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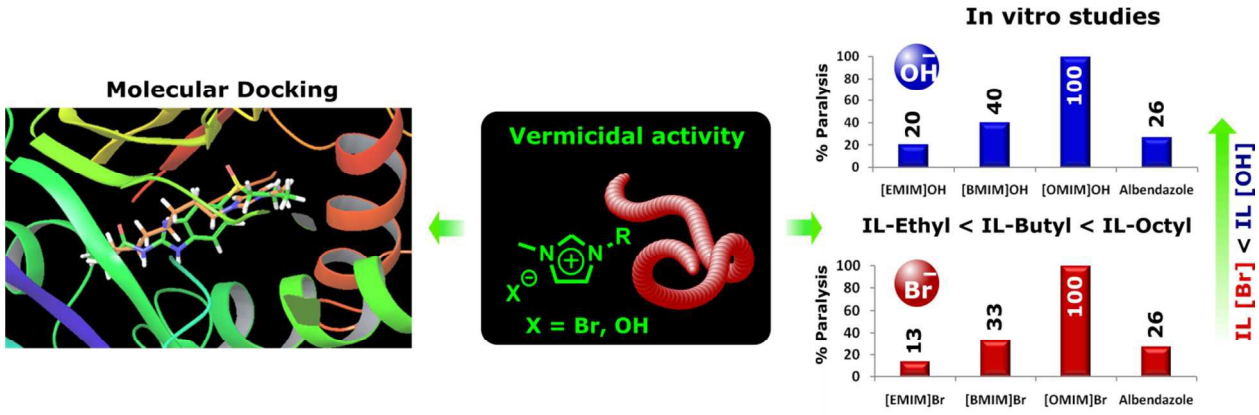
Graphical Abstract



Evaluation of Imidazolium-based Ionic Liquids towards Vermicidal Activity: *In vitro & In silico studies*

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Abstract

A series of six different ionic liquids (ILs) of the kind 1-alkyl-3-methylimidazolium bromide/hydroxide (**IL-Br** and **IL-OH**) tailored with different *N*-alkyl side chains (ethyl, butyl, octyl) were synthesized and evaluated for their vermicidal activity against Indian earthworm, *Pheretima posthuma*. The percentage of paralysis and mortality of earthworms against ILs were recorded in dose dependent (at different concentrations 2, 4, 8 and 16 mM) and time dependent manner. ILs with hydroxide as counter anion (**IL-OH**) showed higher vermicidal activity compared to their bromide counter parts (**IL-Br**). Moreover, ILs with the longest alkyl chain (octyl) are observed with significant vermicidal activity compared to the rest (ethyl and butyl) as well as the standard drug, Albendazole. Furthermore, theoretical modeling was carried out to visualize the preferential docking positions of these ILs into the active site of β-Tubulin. Fascinatingly, it was found that ILs with longest alkyl side chain showed remarkable vermicidal activity compared to the rest and the molecular docking studies not only validated the

experimental results but also showed per residue interaction analysis between ILs and different components of β-Tubulin.

Key words

Vermicidal activity, Ionic liquids (ILs),*Pheretima posthuma* earthworms, Percentage of paralysis, Percentage of mortality, Molecular docking, β-Tubulin

1. Introduction

Ionic liquids (ILs) are well known as green solvents[1] which are widely used as benign alternatives to conventional organic solvents and has gained considerable global attention due to their diverse applications in various fields of chemical sciences.[2-7] Since the past few decades, they have attracted significant attention of the researchers owing to their unique physiochemical properties[8] such as low vapour pressure, recyclability, non-volatility, and non-combustibility. In general, ILs are composed of organic cations such as imidazolium,[9,10] pyridinium,[10-12] phosphonium,[13] and ammonium;[14]and inorganic anions such as halides, triflates, and natural amino acids. ILs are prominently known for their "tunable nature" through which their properties can be easily tuned by independently varying the counter anions and alkyl side chain length.[15] In this context, various scientists have explored the potential application of ILs as solvents in organic reactions[16] and polymerization processes[17] and as electrolytes in analytical chemistry[18] by tuning their physiochemical properties. Furthermore, ILs are found to possess significant biological activity against a variety[9-12, 14] of bacteria and fungi as well as human tumour cell lines.[13, 19] Interestingly, the activity of ILs was noticed to increase with an increase in the *N*-alkyl side chain length and variation in the counter anions. For instance, *Pernak et al* observed an increase in the antimicrobial activity of different systems of ILs such as imidazolium,[9,

[10]pyridinium,[10-12] ammonium,[14] and benzimidazolium[12] type with an increase in the alkyl chain length against several micro-organisms. Moreover, a notable change in their antimicrobial activity has also been observed by varying the counter anions such as halides, acetate, tetrafluoroborate, nitrate, and perchlorate.[11]This tunable property of ILs tailored with biologically active cations or anions can greatly add to their administration in Active Pharmaceutical Ingredients (APIs), where the therapeutic effects of both the cationic and anionic part in ILs are expected to influence largely which has been elaborately discussed in a review byFerraz and co-workers.[20-22]Though the toxicity of ILs is the most debated topic in their application as API, several efforts are being made to afford ILs with a balance between their toxicity and biochemical and biopharmaceutical properties. Hence, the tuning of biological activity of ILs by varying their solubility, counter anions and alkyl chain length would reflect their therapeutic application in a greater perspective.[21, 22]

On the other hand, vermicidal infections are most common and widespread infections prevalent in rural areas than that of urban areas due to poor hygienic conditions and affect a large population of the world. A usual fatal disease, Helminthiasis caused by helmintic worms such as pin worm, hook worm, round worm and tape worms cause major part of the damage such as Pneumonia, Eosinophilia, Malnutrition, and Anaemia.[23,24]Albendazole is a standard anthelmintic drug, widely administered for the treatment of helmintic infections. However, these helmintic parasites are getting resistant to the currently available standard anthelmintic drugs.[25] Hence, there is an increasing need for the implementation of more effective drugs with excellent therapeutic activity for the elimination of these harmful parasites. This necessity has motivated us to explore the vermicidal activity of imidazolium-based ILs tailored with varying alkyl chain length and counter anions. Though, there are several reports regarding the antimicrobial, anti-

biofilm, and antitumor activity of ILs; to the best of our knowledge, the vermicidal activity of ILs has been unexplored till date. In this contribution, we have investigated the vermicidal activity of a series of ILs of the kind 1-alkyl-3-methylimidazolium bromide/hydroxide (**IL-Br** and **IL-OH**) *N*-alkylated with different side chain lengths and compared with the standard drug, Albendazole. The experiments carried out *in vitro* conditions showed a significant variation in vermicidal activity of ILs with respect to the change in alkyl side chain length as well as counter anions. Moreover, the binding energy values obtained from the molecular docking studies were found to be in good agreement with the experimental results.

2. Experimental

2.1 Materials and methods

1-Methylimidazole, *n*-ethyl bromide, *n*-butyl bromide, *n*-octyl bromide, and deuterated solvents were purchased from Sigma-Aldrich and used as received. Albendazole was purchased from Hi-media, India. All the organic solvents were purchased from SD Fine and distilled prior to usage. ^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER (400 MHz) in appropriate deuterated solvents. UV-spectra were recorded on Jasco V-670 instrument. LC-MS spectra were recorded on Quattro LC and ESI mass spectra were recorded on Thermo F Innigan.

2.2 Synthesis of ionic liquids (ILs)

The three ILs with bromide as counter anion (IL-Br) namely 1-ethyl-3-methylimidazolium bromide [EMIM]Br, 1-butl-3-methylimidazolium bromide [BMIM]Br and 1-octyl-3-methylimidazolium bromide [OMIM]Br were prepared according to literature report.[26] A representative procedure for the synthesis of [EMIM]Br is as follows: A dry flask was sealed after charging with 0.98 g (12 mmol) of 1-methyl imidazole and 1.526 g (14 mmol) of *n*-ethyl

bromide and was heated in an oil bath maintained at 140 °C for 10 minutes and cooled to room temperature. Once again the above reaction mixture was heated at the same temperature for 10 minutes and then cooled to room temperature. The obtained IL was washed with ethyl acetate (3 x 25 mL) to remove unreacted starting materials, vacuum dried and characterized using NMR and mass spectroscopy. Initially [EMIM]Br and [EMIM]Br were obtained as light yellow solids, which became viscous liquids during the manipulation due to their hygroscopic nature. The rest of ILs were prepared in similar fashion. ILs with hydroxide as counter anion such as **(IL-OH)**1-ethyl-3-methylimidazolium hydroxide [EMIM]OH, 1-butyl-3-methylimidazolium hydroxide [BMIM]OH and 1-octyl-3-methylimidazolium hydroxide [OMIM]OHwere prepared as per the literature report with minor modifications.[27] Initially, 3 g of the Amberlite® IRA-400 chloride resin was taken in a round bottomed flask containing 100 mL of 1N NaOH and stirred for 12 hours. After anionic exchange, the resin was washed with double distilled water till pH 7 was reached and then, filtered and dried which resulted resin with hydroxide anions. A representative example for the synthesis of [EMIM]OH is as follows: 5 g of [EMIM]Br was dissolved in sufficient amount of methanol and passed slowly through a column packed with Amberlite® IRA-400 hydroxide resin. The solvents were rotar evaporated from the collected solution and then vacuum dried which afforded 1-ethyl-3-methylimidazolium hydroxide as a viscous liquid in quantitative yields. The other two ILs ([BMIM]OH, [OMIM]OH) were prepared in similar fashion by simple anion metathesis from their bromide counter parts (**Scheme 1**).

2.3 Vermicidal activity

2.3.1 Test worms

Indian adult earthworms, *Pheretima posthuma* were employed for the screening of vermicidal property of ILs due to their physiological and anatomical resemblance with intestinal parasitic worms and round worms of human beings. The earthworms were collected from Christian Medical Hospital, Vellore, India and cleaned with saline to remove all faecal matter.

2.3.2 Positive and negative control

ILs and Albendazole were used as positive control and sterilised distilled water was used as negative control against the earthworms.

2.3.3 Vermicidal assay

The vermicidal activity was performed according to the literature procedure with minor modifications.[28] Earthworms of the size 8±1 cm were employed for the activity. The worms were acclimatized to the laboratory conditions before experimentation. The earthworms were divided into five groups containing five worms in each group were placed in each petriplate and four different concentrations i.e.,2, 4, 8 and 16 mM of ILs were employed for this activity. Each test sample (10 mL) of respective concentration was poured into each petriplate containing earthworms. The experiment was performed in triplicates to confirm the results and the mean values were taken. Earthworms after being exposed to ILs and positive control exhibited paralysis. After getting paralysed, some of the earthworms died due to high vermicidal activity of the test samples. The worms which showed little movement upon gentle stimulation were identified as paralyzed worms; whereas, those with no movement were transferred to a beaker containing water at 50 °C which stimulated the movements if the worms were alive. Therefore,

those worms which ultimately showed no movement were identified as dead ones. The percentage of paralysis and percentage of mortality were noted at different time intervals of 15, 30, 45, 60, 75, 90, and 120 minutes.

2.4 Molecular docking

Molecular docking is a routinely adopted theoretical modeling approach to predict the preferred orientation of small molecules into their protein targets which when bound to each other will form a stable complex. This knowledge is very essential to predict the strength of association between two molecules. Therefore, in order to understand the relative difference in binding affinity among the synthesized ILs, molecular docking studies were performed on the X-ray crystal structure of β-tubulin bound to Albendazole Sulphoxide (www.rcsb.org; PDB ID: 1OJ0).The program Glide (Grid-Based Ligand Docking with Energetics) incorporated in the Schrödinger molecular modeling package (Schrödinger, Inc., USA, *version- 2014-14*) installed on a Windows workstation with an Intel (R) xenon 2.8 GHz processor and 32 GB physical memory was used to perform the molecular docking study.[29,30] The details of the experimental set-up for the molecular docking study can be found in the supporting information.

In order to ensure that the docking protocol correctly predicts the binding modes of the molecules to its target receptor β-tubulin, the protocol adopted herein was validated by reproducing the experimentally observed binding pose of the native ligand, Albendazole. It was observed that the best scoring docking solutions for the native ligand were all within 1.0 Å RMSD of the crystal structure. The best scoring pose for Albendazole produced by docking has been compared with the crystal structure in **Fig. 1**.

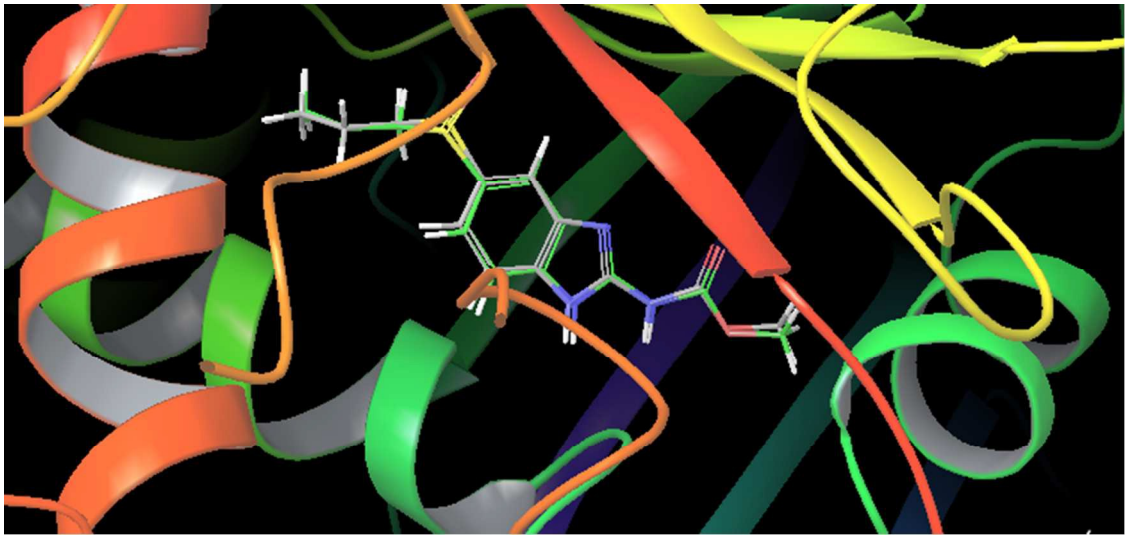
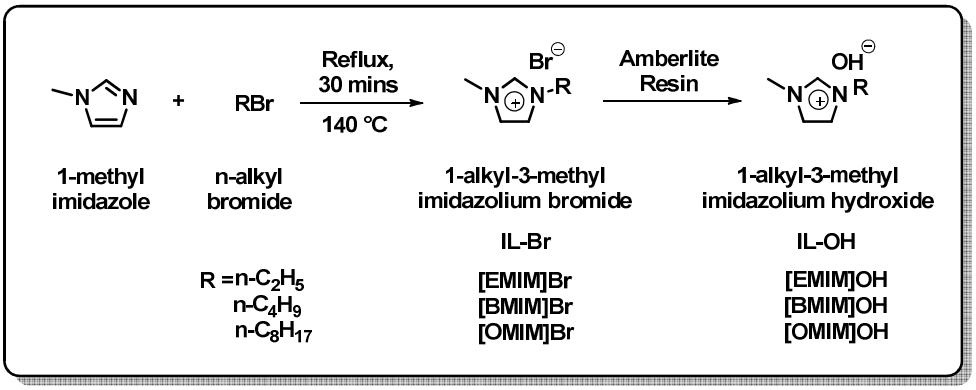


Fig. 1 Overlay of the X-ray conformation of Albendazole over its best docked conformation with it target receptor β-tubulin.

3. Results and discussion

3.1 Vermicidal activity

Imidazolium-based ILs [EMIM]Br, [EMIM]OH, [BMIM]Br, [BMIM]OH, [OMIM]Br and [OMIM]OH were prepared as per the procedure (**Scheme 1**) in their purest form.



Scheme 1. Synthetic scheme for imidazolium-based ILs used in this study.

Albendazole is the most widely administered anthelmintic drug containing an uncharged imidazole ring unlike ILs which contain an imidazolium moiety as shown in Fig. 2. This has prompted us to explore the vermicidal activity of six different imidazolium-based ILs by varying the alkyl chain length (ethyl, butyl and octyl) and counter anions (bromide and hydroxide).

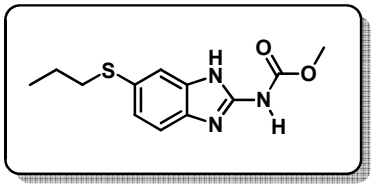


Fig.2 Structure of Albendazole.

Vermicidal activities of ILs were carried out against Indian earthworm *Pheretima posthuma*. Vermicidal effect of the ILs was noted as a function of percentages of paralysis and mortality of

earthworms at four different concentrations i.e., 2, 4, 8 and 16 mM (**Fig. 3** and **4**). 10 mL IL of respective concentration were poured into each Petri plate containing earthworms. Some of the earthworms got paralysed due to vermicidal activity of the employed ILs. The percentage of paralysis and percentage of mortality of earthworms were noted at regular time intervals of 15, 30, 45, 60, 75, 90 and 120 minutes and the values are given in the supporting information (see**Table S1**). The experiments were performed in triplicates.

Paralyses of earthworms were noticed with all the six ILs even at very low concentration of 2 mM. But, the percentage of paralysis of earthworms varied drastically with respect to the concentration as well as the type of IL employed. As expected, the vermicidal activity of ILs increased with increase in IL concentration in all the above experiments. A steady increase in vermicidal activity was noticed (**Fig.3**) with increase in the N-alkyl chain length from ethyl to butyl to octyl in the ILs (same trend was noticed in both **IL-Br** and **IL-OH**). A maximum of 20 % paralysis of earthworms was observed with a low concentration (2 mM) of [OMIM]OH within 15 minutes and the percentage of paralysis reached to 100% within 45 minutes. Also, the counter anion in the ILs has played significant role towards vermicidal activity. From **Fig. 3**and**4,** it is clearly seen that imidazolium systems with hydroxide as counter anion (**IL-OH)** exhibited higher activity compared to their bromide counter parts (**IL-Br)**. The vermicidal activity of ILs is influenced by both N-alkyl side chain as well as counter anion. The mortality of earthworms was noticed with all the six ILs employed. A maximum of 40 % mortality was noticed for [OMIM] OH at 45 minutes with a concentration of 2 mM and the percentage of mortality reached 100 % within 90 minutes. Among six ILs that were applied in the present study, [OMIM] OH showed highest vermicidal activity.

The percentage of paralysis and mortality of the six synthesized ILs were compared with that of standard vermicidal drug, Albendazoleat a uniform concentration of 4 mM in different time intervals (15, 30, 45 and 60 minutes) as illustrated in **Fig. 5**. Here, it is noticed that though all the ILs showed noticeable percentage of paralysis at this concentration in different time intervals; impressively, [OMIM]OH alone reached 100 % paralysis within 45 minutes whereas Albendazole showed a maximum of 20 % in 120 minutes. On the other hand, [OMIM]OH alone showed 20, 40 and 80 % mortality in 30, 45 and 60 minutes whereas the rest of ILs and Albendazole showed 0 % mortality at this concentration and time intervals. These imidazolium-based ILs exhibited significant vermicidal activity even at low concentration (0.5g/mL) compared to the plant extracts such as *Tamarindus indica* (15 mg/mL)[31] and *Piper betle* (50 mg/mL)[32] which showed considerable activity at high concentrations.

The physiochemical characteristics such as hydrophobicity, lipophilicity and concentration of the anthelmintic compound and the structural features of the parasite are responsible factors for the diffusion through the bio-membrane of parasitic worm.[33] Though imidazolium-based ILs with bromide and hydroxide anions are water soluble (hydrophilic), the degree of hydrophobicity (hydrocarbon ratio) increases with increase in the alkyl chain length in the cationic position.ILs with the longest alkyl chain (octyl) are observed with utmost vermicidal activity compared to the rest (ethyl and butyl) which may be ascribed to the increase in the degree of hydrophobicity with respect to the alkyl chain length (hydrocarbon ratio). We believe that this elevated levels of hydrophobicities in [OMIM]Br/OH ILs may enhance their easy diffusion across the phospho-lipid bilayer of parasitic worms compared to the rest with lower hydrophobicity. Here, we highlighted the potentialof ILs as active anthelmintic agents and moreover, their synthesis is much easier, less tedious and economic compared to that of plant extracts. As ILs possess a

unique property i.e., “tunable nature”, the vermicidal activity of ILs could be regulated by tailoring them with desired counter anions and alkyl side chains. Further studies can be made to explore the drug design, mechanism of action, structure-activity relationship, prosperities and consequences of employing imidazolium-based ionic liquid in therapeutic applications.

On the other hand, a possible explanation about the biochemical mode of action of the anthelmintic drugs on the parasitic worms may be given according to the literature reports.[34-42] The anthelmintic drug suppresses the β-tubulin polymerase enzyme consequently leading to the decrease in the uptake of glucose which eventually results in the death of parasite due to the exhaustion of glycogen levels/storage. As the alkyl chain length increases, the hydrophobicity of IL increases and thereby it damages the cell wall of the earthworm which results in the damage of the cutaneous layer of the worm and the subsequent leakage of the body fluids eventually leads to death of the worm. Earthworms have been specifically chosen for the vermicidal activity as they fall under a higher phylum than platyhelminthis and nematihelminthis which have psuedoseal. This report motivated us to evaluate the mode and relative strength of binding for the ILs against the β-tubulin structure using the molecular docking approach.

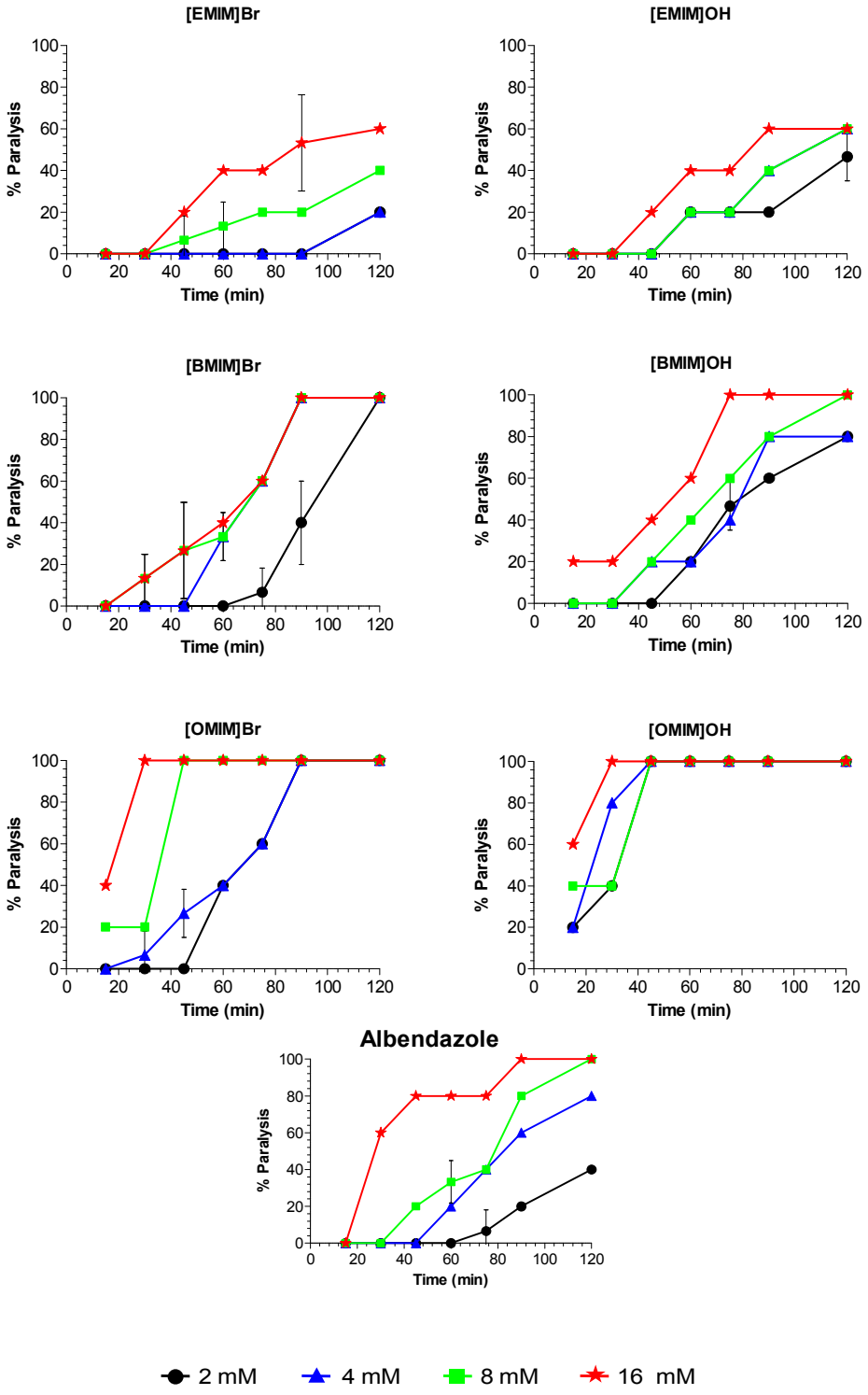


Fig. 3 Graphical representation of percentage paralysis of earthworms on treatment of ILs ([EMIM]Br, [EMIM]OH, [BMIM]Br, [BMIM]OH, [OMIM]Br [OMIM]OH) and Albendazole at four different concentrations 2, 4, 8 and 16 mM as a function of time.

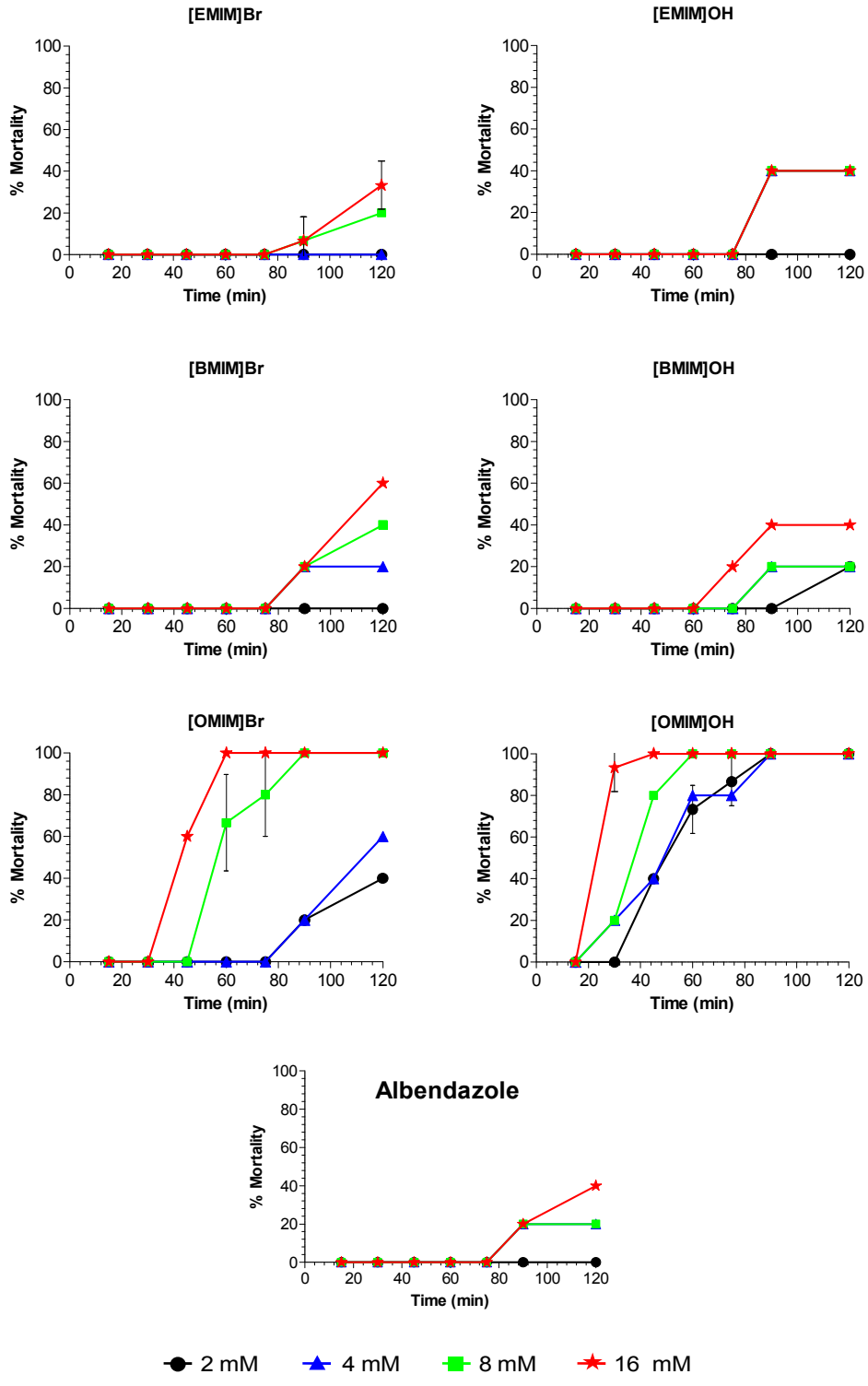


Fig. 4 Graphical representation of percentage mortality of earthworms on treatment of ILs [EMIM]Br, [EMIM]OH, [BMIM]Br, [BMIM]OH, [OMIM]Br,[OMIM]OH and Albendazole at four different concentrations 2, 4, 8 and 16 mM as a function of time.

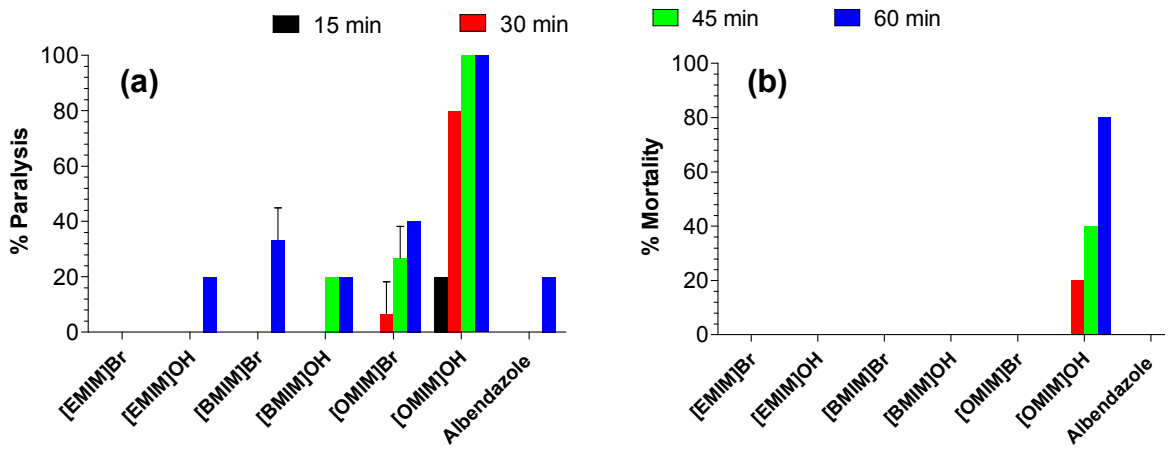


Fig. 5 Comparison of(**a**) percentage of paralysisand (**b**) percentage of mortality of earthworms against six ILs and Albendazoleat 4 mM concentration in 15, 30, 45 and 60 minutes.

3.2 Molecular Docking

Table 1. Quantitative analysis of the docking study– Glide score, Glide energy and non-bonded interactions (van der Waals and electrostatic) regarding the vermicidal activity of ILs.

S.No	ILs/Employed drug	% Paralysis of earthworms after 15 minutes#	Glide score	Glide Energy (kcal/mol)	vdW energy (kcal/mol)	Columbic energy (kcal/mol)
1	[EMIM]Br	0	-6.083	-105.428	-78.076	-27.352
2	[EMIM]OH	20	-9.15	-116.465	-86.698	-29.766
3	[BMIM]Br	33	-11.487	-123.892	-93.288	-30.604
4	[BMIM]OH	20	-10.414	-121.656	-89.944	-31.712
5	[OMIM]Br	40	-12.272	-125.856	-94.231	-31.625
6	[OMIM]OH	100	-14.504	-149.786	-113.956	-35.83
7	Albendazole	20	-13.034	-129.866	-98.346	-31.52

= experimentally determined at a concentration of 4 mM

A very good harmony was observed between the experimentally observed vermicidal activity and the theoretical predictions from docking study with the most active ILs showing the highest binding affinity (docking scoreand binding energy) while those exhibiting weakervermicidal

activity were also predicted to have a lower docking score as well as the binding energy. Additionally the molecular docking study could also provide an insight into the mode of interaction between the ILs and the β-tubulin receptor present in the earthworms.The variation observed in the binding affinity of these ILs towards the β-tubulin receptor has been interpreted based on three main parameters- Glide score (docking score), Glide energy (binding energy) and the non-bonded interactions (van der Waals and Columbic) (**Table 1**). The ionic liquids are ranked on the basis of the Glide score and their vermicidal activity expressed as percentage paralysis of earthworms after 15 minutes.**Fig.6** shows the correlation plots of the percentageparalysis of earthworms observed after 15 minutes (as a representative case)versus the Glide docking score of the IL-Br, IL-OH along with standard drug Albendazole. It is clear from the plots that the ILs with the longest alkyl chain (octyl) are observed with utmost docking score compared to those with ethyl and butyl. Also the ILs with hydroxide as counter anion (**IL-OH**) showed higher docking score compared to their bromide counter parts (**IL-Br**).

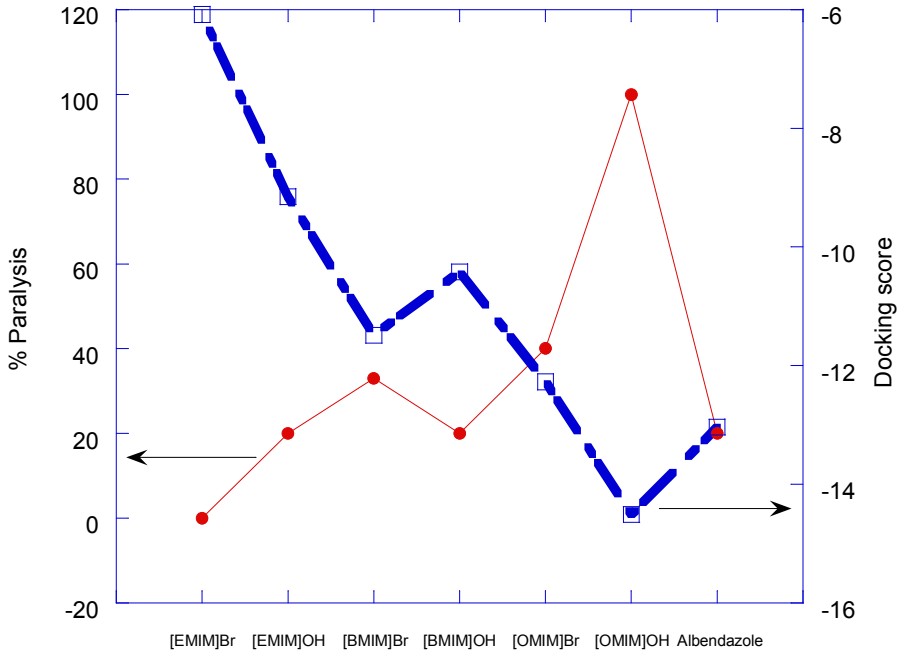


Fig. 6 The correlation plots of the percentage paralysis of earthworms (after 15minutes) and the Glide docking score of the IL-OH, IL-Br and Albendazole.

A detailed per-residue interaction analysis between the β-tubulin receptor and the different ILs was carried out to gain an insight into factors governing the variation observed in the experimental vermicidal activity. However, for the sake of brevity we have compared the results only for the most active ionic liquid **[OMIM]OH**, and the least active ionic liquid having hydroxide as counter anion **[EMIM]OH**. Analysis of the docking poses of **[OMIM]OH** and **[EMIM]OH** shows that they occupy the binding site of β-tubulin in the coordinates very close to that of Albendazole in the crystal structure (**Fig. 7**). The RMSD between imidazolium scaffolds for both ILs was observed to be 0.4Å. However, it was the associated side chain with these ILs that contributed to the variation in the observed binding affinity. The binding site analysis clearly deciphers that **[OMIM]OH** occupies a large space in the binding site owing to its extended alkyl chain compared to **[EMIM]OH** resulting in additional thermodynamic interactions and higher

proximity to the active site residues consequently resulting in higher docking score (-14.504) than **[EMIM]OH** (-9.15). Inspection of the non-bonded interaction energies revealed that for both the ILs, the van der Waals interaction energy component dominated over the electrostatic component in the overall binding energy (Table 1). The per residue interaction analysis for **[OMIM]OH** showed that the enhanced binding affinity towards β-tubulin is attributed to the extensive network of favourable van der Waals and electrostatic interactions observed with the residues comprising the active site (Table 2) (**Fig.7**). Furthermore a very significant π-π stacking interaction was observed between the heterocyclic scaffold of [OMIM]OH and the phenyl rings in Phe200 and Phe167 adding to the stability of the complex.

Even after defining a sufficiently large grid of 14×14×14Å to explore a large portion of the receptor, interesting all the ionic liquids were seen to occupy the binding site of β-tubulin in the coordinates very close to that of Albendazole suggesting this should be their preferred active site (Fig. 8). Furthermore comparison of the binding mode of the most active ionic liquid [OMIM]OH with Albendazole showed that the linear backbone of the [OMIM]OH resulted in a closer association with the β-tubulin structure. This could be one reason for the relatively tighter binding observed for [OMIM]OH (-149.786kcal/mol) over Albendazole (-129.866 kcal/mol). Furthermore the distances between the interacting residues and the two ligands ([OMIM]OH and Albendazole) was also measured to gauze the proximity with the receptor surface. The measurements observed for [OMIM]OH and Albendazole are given in table 2. The difference binding affinity can be further explained in terms of the specific bonded and non-bonded per-residue interactions observed with the residues in the active site (Table 2). It is noteworthy that while the ionic liquids were anchored into the active site through two prominent pi-pi stacking interactions via Phe200 and Phe167, one of these interactions i.e. with Phe167 was missing in

case of Albendazole. Therefore, relative higher values observed for the per-residue interactions along with more proximity to the binding site residues may increase the binding affinity of the [OMIM]OH significantly as indicated by a higher docking score as compared to that of the Albendazole.

On the other hand, a similar per residue interaction analysis for **[EMIM]OH** with the residues comprising the active site was carried out to obtain the quantitative estimate of these thermodynamic interactions(Table 2) (**Fig.9**). It is clear from the per-residue interaction energy breakup that significantly higher values observed for **[OMIM]OH** contributed to its stronger binding affinity to β-tubulin over **[EMIM]OH** which is in agreement with the observed vermicidal activity.

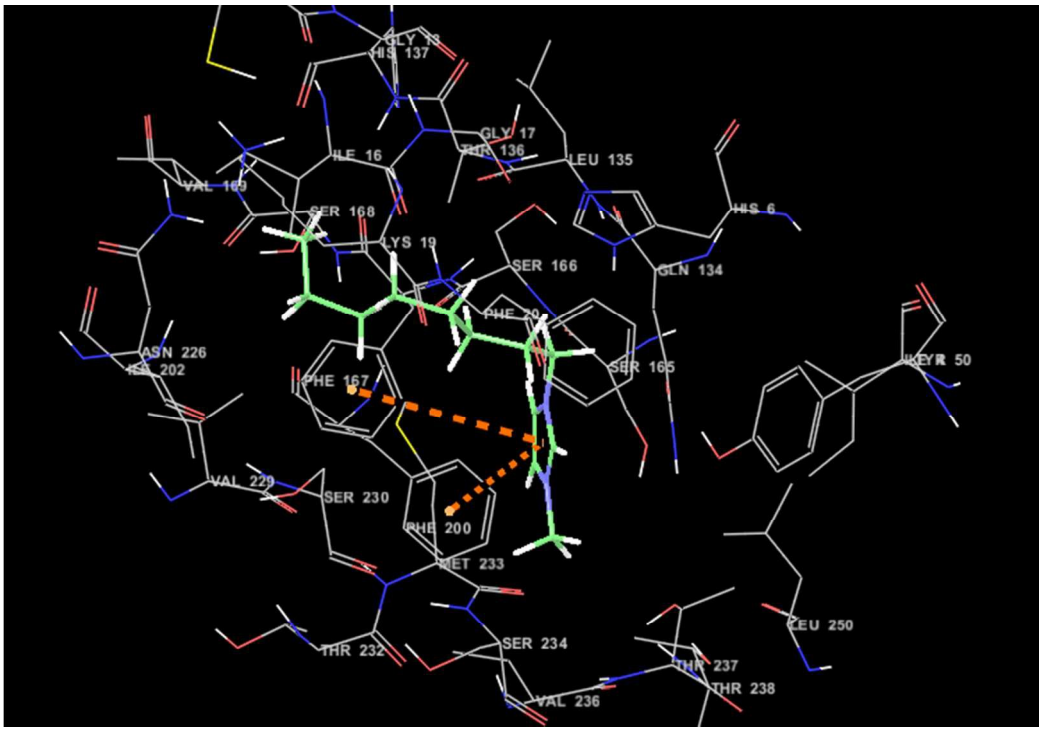


Fig. 7 Binding mode of [OMIM]OH into the active site of β-tubulin ((image displays the interactions with the residues within 5Å distance of [OMIM]OH). Dotted lines represent the pi-pi interactions.

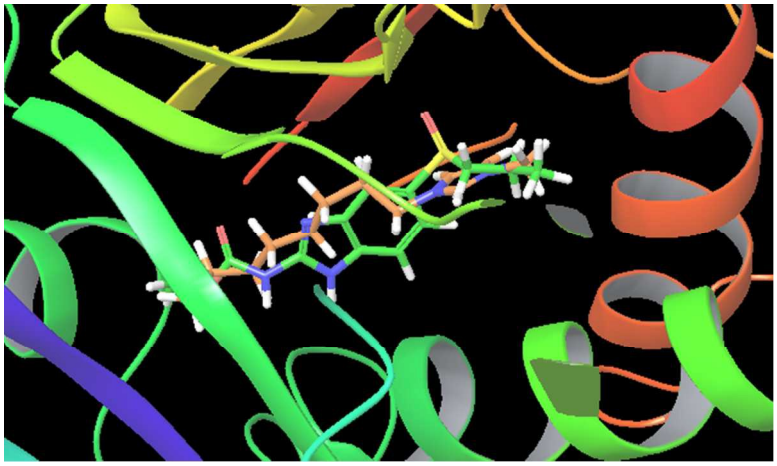


Fig. 8 Overlay of the docking poses at the bonding sites of [OMIM]OH and Albendazole with β-tubulin.

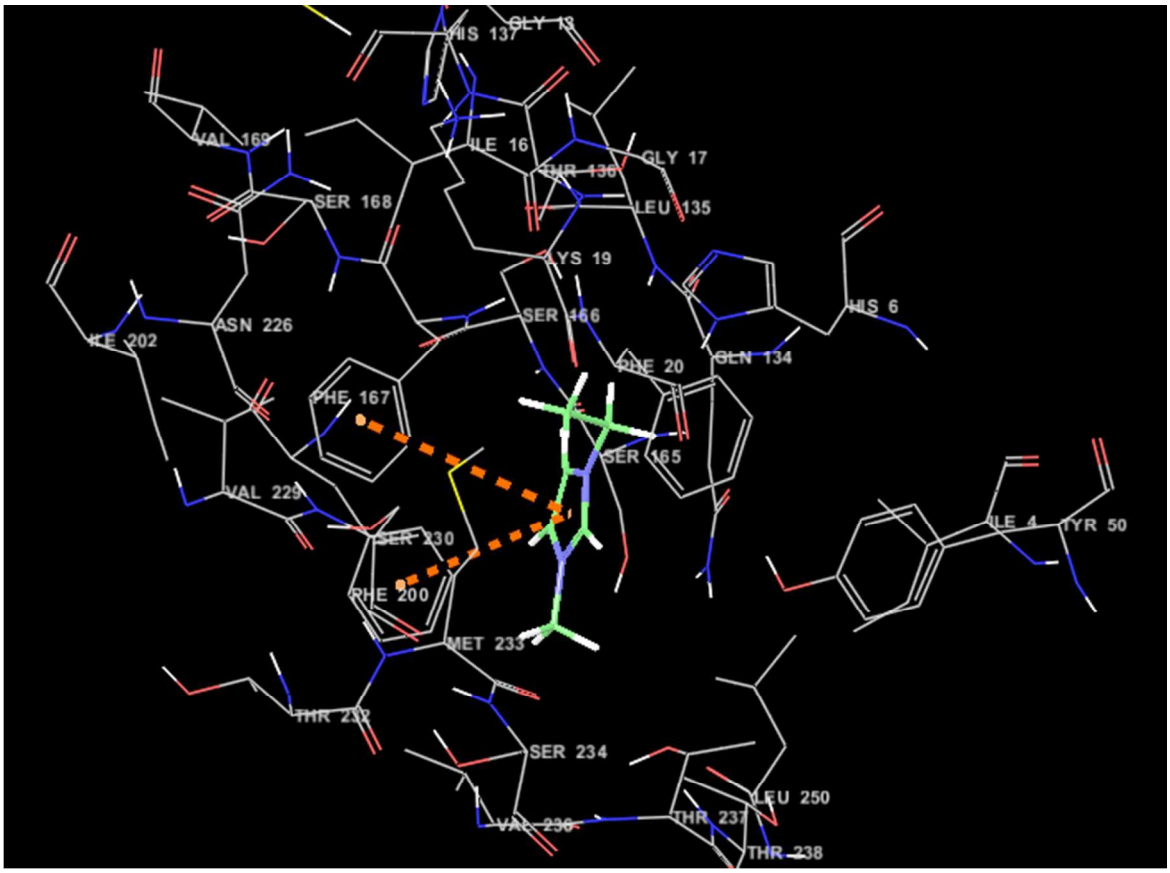


Fig. 9 Binding mode of [EMIM]OH into the active site of β-tubulin. (Image displays the interactions with the residues within 5Å distance of [EMIM]OH). Dotted lines represent the pi-pi interactions.

Table 2: Results of per-residue interaction analysis and distance between interacting residues and ligands from molecular docking studies observed for the ionic liquids and compared with the reference standard Albendazole.

	Per-residues interactions			Distance between interacting residues and ligands (Å)
	van der Waals (kcal/mol)	**Electrostatic (kcal/mol)**	**Pi-Pi Stacking**	
[OMIM]OH	Val236 (-3.721), Met233 (-3.74), Thr232 (-2.159), Ser230 (-2.176), Phe200 (-3.447), Phe167 (-3.636), Ser165 (-1.93), His137 (-2.035), Thr136 (-2.642), Leu135 (-2.228), Gln134 (-4.165), Phe20 (-3.106), Ile16 (-2.095), Lys19 (-2.028), Gly13 (-3.021) and Ile4(-2.157)	Val236 (-1.89), Met233 (-1.93), Ser230(-2.15), Phe200(-1.956), Ser165 (-1.761), His137 (-1.217), Thr136(-2.31), Gln134(-1.468), Phe20(-1.465), Ile16(-1.524), Gly13(-1.46)	Phe200 and Phe167	Val236 (1.856Å), Met233 (1.3Å), Thr232 (2.704Å), Ser230 (2.671Å), Phe200 (1.88Å), Phe167 (1.129Å), Ser165 (1.678Å), His137 (2.22 Å), Thr136 (1.925Å), Leu135 (1.798Å), Gln134 (1.392Å), Phe20 (1.858Å), Lys19 (3.447 Å), Ile16 (2.951Å), Gly13 (2.274Å) and Ile4(5.409Å)
[EMIM]OH	Val236 (-2.462), Met233 (-2.209), Thr232 (-1.205), Ser230 (-1.089), Phe200 (-2.804), Phe167 (-2.045), Ser165 (-1.053), His137 (-1.00), Thr136 (-1.315), Leu135 (-1.107), Gln134 (-3.581), Phe20 (-2.133), Ile16 (-1.268), Lys19 (-1.026), Gly13 (-1.014) and Ile4(-1.167)	Val236 (-1.021), Met233 (-1.038), Ser230(-1.323), Phe200(-1.471), Ser165 (-1.156), His137 (-1.008), Thr136(-1.188), Gln134(-1.277), Phe20(-1.01), Ile16(-1.103), Gly13(-1.116)	Phe200 and Phe167	-n.d-
Albendazole	Val236 (-3.295), Met233 (-3.49), Thr232 (-1.814), Ser230 (-1.82), Phe200 (-2.773), Phe167 (-3.517), Ser165 (-1.719), His137 (-1.597), Thr136 (-1.199), Leu135 (-1.484), Gln134 (-3.911), Phe20 (-2.489), Lys19 (-1.98), Ile16 (-1.839), Gly13 (-2.086) and Ile4(-1.833),	Val236 (-1.124), Met233 (-1.654), Ser230(-1.602), Phe200(-3.303), Ser165 (-1.562), His137 (-1.15), Thr136(-2.01), Gln134(-1.369), Phe20(-1.201), Ile16(-1.238), Gly13(-1.321)	Phe200	Val236 (2.337Å), Met233 (2.512Å), Thr232 (4.57Å), Ser230 (5.408Å), Phe200 (3.026Å), Phe167 (2.468Å), Ser165 (2.619Å), His137 (3.652Å), Thr136 (3.051Å), Leu135 (3.826Å), Gln134 (2.773Å), Phe20 (3.003Å), Lys19 (4.047Å), Ile16 (4.021Å), Gly13 (4.953Å) and Ile4(5.451Å)

4. Conclusion

In this study, vermicidal activity of imidazolium-based ILs against Indian earthworm *Pheretima posthuma* was carried out. Paralyses of earthworms were noticed with all the six ILs that were employed for this study, even at low concentrations2 mM. As the *N*-alkyl side chain length increased from ethyl to octyl in both **IL-Br** and **IL-OH**, an increase in vermicidal activity is noticed (IL-Octyl> IL-Butyl > IL-Ethyl). Also, **IL-OH** exhibited significant vermicidal activity

compared to **IL-Br**. Hence, it is obvious that the vermicidal activity of ILs is strongly dependent on nature of the *N*-alkyl side chain length as well as counter anion. [OMIM]OH showed highest activity than remaining ILs and the standard anthelmintic drug, Albendazole. The experimental findings were in good agreement with the molecular docking studies which clearly explained the influence of the extended alkyl chain associated with the imidazolium scaffold on the binding affinity between ILs and β-Tubulin receptor. Moreover, the electrostatic interactions due to the imidazolium ring were dominated by the van der Waals interactions due to the alkyl side chains in case of per residue interaction analysis. This investigation represents imidazolium-based ILs as a potential group of anthelmintic compounds and we envisage that they may find possible therapeutic application to control helmintic infections.

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Supporting information

The following information can be seen in the supporting information: percentage of paralysis and percentage of mortality of earthworms due to employed imidazolium-based ILs at different concentrations and time (**Table S1**); Spectral data (^{1}H NMR and mass) of synthesized ILs.

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