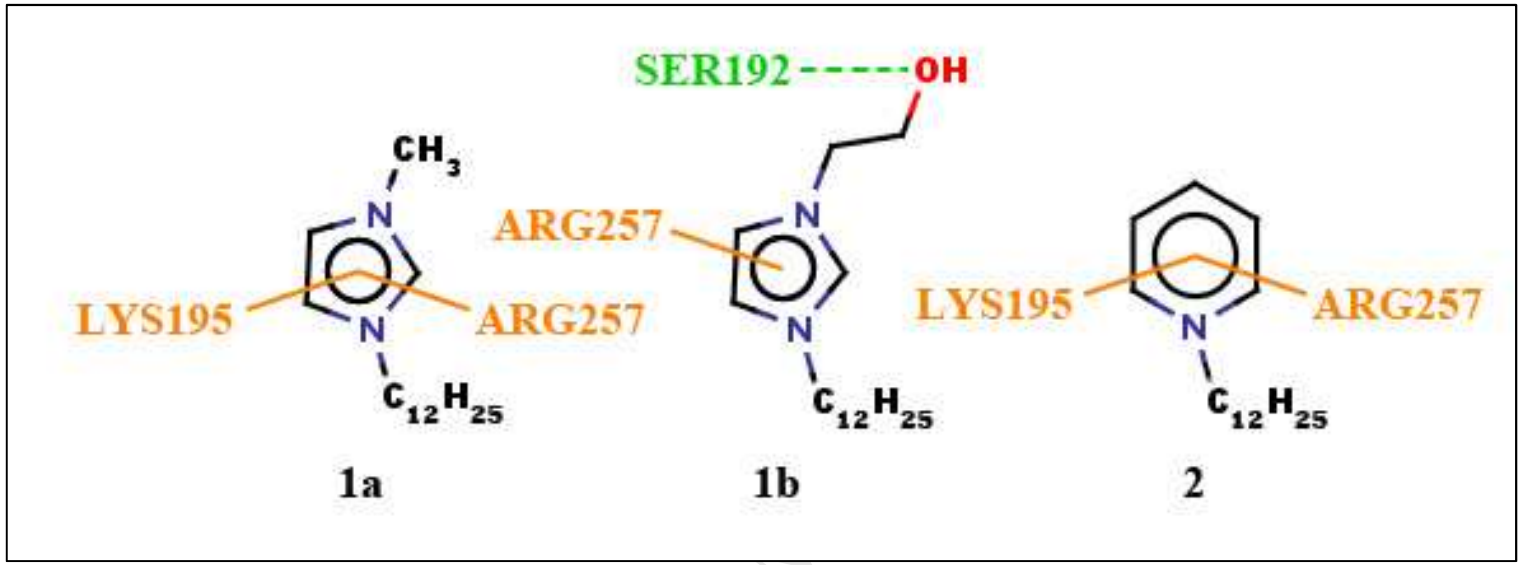

Synthesis, spectroscopic and molecular docking studies of imidazolium and pyridinium based ionic liquids with HSA as potential antimicrobial agents

Abstract

The interaction between human serum albumin (HSA) and synthesized imidazolium and pyridinium based ionic liquids (ILs), as good potential microbial growth inhibitors, was investigated by spectroscopic techniques combined with molecular docking analysis. All compounds were significant active against the tested bacterial and fungal strains. FT-IR spectroscopy indicated that the interaction of HSA with ILs generates considerable changes in protein secondary structure. The results of the molecular docking study showed that the studied ILs are able to firmly bind in the subdomain IIA of HSA with almost equal binding affinity (about -6.23 kcal/mol). Investigated HSA–ILs complex binds through hydrogen bonding or/and cation-π interactions. This study provides a better understanding of the binding of imidazolium and pyridinium based ILs to HSA and opens the way for their further biological and pharmaceutical investigations as candidates with antimicrobial properties.

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1. Introduction

Ionic liquids (ILs) are a recent and promising class of low-temperature liquid salts (<100 °C) consisting of ions (anions and cations) and widely used in various areas of life.

ILs show great potential in the energy industry [1], in chemical and electrochemical industries [2], for analytical processes, catalysis, synthesis [3-5], for the use in batteries [6-9] and fuel cells [10-13], sensors, solar panels [14], solid-state photocells, as thermal fluids, lubricants [15], paint additives, coating [16], hydraulic fluids, ionogels and in other applications [17,18].

One of the most important applications of ILs is in the pharmaceutical industry. ILs can be specially adapted for providing certain required characteristics and improving existing ones, such as stability, solubility, permeability, acidity/basicity, viscosities and other properties [19-21]. Thus, during the last years the use of ILs as drug delivery agents and in combination with the prodrugs opens up new possibilities in the treatment of diseases [22-27]. Some ionic liquids show a potential as an effective anticancer drugs [28-31]. Many ILs exhibit antimicrobial activity [19,24,32-35] and used as antiseptics in bioactive materials [29,36].

Human serum albumin (HSA) is unique and the most abundant plasma protein, one of the main functions of which is transportation [37]. In the basis of albumin transport function is the ability to reversibly bind many kinds of ligands with different chemical structure, such as biologically active substances [38], fatty acids [39], inorganic ions [40], drugs and their metabolites [41,42]. This binding leads to an increase in the solubility of the ligands in the plasma, decreases their toxicity and protects them from enzymatic and oxidative degradation. Therefore, the molecular aspects of albumin-ligand interactions invariably have a great interest for the scientists and specialists who deal with the drug discovery and development of new biologically active molecules.

In this paper, we represent the antimicrobial evaluation of long-chain imidazolium and pyridinium based ionic liquids. The said ionic liquids are reported to have various antimicrobial activity due to their optimal amphiphilic properties and

high surface activity [43]. The results of molecular interactions studies between the synthesized ILs as potential pharmaceutical agents and HSA using FT-IR spectroscopic techniques and molecular docking analysis are also presented and discussed.

2. Experimental

2.1. Materials

HSA was purchased from Sigma-Aldrich Chemicals Company (USA). The HSA and drug solutions were dissolved in phosphate buffer (10 mM and pH 7.4). The working concentration of protein was 50 μM.

The following chemicals were used for the synthesis of ionic liquids: pyridine (99.5%, Labscan), 1-methylimidazole (99%), 1-(2-hydroxyethyl)imidazole (97%), 1-chlorododecane (98%,), ethyl acetate, hexane, methylene chloride, and sodium sulfate (Sigma-Aldrich).

2.2. Synthesis of ionic liquids

The ionic liquids were synthesized according to a procedure described in the literature [44] (Fig. 1).

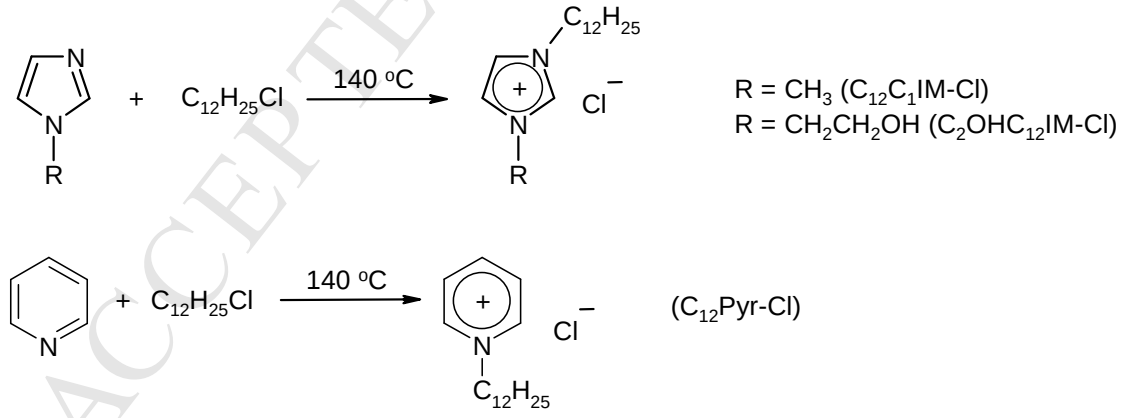


Fig. 1. Synthesis of ionic liquids.

Compound 1a. *1-dodecyl-3-methylimidazolium chloride ([$C_{12}C_1$IM]Cl)*

The stirred mixture of 1-methylimidazole (5 g, 0.06 mol) and 1-chlorododecane (14.3 g, 0.07 mol) was heated at 140 °C for 24 h under an argon atmosphere. The

obtained low melted product was purified by recrystallization from hexane-ethyl acetate mixture (4:1 (v/v)). It has melting point of 46-47 ^{o}C.

^{1}H NMR (300 MHz, DMSO-d_6, 25 ^{o}C, TMS): δ = 0.85 (t, 3H, CH_3), 1.24 (m, 18H, $CH_3(CH_2)_9$), 1.79 (m, 2H, NCH_2CH_2), 3.91 (s, 3H, NCH_3), 4.21 (t, 2H, NCH_2), 7.84 (br s, 1H, C_4-H), 7.92 (br s, 1H, C_5-H), 9.57 (s, 1H, C_2-H).

Compound 1b. *1-(2-hydroxyethyl)-3-dodecylimidazolium chloride ($[C_2OHC_{12}IM]Cl$)*

The mixture of 1-(2-hydroxyethyl)imidazole (2 g, 0.017 mol) and 1-chlorododecane (4.2 g, 0.02 mol) was stirred at 140 ^{o}C for 24 h under an argon atmosphere. The obtained semi-solid product was purified by washing with hexane-ethyl acetate mixture (4:1 (v/v), 3x30 ml). Residual solvents were removed in vacuum 10 mbar at 70-80 ^{o}C for 12 h. The ionic liquid has melting point of 28-29 ^{o}C.

^{1}H NMR (400 MHz, DMSO-D_6): δ = 0.85 (t, 3H, CH_3), 1.25 (m, 18H, $CH_3(CH_2)_9$), 1.79 (m, 2H, NCH_2CH_2), 3.38 (br s, 1H, OH), 3.72 (m, 2H, NCH_2CH_2OH), 4.16-4.23 (m, 4H, NCH_2), 7.78 (br s, 1H, C_4-H), 7.81 (br s, 1H, C_5-H), 9.27 (s, 1H, C_2-H).

Compound 2. *1-dodecylpyridinium chloride ($[C_{12}Pyr]Cl$)*

The mixture of pyridine (5 g, 0.06 mol) and 1-chlorododecane (14.3 g, 0.07 mol) was stirred at 140 ^{o}C for 24 h under an argon atmosphere. The solid product was purified by recrystallization from hexane-ethyl acetate mixture (4:1 (v/v)). It has melting point of 92-93 ^{o}C.

^{1}H NMR (400 MHz, DMSO-d_6, 25 ^{o}C, TMS): δ = 0.84 (t, 3H, CH_3), 1.24 (m, 18H, $CH_3(CH_2)_9$), 1.90 (m, 2H, NCH_2CH_2), 4.66 (t, 2H, NCH_2), 8.18 (t, 2H, C_3-H, C_5-H), 8.62 (t, 1H, C_4-H), 9.22 (d, 2H, C_2-H, C_6-H).

2.3. Antimicrobial assay

Antibacterial and antifungal properties of the ILs were tested by using standard disk diffusion method in Mueller-Hinton agar and Sabouraud agar, respectively [45]. The tested bacterial strains were *Staphylococcus aureus* ATCC 25923, *Pseudomonas*

aeruginosa ATCC 27853 and fungi strain was *Candida albicans* M 885 ATCC 10231. The microbial load was $1 \cdot 10^5$ colony-forming units (CFU) in 1 ml.

All ILs were investigated in concentrations of 1.0 %, 0.1 % and were dissolved in water. The tested compounds (0.02 ml) were applied to paper disks (6 mm) which were placed on each agar plate. The plates were then incubated at 37 °C for 24 h. The tests were repeated three times. The antimicrobial activity of ILs was assessed by measuring zone diameter of the growth inhibition (in mm).

2.4. FT-IR spectroscopy experimental procedure

The FT-IR measurements were obtained on a Bruker VERTEX 70 spectrometer equipped with KBr beamsplitter, RT-DLaTGS detector and diamond attenuated total reflection (ATR) accessory at ambient temperature (20±1 ºC). The spectrometer was continuously purged with dry air during the measurements. The absorption spectra were obtained in the wave number rage of 400-4000 cm^{-1}. A spectrum was taken as an average of 100 scans to increase the signal to noise ratio, with spectral resolution 2 cm^{-1}. Base-line correction, normalization and band deconvolution were performed for all the spectra by OPUS software. The FT-IR spectrum of free HSA and HSA-ionic liquid complexes were obtained in the region of 1400-1800 cm^{-1}. The FT-IR spectrum of free HSA was acquired by subtracting the absorption spectrum of the phosphate buffer from the spectrum of the protein solution. For the net interaction effect, the difference spectra of (HSA−IL complex) were generated using the featureless region of the HSA solution 1800-2100 cm^{-1} as an internal standard [46]. The obtained spectral differences were used to investigate the nature of HSA−IL interaction.

2.5. Molecular docking

We used AutoDock Tools (ADT) (ver. 1.5.6) [47] to prepare the docking compatible structure formats of the protein, ligands and grid box creation. The crystal structure of the human serum albumin (PDB code 1AO6) [48] was used in our study. The structure of A-subunit of HSA was selected and stored as a pdb file by Accelrys DS (ver. 2.5) [49]. To add only polar hydrogens in ADT, we used the noBondOrder

method and renumbered atoms to include new hydrogens. The partial charges were calculated and added using Gasteiger method, the prepared file was saved in PDBQT format. We used the ChemAxon Marvin Sketch 5.3.7 program [50] for creation and optimization structures of ligands **1a**, **1b**, **2**. These structures were saved in Mol2 format. Partial charges and torsions angles of ligands were changed by ADT, and the resulting files were saved in PDBQT format. AutoGrid was used for the preparation of the grid map using a grid box. The box center (x = 25.90, y = 35.70, z = 34.20) was set to the atom CD2 of the tryptophan amino acid residue (Trp214) in the original pdb file. A grid of 40×40×40 points was used with grid spacing of 0.375 Å. For docking we used AutoDock Vina 1.1.2 [51]. The time to dock one ligand was about 3-5 min. All operations were performed on a Windows XP SP3 computer with an Intel Core i3 CPU (3.20 GHz) and 2 GB of RAM. The software package Accelrys DS was used for illustration and to study protein-ligand interactions. In our study, we used the IIA binding site of A-subunit of HSA, because, according to the literature [52-54], the formation of complexes between ILs and HSA occurs in this region.

3. Results and discussion

3.1. Antimicrobial studies

The antimicrobial activity of imidazolium and pyridinium based ionic liquids (Fig. 1) is shown in Table 1. The results of antibacterial and antifungal tests of compound **1a** were published previously [55,56].

Table 1
Antimicrobial activity of studied compounds.

№	ILs	Conc.	Diameter of the inhibitory zone, mm		
			Staphylococcus aureus ATCC 25923	*Pseudomonas aeruginosa* ATCC 27853	*Candida albicans* ATCC 10231
1a	$[C_{12}C_1IM]Cl$	1%	35	31	54
		0,1%	22	24	38
1b	$[C_2OHC_{12}IM]Cl$	1%	29	28	38
		0,1%	13	0	22
2	$[C_{12}Pyr]Cl$	1%	31	33	59
		0,1%	19	19	42

In general, the most active compounds against *St. aureus*, *P. aeruginosa* and *Candida albicans* were $[C_{12}C_1IM]Cl$ and $[C_{12}Pyr]Cl$, while compound

$[C_2OHC_{12}IM]Cl$ showed slightly lower activity against these tested microorganisms. It can be noted that the presence of 2-hydroxyethyl group in dodecylimidazolium chloride molecule at position 1 led to a decrease in antimicrobial activity of this compound, but the presence of methyl group at the same position increases the properties of substance.

3.2. Spectroscopic studies

FT-IR spectroscopy is a powerful technique for the study of hydrogen bonding [57] and has been identified as one of the few techniques that is established in the determination of protein secondary structure at different physiological systems [58,59]. The information on the secondary structure of proteins could be deduced from infrared spectra. Proteins exhibit a number of amide bands, which represent different vibrations of the peptide moiety. The amide group of proteins and polypeptides presents characteristic vibrational modes (amide modes) that are sensitive to the protein conformation and largely been constrained to group frequency interpretations [60].

The modes most widely used in protein structural studies are amide I and amide II. Amide I band ranging from 1700 to 1600 cm^{-1} and arises principally from ν (C=O) stretching vibrations, has been widely accepted to be used [59]. The amide II band is primarily δ (N-H) bending vibrations with a contribution from ν (C-N) stretching mode. Amide II ranging from 1600 to 1480 cm^{-1} is much less intensive than amide I, therefore spectral range of amide I is predominately used in analysis of protein secondary structure changes [53]. The peak positions in the spectral region 1700 – 1600 cm^{-1} of the amide I bands are β-antiparallel (1700-1687 cm^{-1}), turn (1687-1672 cm^{-1}), α-helix (1672-1643 cm^{-1}), random coil (1643-1627 cm^{-1}), and β-sheet (1627-1610 cm^{-1}).

Analysis of the secondary structure of HSA and complexes with ionic liquids was carried out on the bases of the method previously reported [53]. The shift in the peak position of amide I band of HSA from 1651 to 1639 cm^{-1} after addition of ionic liquids (Fig. 2 a-c) indicates the change in secondary structure of HSA due to interaction with various types of ILs.

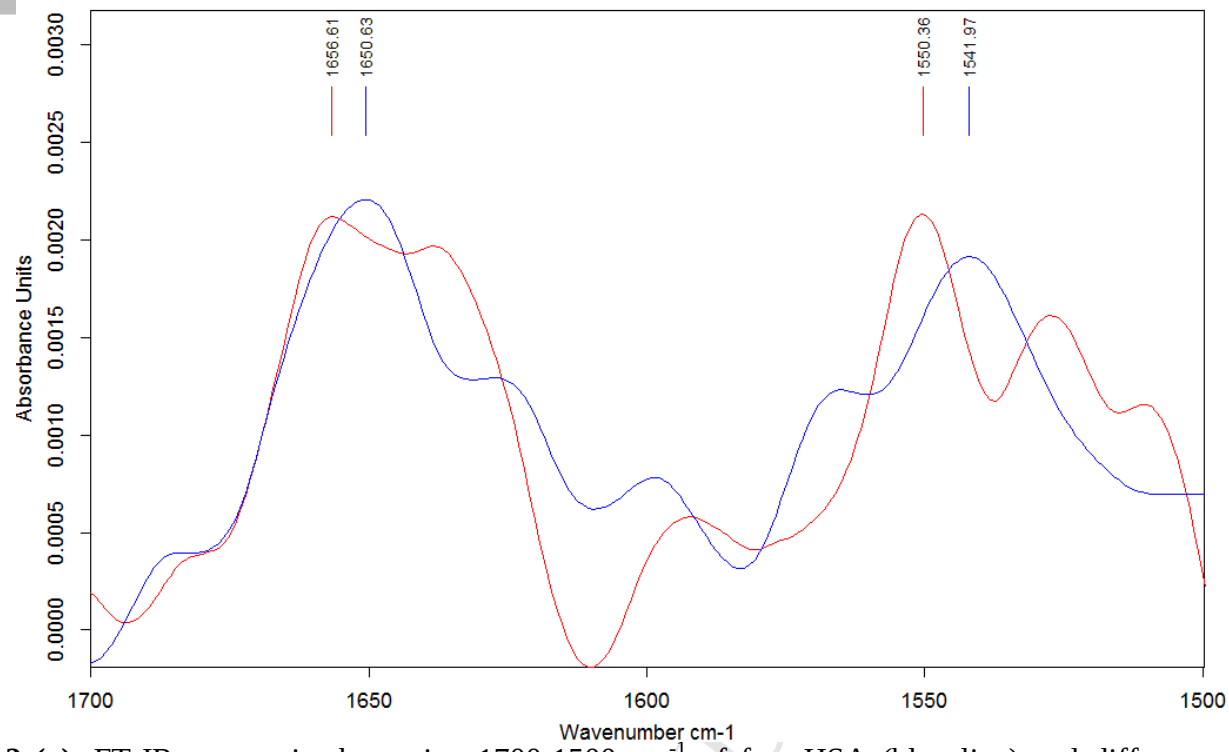


Fig.2 (a). FT-IR spectra in the region 1700-1500 cm^{-1} of free HSA (blue line) and difference spectra of HSA–$[C_{12}C_1IM]Cl$ complex (red line). HSA = 50 µM, $[C_{12}C_1IM]Cl$ = 0.01 M.

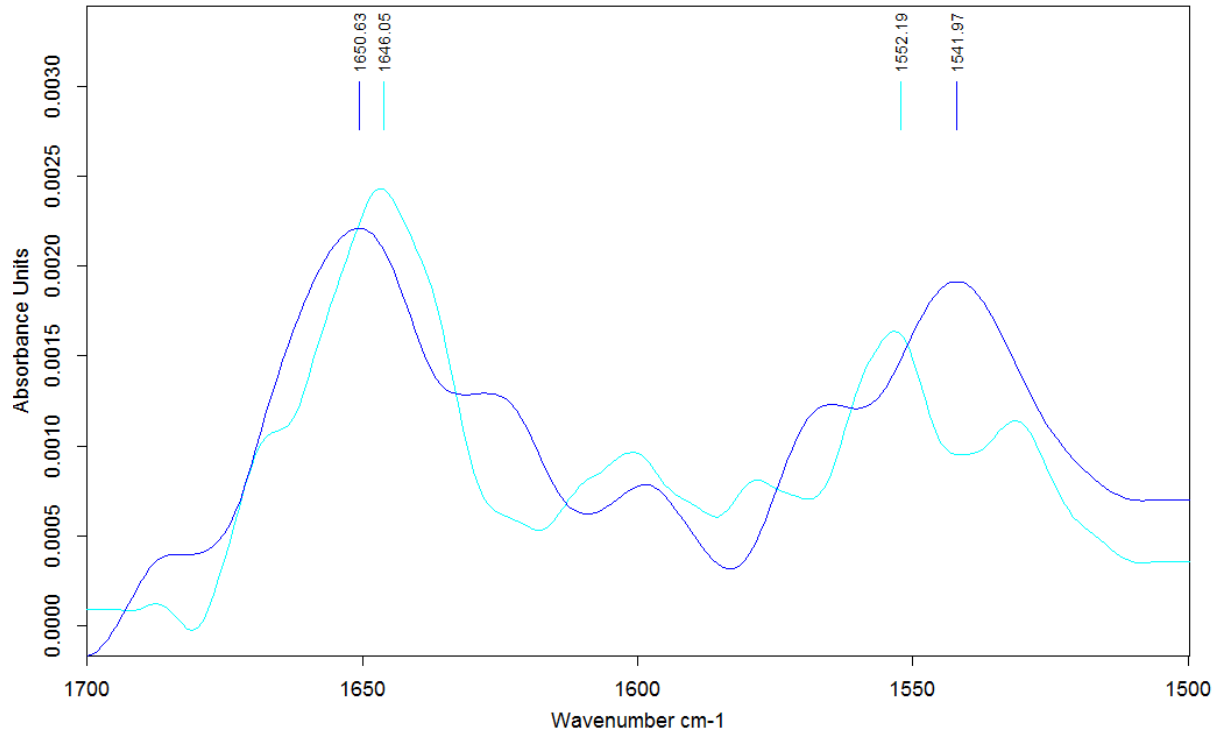


Fig.2 (b). FT-IR spectra in the region 1700-1500 cm^{-1} of free HSA (blue line) and difference spectra of HSA–$[C_2OHC_{12}IM]Cl$ complex (sky blue line). HSA = 50 µM, $[C_2OHC_{12}IM]Cl$ = 0.01 M.

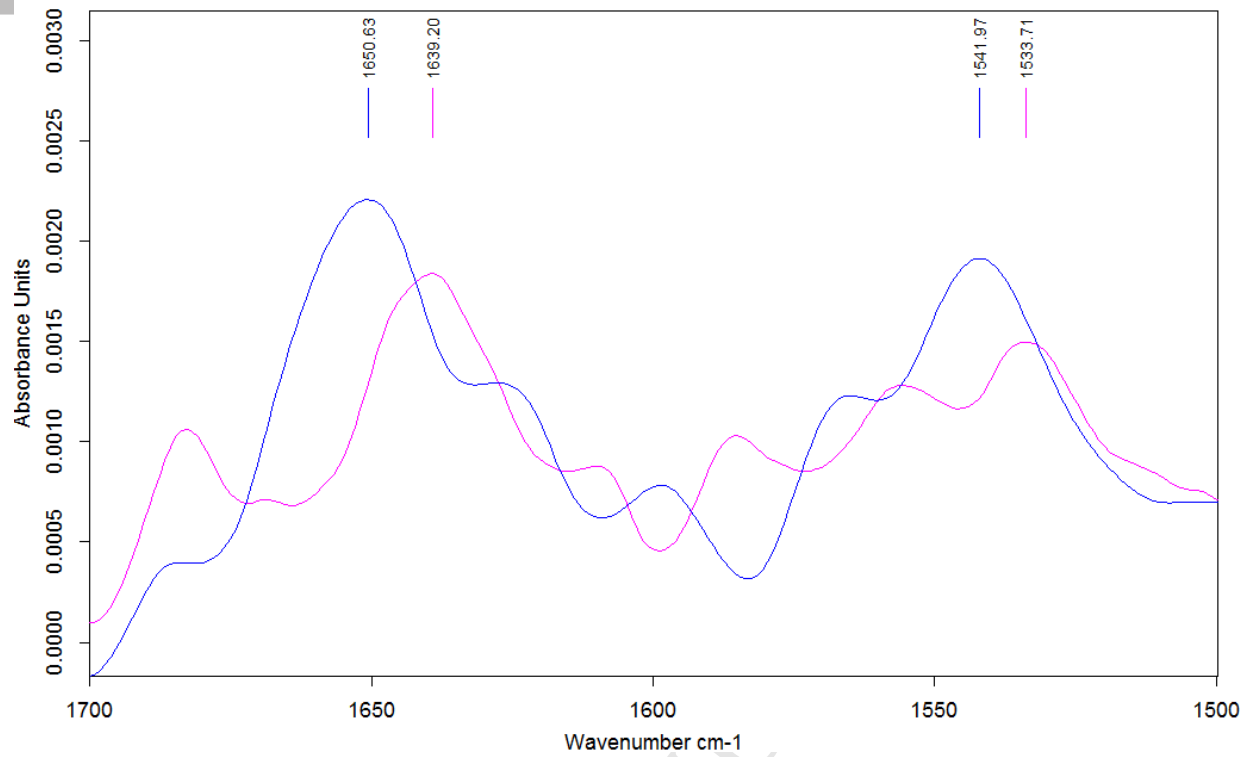
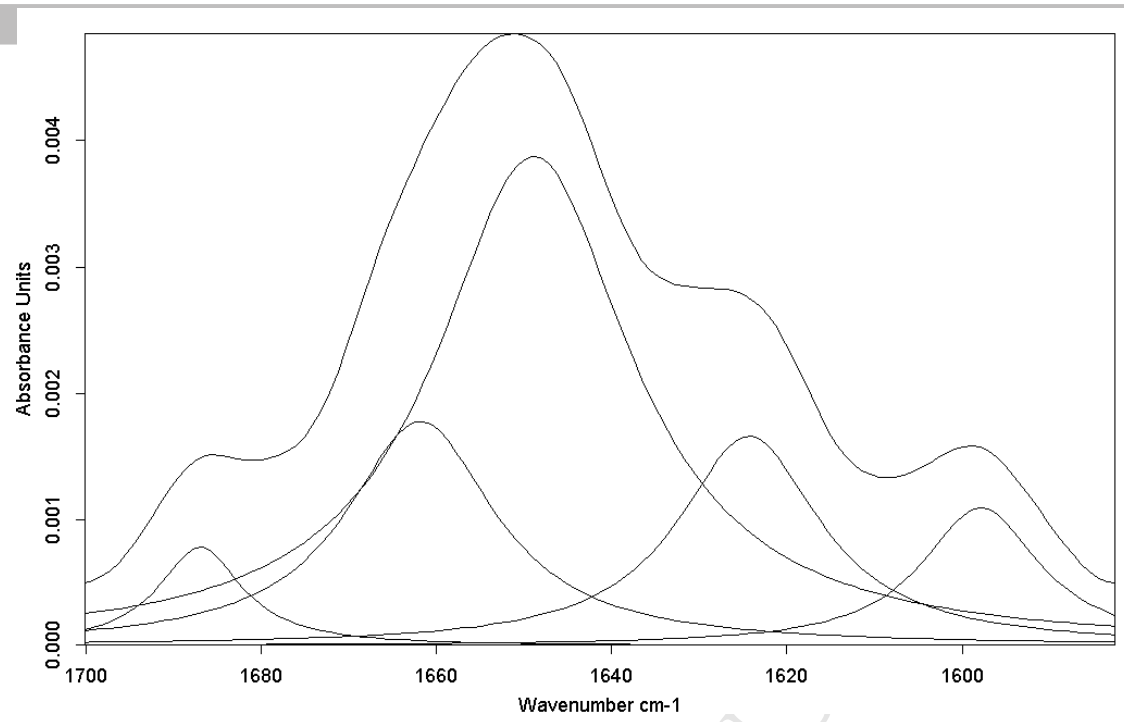


Fig.2 (c). FT-IR spectra in the region 1700-1500 cm^{-1} of free HSA (blue line) and difference spectra of HSA–[C_{12}Pyr]Cl complex (pink line). HSA = 50 μM, [C_{12}Pyr]Cl = 0.01 M.

All the major peaks for the HSA and their complexes with ILs in the deconvoluted spectral region were resolved (Fig. 3) and the area of all the component bands assigned to a given conformation were then summed up and divided by the total area thus obtaining of percentage of each secondary structure form listed in Table 2.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Wavenumber cm-1

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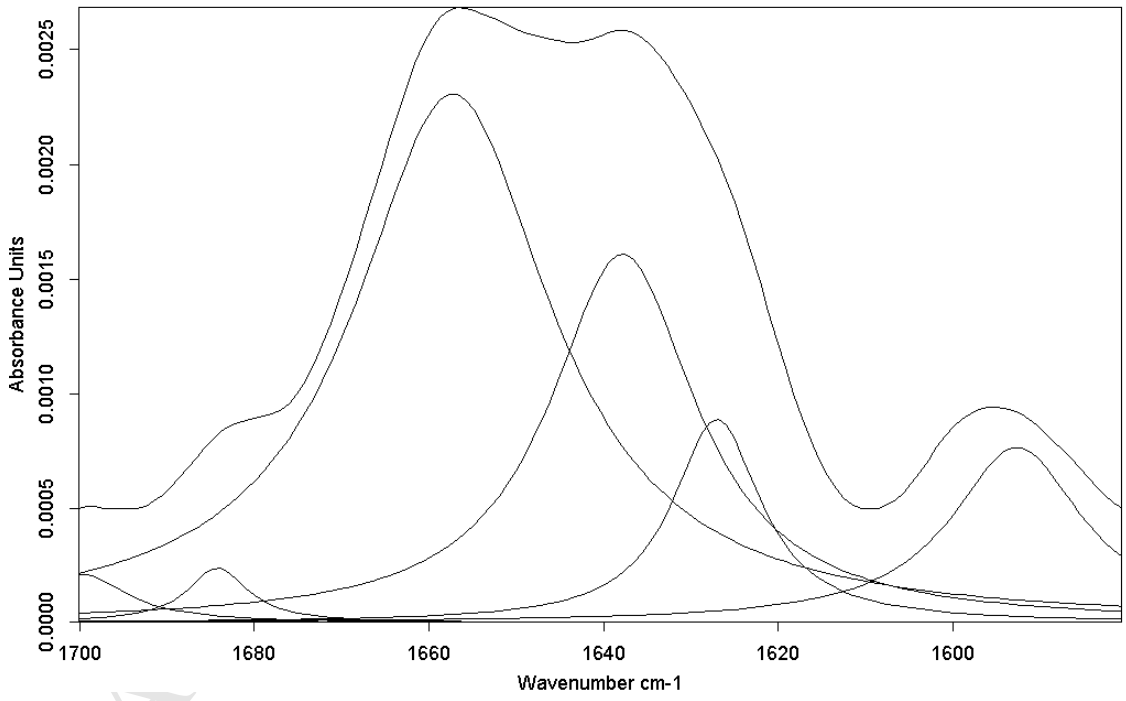
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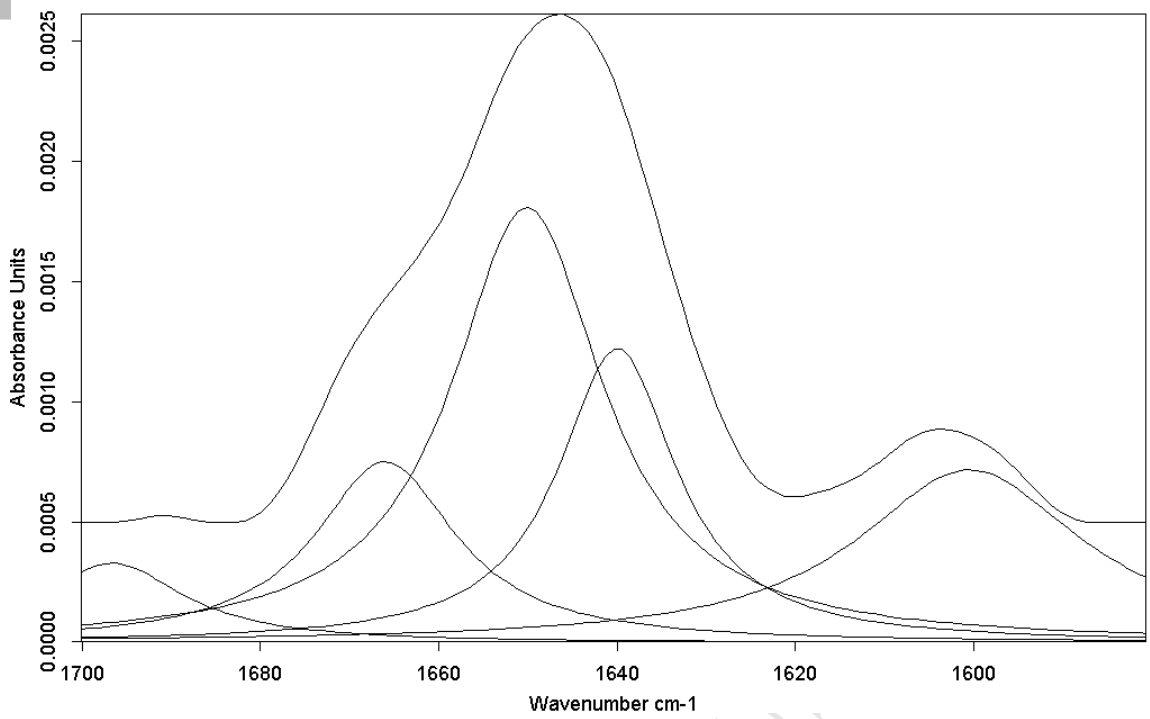
(b)



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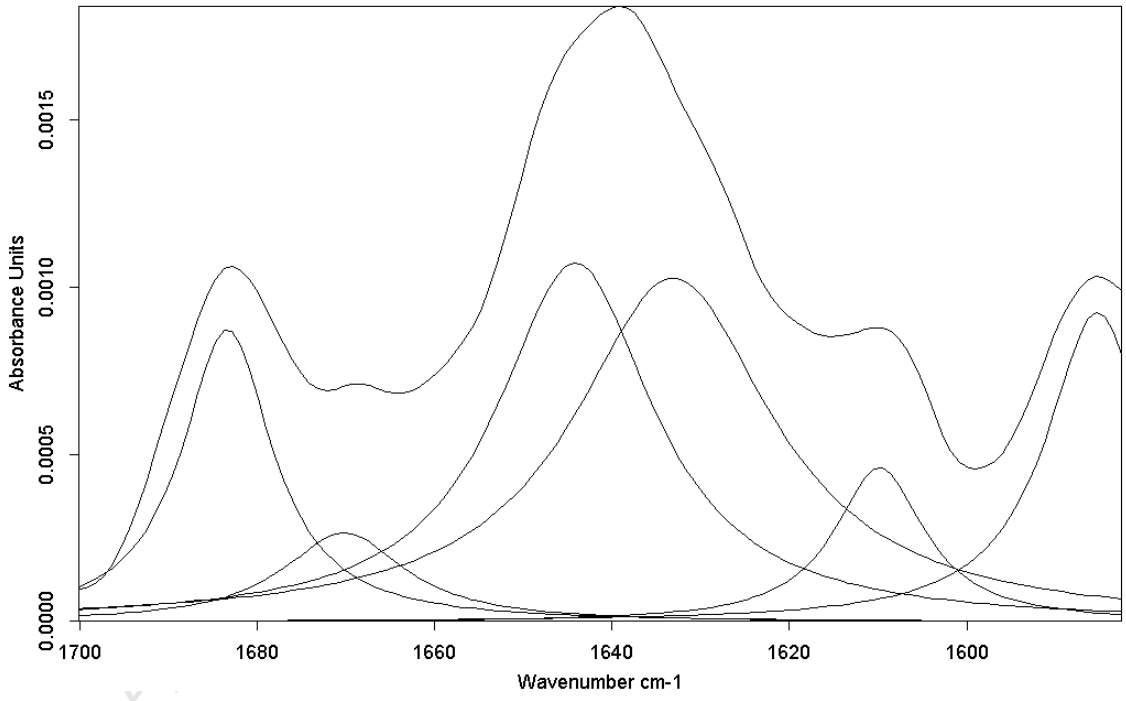

(d)

Fig.3. Curve fitting of amide I region (1700–1580 cm^{-1}) of FTIR spectrum:

(a) free HSA (50 μM); (b) HSA–[$C_{12}C_1IM$]Cl (HSA = 50 μM, [$C_{12}C_1IM$]Cl = 0.01 M); (c) HSA–[$C_2OHC_{12}IM$]Cl (HSA = 50μM, [$C_2OHC_{12}IM$]Cl = 0.01 M); (d) HSA–[$C_{12}Pyr$]Cl (HSA = 50 μM, [$C_{12}Pyr$]Cl = 0.01 M).

Table 2

Band assignment in absorption spectra of HSA with different ILs for amide I region.

Assignment	System			
	HSA (%)	HSA–$[C_{12}C_1IM]Cl$ (%)	HSA–$[C_2OHC_{12}IM]Cl$ (%)	HSA–$[C_{12}Pyr]Cl$ (%)
β-Antiparallel (1687-1700)	1687 (4.9)	1700 (13.4) 1684 (17.9)	1697 (8.4)	1683 (9.6)
Turn (1672-1687)			1666 (18.2)	1670 (39.8)
α-Helix (1643-1672)	1661 (20.0)	1657 (5.7)	1650 (47.9)	-
Random coil (1627-1643)	1648 (57.2)	1637 (29.4)	1639 (25.5)	1644 (20.5)
β-Sheet parallel (1610-1627)	1624 (17.9)	1627 (33.6)	-	1633 (24.9) 1610 (5.2)

As can be seen from Table 2, interaction with different ILs changes HSA secondary structure in varied ways. Comparison Fig.3a with Fig.3b-d reveals that titled interactions have crucial effect on FTIR spectra changing both wavenumbers and intensities of appropriate amide I bands. Addition of $[C_{12}Pyr]Cl$ decreases ν(C=O) of β-antiparallel form from 1687 (in free HSA) to 1683 cm^{-1} with simultaneous two-fold increase of percentage of this form. Moreover, interaction with $[C_{12}Pyr]Cl$ invokes generation of the turn structure (new band appears at 1670 cm^{-1}). However, the form α-helix disappears completely. Percentage of random coil diminishes from 57.2 to 20.5 while percentage of β-sheet parallel increases. Interaction with $[C_{12}C_1IM]Cl$ also increases percentage of β-antiparallel form in secondary structure of HSA, meanwhile the main band shifts to lower wavenumbers (from 1687 to 1684 cm^{-1}) with simultaneous appearance the band at 1700 cm^{-1} which means that some parts of β-antiparallel form are embedded into hydrophobic surroundings. Bands corresponding to α-helix and random coil forms shit to lower wavenumbers due to intermolecular hydrogen bond formation and their percentage decreases also. At the same time the β-sheet parallel form increases both in wavenumber and quantitatively (from 17.9 to 33.6%). Finally, interaction of HSA with $[C_2OHC_{12}IM]Cl$ results in increase of β-antiparallel form percentage with simultaneous shift of corresponding band to higher wavenumbers. Percentage of both turn and α-helix form also increases whereas content of random coil form significantly decreases. In contrast with all other systems the band corresponding to β-sheet parallel form is absent.

In conclusion we may state that interaction of HSA with different ILs generates considerable changes in protein secondary structure. In some cases (system HSA–$[C_{12}C_1IM]Cl$, HSA–$[C_2OHC_{12}IM]Cl$) β-antiparallel form partly or completely becomes imbedded into hydrophobic surroundings and corresponding band is shifted to higher wavenumbers. Interaction of HSA with $[C_{12}Pyr]Cl$ and $[C_2OHC_{12}IM]Cl$ generates appearance of turn form, whereas interaction with $[C_{12}Pyr]Cl$ ionic liquid results in complete disappearance of α-helix form. The same effect is observed for β-sheet parallel form of HSA–$[C_2OHC_{12}IM]Cl$ complex.

3.3. Molecular docking

Molecular docking studies of IL **1a** show the formation of a strong protein-ligand complex (E = –6.2 kcal/mol), depicted in Fig. 4. Two cation-π interactions were formed. The first interaction (3.79 Å length) is formed between the imidazole ring of the ligand and the NH-group of amino acid residue Lys195. The second interaction (5.89 Å length) is formed between the imidazole ring of the ligand and the NH-group of Arg257. The presence of two cation-π bonds in the protein-ligand system indicates the stability of this complex.

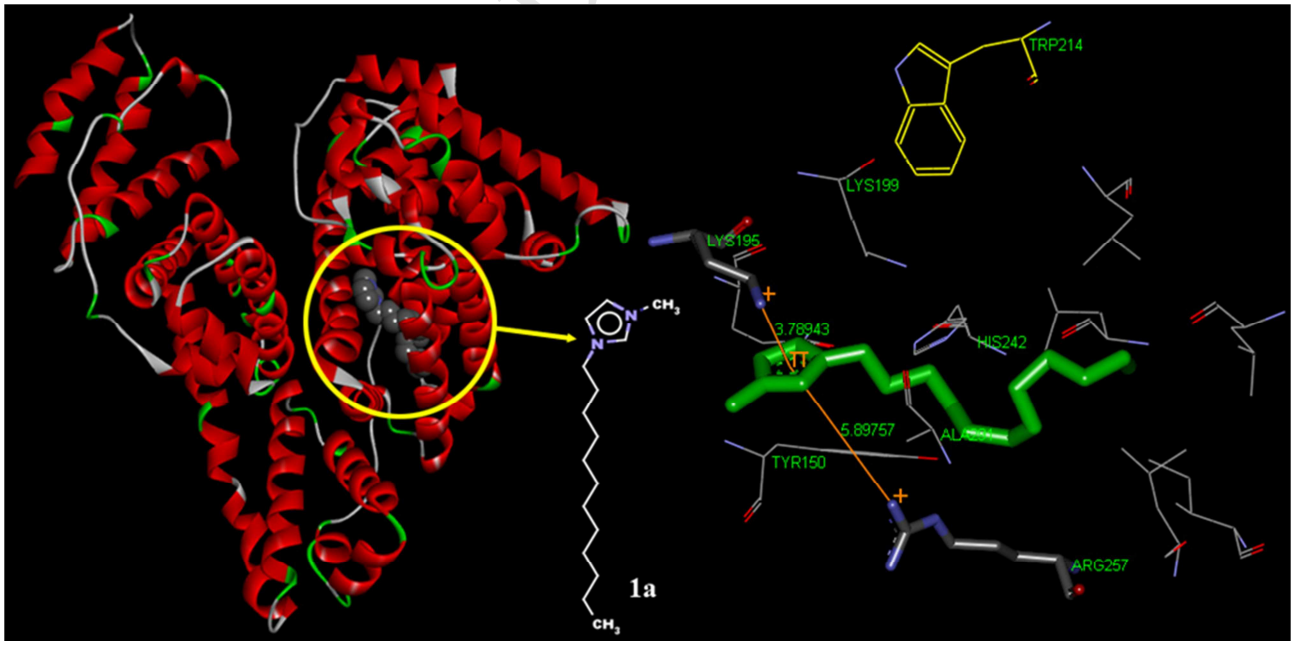

Fig. 4. Molecular docking of imidazolium ionic liquid $[C_{12}C_1IM]Cl$ and HSA.

The results of molecular docking of compound **1b** (Fig. 5) indicate the formation of a 2.83 Å length hydrogen bond between the hydroxy group of compound **1b** and amino acid residue Ser192 and formation of the cation-π interaction of 4.79 Å length between the aromatic ring of the ligand and amino acid residue Arg257. The predicted binding affinity of this complex is -6.2 kcal/mol. The presence of hydrogen bond and cation-π bond indicates the formation of sufficiently stable ligand-receptor complex.

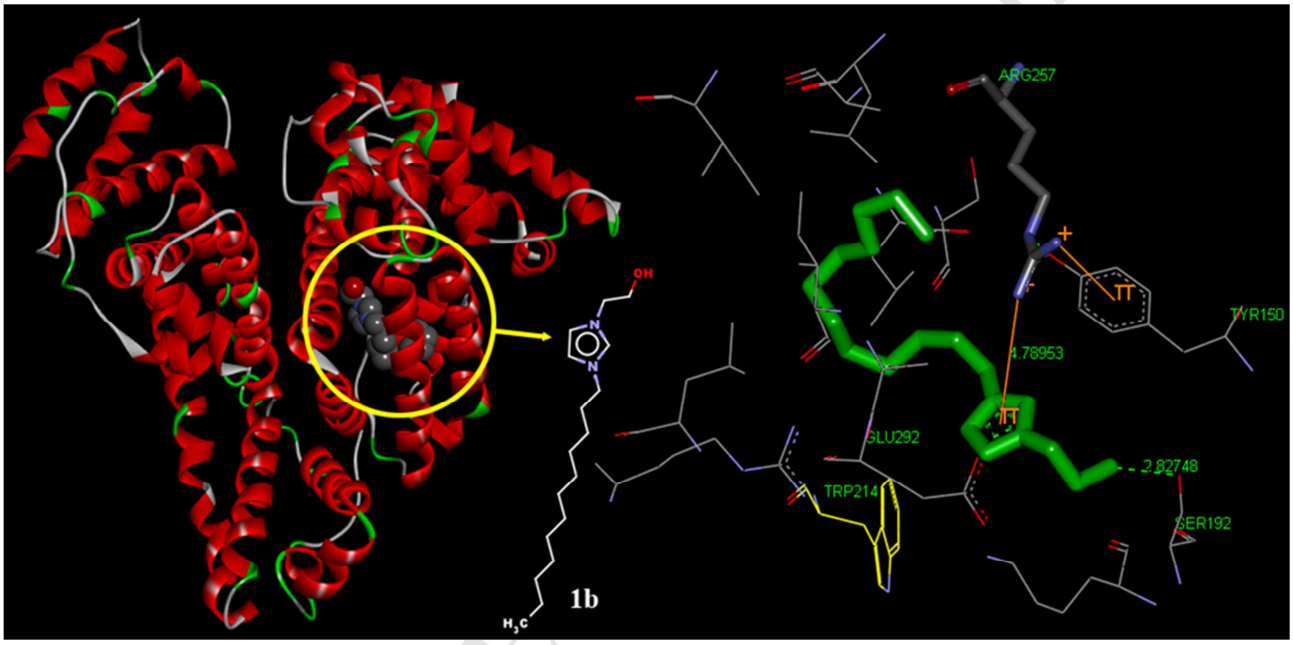

Fig. 5. Molecular docking of imidazolium ionic liquid [$C_2OHC_{12}IM$]Cl and HSA.

Molecular docking of the compound **2** and protein indicates the presence of two cation-π bonds: 4.31 Å and 5.48 Å length (Fig. 6). The first bond is between the pyridine ring of the compound **2** and the NH-group of amino acid residue Lys195. The second bond is between the pyridine ring of the compound **2** and the NH-group of Arg257. The predicted binding energy of the complex is –6.3 kcal/mol. The stability of this complex is determined by the presence of two cation-π bonds, similarly as in the first protein-ligand system with compound **1a**.

Thus, we suppose that the binding region of studied ILs is located about 10-12Å from Trp214, between amino acid residues Lys195 and Arg257 (Fig. 7).

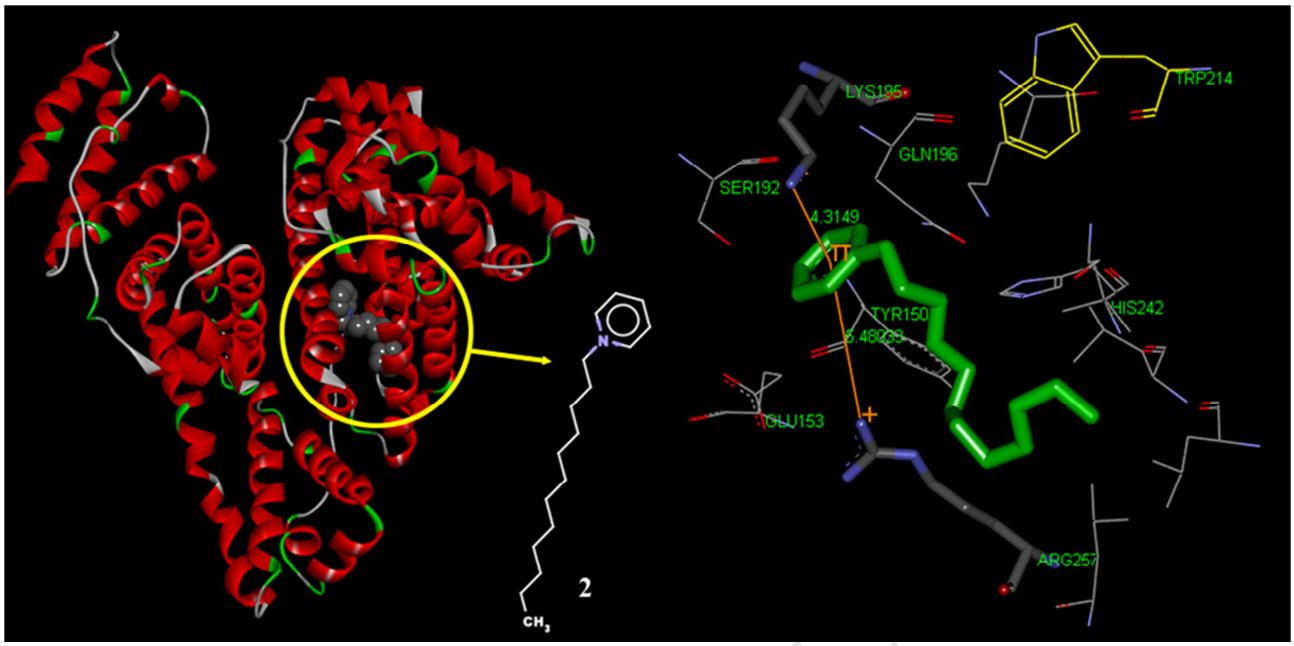

Fig. 6 Molecular docking of pyridinium ionic liquid [C_{12}Pyr]Cl and HSA.

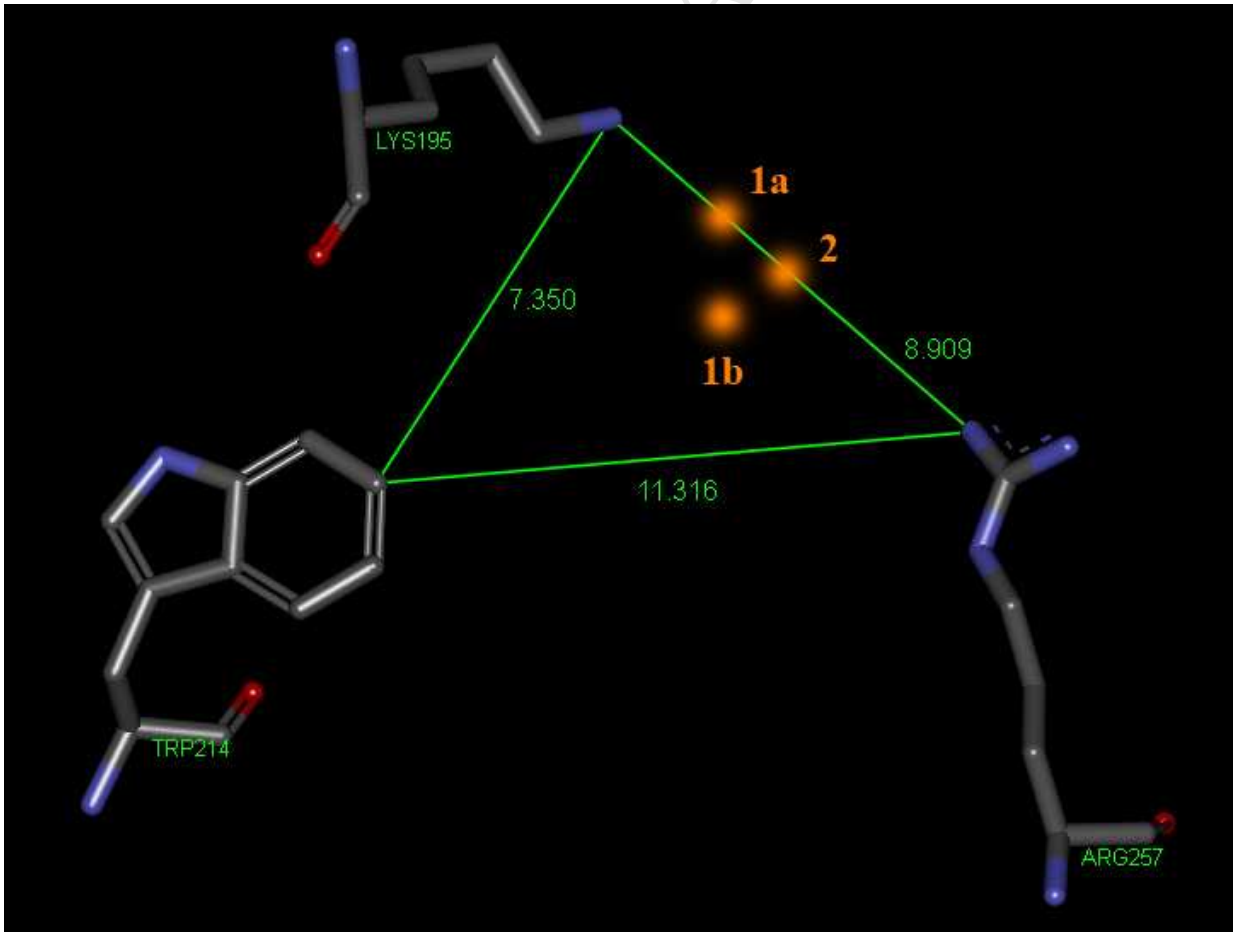

Fig.7 The binding region of studied ILs.

4. Conclusions

The aim of this study was to evaluate the antimicrobial activity of imidazolium and pyridinium based ionic liquids and the interaction between ILs and HSA using FT-IR spectroscopy and molecular docking method. All compounds showed good antibacterial and antifungal activities. The results obtained from FT-IR spectra indicated that the binding of ILs to HSA induce the conformational changes in protein secondary structure. The molecular docking results displayed that 1-dodecyl-3-methylimidazolium chloride, 1-(2-hydroxyethyl)-3-dodecylimidazolium chloride, 1-dodecylpyridinium chloride are able to bind in the II-A binding site HSA with predicted binding affinities of -6.2, -6.2, -6.3 kcal/mol respectively. We believe that binding region between amino acid residues Lys195 and Arg257 is typical for interaction HSA with ILs that have a similar structure and can form the quite stable complexes. The obtained results can be a useful supplement to investigate and develop future pharmaceutical applications of imidazolium and pyridinium based ILs as potential antimicrobial agents.

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Highlights

- Antimicrobial activities of the ionic liquids against *S. aureus* (ATCC 25923), *Ps. aeruginosa* (ATCC 27853) and *C. albicans* (M 885 ATCC 10231) were investigated.

- The interaction between HSA and ionic liquids was studied by spectroscopic techniques and molecular docking analysis.

- The secondary structure of protein has been changed upon the interaction with investigated compounds.

- Investigated ILs binds in IIA binding site of A-subunit of HSA molecule.