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ARTICLE TYPE

1-Butylimidazole derive ionic liquids: Synthesis, characterization and their evaluations of antibacterial, antifungal and anticancer activities

Prabodh Ranjan, Bheru Singh Kitawat and Man Singh*

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A series of new 1-butylimidazole based ionic liquids (**3a-3h**) has been synthesized by the quaternization reaction of 1-butylimidazole with different alkyl and alkoxy substituted aryl halides under microwave solvent-free approach with ≈ 82-95 % yield. The reaction under solvent-free approach allows for the preparation of a variety of ILs with better yield and purities, making any further purification unnecessary. Structures were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, LCMS (Q-TOF). ILs were screened against the gram-positive (*S. aureus* and *B. subtilis*) and gram-negative bacterial (*E. coli* and *P. aeruginosa*) strains. Compounds **3b**, **3c**, **3e** and **3f** showed good activity against both *S. aureus* and *B. subtilis* strains, while those of **3a** and **3c** exhibited good activity against *P. aeruginosa* strains. ILs **3a**, **3b**, **3d** and **3e** showed better antifungal activity against the *C. albicans* strain. Additionally, compounds were tested for their *in vitro* anticancer activity against the MCF-7 and MDA-MB-435 cell lines by SRB assay protocol to estimate cell growth, where compounds **3a**, **3d** and **3e** demonstrated 50-60 % activity against MDA-MB-435 cell line with GI_{50} values 67.2, 52.5 and 57.9 μM respectively as compared to standard adriamycin (GI_{50} 24.4 μM).

Introduction

Ionic liquids (ILs) are fused salts constituting cations and anions moieties[1] possess some fundamental properties, for example negligible vapor pressure, thermal stability up to 300 ºC, non-flammable, high ionic conductivities,[2-4] wide electrochemical stability window,[2,3] high polarity, which enables wide kinetic controls and immiscible with numerous organic solvents.[5] For last few decades ILs recognized as "designer solvent"[6-8] because of its scope to tune the physical, chemical and biological properties to get desired task-specific ionic liquids (TILs) for a multitude of applications.[9-12] These unique properties of ILs open wide scope in different domains of chemistry that makes them excellent candidate for use in "Green synthesis" as green solvents.[13-15]

Moreover, despite the promising results evidenced by the many studies in which they have been used as solvents or catalyst for chemical synthesis[16,17] electrolytes for electrochemical devices,[18] super capacitors[19,20] engineering and physical chemistry.[21,22] Their widespread application is still hampering by doubts related to some practical drawbacks: (i) cost and possible toxicological concerns, (ii) problems related to product isolation and (iii) catalyst recovery. To overcome at least some of these drawbacks more recently ether functionalized ILs, the so-called TSILs, have been synthesized.[23,24] For a past decade numerous green route involving: reaction under solvent-free condition[25] using non-classical techniques such as ultrasonication[26] microwaves and others[27, 28] have been adopted for synthesis of ILs. Nowadays, use of microwave (MW) assisted synthesis leads to provide green synthetic route, rapid reactions, with large reduction in reaction time (from hours to minutes), maintain uniform temperature and pressure during course of reaction and provide better yields.[29] Additionally, biological activities of several ILs have been investigated and explored. Therefore, the literature revealed that many synthetic ILs and their analogues display a wide spectrum of biological activities like those of antibacterial,[30,31] anticancer,[32,33] antifungal and others.[34-36] For this reason, they are an object of continuously growing interest, in academia as well as industry. Also, ILs may cause environmental menaces to aquatic ecosystems and living organisms because of lack of toxicity data of designed ILs or those yet to be design.[37] Thus, toxicity data of ILs must investigate concerning their biological as well as environmental impact. [38,39]

In general, earlier studies reveal that the biological activity depends on the alkyl chain length of cationic head group and functional group present in their chain as well as presence of elements (nitrogen, sulphur, and oxygen) in the ring.[40,41] Thus, the structural modification can makes them more potent substances with greater SAR (structural activity relationship).[42]

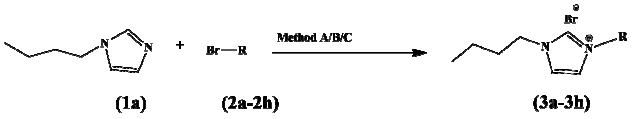
Considering the advantages of microwave solvent-free approach and continuing our investigations on new methodologies for the synthesis of new heterocyclic ring bearing compounds,[43] herein we wish to report an efficient and practical procedure for the synthesis of a series of 1-butylimidazole based ILs. Additionally, the potential biological activities *viz* antibacterial, antifungal and anticancer have been investigated of ILs against a panel of microorganisms and cell lines respectively.

Results and discussions

Synthesis and analytical characterizations

Aim of our study is to synthesize novel 1-butylimidazole based ILs (**3a-3h**) by altering the alkyl or alkoxy substituted aryl bromide thorough microwave dielectric heating as well as heating method. 1-butylimidazole reacts to different alkyl or substituted aryl bromide to deliver the 1-butyl-3-(n-aryl alkyl)-1*H*-imidazole-

3-ium bromide (**3a-3h**), according to Scheme 1. Optimized conditions for ILs synthesis under MW solvent-free, MW with solvent and heating method (or conventional heating method) are summarized in Tables 1-3, respectively. The comparisons between MW assisted methods and heating methods are summarized in Table 4. Reaction in solvent-free MW assisted method produced good yield as compared to heating and MW with solvent method (Table 4).



Method A = MW, 140 °C, 350 Watt, 20-25 min., solvent-free
Method B = MW, 120 °C, 350 Watt, 20-25 min., toluene
Method C = 140 °C, stirr, 3 hrs.

Scheme 1: General scheme for the synthesis of 1-butyl-3-(n-aryl alkyl)-1*H*-imidazole-3-ium bromide (**3a-3h**)

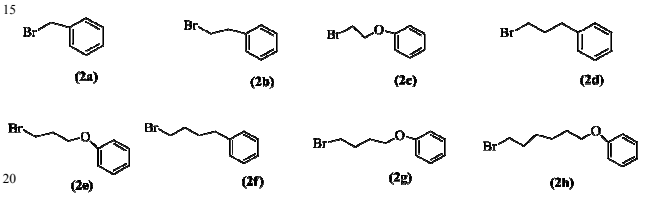


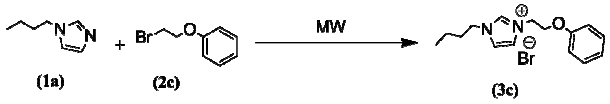
Figure 1: Structure of reactants (**2a-2h**) used in the ILs synthesis

Optimization of reaction conditions: Initially, reaction between equimolar amount of 1-butylimidazole (**1a**) and (2-bromoethoxy) benzene (**2c**) react to deliver product **(3c)**, was considered as a model reaction to screen the reaction conditions for methods A-C, which have been discussed below.

Method A: MW solvent-free approach

Initially, the model reaction was conducted under MW solvent-free condition at 200 W power and 100 ˚C, completed in 25 min and produced 77% yield of **3c** (Table 1, entry 1). Further to optimized MW power, reaction was performed at 250, 300, 350 and 400 W for 25 min, which on result produced **3c** with yields 79, 84, 87 and 87% respectively (Table 1, entries 2-5).

Table 1: Optimization of reaction conditions under MW solvent-free condition



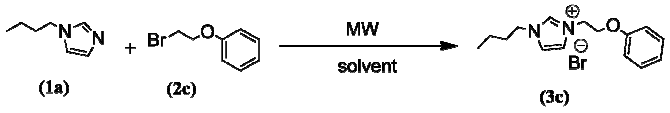
Entry	Power (W)	Temp. (˚C)	Time (min)	Yield (%)
1	200	100	25	77
2	250	100	25	79
3	300	100	25	84
4	350	100	25	87
5	400	100	25	87
6	350	120	25	88
7	350	140	25	90
8	350	160	25	91

Based on the yield comparisons of **3c** at different power it was found that at 350 and 400 W, no better increment in yield was observed. Thus, the 350 W was chosen as optimum microwave power. Next, the effect of temperature were observed and optimized by conducting model reaction at 120 ˚C, 140 ˚C and 160 ˚C, at which yield quaternization was increased 88%, 90% and 91% respectively (Table 1, entries 6-8). On increasing temperature from 140 ˚C to 160 ˚C, no better yield increment was noted. Thus, the suitable reaction conditions for model reaction were suited as microwave power 350 W and temperature 140 ˚C. Scope and versatility of optimized conditions under microwave solvent-free approach was studied by preparing a series of new eight ILs (**3a-3h)** by using different substitution of alkyl and alkoxy bromide (**2a-2h**). It was found that ILs having oxygen in their alkyl chain produced better yield in comparison to alkyl chain. The overall yield of ILs was found in the range of 82-90% depending on the substitution alkyl chain on phenyl ring. The structures of ILs were confirmed using various spectroscopic techniques.

Method B: MW with solvent approach

In this method, to study the effect of solvent on reaction conditions such as MW power, temperature and yield, model reaction was conducted using acetonitrile (ACN), toluene, ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) as solvent at MW at 300 W and 100 ˚C for 25 min produced **3c** with yield of 76, 80, 75 and 70% respectively (Table 2, entries 1-4). The reaction was not proceed, when DCM was used as a solvent (Table 2, entry 5).

Table 2: Optimization of reaction conditions under MW with solvents



Entry	Solvent	Power (W)	Temp (˚C)	Time (min.)	Yields (%)
1	ACN	300	100	25	76
2	Toluene	300	100	25	80
3	EtOH	300	100	25	75
4	MeOH	300	100	25	70
5	DCM	300	100	25	-
6	Toluene	350	100	25	81
7	Toluene	300	120	25	84
8	Toluene	250	120	25	77

We found that at 300 W and 100 ˚C temperature using toluene as a solvent produced higher 80% yield of **3c** (Table 2, entry 2) as compared to other solvents. Thus, further reaction conditions were optimized considering toluene as a solvent. The reaction was carried out at 350 W and 100 ˚C gave 81% yield (Table 2, entry 6) comparatively 1% lead as compared to 300 W (Table 2, entry 2). Further at 300 W power and temperature 120 ˚C, gave 84% yield (Table 2, entry 7). Reaction was also conducted at 250 W and 120 ˚C, gave 77% yield (Table 2, entry 8), which was a lower yield in comparison to 300 W. Therefore, considering these results, the optimized conditions were set as toluene as solvent,

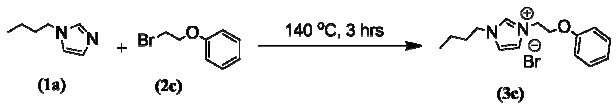
300 W MW power and temperature 120 ˚C to synthesized a series of ILs (**3a-3h)**.

Method C: Heating method (solvent-free)

Equimolar amount of reactants (**1a** and **2c**) was refluxed at 100 ºC, 120 ºC, 140 ºC and 160 ºC temperature with constant stirring at variable times from 2 to 5 h. To, optimize the reaction duration, the reaction mixture was refluxed at 100 ºC for 5, 4, 3 and 2 h with 70, 69, 68, and 62% yields respectively (Table 3, entries 1- 4). Further, the reaction mixture was reflux at 120 ºC for 4, 3 and 2 h with 73, 72 and 69% yields respectively (Table 3, entries 5-7).

It is seen that, with the increase in temperature and reaction duration has increased the yields of **3c**, whereas the yields of **3c** decreased on shortening the reaction duration. Fascinatingly, it was observed that reaction carried out at temperature 100 ºC and 120 ºC for 3 h had resulted good yield with respect to other reaction times. About 1% or 2% increase in yield with respect to the other times duration at the same temperature (100 ºC or 120 ºC) was found. Thus, the 3 h of reaction duration were chosen as best for this method. After optimizing the reaction duration, the reaction temperature was optimized; by performing the reaction at 140 ºC and 160 ºC for 3 h which yielded 84 and 85%.

Table 3: Optimization of reaction conditions through heating method



(1a) (2c) (3c)

Entry	Temp.	Method	Solvents	Time (hrs.)	Yield (%)
1	100 ºC	Reflux	-	5	70
2	100 ºC	Reflux	-	4	69
3	100 ºC	Reflux	-	3	68
4	100 ºC	Reflux	-	2	62
5	120 ºC	Reflux	-	4	73
6	120 ºC	Reflux	-	3	72
7	120 ºC	Reflux	-	2	69
8	140 ºC	Reflux	-	3	84
10	160 ºC	Reflux	-	3	85

Interestingly, the reaction performed at 160 ºC take leads of 1% over the reaction performed at 140 ºC for the same period of time. Thus, the suitable reaction condition for solvent free heating method for the model reaction was 140 ºC and 3 h of reaction duration. Scope and versatility of optimized conditions for solvent-free heating method was studied by preparing a series of new eight ILs (**3a-3h**) by using different substitution of alkyl bromide (**2a-2h**).

Thus, considering these optimized conditions a series of ILs have been synthesized. Hence, it could be conclude, that in non-conventional methods A and B under MW irradiation, there is considerable rate enhanced bringing down the reaction time from hours to minutes with improved yield and purity of the products in comparison of heating method. The reaction time and yield comparisons of all methods are given in Table 4.

Table 4: Comparison between MW methods and Heating method

ILs	MW solvent-free		MW with solvent		Heating method (solvent free)	
	Time (min.)	Yield (%)	Time (min.)	Yield (%)	Time (hrs.)	Yield (%)
3a	25	92	30	85	3	82
3b	25	85	30	84	3	79
3c	25	90	30	85	3	84
3d	25	84	30	74	3	81
3e	25	93	30	79	3	85
3f	25	86	30	82	3	83
3g	25	82	30	79	3	81
3h	25	95	30	78	3	87

It was found that higher MW irradiation power did not increase the product yield. Further, it was observed an improvement in the yield when reaction takes place in the absence of solvent under MW. Therefore, the MW solvent-free method has been standardized and offered new vistas towards simplification of laboratory techniques without the use of stirrers, reflux condenser, water separators and use of noxious solvents. The identity of synthesized ILs by heating method and microwave induced methods was established by their co-TLC and super imposable IR spectra. For each method the IL (**3c)** was confirmed by using proton NMR, LCMS, FTIR spectra.

ILs derived from imidazole may have varied structures depending on the nature of cationic and anionic parts. In present research work, the cationic part of the ILs consists of 1-butylimidazole and has been kept fixed while the anionic part has been varied by using alkyl bromide chains of different lengths. The purpose behind synthesis of imidazole based ILs with varying structures is to comprehend the nature of these novel solvents. The prevailing methods of preparations of ILs such as 1,3-disubstituted imidazolium halides, generally require longer reaction times along with considerable contamination with halide ions (X= Br^-, Cl^-, I^-, F^-). Besides, additional steps are required to meet the quaternization reaction for converting the halide containing precursors to other ILs. Purification of ILs is a matter of foremost concern, as impurities have been found to significantly affect the biological as well as physicochemical properties of these ILs which in turn regulate their domain of applications.[23, 24]

The structures of synthesized ILs (**3a-3h**) were confirmed with ^1H NMR, ^{13}C NMR, FTIR and LCMS (see ESI†). The quaternization reaction of 1-butylimidazole with different alkyl halides were established by their FTIR spectra, showed the presence of –C–N bond stretching in region of 1120 to 1290 cm^{-1} due to formation of quaternary nitrogen of imidazole ring and oxygen in aliphatic chain, while peaks at 1630, 1587, 1560 cm^{-1} are due to –C=N bond of five membered imidazolium ring. The C-N is experiencing an addition that leads of oxygen electron, thus affects the bond stretching of the –C=N bond. Additionally, the peaks at 1497, 1457, 1406 cm^{-1} are due to –C=C– bond of phenyl and imidazolium rings. Oxygen atom present in few ILs (**3c**, **3e**, **3g**, **3h**) showed peaks at 1174 cm^{-1} due to ether linkage (–C-O-C–). The aliphatic -CH_2 and-CH_3 appeared in the region of

2830 to 2970 and 3070 to 3134 cm^{-1} respectively of all the ILs. Positive mode ESI-MS spectra of the synthesized ILs (**3a-3h**) exhibited the $[M-Br]^+$ molecular ion peaks, confirming their molecular masses. ^1H NMR spectra of ILs (**3a**, **3b**, **3d**, **3e** and **3g**), two methylenic protons of imidazole ring appeared as doublet from δ 7.70 to 7.92 ppm with coupling constant J = 9-12 Hz, while in the ILs (**3c**, **3f** and **3h**) methylenic protons appeared as singlet. The single proton of imidazole ring as a singlet appeared in the downfield in the range of δ 9.0 to 9.9 ppm. The differences in the proton shifting of imidazole ring could be attributed to alkyl chain lengths in ILs. For ILs (**3a-3h**), the five aromatic protons appeared as multiplet in the desired aromatic range. The 9H protons of butyl chain in all ILs (**3a-3h**) as -$CH_3CH_2CH_2CH_2$- attached to imidazole ring were observed in upper field where the terminal 3H proton of -CH_3 appeared as a triplet in the region of δ 0.90 ppm with coupling constant J = 7.5 Hz, 2H of – $CH_3\mathbf{CH_2}CH_2CH_2$- as sextet at δ 1.10 to 1.30 ppm (J = 7.5 Hz), 2H of $CH_3CH_2\mathbf{CH_2}CH_2$- as quintet at δ 1.52 to 1.84 ppm (J = 7.5 Hz) and 2H of $CH_3CH_2CH_2\mathbf{CH_2}$- as triplet at δ 3.93 to 4.30 ppm (J = 7.5 Hz) respectively. The -CH_2 protons of alkyl chains attached with quaternary nitrogen of imidazole ring were appeared in the range of the δ 1.0 to 4.9 ppm as per their chemical shift in the standard splitting pattern as triplet and quintet.

^{13}C NMR spectra provided a final structural elucidation of ILs (**3a-3h**). Therefore, signals due to formation of quaternary nitrogen in 1-butylimidazolium based ILs were found in the range of at δ 48.50 ppm to 55.96 ppm. So the ILs (**3f**) and (**3h**) appeared in downfield at δ 55.97 ppm, while ILs (**3b**) and (**3e**) appeared in upper field at δ 48.57 ppm and δ 48.54 ppm respectively. The two carbons (-CH=CH-) of imidazole ring in ILs were found at δ 119.0 ppm to 125.0 ppm while third carbon as -N-C=N- appeared in the range of δ 134.70 ppm to 136.57 ppm respectively. In general, the aromatic phenyl ring carbons appeared in the range of 114.4 ppm to 131.0 ppm, where the alkyl substituted carbon of phenyl ring appeared in downfield at δ 134.0 to 163.0 ppm.

The UV-Vis spectra are exclusively recorded for the eight different ILs using DMSO, THF, ACN, MeOH, EtOH, $CHCl_3$ solvents. For each solvent 1mM of ILs was used to determine the electronic transitions.[43] The experiment was carried out at rt for ILs (**3a-3h**) in the range of the 200 to 600 nm and illustrated in Figure 2 (see ESI‡ for absorption spectra of 3b, 3c, 3e-3h). The extinction coefficient (ε) was calculated for each band which is the characteristic molecular property of ILs owing to their different electronic transitions. The quantitative relation between absorbance, extinction coefficient, concentration and path length is given by the Beer–Lambert law. In a row to study the absorption spectra of eight different ILs in six solvents, the absorption spectra of ILs (**3a-3h**) have been recorded using DMSO, EtOH, $CHCl_3$, MeOH, ACN and THF.

Each IL showed two bands in the region of 200 to 300 nm, which are attributed to their $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions respectively. The $n\rightarrow\pi^*$ transition is due to lone pair of nitrogen present in imidazole ring and oxygen in aliphatic chain, while $\pi\rightarrow\pi^*$ transition is attributed to the conjugated aromatic phenyl ring and imidazole ring and oxygen in aliphatic chain, while $\pi\rightarrow\pi^*$ transition is attributed to the conjugated aromatic phenyl ring and imidazole ring. The effect of different solvents on electronic transitions of ILs can be seen from their electronic transition spectra (Figure 2), where the DMSO showed maximum absorbance or hyperchromic shift as compared to other solvents, while MeOH showed least absorbance. In general it was found that DMSO showed red shift of ≈ 30 nm (250 nm) in comparison to MeOH (230 nm). The descending order of the ILs (**3b-3h**) can be arranged on the basis their absorbance as (**3a**): DMSO > ACN > THF > $CHCl_3$ > EtOH > MeOH. In order of their decreasing polarity of the solvents (**3b**): DMSO > THF > ACN > MeOH > $CHCl_3$ > EtOH, (**3c**): DMSO > ACN > $CHCl_3$ > THF > MeOH ≈ EtOH, (**3d**): DMSO > ACN > $CHCl_3$ > THF > MeOH > EtOH,

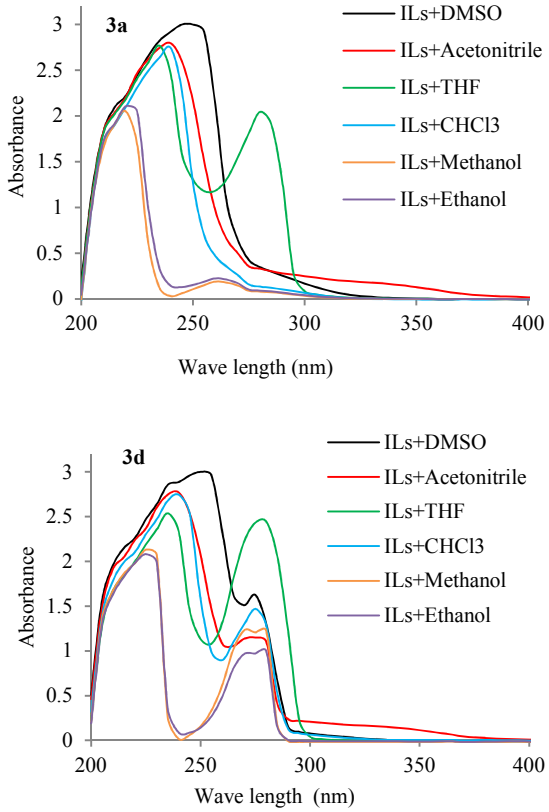


Figure 2: UV-Vis absorption spectra of ILs 3a and 3d

(**3d**): DMSO > ACN > $CHCl_3$ > THF > MeOH > EtOH, (**3e**): $CHCl_3$ > DMSO > ACN > EtOH > THF > MeOH, (**3f**): DMSO > ACN > $CHCl_3$ > THF > MeOH > EtOH, (**3g**): DMSO > ACN > THF ≈ MeOH > $CHCl_3$ > EtOH, (**3h**): DMSO > ACN > $CHCl_3$ > THF > EtOH > MeOH. Thus the extinction coefficient and lambda value of solute in solvents decrease with decrease in polarity and dielectric constants of the solvents. Thus, the differences in electronic transitions might result from the different conjugation degrees and different electronic environment of the ILs. The effect of different solvents on electronic transitions of ILs can be seen from their electronic

transition spectra (Figure 2), where the DMSO showed maximum absorbance or hyperchromic shift as compared to other solvents, while MeOH showed least absorbance.

Biological activities

Antibacterial

Initially, to investigates compounds as antibacterial agent, due to presence of ether functionality substituted aromatic ring and nitrogen containing five membered imidazole ring, were screened for their antibacterial activity against human pathogenic gram-positive (*S. aureus* and *B. subtilis*) and gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains. Compounds were screened at 100 µg mL^{-1} concentrations by Agar disc diffusion method and the zones of inhibition illustrated in Table 5.

Table 5: Antimicrobial and antifungal activity of ILs in terms of zone of inhibition (mm)

Strain ↓	Entry								
	Ionic Liquids						Standards		
	3a	3b	3c	3d	3e	3f	S1	S2	S3
S. aureus	9	15	21	8	16	13	30	22	NA
B. subtilis	9	14	19	10	12	13	26	21	NA
E. coli	-	8	-	8	9	-	29	21	NA
P. aeruginosa	18	11	17	-	13	-	24	22	NA
C. albicans	12	11	-	13	10	-	NA	NA	15
A. niger	-	-	-	-	-	-	NA*	NA	14

Data represent is mean of three replicates for each concentration
*Diameter in mm calculated by Vernier Caliper
'-' means no zone of inhibition, NA for not applicable
S1 =Chloramphenicol, S2 = Ciprofloxacin and S3 = Amphotericin-B

It is evident from the zone of inhibitions data that ILs **3b**, **3c**, **3e** and **3f** showed good activity against both gram positive strains having 15, 21, 16 and 13 mm and 14, 19, 12 and 13 mm zones of inhibitions against *S. aureus* and *B. subtilis* strains respectively (Table 5). The rest of ILs **3a** and **3d** displayed moderate activities against both gram-positive strains as compared to standards chloramphenicol (30 mm) and ciprofloxacin (22 mm). On other hand ILs **3b**, **3d** and **3e** showed moderate activities against *E. coli* strain in comparison to standard drugs, while **3a**, **3c** and **3f** showed no activity against *E. coli* strain. ILs **3a** and **3c** showed good activity against *P. aeruginosa* with zone of inhibitions 18 and 17 mm respectively. The ILs **3b** and **3e** showed moderate activities, while **3d** and **3f** showed no activities against *P. aeruginosa* strain (Table 5).

Thus, it is clear that the switching in alkyl chain of ILs seems to be responsible for their higher and lower antibacterial activities, because the rest of the structures *viz* imidazole and phenyl ring were kept constant. The ILs having two and three $–CH_2$ chain (**3b** and **3d**) and $–CH_2$-O- (**3c** and **3e**) showed good activity. The introduction of oxygen as ether linkage in alkyl chain increases antibacterial activities in comparison to normal alkyl chain (**3b** versus **3c**). It was also an evident that on increasing alkyl chain length unto four $–CH_2$, lower the activities (**3b** versus **3f**), attributed to their hydrophobic effect. Thus, the introduction of oxygen in alkyl chain increase the ability to irritate the cell membranes or inhibition of bacterial growth could be dependent on the ability of the ILs to cross the outer membrane in gram-negative bacteria and the cell wall in gram-positive bacteria.[44]

Antifungal activity

Antifungal activity of ILs was determined by Agar disc diffusion method against *C. albicans* and *A. niger* at 100 µg mL^{-1} concentration and zone of inhibitions were determined and summarized in Table 5. Compounds **3a**, **3b**, **3d** and **3e** with zone of inhibitions 12, 11, 13 and 10 mm respectively, showed good activity against *C. albicans*, while all the ILs (**3a-3f**) showed no activities against *A. niger* strain (Table 5). The zone of inhibitions was compared to standard drug Amphotericin-B having 15 mm zone of inhibition. From the results of antifungal activity no significant effect of oxygen was found, whereas it showed good activities against bacterial strains.

Anticancer Activity

The *in vitro* anticancer activity of five ILs **3a**, **3b**, **3d**, **3e** and **3g** was performed against the MCF-7 and MDA-MB-435 at four dose levels of 0.1, 1.0, 10 and 100 µM in DMSO and the test consisted of a 48 h continuous drug exposure protocol using SRB assay to estimate cell growth. Suitable positive controls were run in every experiment, which was repeated thrice and a graph was plotted against percentage control growth and concentration (Figures 3 and 4) to calculate numerous parameters.

Results are given in terms of GI_{50} (concentration of drug that produces 50% inhibition of the cells), TGI (concentration of the drug that produces total inhibition of the cells) and LC_{50} (concentration of the drug that kills 50% of the cells) values that were calculated from the mean graph (Figures 3 and 4) and given in Table 6. Adriamycin (ADR), which is a chemotherapy drug often used to kill cancerous cell, was used as the standard anticancer drug. Reported parameters are given in Table 6. The compounds which have GI_{50} values of ≤ 0.1 µM and 24.4 µM were considered to demonstrate anticancer activity against MCF-7 and MDA-MB-435 cell lines respectively. The GI_{50} of each ILs against the MCF-7 cell line were found more than 100 µM, hence these ILs were found inactive, while, ADR showing better result with GI_{50} value (< 0.1 µM).

Further, ILs were evaluated against MDA-MB-435 cell line at same experimental conditions. The ILs **3a**, **3d** and **3e** demonstrated 50-60 % activity having GI_{50}values 67.2, 52.5 and 57.5 µM respectively as compared to standard ADR (GI_{50} 24.4 µM). The GI_{50} of **3b** and **3g** were found 96.3 and more than100 µM respectively, which claim them inactive against MDA-MB-435 cell line. The trend of GI_{50} value of ILs and ADR against MDA-MB-435 cell line noted as **ADR** > **3d** > **3e** > **3a** > **3b** > **3g**.

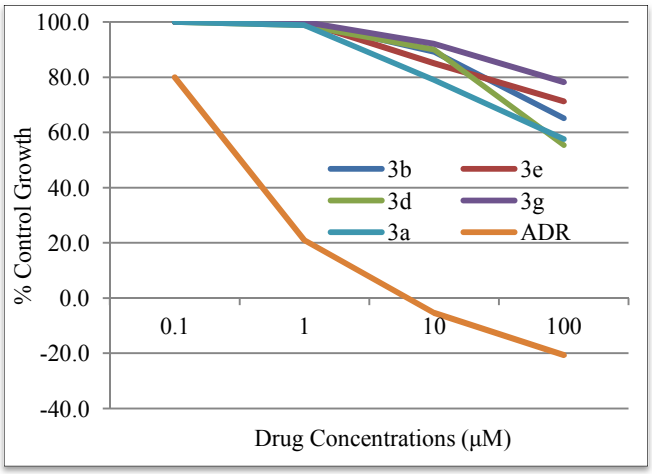


Figure 3: Curve between percentage control growth and concentrations of drugs on MCF-7 cell line

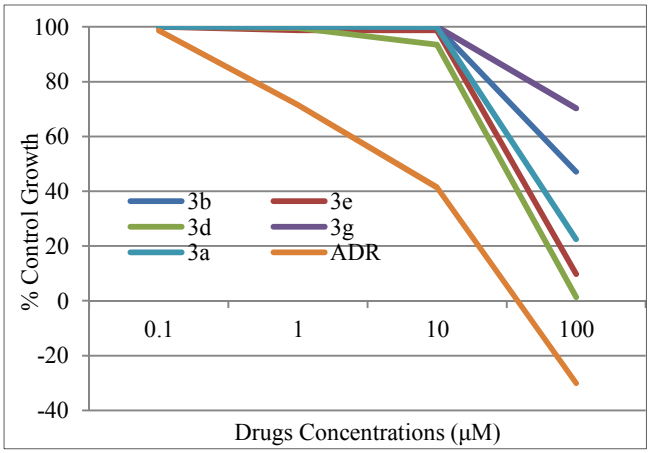


Figure 4: Curve between percentage control growth and concentrations of drugs on MDA-MDMB-435 cell line

Table 6: *In-vitro* testing expressed as growth inhibition of human cancer cell lines MCF-7 and MDA-MB-435 of ILs

ILs	MCF-7			MDA-MB-435		
	LC_{50}*	TGI*	GI_{50}*	LC_{50}*	TGI*	GI_{50}*
3a	>100	>100	>100	>100	>100	67.2
3b	>100	>100	>100	>100	>100	96.3
3d	>100	>100	>100	>100	>100	52.5
3e	>100	>100	>100	>100	>100	57.9
3g	>100	>100	>100	>100	>100	>100
ADR	>100	60.3	<0.1	>100	70.8	24.4

Data represent is mean of three replicates for each concentration DMSO was used as a solvent

*LC_{50} = concentration of drug causing 50% cell kill

*TGI = concentration of drug causing total inhibition of cell growth

*GI_{50} = concentration of drug causing 50% inhibition of cell growth

*GI_{50} values of ≤ 0.1 μM and 24.4 μM are considered to demonstrate anticancer activity against MCF-7 and MDA-MB-435 cell lines respectively.

In general the LC_{50} which is a parameter of cytotoxicity and reflects the molar concentration needed to kill 50% of the cells were also found more than 100 μM for ILs as well as ADR for both cell lines. Thus, it can be concluded that ILs **3d** having two $–CH_2$ alkyl chain and **3e** with additional oxygen in $–CH_2$ chain demonstrated 50% activity, while increasing the alkyl chain length up to four $–CH_2$ in IL (**3g**) decreases activity against the MDA-MB-435 cell line. These differences might be due to hydrophobic effect of alkyl chain length.

Conclusion

The present study reports the synthesis of imidazole based ILs under MW solvent-free approach proved to be compatible, efficient, eco-friendly and green route of synthesis. The solvent-free synthesis is better than the solvent free Heating method with shorter reaction time (hrs to min), better yield, simpler work-up and afforded 82-95% yield of ILs (**3a-3h**). Their characterization was made using various spectral techniques. Further, ILs (**3b** and **3d**) and (**3c** and **3e**), possessed good antibacterial activities against the gram-positive (*S. aureus* and *B. subtilis)* and gram-negative (*E. coli* and *P. Aeruginosa*) strains respectively. ILs **3a**, **3b**, **3d** and **3e** showed good activities against *C. albicans* fungal strain, whereas none of the ILs expressed activity against *A. niger*. Additionally, ILs **3a**, **3d** and **3e** showed ≈ 50 % activity against MDA-MB-435 cell line based on their GI_{50} values. The cytotoxicity data based on their LC_{50} values were found in the range of the standard drug adriamycin. Thus, the synthesized ILs could be used as a catalyst, solvent, electrolyte and other domains of chemical and medical sciences to design novel TILs by modulating their anionic and cationic parts.[11]

Experimental

Material and methods

Chemicals used were of analytical reagent grade procured from Sigma- Aldrich. Ethyl acetate (EA), DCM, ACN, toluene, EtOH, DMSO, cyclohexane and EtOH solvents were of Rankem, India and used without further purification.

The reactions were carried out using Anton Paar Synthos 3000 microwave reaction system in close vessels. Reaction progress was monitored through TLC (Merck, silica GF257) using EtOH and ethyl acetate (1:9 V/V) solvent system and spot were visualized under UV light (RICO scientific industries, Model RSUV-5). FTIR spectra were recorded with a Perkin Elmer spectrum 65 FTIR spectrometer using KBr plates, characteristic wave numbers are given in cm^{-1}. Mass spectral analysis was accomplished with Agilent Technologies G6520B LCMS (Q-TOF) mass spectrometry with +ESI ionization method. The 0.02% trifloroacetic acid in water and acetonitrile: EtOH (60:40 v/v) was run as mobile phase in the ratio of 30:70% v/v, on an Agilent zorbax 300 SB-C18 column (3.5 mm, 4.6 × 50 mm) with flow rate of 0.5 mL min^{-1}. ^1H and ^{13}C NMR spectra were recorded at room temperature (rt) in 5 mm tube using Bruker Avance III-500 MHz spectrometer in deuterated or $CDCl_3$ and DMSO-d_6, TMS as an internal standard. The chemical shifts are given in δ ppm and coupling constant (J) in Hz. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (q), sextet (s) and multiplet (m). The absorption transition

(λ_{max}) was recorded in EtOH at r.t. ranging from 200–600 nm using Analytical UV spectro 2060 plus. The sample was scanned using 1 cm path length cuvette.

Synthesis of ILs

A series of eight novels ILs (**3a-3h**) have been synthesized according to Scheme 1, using 1-butylimidazole (**1a**) as cationic moiety and substituted aryl alkyl and alkoxy bromide as anionic moieties (**2a-2h**). Three different methods such as MW assisted solvent-free method (method A), MW assisted solvent using solvent phase method (method B) and solvent free Heating method (method C).

Method (A): MW assisted solvent-free approach

Equimolar ratio of 1-butylimidazole (**1a**, 0.04 mol) and aryl alkyl bromide (**2a-2h**, 0.04 mol) as reactants were taken into the teflon reaction vials and the reaction has been carried out for 25 min at 350 W, 20 bar pressure and temperature 140 ºC. Reaction was continuously monitored with TLC, till its completion at after each 60 sec interval. After completion, the ILs were washed with 10 mL of EA for 4-6 times, and then ILs was dried at 60 ^{o}C for 1 h on a rotary evaporator under reduced pressure. Further, ILs was kept under vacuum for overnight to ensure the complete removal of moistures and EA, before taking the spectroscopic measurements. All ILs were viscous with light yellowish color. The yield and reaction time is given in Table 4.

Method (B): MW assisted synthesis with solvent approach

Equimolar ratio of 1-butylimidazole (**1a**, 0.04 mol) and aryl alkyl bromide (**2a-2h**, 0.04 mol) were taken into teflon reaction vials followed by 10 mL of toluene were added to the reaction vials and the reactions were performed for 30 min at 300 Watt, 20 bar, and 120 ºC. The reaction was continuously monitored with TLC till completion. After completion, toluene was taken off from the products with the help of rotary evaporator and then the ILs were washed with 10 mL of ethyl acetate for 4-6 times, after that ILs were dried at 60 ºC for 1 h on a rotary evaporator under reduced pressure. The ILs was further kept in vacuum oven for overnight to ensure the complete removal of moistures and ethyl acetate, before taking the spectroscopic measurements. ILs obtained were viscous with light yellowish color. The yield and reaction time is given in Table 4.

Method (C): heating method (solvent-free)

To a 50 mL round bottom flask equimolar ratio of 1-butylimidazole (**1a**, 0.04 mol) and aryl alkyl bromide (**2a-2h**, 0.04 mol) were taken and the reactions system was reflux for 3 h at 140 °C with constant stirring. The reaction progress was continuously monitored at regular interval with TLC till completion. After completion the newly synthesized ILs was washed with 10 mL of EA for 4-6 times. The ILs were dried at 60 ºC for 1 h on a rotary evaporator under reduced pressure, were kept in vacuum oven overnight to ensure the complete removal of moistures and EA. All ILs were viscous with light yellowish color. Reaction time and yield of the novel ILs **(3a-3h)** synthesized by all the three methods are compared in the Table 4.

3-benzyl-1-butyl-1*H*-imidazol-3-ium bromide (3a)

Chemical Formula: $C_{14}H_{19}BrN_2$; Color: Light yellowish; State: liquid; FTIR (KBr, cm^{-1}): ν_{max} 3133, 3070 (-CH str., -CH_3); 2968, 2936, 2877 (-CH str., -CH_2); 1629, 1562 (-C=N, imidazole); 1499, 1460, 1408 (-C=C); 1365, 1322, 1278, 1211 (C-C); 1160 (C-N); 1108, 1049, 1029 (-C-H, bending); 820, 757, 714 (str., -CH_2). UV-Vis [λ in nm (ε in M^{-1} L^{-1})**]:** DMSO [250 (3000)]; EtOH [220, 260 (2108, 190)]; THF [235, 280 (2770, 2046)]; EtOH [220 (2151)]; $CHCl_3$ [240 (2745)]; ACN [240 nm (2796)]. ^{1}H NMR **(**500 MHz, DMSO-d_6**):** δ 0.89 (t, 3H, *J*= 7.5 Hz, -CH_3); 1.25 (sex, 2H, *J* =7.5 Hz, -CH_2); 1.79 (q, 2H, *J* =7.5 Hz, -CH_2); 4.23 (t, 2H, *J* = 7.0 Hz, -CH_2); 5.53 (s, 2H, -CH_2); 7.39 -7.50 (m, 5H, Ar ring); 7.92 (d, 2H, J = 10.5 Hz, imidazole ring); 9.60 (s, 1H, imidazole ring). ^{13}C NMR **(**125 MHz, DMSO-d_6**):** δ 13.25 (-CH_3), 18.75, 31.26, 48.55, 55.95 (-CH_2), 122.44, 122.78, (C_2, C_3 imidazole ring), 127.99, 128.32, 128.40, 128.93, 128.95 (C_2,-C_6, Ar ring), 134.95 (C_1, Ar ring), 136.09 (C_1, imidazole ring). +ESI-MS (*m/z*)**:** Calculated for $C_{14}H_{19}N_2^+$ (M-Br)$^+$ 215. 1543, found 215.1515.

1-butyl-3-phenethyl-1*H*-imidazol-3-ium bromide (3b)

Chemical formula: $C_{15}H_{21}BrN_2$; Color: Light yellowish; State: Liquid; FTIR (KBr, cm^{-1})**:** v_{max} 3137, 3062 (-CH str., -CH_3); 2964, 2932, 2873 (-CH str., -CH_2); 1629, 1606 (-C=N, imidazole), 1562, 1495,1456 (-C=C); 1361, 1333 (C-C); 1164 (-C-N); 1085, 1033, 860 (-C-H, bending); 753, 706 (C-H, -CH_2). UV-Vis [λ in nm (ε in M^{-1} L^{-1})**]:** DMSO [280, 290 (3046, 2959)]; EtOH [280 (252)]; THF [280 (2018)]; EtOH [280 (319)]; $CHCl_3$ [280 (257)]; ACN [280 (437)]. ^{1}H NMR **(**500 MHz, DMSO-d_6**):** δ 0.90 (t, 3H, *J*= 7.5, -CH_3); 1.26 (sex, 2H, *J* = 7.5 Hz, -CH_2); 1.77 (q, 2H, *J* =7.5 Hz, -CH_2); 2.59 (t, 2H, *J* = 7.5 Hz, -CH_2); 4.16 (t, 2H, *J* = 7.0 Hz, -CH_2); 4.20 (t, 2H, *J* = 7.5 Hz, -CH_2); 7.31-7.19 (m, 5H, Ar ring); 7.83 (d, 2H, J = 12.0, imidazole ring); 9.27 (s, 1H, imidazole ring). ^{13}C NMR (125 MHz, DMSO-d_6**):** δ 13.26 (-CH_3); 18.76, 30.70, 31.44, 48.57 (5 -CH_2); 122.42, 122.43 (C_2, C_3, imidazole ring); 126.07 (C_2, C_4, C_6 Ar ring); 128.30 (C_3, C_5, Ar ring); 136.01 (C_1, imidazole ring); 140.43 (C_1, Ar ring). +ESI-MS (*m/z*)**:** Calculated for $C_{15}H_{21}N_2^+$ (M-Br)$^+$ 229. 1699, found 229.1661.

1-butyl-3-(2-phenoxyethyl)-*1H*-imidazol-3-ium bromide (3c)

Chemical formula: $C_{15}H_{21}BrN_2O$ Color: Light yellowish; State: liquid; FTIR (KBr, cm^{-1}): v_{max} 3143, 3084 (-CH str., -CH_3); 2958, 2934, 2871 (-CH str., -CH_2); 1599, 1587, 1564 (-C=N, imidazole); 1493, 1469 (-C=C); 1387, 1359 (C-C); 1296, 1237 (-C-N); 1174 (-C-O); 1083, 1056 (-C-H, bending); 914, 760, 697 (str., -CH_2). UV-Vis [λ in nm (ε in M^{-1} L^{-1})]: DMSO [250, 280 (3222, 2699)]; EtOH [230, 275 (2538, 2036)]; THF [235, 275 (2770, 2678)]; EtOH [230, 275 (2538, 1896)]; $CHCl_3$ [240, 275 (2796, 1682)]; ACN [245, 275 (3046, 2244)]. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (t, 3H, *J*= 7.5, -CH_3); 1.30 (sex, 2H, *J* = 7.5 Hz, -CH_2); 1.84 (q, 2H, *J* =7.5 Hz, -CH_2); 4.85-4.30 (m, 6H, -CH_2); 6.90 (d, 2H, *J*=8.0 C_2, C_6, Ar ring); 6.94 (d, 2H, *J*= 7.0 C_3, C_5, Ar ring); 7.23 (t, 1H, *J*= 7.5, C_4 Ar ring); 7.80, 7.56 (s, 2H, C_2, C_3 imidazole ring); 9.93 (s, 1H C_1, imidazole ring). ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.32 (-CH_3); 19.24, 31.86, 49.24, 49.63, 66.04 (-CH_2); 114.41 (C_2, C_6, Ar ring); 121.51 (C_4, Ar

ring); 122.03, 123.20 (C_2, C_3 imidazole ring); 129. 49 (C_3,C_5, Ar ring); 136.52 (C_1, imidazole ring); 157.43 (C_1, Ar ring). +ESI-MS **(*m/z*):** Calculated for $C_{15}H_{21}N_2O^+$ $(M\text{-}Br)^+$ 245.1648, found 245.1618.

1-butyl-3-(3-phenylpropyl)-1*H*-imidazol-3-ium bromide (3d)

Chemical Formula: $C_{16}H_{23}BrN_2$; Color: Light yellowish; State: Liquid; FTIR (KBr, cm^{-1}): ν_{max} 3139, 3072 (-CH str., $-CH_3$); 2962, 2934, 2871 (-CH str., $-CH_2$); 1627, 1603, 1564 (-C=N, imidazole); 1497, 1457 (-C=C); 1410, 1363, 1335 (C-C); 1166 (C-N); 1083, 1032 (-C-H, bending); 855, 752, 705 (str., $-CH_2$). UV-Vis [λ in nm (ε in M^{-1} L^{-1})]: DMSO [250, 275 (3000, 1625)]; EtOH [225, 280 (1767, 998)]; THF [235, 280 (2538, 2444)]; EtOH [225, 280 (2131, 1226)]; $CHCl_3$ [240, 275 (2745, 1470)]; ACN [240, 275 (2770, 1149)]. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.86 (t, 3H, $-CH_3$); 1.10 (sex, 2H, J = 7.5 Hz, $-CH_2$); 1.68 (q, 2H, J = 7.0 Hz, $-CH_2$); 3.14 (t, 2H, J = 7.0 Hz, $-CH_2$); 4.11 (t, 2H, J = 7.0, $-CH_2$); 4.45 (t, 2H, J = 7.0, $-CH_2$); 7.16 (d, 2H, J = 7.5 Hz, C2, C6 Ar ring); 7.23 (t, 2H, J = 7.0, C3, C5 Ar ring); 7.29 (t, 1H, J = 7.5 Hz, Ar ring); 7.78 (s, 2H, C2, C3, imidazole ring); 9.08 (s, 1H, imidazole ring). ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.21 ($-CH_3$); 18.54, 30.68, 31.23, 35.21($-CH_2$); 48.44, 49.83 ($-CH_2$); 122.41 (C_2, C_3 imidazole ring); 126.81 (C_4, Ar ring); 128.56 (C_2, C_3, C_5, C_6, Ar ring); 135.9 (C_1, imidazole ring); 136.7 (C_1, Ar ring). +ESI-MS (*m/z*): Calculated for $C_{16}H_{23}N_2^+$; $(M\text{-}Br)^+$ 243.1831, found 243.1832.

1-butyl-3-(3-phenoxypropyl)-1*H*-imidazol-3-ium bromide (3e)

Chemical formula: $C_{16}H_{23}BrN_2O$; Color: Light yellowish; State: Liquid; FTIR (KBr, cm^{-1}): ν_{max} 3139, 3072 (-CH str., $-CH_3$); 2962, 2934, 2871 (-CH str., $-CH_2$); 1631, 1599 (-C=N, imidazole); 1591, 1568, 1493, 1473 (-C=C); 1390, 1335 (C-C); 1237 (C-N); 1174 (-C-O, C-O-C); 1115, 1083, 1044 (-C-H, bending); 953, 823, 882, 756, 693 (C-H, $-CH_2$). UV-Vis [λ in nm (ε in M^{-1} L^{-1})]: DMSO [245, 275 (3000, 1442)]; EtOH [230, 275 (2409, 1216)]; THF [235, 280 (2377, 2208)]; EtOH [230, 275 (2201, 1224)]; $CHCl_3$ [245, 255 (3046, 3000)]; ACN [240, 275 (2569, 1227)]. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.87 (t, 3H, J= 7.5 Hz, $-CH_3$); 1.22 (sex, 2H, J = 7.5 Hz, $-CH_2$); 1.74 (q, 2H, J =7.5 Hz, $-CH_2$); 2.29 (q, 2H, J = 6.5 Hz, $-CH_2$); 4.01 (t, 2H, J = 6.0 Hz, $-CH_2$); 4.16 (t, 2H, J = 7.0 Hz, $-CH_2$); 4.37 (t, 2H, J = 7.0 Hz, $-CH_2$); 6.89 (d, 2H, J = 8.0 Hz, C2, C6 Ar ring); 6.94 (t, 1H, J = 7.5 Hz, C4); 7.28 (t, 2H, J = 7.5 Hz, C3, C5 Ar ring); 7.85 (d, 2H, J = 18.0 Hz, C_2, C_3, imidazole ring); 9.31 (s, 1H, imidazole ring). ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.24 ($-CH_3$); 18.75, 28.89, 30.96, 46.53, 48.54, 64.39 ($-CH_2$); 114.34 (C_2, C_6, Ar ring); 120.75 (C_4, Ar ring); 122.50 (C_2, C_3, imidazole ring); 129.47 (C_3, C_5, Ar ring); 136.16 (C_1, imidazole ring); 158.11 (C_1, Ar ring).+ESI-MS (*m/z*): Calculated for $C_{16}H_{23}N_2O^+$ $(M\text{-}Br)^+$ 259.1805, found 259.1774.

1-butyl-3-(4-phenylbutyl)-1*H*-imidazol-3-ium (3f)

Chemical formula: $C_{17}H_{25}BrN_2$ Color: Light yellowish; State: Liquid; FTIR (KBr, cm^{-1}):ν_{max} 3133, 3066, 3027 (-CH str., $-CH_3$); 2964, 2936, 2865 (-CH str., $-CH_2$); 1606, 1566 (-C=N, imidazole), 1495, 1456 (-C=C-); 1369, 1329 (C-C); 1164 (C-N); 1116, 1029 (-C-H, bending); 860, 753, 702 (C-H, $-CH_2$). UV-Vis [λ in nm (ε in M^{-1} L^{-1})]: DMSO [250, 275 (3000, 1625)]; EtOH [225, 280 (2081, 998)]; THF [235, 280 (2538, 2444)]; EtOH [225, 270, 280 (2131, 1232, 1226)]; $CHCl_3$ [240, 275 (2745, 1470)]; ACN [240, 275 (2770, 1149)]. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.88 (t, 3H, J= 7.5 Hz, $-CH_3$); 1.23 (sex, 2H, J =7.5 Hz, $-CH_2$); 1.52 (q, 2H, J = 7.5 Hz, $-CH_2$); 1.858-1.746 (m, 4H, $-CH_2$); 2.60 (t, 2H, J = 7.5 Hz, $-CH_2$); 4.25-4.18 (m, 4H, $-CH_2$); 7.28-7.15 (m, 5H, C_2-C_6 Ar ring); 7.87 (s, 2H, C_2, C_3, imidazole ring); 9.41 (s, 1H, imidazole ring). ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.24 ($-CH_3$); 18.74, 27.41, 28.99, 31.25, 34.29, 48.54, 55.97 ($-CH_2$); 119.36, 122.44 (C_2, C_3, imidazole ring); 125.78 (C_4, Ar ring); 128.24 (C_2, C_3, C_5, C_6, Ar ring); 135.96 (C_1, imidazole ring); 141.57 (C_1, Ar ring). +ESI-MS (m/z): +ESI-MS (*m/z*): Calculated for $C_{17}H_{25}N_2^+$ $(M\text{-}Br)^+$ 257.2012, found 257.1996.

1-butyl-3-(4-phenoxybutyl)-1*H*-imidazol-3-ium bromide (3g)

Chemical formula: $C_{17}H_{25}BrN_2O$ Color: Light yellowish; State: Liquid; FTIR (KBr, cm^{-1}): ν_{max} 3139, 3076 (-CH str., $-CH_3$); 2962, 2934, 2875 (-CH str., $-CH_2$); 1627, 1599 (-C=N, imidazole); 1587, 1564, 1493, 1473 (-C=C); 1390, 1335 (C-C); 1296 (-C-N); 1245, 1166 (-C-O, C-O-C); 1119, 1083, 1032 (-C-H, bending); 760, 693 (C-H, $-CH_2$). UV-Vis [λ in nm (ε in M^{-1} L^{-1})]: DMSO [250 (3000)]; EtOH [225 (2032)]; THF [235, 280 (2721, 2569)]; EtOH [240 (2721)]; $CHCl_3$ [225 (2092)]; ACN [240 (2770)]. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.9 (t, 3H, J= 7.5 Hz, $-CH_3$); 1.25 (sex, 2H, J = 7.5 Hz, $-CH_2$); 1.70 (q, 2H, J =6.5 Hz, $-CH_2$); 1.80 (q, 2H, J = 7.5 Hz, $-CH_2$); 1.97 (q, 2H, J = Hz, $-CH_2$); 3.99 (t, 2H, J = 6.0, $-CH_2$); 4.17 (t, 2H, J = 7.0, $-CH_2$); 4.26 (t, 2H, J = 7.0, $-CH_2$); 6.93 (t, 3H, J = 7.5 Hz, C_2, C_4, C_6 Ar ring); 7.29 (t, 2H, J = 8.0, C_3, C_5 Ar ring); 7.84 (d, 2H, J = 10.0, imidazole ring); 9.28 (s, 1H, imidazole ring). ^{13}C NMR (125 MHz, DMSo-d_6): δ 18.50 ($-CH_3$); 24.02, 30.59, 31.55, 35.93, 36.49, 53.81, 71.76 ($-CH_2$); 119.61 (C_2, C_6, Ar ring); 125.77 (C_2, C_3, imidazole ring and C_4, Ar ring); 127.64 (C_3, C_5, Ar ring); 134.70 (C_1, imidazole ring); 163.63 (C_1, Ar ring). +ESI-MS (*m/z*): Calculated for $C_{17}H_{25}N_2O^+$ $(M\text{-}Br)^+$ 273.1961, found 273. 1943.

1-butyl-3-(6-phenylhexyl)-1*H*-imidazol-3-ium bromide (3h)

Chemical Formula: $C_{19}H_{29}BrN_2O$; Color: Light yellowish; State: Liquid; FTIR (KBr, cm^{-1}): ν_{max} 3137, 3073 (-CH str., $-CH_3$); 2940, 2864 (-CH str., $-CH_2$); 2469, 2059 ($-CH_2$, bending); 1605, 1601, 1585, 1564 (-C=N, imidazole); 1496, 1468 (-C=C); 1387, 1335 (C-C); 1299, 1247 (C-N); 1170 (-C-O-C-); 1118, 1082, 1033 (-C-H, bending); 885, 756, 695 (str., $-CH_2$). UV-Vis [λ in nm (ε in M^{-1} L^{-1})]: DMSO [245, 255, 275 (2959, 2921, 1411)]; EtOH [225, 225 (2137, 1031)]; THF [235, 275 (2658, 2469)]; EtOH [225, 275 (2119, 1164)]; $CHCl_3$ [240, 275 (2699, 1091)]; ACN [240, 275 (2721, 1164)]; ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.9 (t, 3H, J= 7.5 Hz, $-CH_3$), 1.26 (sex, 4H, J =7.0 Hz, $-CH_2$); 1.43 (d, 2H, J= 7.0 Hz, $-CH_2$); 1.69 - 1.83 (m, 6H, $-CH_2$); 3.93 (t, 2H, J = 6.0, $-CH_2$); 4.20 (d, 4H, J= 7.0 Hz, $-CH_2$); 6.90 (t, 3H, J= 3.0 Hz, C_2, C_4, C_6 Ar ring); 7.27 (t, 2H, J = 6.0 Hz, C_3, C_5 Ar ring); 7.88 (s, 2H, C_2, C_3, imidazole ring); 9.42 (s, 1H, imidazole ring). ^{13}C NMR (125 MHz, DMSO-d_6): δ13.23 ($-CH_3$); 18.74, 24.86, 25.20, 28.40, 29.22, 31.27, 48.61, 55.96, 67.03 ($-CH_2$); 114.32 (C_2, C_6, Ar ring); 120.31 (C_4, Ar ring); 122.41 (C_2, C_3, imidazole ring); 129.41 (C_3, C_5, Ar ring); 135.95 (C_1, imidazole ring); 158.55 (C_1, Ar ring). +ESI-MS (*m/z*): Calculated for $C_{19}H_{29}N_2O^+$ $(M\text{-}Br)^+$ 301.2274, found 301.2252.

Antibacterial activity

The synthesized ILs **3a-3f** were evaluated against the human pathogenic gram-positive *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2250) and gram-negative *Escherichia coli* (NCIM 2109), *Pseudomonas aerugenosa* (NCIM 2036) bacterial strain by Agar disc diffusion method.[45] Stock solution of 1000 µg/mL of each compound was prepared in DMSO. Assay carried out by taking concentration of 100µg/mL. Hi-media antibiotics disk: chloramphenicol and ciprofloxacin were used as reference drugs. Nutrient agar microbiological media used for gram-positive and gram-negative strains was obtained from Hi-media (India) and its composition (grams per liter) has sodium chloride, 5.0; beef extract 10.0; peptone 10.0 (pH 7.2).The zone of inhibition was measured in millimeter (mm) for ILs (**3a-3f**) after 24 h incubation at 37 °C and pH 7.2.

Antifungal activity

The synthesized ILs were screened against human pathogenic fungal strain *viz*. *Candida albicans* (NCIM 3471) and *Aspergillus niger* (NCIM 545) by Agar disc diffusion method at 100 µg/mL.[45] Potato dextrose agar (Hi-media, India) was used as medium for *A. niger* and its composition (gram per liter) has potatoes infusion, 200.0; dextrose 20.0 (pH 5.2). Microbiological media for *C. albicans* used was MGYP (all ingredients of Hi media) and its composition (gram per liter) has malt extract, 3.0; glucose, 10.0; yeast extract, 3.0; peptone, 5.0 (pH 6.4). The zone of inhibition was measured in millimeter (mm) of **3a-3f** after 24 h of incubation at 37 °C. Amphotericin-B was used as standard drug to compare zone of inhibitions. The samples were prepared in DMSO as a solvent.

Anticancer Activity

The *in vitro* anticancer activity of ILs **3a**, **3b**, **3d**, **3e**, **3g** was performed on human malignant breast cancer cell line MCF-7 and MDA-MB-435 at four dose levels of 0.1, 1.0, 10 and 100 µM in DMSO. The test consisted of a 48 h continuous drug contact protocol using sulforhodamine B (SRB) assay to estimate cell growth. Experimental procedure followed as per NCI SRB assay protocols.[46] Briefly, this assay relies on the uptake of the negatively charged pink aminoxanthine dye, sulphorhodamine-B (SRB) by basic amino acids in the cells. The greater the number of cells, greater the amount of dye is taken up and, after fixing, when the cells are lysed, the released dye gives a greater absorbance. The SRB assay was found to be more dependable, sensitive, simple, reproducible and more rapid than the formazan-based assays and gives best results.[47] Appropriate positive controls were run in each experiment and each experiment was repeated thrice and a graph was plotted against percentage control growth and concentration (Figure 2 and 3) to calculate various parameters. Results are given in terms of GI_{50} (concentration of drug that produces 50% inhibition of the cells), TGI (concentration of the drug that produces total inhibition of the cells) and LC_{50} (concentration of the drug that kills 50% of the cells) values were calculated from mean graph. The results of anticancer activities are given in Table 6.

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Notes and references

[a]School of Chemical Sciences, Central University of Gujarat, Gandhinagar-382030 (India). Fax: +91-079-232-60076; Tel: +91-079-232-60210; E-mail: mansingh50@hotmail.com

†Electronic Supplementary Information (ESI) available: [Copies of ^{1}H and ^{13}C NMR spectra are given in supplementary information].

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