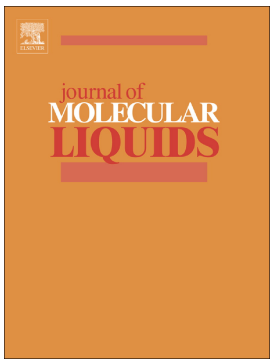

Novel phenethylimidazolium based ionic liquids: Design, microwave synthesis, in-silico, modeling and biological evaluation studies

Abderrahim Titi, Saud M. Almutairi, Abdulwahed F. Alrefaei, Salim Manoharadas, Bakheet A. Alqurashy, Pramod K. Sahu, Belkheir Hammouti, Rachid Touzani, Mouslim Messali, Imran Ali

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Dear Prof. Schröer,

Good day and Greetings…

Thank you very much for giving us a chance to revise our manuscript. The manuscript is revised as per the suggestions made by the learned reviewers. The changes made are highlighted in red color. The revised manuscript is uploaded herewith for publication in Journal Molecular Liquids.

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Title: Novel phenethylimidazolium based ionic liquids: Design, microwave synthesis, in-silico, modeling and biological evaluation studies

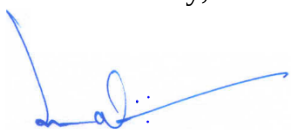
Novelty:

- Economic microwave synthesis of novel phenethylimidazolium based ionic liquids.
- Antimicrobial activity of the synthesized ILs against resistant Gram-positive and Gram-negative bacteria.
- Anticancer screening of the synthesized ILs against MCF-7, HepG-2 and CACO2 cell lines.
- In-silico physico-chemical analysis to predict molecular properties.
- Docking with DNA to determine the working mechanism.

Looking forward to hearing from you a positive response.

Thanking you with regards,

Yours sincerely,



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Novel phenethylimidazolium based ionic liquids: Design, microwave synthesis, *in-silico*, modeling and biological evaluation studies

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Abstract:

An eco-friendly preparation method for the novel bioactive imidazolium ionic liquids halides (ILs) was developed under microwave-assisted conditions. Synthesized ILs were characterized by spectroscopic techniques. Selected ILs were investigated for their antimicrobial activity against highly resistant Gram-positive and Gram-negative bacterial strains. Overall, 3-(2-chlorobenzyl)-1-phenethyl-1H-imidazol-3-iumchloride (**4)** showed high antimicrobial activity against the *Staphylococcus aureus* strain in the inhibition zone tests and displayed low MIC and MBC levels against almost all tested bacteria. Furthermore, the ILs were screened *in vitro* against human hepatocellular carcinoma (HepG-2), human breast adenocarcinoma (MCF-7), and human colon carcinoma (Caco-2) cell lines. The screening results showed excellent to moderate anticancer activity across the ILs. Among the synthesized ILs, Overall, 3-(2-chlorobenzyl)-1-phenethyl-1H-imidazol-3-ium chloride (**4)** and 1-phenethyl-3-(3-phenoxypropyl)-1H-imidazol-3-ium bromide **(7)** were found to exhibit the most promising ant proliferative effects and had the lowest IC_{50} values. The docking study suggested strong interaction of ILs with DNA with binding energy ranging from -4.9 to -4.1 kcal/mol. ILs **4** and **7** were most strongly bonded with -4.9 and -4.8 kcal/mol binding energy; confirming *in vitro* anticancer results.

Keywords: Novel ionic liquids; Microwave-assisted*;* In silico prediction; In vitro anticancer activity; DNA modeling.

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1. Introduction

Nowadays, scientists have focused on developing alternatives to environmentally harmful compounds due to greater environmental consciousness. In this regard, imidazolium-based halide ionic liquids (ILs) are alternatives that have great properties including zero-vapor pressure, excellent conductivity, remarkable chemical and electrochemical stabilities and no flammability [1-3]. The composition of ILs contain anions such as halides or fluorinated anions [4–5]. Scientists have synthesized a variety of ILs using different combinations of cations and anions. Recently, ILs have been widely used in many applications, such as media for electrodeposition of metals [6], a catalyst and biocatalyst [7–8], and potential corrosion inhibitors [9–11]. ILs have also been used in the polymer [12] and food chemical science [13], as well as electrolytes for batteries [14,15] and in many other areas of research. Moreover, ILs have also been investigated for their biological activity and toxicity [16] and have been found to display broad activity against both environmental and clinically important microorganisms [17,18]. Environmentally benign approaches, such as microwave irradiation, have gained attention in recent years for the clean synthesis of ILs. With such an approach, the reaction time could be reduced compared with that required in the conventional preparation of ILs [19,20].

On the other hand, there is a great demand for the search of new antibacterial agents as bacteria are becoming antibiotics resistant continuously. Besides, scientists are exploring new anticancer drugs due to the lack of proper and exact medicine for treating cancer; especially at the late stage of cancer [21-26]. ILs have been found as bioactive molecules [27,28] and expected to be good candidates for many biological actions (medicines). In view of these facts, the efforts are made to synthesize a variety of new ILs via most eco-friendly microwave synthesis. These ILs were characterized by various spectroscopic techniques and tested as antibacterial and anticancer agents. Finally, DNA docking was carried out with the most active ILs to understand the mechanism of their biological activities. The results of these findings are given in this article.

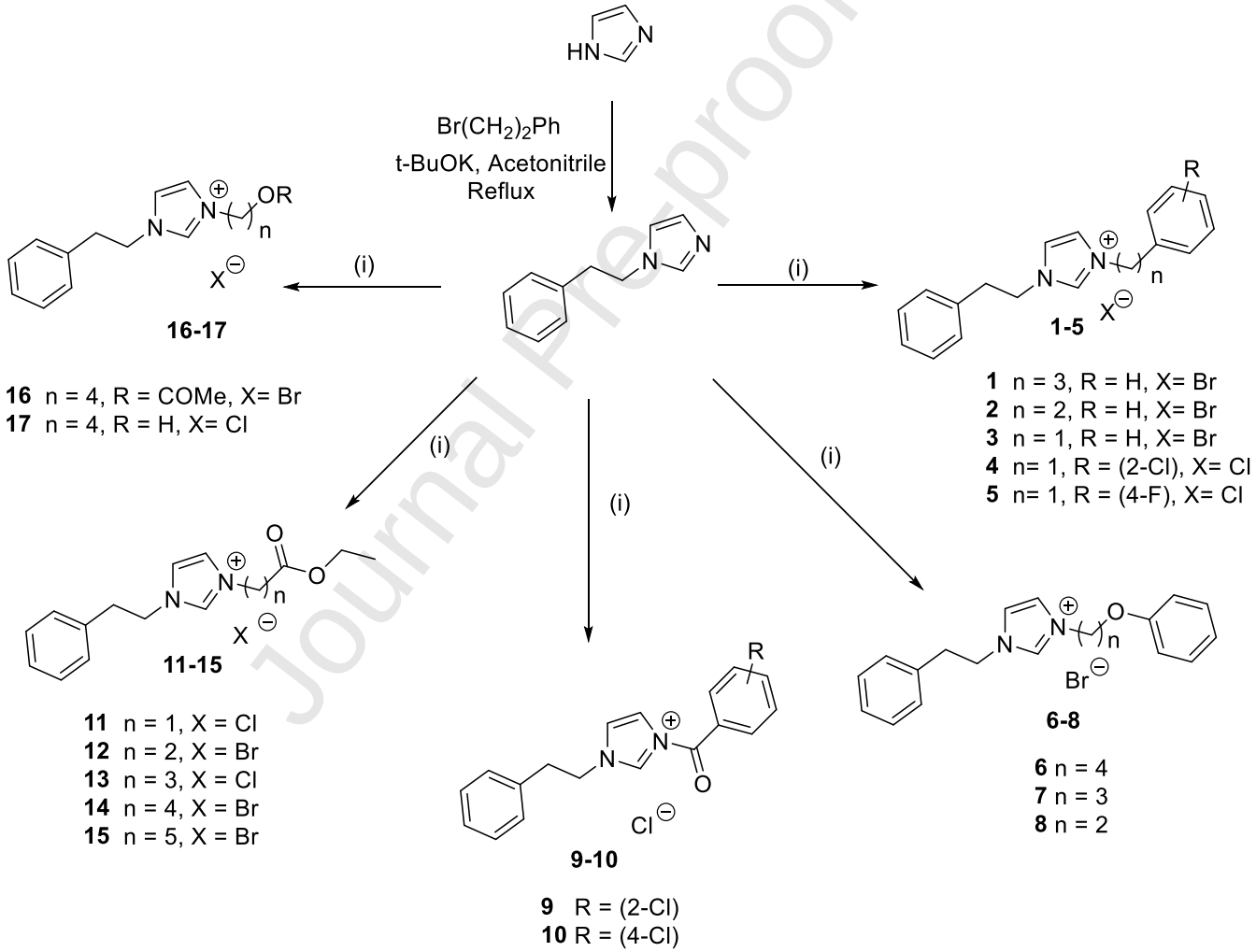
2. Results and discussion

Novel room temperature imidazolium-based ionic liquids were synthesized using microwave irradiation to reduce or eliminate the related costs, disposal requirements, and

hazards associated with volatile organic synthesis methods. Additionally, select synthesized ILs were evaluated for their antibacterial and antitumor activity.

2.1. Chemistry

The ILs were synthesized via the conventional procedure. First, 1-phenethyl-*1H*-imidazole was synthesized by treating imidazole with butyl bromide in acetonitrile in the presence of t-BuOK. Then, the initial phenethylimidazolium based derivatives (**1–5**) containing aliphatic chains were prepared by treating 1-phenethyl-*1H*-imidazole with the appropriate alkyl halides in toluene at 80 °C for 18 h (Scheme 1).



Scheme 1. (i) N-alkylation of 1-phenethyl-1H-imidazole leading to ILs **1-17**: conventional preparation and microwave irradiation conditions. CP: RBr, toluene, 80 °C, 18 h; MW: RBr, toluene, 80 °C, 20 min.

The quaternization reaction led to the production of the imidazolium bromides **1**–**5** when the nitrogen lone pair of 1-phenethyl-*1H*-imidazole reacted with the alkyl halides. The resulting yields of the oils were 69%–79% (**Table 1)**. Microwave irradiation reactions were used for the synthesis of 1-phenethyl-1H-imidazolium-based ILs **1**–**5**. 1-Phenethyl-*1H*-imidazole and the appropriate alkyl halide were treated with microwave irradiation in a closed vessel. IL formation was confirmed when two phases were formed, as the ILs are insoluble in toluene. A short irradiation period was required for completion, and good yields were achieved.

Table 1. IL yields from the conventional and microwave-assisted preparation methods.

Ionic liquid	Alkyl halide RX	Yield (%) of the Quaternization Step	
		CP	MW
1	$Ph(CH_2)_3Br$	71	87
2	$Ph(CH_2)_2Br$	72	88
3	$PhCH_2Br$	73	89
4	(2-Cl)-$PhCH_2Cl$	69	86
5	(4-F)-$PhCH_2Cl$	69	87
6	$PhO(CH_2)_4Br$	72	85
7	$PhO(CH_2)_3Br$	72	83
8	$PhO(CH_2)_2Br$	73	87
9	(2-Cl)-PhCOCl	78	90
10	(4-Cl)-PhCOCl	76	87
11	EtO_2CCH_2Cl	79	89
12	$EtO_2C(CH_2)_2Br$	78	88
13	$EtO_2C(CH_2)_3Cl$	74	89
14	$EtO_2C(CH_2)_4Br$	70	86
15	$EtO_2C(CH_2)_4Br$	73	89
16	$MeCO_2(CH_2)_4Br$	72	88
17	$HO(CH_2)_4Cl$	70	84

Conventional procedure (CP) and microwave (MW) irradiation conditions.

To establish the structures of ILs **1**–**5**, 1-phenethyl-3-(3-phenylpropyl)-1H-imidazol-3-ium bromide (**1**) was chosen for the discussion of spectral data. The structure of 1-phenethyl-3-(3-phenylpropyl)-1H-imidazol-3-ium bromide (**1**) was confirmed using ^{1}H NMR. A quintet at 2.11 ppm and one multiplet around 1.27 ppm were due to the methylene protons of $CH_3CH_2CH_2$, and a quintet around 1.86 ppm was due to the methylene protons of $CH_2CH_2CH_2$. Since IL **1** contained 4 other methylene groups, the protons from these groups corresponded to triplets at 2.53 and 3.17 ppm for the CH_2CH_2Ph protons and 4.19 and 4.55 ppm for the NCH_2CH_2 protons. The singlet at 9.90 was due to the aromatic region around the N*CH*N imidazolium proton. The

^{1}H NMR spectrum for **1** also contained two additional signals at 7.05 and 7.20 ppm corresponding to the imidazolium ring protons (N*CHCH*N).

In the ^{13}C NMR spectrum of IL **1**, the five signals at δC 49.3 ppm were due to the imidazolium ring, and the peak at 50.9 ppm was due to NCH_2. The peaks at 31.4, 32.1, 29.8, and 36.3 ppm corresponded to the three other methylene groups. Furthermore, the signals corresponding to the aromatic carbons were in the range of 121.9–139.6 ppm. The findings from the ^{13}C NMR spectrum were confirmed with APT-^{13}C-NMR. Under similar reaction conditions, ILs **6**–**17** were synthesized. These ILs were modified with different substituents, such as ethers, ketones, esters, and alcohols. The last IL synthesized in this work was 3-(4-hydroxybutyl)-1-phenethyl-1H-imidazol-3-ium chloride (**17**). The structures of ILs **6**–**17** were also confirmed by spectroscopic analysis. Two ILs were selected from **6**–**17**, namely, 3-(4-chlorobenzoyl)-1-phenethyl-1H-imidazol-3-ium chloride (**10**) and 3-(4-ethoxy-4-oxobutyl)-1-phenethyl-1H-imidazol-3-ium chloride **(13)**, for the discussion of the spectral data to verify the quaternization reaction.

^{13}C-NMR analysis was used to establish the structure of IL **10**. A signal at 160.6 ppm corresponded to the carbonyl group (CO). In the H-NMR spectrum, IL **13** showed a triplet and a quartet around 1.19 and 4.06 ppm, respectively. Furthermore, the C-NMR spectrum of IL **13** had a peak at 172.1 ppm corresponding to the carbonyl group (CO) and peaks at 60.8 ppm and 14.1 ppm corresponding to the CH_2 and CH_3 of the ester group (OCH_2CH_3), respectively. The ^{13}C NMR spectrum of IL **13** showed peaks at 48.8 and 51.0 ppm due to the 2 NCH_2CH_2 carbons. The functional groups of ILs **10** and **13** were also confirmed by FT-IR spectroscopy. The absorption bands at 1685 and 1742 cm^{-1} were designated to the ketone and ester carbonyl groups (C=O), respectively.

2.2. Antimicrobial activity

The inhibition zone (IZ) assay of the compounds was performed against bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli* MC4100, and *Escherichia coli* XL-1 Blue. As shown in Table 2, the most potent activity was exhibited by **4**against *S. aureus*, with an IZ diameter of 22 mm. An IZ diameter of 19 mm was observed against *S. aureus* with **1**. Interestingly, **1**, **2**, **4,** and **5** exhibited antibacterial activity against all the tested bacterial strains, particularly with strong activity against Gram-positive bacterial

strains. However, no activity was observed from **16** and **17**, apart from the moderate activity against *E. coli* MC4100. In comparison with **16** and **17**, **15** had moderate antimicrobial activity against all the bacterial strains.

Table 2. IZ diameter measurements (mm) of select ILs against various bacterial strains.

Sample	S. aureus	B. subtilis	K. pneumoniae	P. aeruginosa	E. coli MC4100	E. coli XL1-Blue
1	19	12	11	8	10	10
2	14	9	10	6	12	10
4	22	12	12	9	14	14
5	16	13	13	10	14	13
11	9	0	0	0	8	0
14	9	0	9	0	11	6
15	12	9	12	8	13	9
16	0	0	0	0	9	0
17	0	0	0	0	7	0

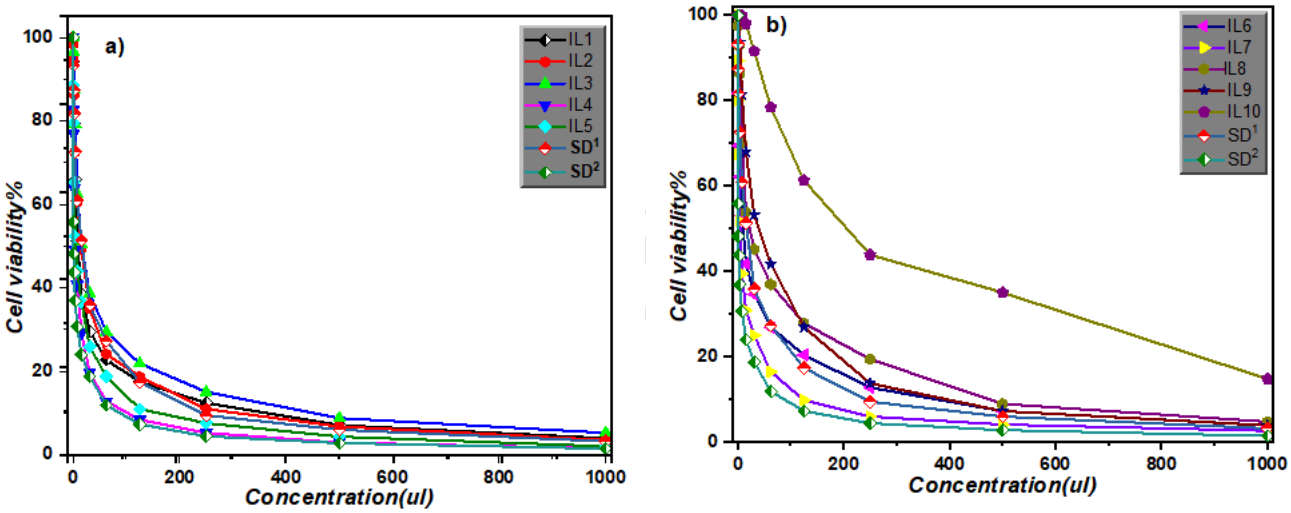
The IZ assay results prompted us to compare the minimum inhibitory concentration (MIC) of the ILs against the bacterial strains with that of the antibiotic control. IL **4**, which had high antimicrobial activity against the *S. aureus* strain in the IZ tests, had MIC and minimum bactericidal concentration (MBC) levels of 12 and 24 µg/ml, respectively (Table 3). Interestingly, the activity of **4** was higher than that of the antibiotic control (Ampicillin: AMP), as the MIC and MBC of AMP against *S. aureus* were both 24 µg/ml. Moreover, ILs **16** and **17** did not show any MIC or MBC against the tested strains besides *E. coli* MC4100. The maximum MIC or MBC tested among the ILs was 384 µg/ml, which was exhibited by ILs **2**, **11**, and **17** against *P. aeruginosa*, *E. coli*, and *E.coli*, respectively.

Table 3. MIC and MBC levels of select ILs against various bacterial strains.

Sample	S. aureus		B. subtilis		K. pneumoniae		P. aeruginosa		E. coli MC4100		E. coli XL1-Blue	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	12	12	96	96	96	96	192	192	96	192	48	48
2	24	24	192	192	192	384	384	384	12	24	48	48
4	12	24	96	96	96	96	192	192	24	24	24	24
5	48	48	96	96	96	192	96	96	48	96	48	48
11	96	192	-	-	-	-	-	-	384	384	-	-
14	96	96	-	-	192	192	-	-	24	24	384	384
15	48	96	48	48	96	96	192	192	24	24	96	96
16	-	-	-	-	-	-	-	-	192	192	-	-
17	-	-	-	-	-	-	-	-	384	384	-	-
AMP	24	24	24	24	-	-	-	-	96	96	96	96

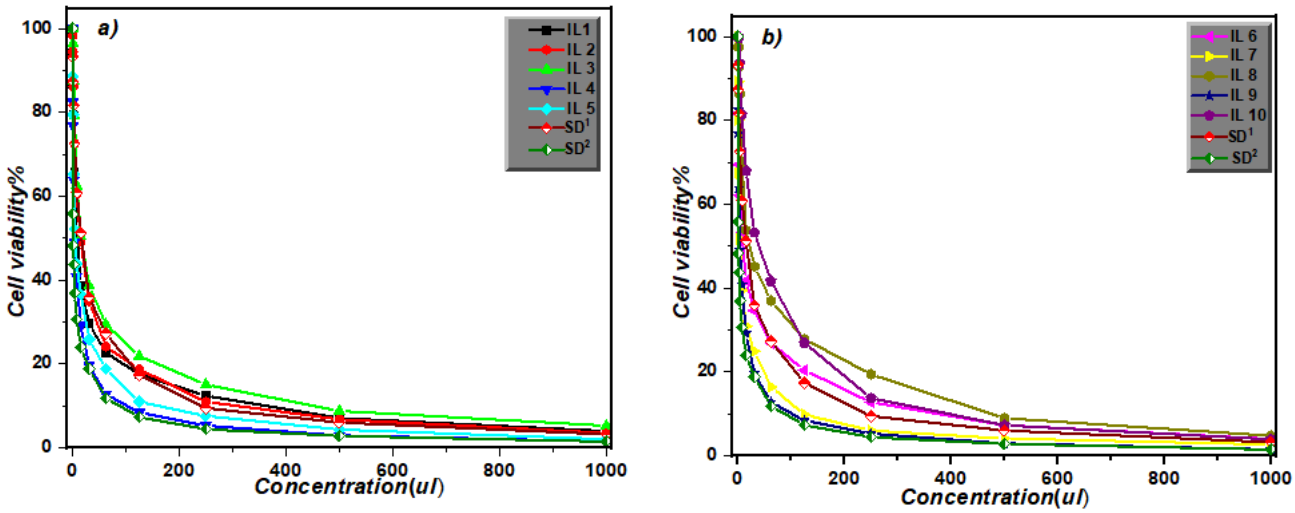
2.3.Cytotoxic activity of ILs 1–10 against tumor cell lines

A previously reported method by the Regional Center for Mycology & Biotechnology at Al-Azhar University, Egypt [29] was used to evaluate the cytotoxicity in terms of cell growth and cell viability. The results are illustrated in Figures 1–3. The screening was performed with various IL dosages. The screening results showed reductions in bacterial survival when the IL dosage was increased. However, there was minimal improvement with increased dosage within the range of 50–125 µM. Moreover, ILs **4** and **7** were observed to exhibit higher antiproliferative activity within the range of 50–125 µM.



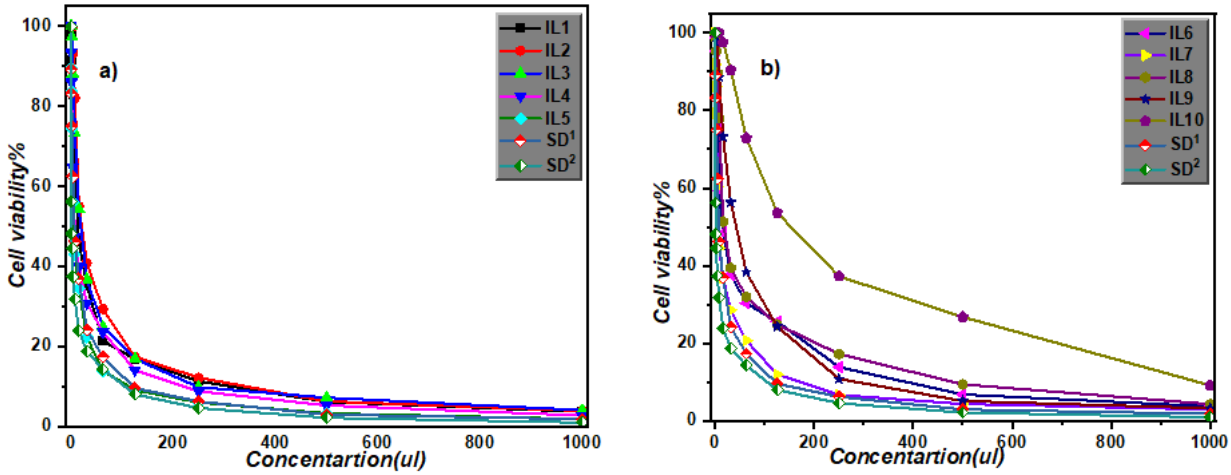
(a) **(b)**

Figure 1 (a) and (b) Cytotoxic activities of ILs **1**–**5** and **6**–**10**, respectively, against HepG-2 cells.



(a) **(b)**

Figure 2 (a) and (b)**.** Cytotoxic activities of ILs **1**–**5** and **6**–**10**, respectively, against MCF-7 cells.



(a) **(b)**

Figure 3 (a) and (b) Cytotoxic activities of ILs **1**–**5** and **6**–**10**, respectively, against Caco-2 cells.

Screening results showed that the IC_{50} values ranged from 3.87 to 224 µM. The results indicated that IL **10** exhibited poor performance overall (Table 4). Based on the IC_{50} values, it was observed that ILs **4** and **7** demonstrated good activity, with IC_{50} values ranging from 3.87 to 7.41 µM. Although, the activities of these ILs were lower than that of **5-FU**, which was used as a

positive control. ILs **1** and **5**, with IC_{50} values ranging from 4.94 to 16 µM, also showed good activity against two of the three cell lines compared to **5-FU**.

Table 4. IC_{50} (µg/mL)values of ILs **1**–**10** against HepG-2, MCF-7, and Caco-2.

Cell line	**5-FU**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
HepG-2	**9.11**	**8.59**	15.1	16.1	**3.87**	**4.94**	9.93	**4.45**	22.5	40	206
MCF-7	**17**	**16**	25.2	25.5	**6.53**	**13.3**	26	**7.41**	30.9	30.7	224
Caco-2	**6.9**	13.8	21	19.4	**5.72**	7.21	14.5	**6.46**	17.4	42.3	153

2.4.*In silico* analysis of ILs 1–17

In the drug designing and development process, Lipinski's Rule of Five is generally used to predict the bioavailability of the target drug. It is assumed that compounds that follow Lipinski's Rule of Five will likely be orally active. Drug property descriptors such as molecular weight (MW) and topological surface area (TPSA) [30,31]were calculated; the results are summarized in Table 5. Molecules that have drug applications usually have molecular weights of less than 500 Da [32, 33]. All ILs did not violate Lipinski's Rule of Five and were expected to be orally active. It was assumed that all ILs were easily diffused, absorbed, and transported due to having molecular weights less than 500 Da [34, 35]. The TPSA (Total Polar Surface Area) values of the ILs were within the range 8.82–35.12 A° and were well below the limit of 160 A°. Permeability property of ILs **1**–**17** considered as liphophilicity (Log P value) were ranging from -2.46 to 0.09 (<5) [36], table 6 and TPSA were calculated as described [37], table 5. Calculated Log P and TPSA were in the acceptable limit, and all the compounds qualified as drug candidates. The calculated values of the molecular properties and bioactivity scores (TPSA, GPCR (G-Protein coupled receptor) ligand, ICM (Ion Channel modulator), KI (Kinase inhibitor), NRL (Nuclear receptor ligand), PI (Protease inhibitor), EI (Enzyme inhibitor) are summarized in Table 5. The molecule has considered biological active possess bioactivity score more than 0.00, values -0.50 to 0.00 are expected to be moderately active and considered as inactive if the score is less than -0.50 [38]. From table 5, it is clear that the investigated compounds were biologically active and produced the physiological actions by interacting with GPCR ligands, nuclear receptor ligands, inhibit protease and other enzymes.

Table 5. *In silico* physicochemical property predictions of ILs **1–17**

Compd.	Calculation of Molecular Properties				Calculation of Bioactivity Scores					
	TPSA	**NONH**	**NV**	**VOL**	**GPCRL**	**ICM**	**KI**	**NRL**	**PI**	**EI**
1	8.82	0	0	295	0.24	0.43	−0.43	−1.11	−0.25	0.34
2	8.82	0	0	278	0.04	0.49	−0.61	−1.27	−0.45	0.37
3	8.82	0	0	261	0.22	0.59	−0.73	−1.32	−0.35	0.44
4	8.82	0	0	275	0.16	0.42	−0.65	−1.21	−0.41	0.26
5	8.82	0	0	266	0.25	0.54	−0.63	−1.20	−0.32	0.40
6	18.05	0	0	321	0.21	0.41	−0.38	−1.00	−0.23	0.27
7	18.05	0	0	304	0.12	0.28	−0.49	−1.06	−0.32	0.22
8	18.05	0	0	287	0.06	0.19	−0.55	−1.09	−0.38	0.25
9	25.89	0	0	277	0.14	0.33	−0.38	−0.89	−0.17	0.14
10	25.89	0	0	277	0.15	0.34	−0.38	−0.90	−0.11	0.16
11	35.12	0	0	251	−0.22	0.19	−0.95	−1.47	−0.60	0.26
12	35.12	0	0	268	−0.01	0.38	−0.74	−1.33	−0.40	0.22
13	35.12	0	0	287	0.10	0.45	−0.66	−1.29	−0.34	0.28
14	35.12	0	0	302	0.14	0.44	−0.60	−1.20	−0.27	0.28
15	35.12	0	0	318	0.15	0.42	−0.56	−1.12	−0.23	0.27
16	35.12	0	0	285	0.17	0.51	−0.59	−1.25	−0.27	0.36
17	29.05	0	0	248	0.13	0.64	−0.62	−1.56	−0.47	0.42
SD[1]	206	7	3	459	0.20	−0.20	−0.07	0.32	0.67	0.66
SD[2]	66	4	0	96	−1.95	−1.24	−1.90	−2.50	−2.66	−1.19
Amp	112	4	0	299	0.04	−0.47	−0.71	0.61	0.87	0.25
Van	530	21	3	1207	−3.94	−3.99	−4.00	−4.01	−3.90	−3.95
RD	220	6	3	756	−2.10	−3.27	−3.04	−2.89	−1.61	−2.42

SD[1]: Doxorubicin; **SD[2]**: Fluorouracil; **Amp**: Ampicillin; **Van**: Vancomycin, and **RD:** Rifampicin

The *in silico* toxicity study is necessary to assess whether a drug is safe for administration. To confirm the toxicity of the synthesized ionic liquids, mutagenicity and carcinogenicity were calculated *in silico*, and the results were tabulated in Table 6. As shown in Table 6, all compounds were safe for carcinogenicity and mutagenicity, except for **16** which was safe for mutagenicity only.

Table 6. *In silico* calculated toxicity risks and logP values of ILs (**1**–**17**).

Compd.	Mw(g/mol)	Toxicity Risks[a]				*cLogP*
		Mut	**Tum**	**Irri**	**Rep**	
1	291	+++	+++	+++	+++	−0.12
2	277	+++	+++	+++	+++	−0.57
3	263	+++	+++	+++	+++	−0.91
4	297	+++	+++	+++	+++	−0.31
5	271	+++	+++	+++	+++	−0.81
6	321	+++	+++	+++	+++	−0.09
7	307	+++	+++	+++	+++	−0.54
8	293	+++	+++	+++	+++	−0.99
9	311	+++	+++	+++	+++	0.09
10	311	+++	+++	+++	+++	0.08
11	259	+++	+++	+++	+++	−2.46
12	273	+++	+++	+++	+++	−2.00
13	287	+++	+++	+++	+++	−1.55
14	301	+++	+++	+++	+++	−1.10
15	315	+++	+++	+++	+++	−0.64
16	287	+++	+++	+++	++	−1.55
17	245	+++	+++	+++	+++	−2.03

Highly toxic: (---), slightly toxic: (++), not toxic (+++).[a] Mut: Mutagenic, Tum: Tumorigenic, Irrit: Irritant, and RE: Reproductive effective.

2.4 Modeling

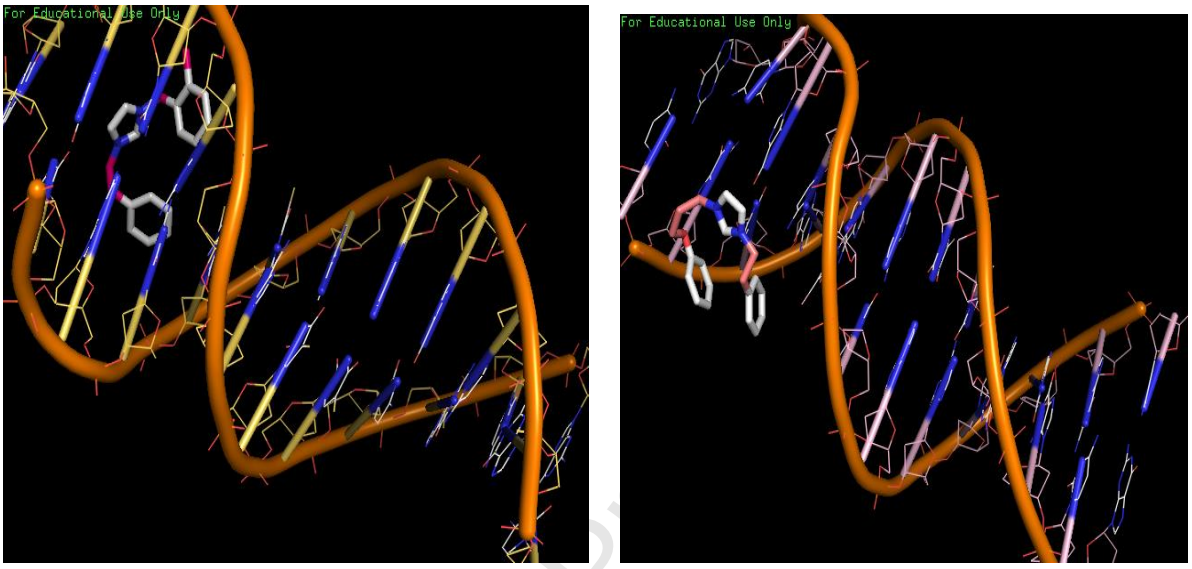
Generally, it is known that most of the anticancer agents work by interacting with DNA [39-41] because mostly caner is occurred by DNA mutation. The modeling study was carried out only for 1-10 ILCs as they were found to be the most active. The results are given in Tables 7 and it is clear from this table that the ILCs interacted with DNA differently. The binding energies of the ILCs with DNA ranged from -4.9 to -4.1 kcal/mol. The common residues involved were dc13, dg14, dc15, dg16, N7, dt8 and dg10. The diverse binding energies of the ILCs with DNA were observed due to their dissimilar configuration. The docking studies showed that the connections among the ILCs and DNA were due to hydrophobic interactions. Besides, hydrogen bonding may be responsible for these interactions. All ILs are π electron-rich molecules due to the presence of benzene rings. Therefore, π-π interactions may be a major contribution. It is important to mention here that only the cation (organic moiety) of ILs interacted with DNA. All

the molecules have benzene rings with nitrogen, chlorine, fluorine and oxygen atoms. The structures of these ILs are almost similar with very little difference. Therefore, the different binding of ILs may be because of the different steric fitting into DNA major groves. Interestingly, the cationic moiety of ILs is attached to DNA molecules by the phosphate negative site of DNA. The strength of the interaction was based on binding energy. ILCs 4 and 7 interacted with DNA stronger than other ones with -4.9 and -4.8 kcal/mol binding energy. Based on these results, ILs 4, and 7 should be more active and effective in cancer treatment because they bind more tightly with DNA as compared to other ILs. And of course, these results are in good agreement with those obtained by anticancer activities. The representative interactions of the most active 4 and 7 ILCs with DNA are shown in Figure 4 while the rest figures are given in supplementary information. These figures also reflected that the ILCs interacted with DNA through major grooves.

Table 7. The DNA modeling results of ILs 1-10.

ILs	Binding energies (kcal/mol)	Hydrophobic interactions	
		Helix A	Helix B
1.	-4.2	Dg10::C17,C20,C16,C18 dc9::C17,C7,C19 dt8::C7,C8,C11	Dc15::C6,C10,C20,C1,C7 dg14::C10,C13 dc16::C1
2.	-4.4	Dc9::C4,C16,C1 dg10::C12,C14 dt8::C4,C1,C2	Dg16::C1,C2 dc15::C1,C7,C19,C3,C8,C11
3.	-4.3	Dt8::C5,C4 dc9::C4,C8,C7 dg10::C18,C17	Dc15::C18,C3,C2,C1 dg14::C10,C13
4.	-4.9	Dt8::C4,C1,C2,C3 dc9::C2,C7,C1,C4 dg10::C12	Dg14::C15,C14 dc15::C13,C10,C5,C4,C15,C14,C6 dg16::C4,C5
5.	-4.6	Dt8::C12,C8 dc9::C7,C9,C12,C3,C4	Dc15::C14,C13 dg14::C16,C1,C6,C10 dg10::C10,C3,C7 dc13::C11,C2
6.	-4.1	Dc9::C17,N1,C19,C8 dt8::C19,C7,C9,C8 dg10::C18,C12,C21	Dg14::O,C20,C13,C12 dc15::C11,C15,C21,C8 dg16::C3
7.	-4.8	Dg10::C20,C15,C16 dc9::C16,C18,C7 dt8::C7,C9,C8,C3	Dc15::C11,C6,C20,C7 dg14::C14,C11,OC17 dc16::C1
8.	-4.1	Dc9::C7,C16,C15 dg10::C14,C18,C15 dt8::C8,C9,C7	Dc15::C10,C18,C7,C5 dg14::C10,O
9.	-4.5	Dc9::C8 dt8::C4	Dg14::C14,C15,C7,C3 dc15::C15,C8,C18,C13 dc13::C11,C7,C3

10.	-4.4	Dc9::C14,C16,C5 dt8::C3,C5 dg10::C17,C14,C15	dc16::C4 Dc13::C9,C13,C1 dc15::C17,C10,C1,C2 dg16::C3,C5



(a) **(b)**

Figure 4. DNA docking models of the most active ILCs (a): **4** and (b): **7**.

3. Materials and methods

3.1. Experimental

Minimal amounts of reagents and solvents were used. The synthesized ILs were characterized by spectroscopic analysis. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured at room temperature with $CDCl_3$ as the solvent. Chemical shifts (δ) were reported in ppm to a scale calibrated to the internal standard, tetramethylsilane (TMS). IR spectra were recorded in NaCl disc on a Shimadzu 8201 PC, FT-IR spectrophotometer (υ_{max} in cm^{-1}). The elemental analyses were given by using the 2400 Series II CHNS/O Elemental Analyzer. The microwave-assisted reactions were performed using a microwave oven with a temperature controller (750 W) and an upper-temperature limit of 100 °C, with nitrogen gas being used as the cooling agent when the temperature inside the reaction chamber increased.

3.2. Synthesis

3.2.1. General procedures for the synthesis of imidazolium halides (1–17) using the microwave method

The alkyl halides (1.1 eq) were added to a solution of 1-pentyl-1H-imidazole (1 eq) in toluene. The solution was then treated with irradiation for 20 min in a closed vessel at 80 °C using a CEM Microwave. The completion of the reaction was indicated by the formation of an oil or solid phase from the initially clear homogenous mixture composed of *N*,*N*-pentylimidazole and the alkyl halide in toluene. The product was either filtered or extracted, and then washed with ethyl acetate. The IL/salt was then dried at reduced pressure.

3.2.2. General procedure for the synthesis of imidazolium halides (1–17) using conventional irradiation

The alkyl bromides (1.1 eq) were added to a solution of 1-pentylimidazole (1 eq) in toluene. The corresponding solutions were placed in a closed vessel at room temperature and stirred at 80 °C for 18 h. After which, the IL was filtered and washed with ethyl acetate. The IL was then filtered again, and ethyl acetate was evaporated to obtain the pure ionic liquid.

3.3 Modeling

DNA is the most likely target of anticancer drugs [42,43] and, hence, a modeling study of ILs with DNA was carried out to understand the mechanism of action. The docking studies of the selected 1-10 compounds were done by Intel® dual CPU (1.86 GHz) with Windows XP operating system. Marwin Sketch software was utilized to draw the structures of 1-10 compounds. The structures were cleaned to 3D and saved in PDB file format [44]. After that, the structure of DNA (pdb ID: 1bna) was downloaded from the protein data bank. Using AutoDock Tools (ADT) 4.2 the structure of DNA to be docked was prepared by assigning Gastegier charges, merging non-polar hydrogen atoms and saving it in PDBQT file format. Docking was performed with AutoDock 4.2 (Scripps Research Institute, USA) considering all the rotatable bonds of the ligand as rotatable and the receptor as rigid [45]. Using the same tool, 1-10 ILs (as ligands) were edited to be saved in PDBQT formate. The grid box size of 60 × 80 × 110 A° with 0.375 A° spacing was used. After saving both files in PDBQT formate, Vina software was used to get binding energy/affinity between receptor (DNA) and ligand (1-10 compounds). After using Vina software, the output file was opened in PyMOL to carry out the molecular docking,

virtual screening and binding site analysis and to get an image of interaction and the bond length of the hydrogen bond between DNA and 1-10 compounds.

4. Conclusion

Seventeen novel RTILs were prepared through quaternization reactions by using microwave irradiation instead of the conventional preparation method. The structures of the ILs were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopies. We determined that the use of microwave irradiation was simple and could both shorten the preparation time and improve the yield of target molecules, thereby fulfilling some principles of green chemistry such as the atomic economy. Based on IC_{50} values, we determined that ILs **4** and **7** demonstrated good activity with IC_{50} values of 3.87–7.41 µM. *In silico* toxicity prediction results showed that all compounds were safe for carcinogenicity and mutagenicity, except for **16**which was safe for mutagenicity only. *In silico* prediction results also proved that all the ILs satisfied Lipinski's Rule of Five to qualify as drug candidates. Molecular property calculations showed that all compounds had LogP values within the acceptable range to be eligible for lipophilicity. TPSA values of ILs were within the range of 8.82–35.12 A° and were well below the limit of 160 A°. The rest of the calculated properties were in good agreement, and all the compounds qualified as drug candidates. DNA modeling results indicated a strong binding of ILs with DNA with binding energy ranging from -4.9 to -4.1 kcal/mol. The results confirmed that the biological activity of ILS is through DNA binding.

Supplementary Materials: Characterization data of all newly synthesized ILS **1-17** included all ^{1}H-NMR, ^{13}C-NMR, DEPT-^{13}C-NMR spectra are associated with this article. Supporting information contains also the methods used for the determination of IZ, MIC and MBC, in-silico analysis and modeling figures.

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Credit author statement

Abderrahim Titi: performed the synthesis of compounds and analyzed the data; **Saud M. Almutairi:** performed the synthesis of compounds and analyzed the data**; Abdulwahed F Alrefaei:** carried out the biological tests; **Salim Manoharadas:** carried out the biological tests; **Bakheet. A. Alqurashy:** analyzed the data; **Pramod K. Sahu:** performed in-silico analysis, Software; **Belkheir Hammouti:** Reviewing; **Rachid Touzani:** Reviewing; **Mouslim Messali:** Conceptualization, Methodology, Visualization, Validation, Writing- Original draft preparation, Methodology, Software, Investigation, Supervision **Imran Ali:** Conceptualization, Methodology, Software, Visualization, Writing- Reviewing and Editing, Validation, Investigation, Supervision.

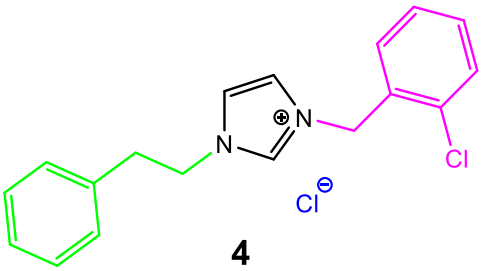
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Novel phenethylimidazolium based ionic liquids: Design, microwave synthesis, *in-silico*, modeling and biological evaluation studies

Graphical Abstract



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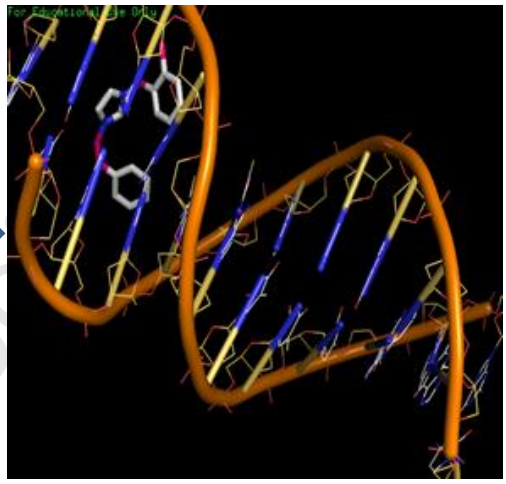
IC50 (MCF-7): 6.53
IC50 (Caco-2): 5.72

In-silico



Highlights

- Economic microwave synthesis of novel phenethylimidazolium based ionic liquids.
- Antimicrobial activity of the synthesized ILs against resistant Gram-positive and Gram-negative bacteria.
- Anticancer screening of the synthesized ILs against MCF-7, HepG-2 and CACO2 cell lines.
- *In-silico* physico-chemical analysis to predict molecular properties.
- Docking with DNA to determine working mechanism.