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Synthesis and evaluation of novel N,N'-disubstituted benzimidazolium bromides salts as antitumor agents

Hasan KÜÇÜKBAY[1,*], Akın MUMCU[1], Suat TEKİN[2], Süleyman SANDAL[2]
[1]Department of Chemistry, Faculty of Science and Arts, İnönü University, Malatya, Turkey
[2]Department of Physiology, Faculty of Medicine, İnönü University, Malatya, Turkey

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Abstract: Novel benzimidazolium bromides salts having (4-methoxyphenyl)ethyl, (phthalimide-2-yl)methyl, 4-nitrobenzyl, 2-phenylethyl, penthyl, or allyl groups were synthesized and their characterizations were conducted by ^{1}H and ^{13}C NMR and IR spectroscopic methods, and microanalysis. In vitro antitumor activities of the novel benzimidazole compounds (**1**–**7**) were determined by using ovarian (A2780) and prostate (PC-3) cancer cell lines. Antitumor properties of all compounds were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. A time-dependent cell viability assay for the tested benzimidazole compounds was performed and the IC_{50} values of the compounds were calculated after treatment for 24 and 48 h. Our results indicate that the tested benzimidazole compounds show antitumor activity against A2780 and PC-3 cell lines ($P < 0.05$).

Key words: Benzimidazole derivatives, antitumor activity, A2780, PC-3

1. Introduction

Cancer is a worldwide health problem, representing the leading cause of mortality and morbidity worldwide and accounting for 13% (8.2 million) of all human deaths in 2012 as indicated by the WHO. Although there are more than 100 types of cancer, the main types of cancer leading to death are lung cancer (1.4 million, 18.4%), gastric cancer (0.866 million, 11.4%), liver cancer (0.653 million, 8.6%), colon cancer (0.677 million, 8.9%), and breast cancer (0.548 million, 7.2%). It was estimated that the number of deaths attributed to cancer would rise to an annual 19.3 million by 2025.[1–4] For this reason, the search for new cancer-treating agent is an important research area in both organic and medicinal chemistry. Many chemical substances having heterocyclic units have been synthesized and evaluated as anticancer drug candidates in recent years. Among the heterocyclic compounds, benzimidazole is an important pharmacophore and has a privileged structure in drug discovery. Many benzimidazole derivatives possess a variety of biological properties such as antiulcer,[5] antihypertensive,[6] antiviral,[7] antihelmentic,[8] antifungal,[9] antibacterial,[10] and antitubercular agents,[11] and several other kinds of therapeutic agent that are still under investigation for their antitumor properties.[5–18] In the literature, various benzimidazole derivatives showed remarkable and promising antitumor properties.[12–26] We have also synthesized and investigated in vitro and in vivo the antibacterial properties of many benzimidazole derivatives for the past two decades and obtained promising results.[27–34] These results encouraged us to synthesize new benzimidazole derivatives and investigate their potential antitumor properties in order to find an effective drug candidate against cancer.

*Correspondence: hkucukbay@inonu.edu.tr

2. Results and discussion

2.1. Synthesis

The compounds **1**–**3** were synthesized from the nucleophilic substitution reaction of 1-[2-(4-methoxyphenyl)ethy] lbenzimidazole (**I**) with 2-phenylethyl bromide, penthyl bromide, and allyl bromide, respectively. The compounds **4**–**7** were synthesized from the nucleophilic substitution reaction of 1-(phthalimide-2-yl)methylbenzimidazole (**II**) with 2-phenylethyl bromide, 4-nitrobenzyl chloride, penthyl bromide, and allyl bromide, respectively. These compounds were characterized by elemental analysis and FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. The general synthesis scheme of the compounds is shown in the Scheme.

2.2. FT-IR spectroscopy

Characteristic $\nu_{(C=N)}$ bands of the benzimidazolium salts (**1**–**7**) in the infrared spectrum were observed between 1560 and 1564 cm^{-1}. In the IR spectra of **4**–**7**, C=O stretching vibrations were observed between 1718 and 1724 cm^{-1}.

2.3. NMR spectroscopy

The benzimidazolium salts are air- and moisture-stable both in the solid state and in solution. The new benzimidazole derivatives (**1**–**7**) were characterized by ^{1}H and ^{13}C NMR, which supported the proposed structures. The NC<u>H</u>N proton signals for the benzimidazolium salts **1**–**7** were observed as singlets at 9.73, 9.71, 9.67, 9.86, 10.18, 9.93, and 9.89 ppm, respectively. As expected, the highest shift to downfield of the NC<u>H</u>N proton signals was observed at the bearing electron withdrawing nitro substituent of compound **5**. These chemical shift values are also parallel to the acidity of the compounds. The value of $\delta[^{13}C\{^{1}H\}]$, N<u>C</u>HN in benzimidazolium salts is usually around 142 ± 4.[35] For benzimidazolium salts **1**–**7** it was found to be 143.1, 142.5, 142.7, 144.1, 145.1, 144.1, and 144.3 ppm, respectively. These values were in good agreement with the previously reported results.[36] The carbonyl carbon (<u>C</u>O) signals for compounds **4**–**7** were observed at 167.3, 167.4, 167.5, and 167.4 ppm, respectively. The detailed ^{1}H and ^{13}C NMR spectral data are given in the experimental section and all spectra for the compounds are depicted in the supplementary file.

2.4. In vitro anticancer activity

The percentages of changes in viability in PC-3 cells after treatment for 24 and 48 h of 1, 5, 25, 50, and 100 μM concentrations of benzimidazole derivatives are shown in Tables 1 and 2, respectively.

Table 1. The cell viability results of A2780 cells after a 24-h treatment with seven (**1**–**7**) new benzimidazole compounds. The changes in cell viability caused by benzimidazole derivatives are compared with the control data. Each data point is an average of 10 viability measurements.

	A2780 (24 h)					
Compound	Control	1 μM	5 μM	25 μM	50 μM	100 μM
1	94.01 ± 1.82	36.67 ± 1.62**	26.80 ± 1.86**	17.21 ± 3.07**	13.13 ± 1.59**	7.40 ± 0.72**
2	94.01 ± 1.82	60.02 ± 4.09*	38.26 ± 3.25**	31.81 ± 4.47**	22.43 ± 3.58**	11.69 ± 2.5**
3	94.01 ± 1.82	51.48 ± 4.75*	42.10 ± 4.57*	31.79 ± 3.41**	21.57 ± 2.05**	17.34 ± 1.3**
4	94.01 ± 1.82	52.64 ± 2.94*	53.67 ± 2.99*	52.12 ± 5.01*	38.74 ± 5.40**	24.07 ± 3.18**
5	94.01 ± 1.82	62.37 ± 13.57*	51.70 ± 5.04*	41.89 ± 3.70**	37.31 ± 7.58**	24.62 ± 3.14**
6	94.01 ± 1.82	84.63 ± 5.98*	71.83 ± 5.47*	52.82 ± 9.58*	43.87 ± 4.69**	28.58 ± 3.45**
7	94.01 ± 1.82	48.25 ± 6.89*	44.97 ± 3.22**	39.55 ± 3.97**	38.68 ± 4.40**	38.53 ± 5.47**

(*P < 0.05, **P < 0.01).

Table 2. The cell viability results of A2780 cells after a 48-h treatment with seven (**1**–**7**) new benzimidazole compounds. The changes in cell viability caused by benzimidazole derivatives are compared with the control data. Each data point is an average of 10 viability measurements.

	A2780 (48 h)					
Compound	Control	1 μM	5 μM	25 μM	50 μM	100 μM
1	90.43 ± 1.91	34.35 ± 2.28**	24.00 ± 2.72**	16.58 ± 2.17**	13.11 ± 2.48**	4.96 ± 0.43**
2	90.43 ± 1.91	56.97 ± 4.86*	33.99 ± 2.51**	27.62 ± 2.15**	17.91 ± 2.47**	10.58 ± 0.91**
3	90.43 ± 1.91	44.77 ± 3.36*	39.85 ± 2.04**	29.13 ± 1.56**	20.01 ± 1.82**	11.81 ± 1.19**
4	90.43 ± 1.91	49.05 ± 2.90*	43.44 ± 2.43*	41.29 ± 2.08**	34.46 ± 2.22**	18.49 ± 1.86**
5	90.43 ± 1.91	59.39 ± 1.71*	49.33 ± 2.49*	39.35 ± 2.35**	28.08 ± 1.79**	16.37 ± 2.16**
6	90.43 ± 1.91	74.97 ± 2.15*	68.16 ± 2.04*	48.45 ± 3.10*	31.08 ± 1.80**	15.68 ± 1.49**
7	90.43 ± 1.91	44.59 ± 3.72*	40.97 ± 1.21**	33.14 ± 2.15**	28.03 ± 2.92**	22.48 ± 2.05**

(*P $<$ 0.05, **P $<$ 0.01).

As can be seen from Tables 1 and 2, the benzimidazole compounds containing a 2-(4-methoxyphenyl)ethyl group (**1**–**3**) exhibit antitumor activity on A2780 cell lines at all tested concentrations except 1 μM (P $<$ 0.05). The benzimidazole compounds containing (phthalimide-2-yl)methyl substituent (**4**–**7**) have antitumor activity on A2780 cell lines at all tested concentrations, except at 1 μM for compound **5** (P $<$ 0.05). Compared to antitumor activity on A2780 with chemical structures, compounds containing 2-(4-methoxyphenyl)ethyl substituent were more active than the others (**4**–**7**) when the results for both 24 and 48 h are taken into consideration. The high activity of these group benzimidazole compounds may result from the phenylethyl-nitrogen skeleton structurally related to hordanine moiety. When compared to the results obtained from a 24-h treatment, stronger cytotoxic activity is observed for the benzimidazole derivatives after a 48-h treatment. The effects of benzimidazole derivatives of 1, 5, 25, 50, and 100 μM concentrations on PC-3 cell viability after a 24-h treatment are given as percentage values in Table 3 and after a 48-h treatment in Table 4. When compared to the results obtained from 24-h and 48-h treatments, similar cytotoxic activity is observed for all benzimidazole derivatives (**1**–**7**) against PC-3 cell lines at 25, 50, and 100 μM, except compound **6**, which shows antitumor activity only 50 and 100 μM after 24-h treatment.

Table 3. The cell viability results of PC-3 cells after a 24-h treatment with seven (**1**–**7**) new benzimidazole compounds. The changes in cell viability caused by benzimidazole derivatives are compared with the control data. Each data point is an average of 10 viability measurements.

	PC-3 (24 h)					
Compound	Control	1 μM	5 μM	25 μM	50 μM	100 μM
1	94.42 ± 2.84	94.05 ± 2.89	84.85 ± 2.68	60.98 ± 3.30**	53.79 ± 4.20**	30.44 ± 7.17**
2	94.42 ± 2.84	91.87 ± 6.49	84.12 ± 4.90	57.11 ± 5.76**	42.54 ± 5.37**	28.17 ± 4.04**
3	94.42 ± 2.84	95.72 ± 3.44	87.30 ± 3.54	79.09 ± 4.42**	78.52 ± 3.14**	64.49 ± 2.21**
4	94.42 ± 2.84	94.81 ± 5.12	93.47 ± 4.32	82.56 ± 5.32**	80.36 ± 3.54**	52.00 ± 2.75**
5	94.42 ± 2.84	92.99 ± 3.34	91.50 ± 4.71	65.74 ± 5.25**	64.85 ± 3.19**	52.39 ± 4.29**
6	94.42 ± 2.84	95.60 ± 3.15	93.84 ± 3.56	90.14 ± 3.93	79.66 ± 4.78**	71.14 ± 5.92**
7	94.42 ± 2.84	93.25 ± 3.61	89.77 ± 6.31	88.30 ± 4.95*	88.50 ± 3.75*	73.62 ± 7.07**

(*P $<$ 0.05, **P $<$ 0.01).

Similar to the result of the A2780 cell lines, the benzimidazol compounds bearing a 2-(4-methoxyphenyl) ethyl group generally exhibit better antitumor activity on PC-3 cell lines than the others (Table 3, compounds **1**, **2**, and **3**) (P $<$ 0.05). A time-dependent cell viability assay for the tested benzimidazole compounds (**1**–**7**)

was conducted and their $LogIC_{50}$ values were calculated after 24- and 48-h treatments. The results are given in Table 5.

Table 4. The cell viability results of PC-3 cells after a 48-h treatment with seven (**1**–**7**) new benzimidazole compounds. The changes in cell viability caused by benzimidazole derivatives are compared with the control data. Each data point is an average of 10 viability measurements.

	PC-3 (48 h)					
Compound	Control	1 μM	5 μM	25 μM	50 μM	100 μM
1	93.19 ± 3.13	90.99 ± 2.22	83.77 ± 2.91	52.41 ± 1.19**	46.90 ± 2.89**	24.22 ± 2.68**
2	93.19 ± 3.13	88.96 ± 4.04	82.60 ± 2.63	50.94 ± 2.69**	38.97 ± 5.26**	19.68 ± 2.07**
3	93.19 ± 3.13	92.69 ± 2.24	86.68 ± 3.71	78.69 ± 4.36**	72.05 ± 3.50**	53.10 ± 5.53**
4	93.19 ± 3.13	93.45 ± 2.16	86.36 ± 6.13	85.97 ± 2.77*	80.99 ± 4.15**	50.26 ± 6.09**
5	93.19 ± 3.13	92.08 ± 3.39	88.90 ± 4.48	62.57 ± 6.21**	44.12 ± 4.43**	42.35 ± 4.68**
6	93.19 ± 3.13	93.02 ± 2.16	91.58 ± 2.21	83.81 ± 2.83*	72.40 ± 3.35**	61.63 ± 3.60**
7	93.19 ± 3.13	93.60 ± 2.57	83.17 ± 2.25	79.44 ± 3.82*	71.27 ± 4.09**	61.84 ± 5.74**

(*$P < 0.05$, **$P < 0.01$).

Table 5. Evaluation of the cytotoxicity and $LogIC_{50}$ values (μM) of benzimidazole compounds (**1**–**7**) of two cancer cell lines (A2780 and PC-3) after 24- and 48-h treatments.

	PC-3 (24 h)	PC-3 (48 h)	A2780 (24 h)	A2780 (48 h)
Compound	$LogIC_{50}$ (μM)	$LogIC_{50}$ (μM)	$LogIC_{50}$ (μM)	$LogIC_{50}$ (μM)
1	1.74	1.58	–0.49	–0.51
2	1.55	1.58	0.07	0.06
3	1.85	2.51	–0.17	–0.28
4	6.10	6.25	–0.53	–0.44
5	1.45	1.45	0.02	0.22
6	2.51	2.22	1.28	1.54
7	5.62	1.77	–1.04	–0.56

2.5. Conclusions

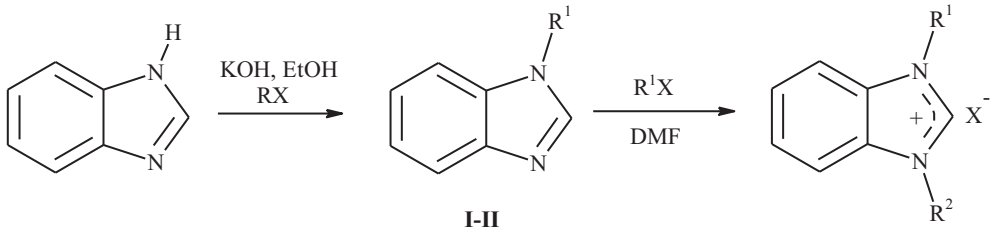
In the present study, new salt-type benzimidazole derivatives having phenylethyl, (4-methoxyphenyl)ethyl, penthyl, allyl, phthalimide-2-ylmethyl, and 4-nitrophenyl substituents at the nitrogen atoms of the imidazole ring were synthesized. They were characterized by ^{1}H NMR, ^{13}C NMR, IR, and microanalysis. Anticancer properties of these compounds were investigated by [3-(4,5-dimethylthiazole)-2-yl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Our results indicate that the new benzimidazole derivatives display potential anticancer activities against ovarian (A2780) and prostate (PC-3) cancer cell lines ($P < 0.05$). Compounds **1**, **2**, and **3** are the most promising compounds in this series and they show high antitumor activity in both cancer cell lines (Figures 1 and 2, compounds **1**, **2**, and **3**).

3. Experimental

3.1. Materials and methods

The starting materials and reagents used in the reactions were supplied commercially by Aldrich, Acros, ABCR, and Merck. The prostate carcinoma (PC-3) and female ovarian (A2780) cancer cell lines were obtained from the American Type Culture Collection (ATCC). Calf serum, trypsin, penicillin, and streptomycin were purchased from Invitrogen (Waltham, MA, USA). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were

recorded using a Bruker DPX-300 high performance digital FT NMR spectrometer and chemical shift values were given as ppm. Elemental analyses were performed by LECO CHNS-932 elemental analyzer. Infrared spectra were recorded with ATR equipment in the range 4000–650 cm^{-1} on a PerkinElmer Spectrum one FTIR spectrophotometer. A microplate reader (BioTek-Synergy HT) was used to measure the absorbance. Melting points were recorded using an Electrothermal-9200 melting point apparatus, and are uncorrected.



I-II

1. R^1 = 2-(4-Methoxyphenyl)ethyl, R^2 = 2-Phenylethyl, X = Br
2. R^1 = 2-(4-Methoxyphenyl)ethyl, R^2 = Penthyl, X = Br
3. R^1 = 2-(4-Methoxyphenyl)ethyl, R^2 = Allyl, X = Br
4. R^1 = (Phthalimide-2-yl)methyl, R^2 = 2-Phenylethyl, X = Br
5. R^1 = (Phthalimide-2-yl)methyl, R^2 = 4-Nitrobenzyl, X = Cl
6. R^1 = (Phthalimide-2-yl)methyl, R^2 = Penthyl, X = Br
7. R^1 = (Phthalimide-2-yl)methyl, R^2 = Allyl, X = Br

Scheme. Synthesis of the benzimidazole derivatives.

3.2. Synthesis of benzimidazolium salts

1-[2-(4-Methoxyphenyl)ethyl]benzimidazole (**I**) and 1-(phthalimide-2-yl)methylbenzimidazole (**II**) used in this work as starting compounds were prepared by treating benzimidazole and 2-(4-methoxyphenyl)ethyl chloride and (phthalimide-2-yl)methyl chloride, respectively, similar to the literature procedure.[37,38]

3.3. General method for the synthesis of compounds 1–3

Equivalent amount of the 1-[2-(4-methoxyphenyl)ethyl]benzimidazole (**I**) and appropriate alkyl halide were refluxed in dimethylformamide (3 mL) for 5 h. Then the mixture was cooled to room temperature and the volatiles were removed under reduced pressure. The residue was crystallized from ethanol/diethyl ether (1:5).

3.3.1. Synthesis of 1-[2-(4-methoxyphenyl)ethyl]-3-phenylethylbenzimidazolium bromide (1)

Yield, 0.73 g, 42%. mp 94–96 °C. Anal. Calculated for $C_{24}H_{27}N_2O_2Br$ (MW = 455.39): C, 63.30; H, 5.98; N, 6.15. Found: C, 63.37; H, 6.05; N, 6.12%. IR (ATR, cm^{-1}): 1564 $\upsilon_{C=N}$. ^{1}H NMR (DMSO-d_6) δ : 9.73 (1H, s, NC<u>H</u>N), 8.00–7.19 (9H, m, Ar-<u>H</u>), 7.11–6.83 (4H, AA'BB' system, $CH_2CH_2C_6\underline{H}_4OCH_3$), 4.75 (2H, t, $C\underline{H}_2CH_2C_6H_5$, J= 7.1 Hz), 4.70 (2H, t, $C\underline{H}_2CH_2C_6H_4OCH_3$, J= 7.2 Hz), 3.70 (3H, s, $OC\underline{H}_3$) 3.20 (2H, t, $CH_2C\underline{H}_2C_6H_4OCH_3$, J= 7.2 Hz), 3.13 (2H, t, $CH_2C\underline{H}_2C_6H_5$, J= 7.1 Hz). ^{13}C NMR (DMSO-d_6) δ : 143.1 (NH<u>C</u>N), 158.7, 137.3, 131.4, 131.3, 130.3, 129.2, 129.1, 127.4, 126.9, 114.5, 114.2, 114.1 ($\underline{C}_6H_4$, $CH_2CH_2\underline{C}_6H_5$, $CH_2CH_2\underline{C}_6H_4OCH_3$), 55.5 ($O\underline{C}H_3$), 48.4 ($\underline{C}H_2CH_2C_6H_4OCH_3$), 48.1 ($\underline{C}H_2CH_2C_6H_5$), 35.3 ($CH_2\underline{C}H_2C_6H_4OCH_3$), 34.3 ($CH_2\underline{C}H_2C_6H_5$).

3.3.2. Synthesis of 1-[2-(4-methoxyphenyl)ethyl]-3-penthylbenzimidazolium bromide (2)

Yield, 1.37 g, 58%. mp 93–96 °C. Anal. Calculated for $C_{21}H_{29}N_2O_2Br$ (MW = 421.37): C, 59.86; H, 6.94; N, 6.65. Found: C, 59.42; H, 6.65; N, 6.63%. IR (ATR, cm^{-1}): 1563 $\upsilon_{C=N}$. ^{1}H NMR (DMSO-d_6) δ :

9.71 (1H, s, NC$\underline{H}$N), 8.12–7.66 (4H, m, Ar-$\underline{H}$), 7.11–6.80 (4H, AA'BB' system, $CH_2CH_2C_6\underline{H}_4OCH_3$), 4.75 (2H, t, $C\underline{H}_2CH_2C_6H_4OCH_3$, J= 7.1 Hz), 4.46 (2H, t, $C\underline{H}_2CH_2CH_2CH_2CH_3$, J= 7.1 Hz), 3.70 (3H, s, $OC\underline{H}_3$), 3.19 (2H, t, $CH_2C\underline{H}_2C_6H_4OCH_3$, J= 7.1 Hz), 1.82 (2H, p, $CH_2C\underline{H}_2CH_2CH_2CH_3$), 1.30 (2H, p, $CH_2CH_2C\underline{H}_2CH_2CH_3$), 1.19 (2H, m, $CH_2CH_2CH_2C\underline{H}_2CH_3$), 0.86 (3H, m, $CH_2CH_2CH_2CH_2C\underline{H}_3$). ^{13}C NMR (DMSO-d$_6$) δ : 142.5 (NH$\underline{C}$N), 158.6, 131.4, 131.3, 130.2, 129.0, 127.0, 114.4, 114.3, 114.1 ($\underline{C}_6H_4$, $CH_2CH_2\underline{C}_6H_4OCH_3$), 55.5 ($O\underline{C}H_3$), 48.5 ($\underline{C}H_2CH_2C_6H_4OCH_3$), 46.9 ($\underline{C}H_2CH_2CH_2CH_2CH_3$), 33.9 ($CH_2\underline{C}H_2C_6H_4OCH_3$), 28.7 ($CH_2\underline{C}H_2CH_2CH_2CH_3$), 28.2 ($CH_2CH_2\underline{C}H_2CH_2CH_3$), 22.0 ($CH_2CH_2CH_2\underline{C}H_2CH_3$), 14.2 ($CH_2CH_2CH_2CH_2\underline{C}H_3$).

3.3.3. 1-Allyl-3-[2-(4-methoxyphenyl)ethyl]benzimidazolium bromide (3)

Yield, 0.79 g, 63%. mp 132–134 °C. Anal. Calculated for $C_{19}H_{23}N_2O_2Br$ (MW = 391.30): C, 58.32; H, 5.92; N, 7.16. Found: C, 58.56; H, 5.39; N, 7.38%. IR (ATR, cm^{-1}): 1560 $\upsilon_{C=N}$. ^{1}H NMR (DMSO-d$_6$) δ : 9.67 (1H, s, NC$\underline{H}$N), 8.09–7.67 (4H, m, Ar-$\underline{H}$), 7.13–6.83 (4H, AA'BB' system, $CH_2CH_2C_6\underline{H}_4OCH_3$), 6.11–5.98 (1H, m, $CH_2C\underline{H}$=CH_2), 5.35 (1H, bd, CH_2CH=C$\underline{H}_{2cis}$, J = 10.6 Hz), 5.23 (1H, bd, CH_2CH=C$\underline{H}_{2trans}$, J = 17.1 Hz), 5.16–5.14 (2H, d, $C\underline{H}_2CH$=CH_2, J = 5.7 Hz), 4.74 (2H, t, $C\underline{H}_2CH_2C_6H_4OCH_3$, J= 7.2 Hz), 3.70 (3H, s, $OC\underline{H}_3$) 3.19 (2H, t, $CH_2C\underline{H}_2C_6H_4OCH_3$, J = 7.2 Hz). ^{13}C NMR (DMSO-d$_6$) δ : 142.7 (NH$\underline{C}$N), 158.7, 131.4, 131.3, 130.3, 129.1, 127.1, 114.5, 114.4, 114.3 ($\underline{C}_6H_4$, $CH_2CH_2\underline{C}_6H_4OCH_3$), 131.5 ($CH_2\underline{C}H$=$CH_2$), 120.5 ($CH_2CH$=$\underline{C}H_2$), 55.5 ($O\underline{C}H_3$), 49.1 ($\underline{C}H_2CH$=$CH_2$), 48.5 ($\underline{C}H_2CH_2C_6H_4OCH_3$), 34.1 ($CH_2\underline{C}H_2C_6H_4OCH_3$).

3.4. General method for the synthesis of compounds 4–7

Equivalent amount of the 1-(phthalimide-2-yl)methylbenzimidazole (**II**) and appropriate alkyl halide were refluxed in dimethylforamide (3 mL) for 5 h. Then the mixture was cooled to room temperature and the volatiles were removed with reduced pressure. The residue was crystallized from ethanol/diethyl ether (1:5).

3.4.1. 1-Phenylethyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (4)

Yield, 0.85 g, 51%. mp 216–217 °C. Anal. Calculated for $C_{24}H_{24}N_3O_4Br$ (MW = 498.37): C, 57.84; H, 4.85; N, 8.43. Found: C, 58.11; H, 4.89; N, 8.31%. IR (ATR, cm^{-1}): 1560 $\upsilon_{C=N}$, 1724 $\upsilon_{C=O}$. ^{1}H NMR (DMSO-d$_6$) δ : 9.86 (1H, s, NC$\underline{H}$N), 8.21–7.15 (13H, m, $C_6\underline{H}_4$, $C_6\underline{H}_5$), 6.33 (2H, s, NC$\underline{H}_2$N), 4.82 (2H, t, $C\underline{H}_2CH_2C_6H_5$, J= 7.4 Hz), 3.21 (2H, t, $CH_2C\underline{H}_2C_6H_5$, J= 7.4 Hz). ^{13}C NMR (DMSO-d$_6$) δ : 167.3 ($\underline{C}$=O), 144.1 (NH$\underline{C}$N), 137.2, 135.6, 131.8, 131.2, 130.9, 129.3, 128.9, 127.3, 127.1, 124.2, 114.3, 114.2 ($\underline{C}_6H_4$, $\underline{C}_6H_5$), 48.2 (N$\underline{C}H_2$N), 47.5 ($\underline{C}H_2CH_2C_6H_5$), 35.2 ($CH_2\underline{C}H_2C_6H_5$).

3.4.2. 1-(4-Nitrobenzyl)-3-(phthalimide-2-yl)methylbenzimidazolium chloride (5).

Yield, 0.90 g, 56%. mp 267–268 °C. Anal. Calculated for $C_{23}H_{17}N_4O_4Cl$ (MW = 448.86): C, 61.54; H, 3.82; N, 12.48. Found: C, 61.28; H, 3.51; N, 12.41%. IR (ATR, cm^{-1}): 1560 $\upsilon_{C=N}$, 1720 $\upsilon_{C=O}$. ^{1}H NMR (DMSO-d$_6$) δ : 10.18 (1H, s, NC$\underline{H}$N), 8.27–7.62 (12H, m, $C_6\underline{H}_4$), 6.40 (2H, s, NC$\underline{H}_2$N), 6.05 (2H, s, C$\underline{H}_2$). ^{13}C NMR (DMSO-d$_6$) δ : 167.4 ($\underline{C}$=O), 145.1 (NH$\underline{C}$N), 148.0, 141.7, 135.5, 131.9, 131.4, 130.9, 129.7, 127.5, 127.3, 124.4, 124.2, 114.6, 114.1 ($\underline{C}_6H_4$), 49.5 (N$\underline{C}H_2$N), 47.6 ($\underline{C}H_2$).

3.4.3. 1-Penthyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (6)

Yield, 1.30 g, 72%. mp 175–177 °C. Anal. Calculated for $C_{21}H_{24}N_3O_3Br$ (MW = 446.34): C, 56.51; H, 5.42; N, 9.41. Found: C, 56.35; H, 5.52; N, 9.48%. IR (ATR, cm^{-1}): 1561 $\upsilon_{C=N}$, 1724 $\upsilon_{C=O}$. ^{1}H NMR (DMSO-d_6) δ : 9.93 (1H, s, NC<u>H</u>N), 8.20–7.68 (8H, m, $C_6\underline{H}_4$), 6.35 (2H, s, NC<u>H</u>$_2$N), 4.56 (2H, t, C<u>H</u>$_2$CH$_2$CH$_2$CH$_2$CH$_3$, J= 7.1 Hz), 1.88 (2H, p, CH$_2$C<u>H</u>$_2$CH$_2$CH$_2$CH$_3$, J= 7.1 Hz), 1.34–1.29 (4H, m, CH$_2$CH$_2$C<u>H</u>$_2$C<u>H</u>$_2$CH$_3$), 0.87 (3H, t, CH$_2$CH$_2$CH$_2$CH$_2$C<u>H</u>$_3$, J= 6.7 Hz). ^{13}C NMR (DMSO-d_6) δ : 167.5 (<u>C</u>=O), 144.1 (NH<u>C</u>N), 135.5, 131.8, 131.3, 131.1, 127.3, 127.0, 124.1, 114.3, 114.1 ($\underline{C}_6H_4$), 47.6 (N<u>C</u>H$_2$N), 47.2 (<u>C</u>H$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 28.9 (CH$_2$<u>C</u>H$_2$CH$_2$CH$_2$CH$_3$), 28.3 (CH$_2$CH$_2$<u>C</u>H$_2$CH$_2$CH$_3$), 22.1 (CH$_2$CH$_2$CH$_2$<u>C</u>H$_2$CH$_3$), 14.3 (CH$_2$CH$_2$CH$_2$CH$_2$<u>C</u>H$_3$).

3.4.4. 1-Allyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (7)

Yield, 1.11 g, 77%. mp 210–212 °C. Anal. Calculated for $C_{19}H_{18}N_3O_3Br$ (MW = 416.27): C, 54.82; H, 4.36; N, 10.09. Found: C, 54.97; H, 4.13; N, 10.12%. IR (ATR, cm^{-1}): 1564 $\upsilon_{C=N}$, 1718 $\upsilon_{C=O}$. ^{1}H NMR (DMSO-d_6) δ : 9.89 (1H, s, NC<u>H</u>N), 8.21–7.67 (8H, m, $C_6\underline{H}_4$), 6.36 (2H, s, NC<u>H</u>$_2$N), 6.13–6.00 (1H, m, CH$_2$C<u>H</u>=CH$_2$), 5.43 (1H, bd, CH$_2$CH=C<u>H</u>$_{2trans}$, J = 15.6 Hz), 5.38 (1H, bd, C<u>H</u>$_2$CH=CH$_{2cis}$, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6) δ : 167.4 (<u>C</u>=O), 144.3 (NH<u>C</u>N), 135.5, 131.8, 131.2, 131.1, 127.3, 127.1, 124.1, 114.4, 114.3 ($\underline{C}_6H_4$), 131.5 (CH$_2$<u>C</u>H=CH$_2$), 120.9 (CH$_2$CH=<u>C</u>H$_2$), 49.3 (<u>C</u>H$_2$CH=CH$_2$), 47.5 (N<u>C</u>H$_2$N).

3.5. Cell cultures

A2780 and PC-3 cell lines were preserved in RPMI-1640 culture medium supplemented with L-glutamine (10% heat-inactivated fetal bovine serum, 100 U/mL penicillin-streptomycin), with addition of 10 mM nonessential amino acids for culture of prostate cancer cells. The cell lines were kept at 37 °C in a 5% CO_2 humidified incubator.

3.5.1. MTT assay

The synthesized benzimidazole compounds were screened for their antitumor activities against different type cancer cell lines (PC-3 and A2780) by MTT assay. The pale yellow tetrazolium salt, MTT, was transformed by active mitochondria to form a dark blue formazan that was determined by a microplate reader.[39]

The MTT method provides a simple way to detect living and growing cells without using radioactivity. Shortly, 15×10^3 prostate and ovarian cancer cells were plated in triplicate in 96-well flat bottom tissue culture plates, and treated with DMSO (for positive control group) and different concentrations (1, 5, 25, 50, and 100 μM) of benzimidazole compounds (**1**–**7**) in DMSO; then cells were incubated for 24 and 48 h at 37 °C in a 5% CO_2 humidified incubator. After 24 and 48 h MTT (0.005 g/mL in phosphate buffer saline) was added to the cell culture and incubated for 3 h. The formazan crystals formed during the reaction of active mitochondria with MTT were dissolved in 0.04 N (100 mL) isopropanol and readings were recorded on a microplate reader using a 570 nm filter. The relative cell viability (%) was expressed as a percentage relative to the untreated control cells. Each value represented an average of 10 measurements. All cellular results were determined against control cells.[40,41]

3.6. Statistical analyses

Quantitative data were presented as mean ± standard deviation (SD). Normal distribution was confirmed by Kolmogorov–Smirnov test. Quantitative data were analyzed using Kruskal–Wallis H test following the Mann–Whitney U test with Bonferroni adjustment as a post-hoc test.

All P values < 0.05 were considered statistically significant. All analyses were done by IBM SPSS Statistics 22.0 for Windows. The LogIC_{50} values were determined by using % cell viability values of compounds by the GraphPad Prism 6 program.

Acknowledgment

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Supplementary Materials

NMR and IR spectra of the new compounds are given at the end of the paper.

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Supporting Information

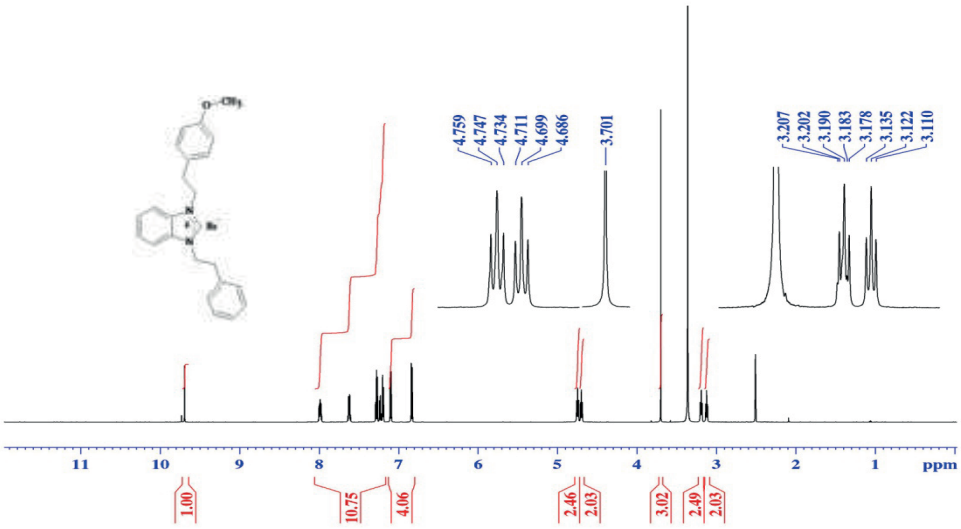

Figure 1. ^{1}H NMR spectrum of 1-[2-(4-methoxyphenyl)ethyl]-3-phenylethylbenzimidazolium bromide (**1**).

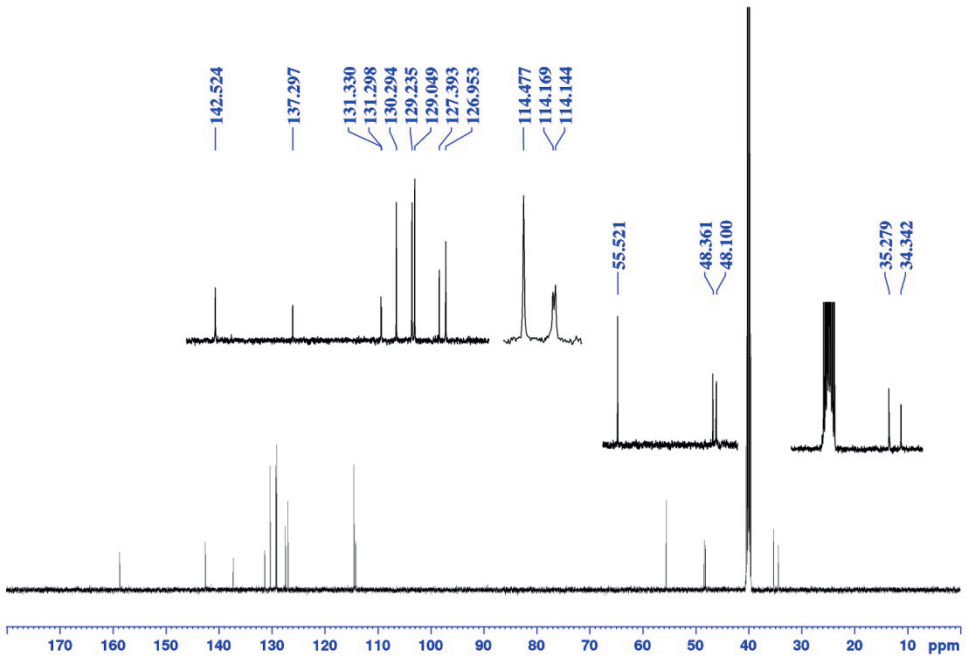

Figure 2. ^{13}C NMR spectrum of 1-[2-(4-methoxyphenyl)ethyl]-3-phenylethylbenzimidazolium bromide (**1**).

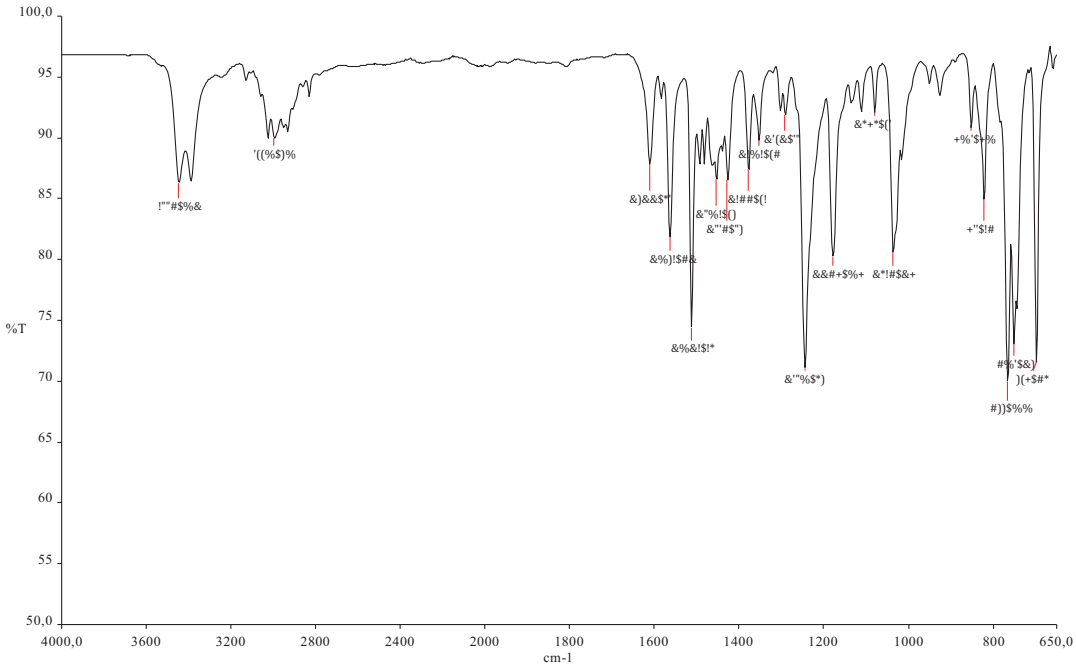

Figure 3. IR spectrum of of 1-[2-(4-methoxyphenyl)ethyl]-3-phenylethylbenzimidazolium bromide (**1**).

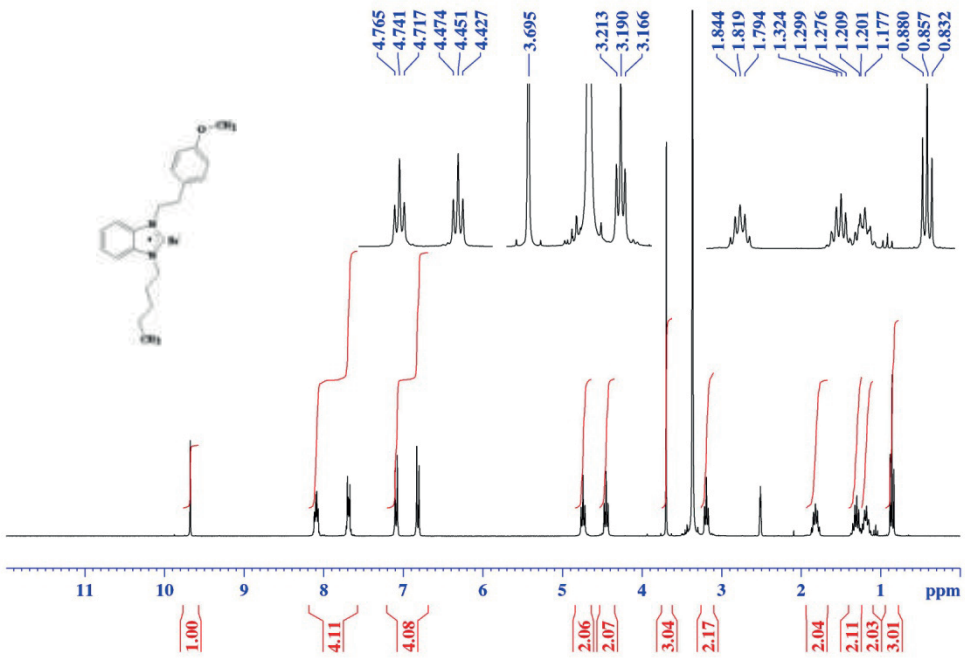

Figure 4. ^{1}H NMR spectrum of 1-[2-(4-methoxyphenyl)ethyl]-3-penthylbenzimidazolium bromide (**2**).

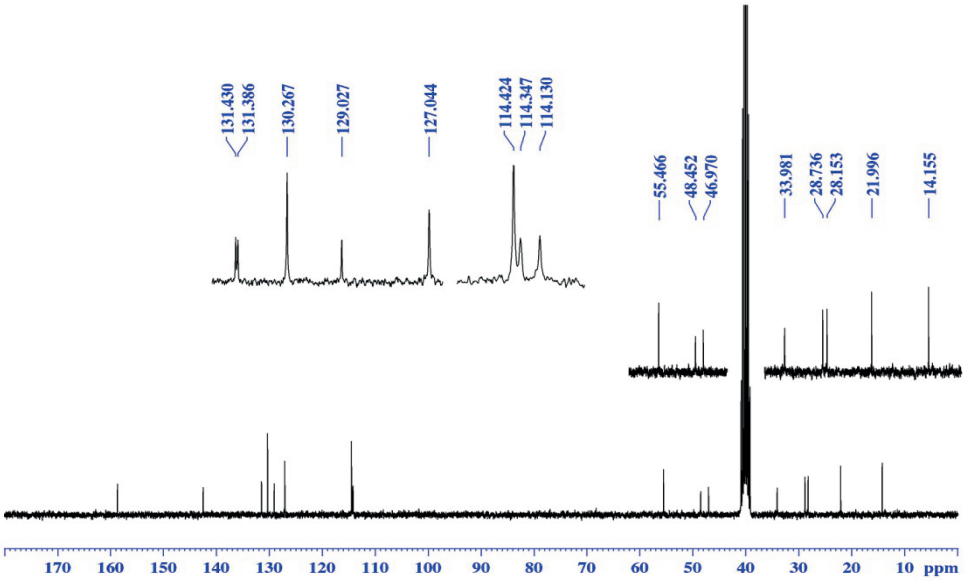

Figure 5. ^{13}C NMR spectrum of 1-[2-(4-methoxyphenyl)ethyl]-3-penthylbenzimidazolium bromide (**2**).

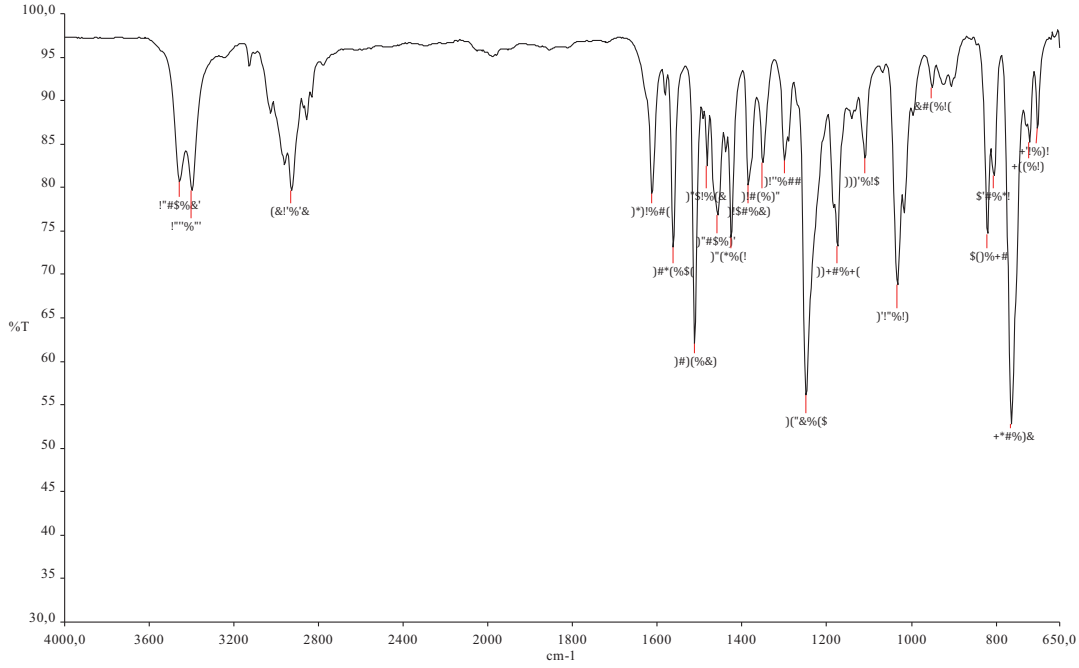

Figure 6. IR spectrum of 1-[2-(4-methoxyphenyl)ethyl]-3-penthylbenzimidazolium bromide (**2**).

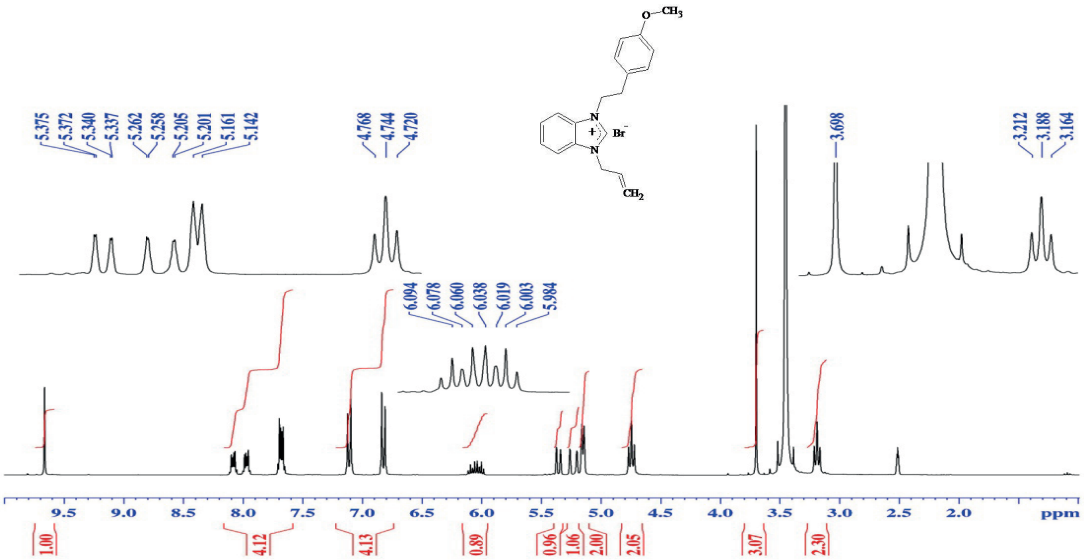

Figure 7. ^{1}H NMR spectrum of 1-allyl-3-[2-(4-methoxyphenyl)ethyl]benzimidazolium bromide (**3**).

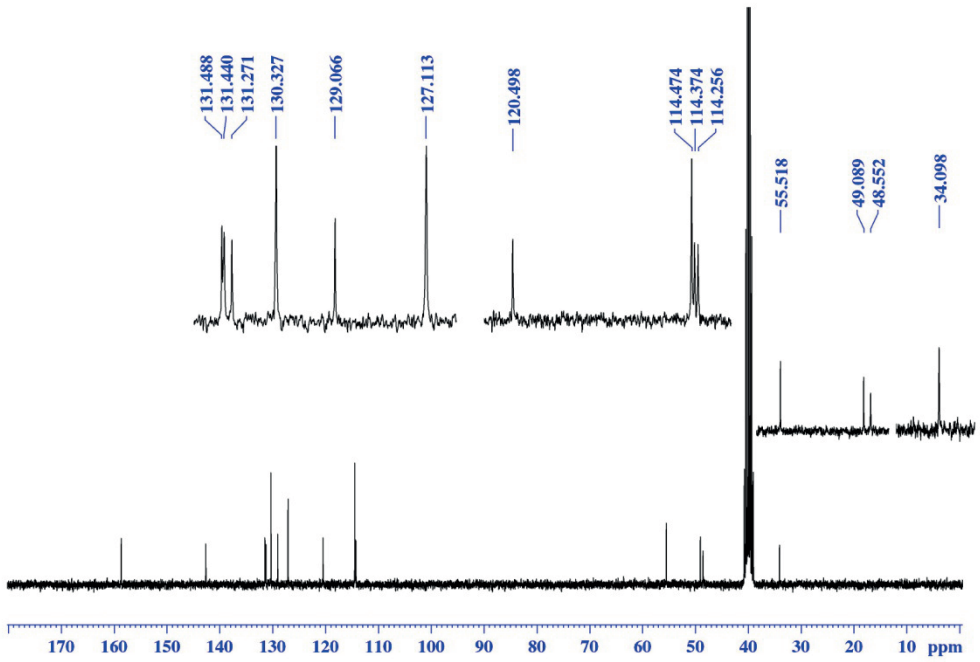

Figure 8. ^{13}C NMR spectrum of 1-allyl-3-[2-(4-methoxyphenyl)ethyl]benzimidazolium bromide (**3**).

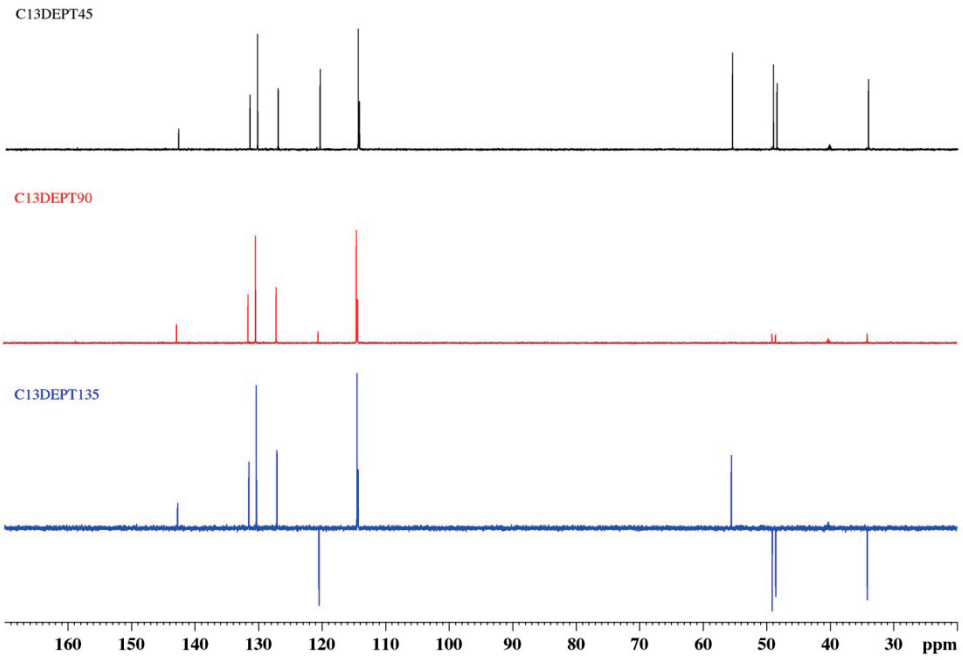

Figure 9. ^{13}C DEPT NMR spectrums of 1-allyl-3-[2-(4-methoxyphenyl)ethyl]benzimidazolium bromide (**3**).

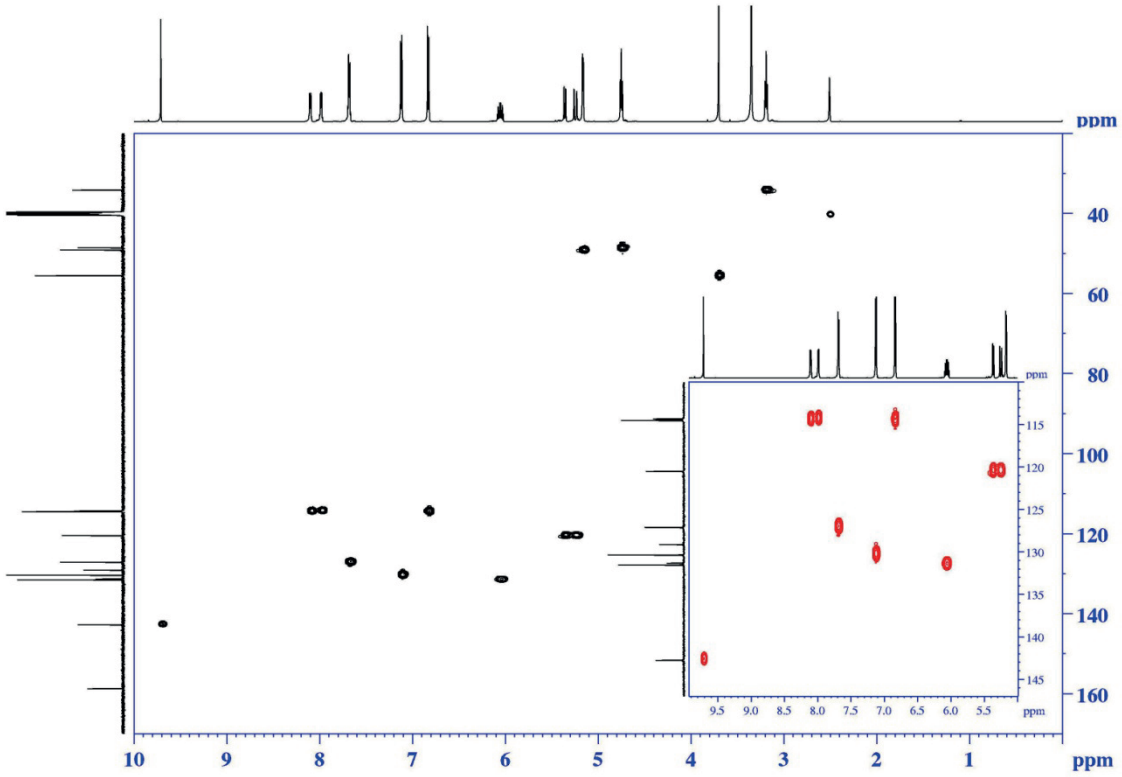

Figure 10. HSQC 2D NMR spectrum of 1-allyl-3-[2-(4-methoxyphenyl)ethyl]benzimidazolium bromide (**3**).

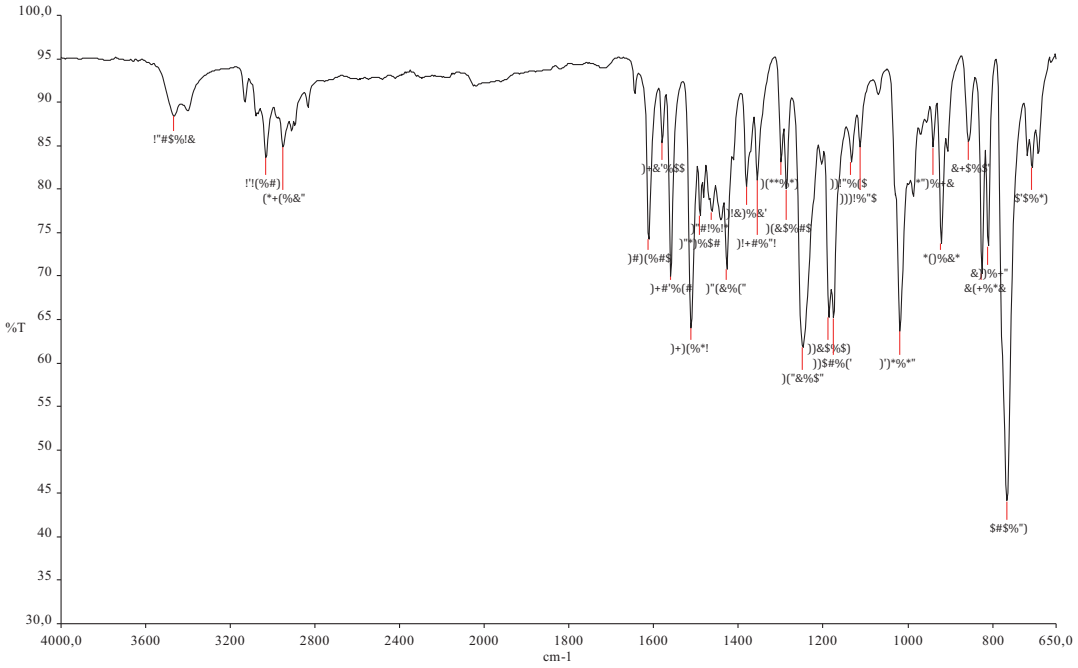

Figure 11. IR spectrum of 1-allyl-3-[2-(4-methoxyphenyl)ethyl]benzimidazolium bromide (**3**).

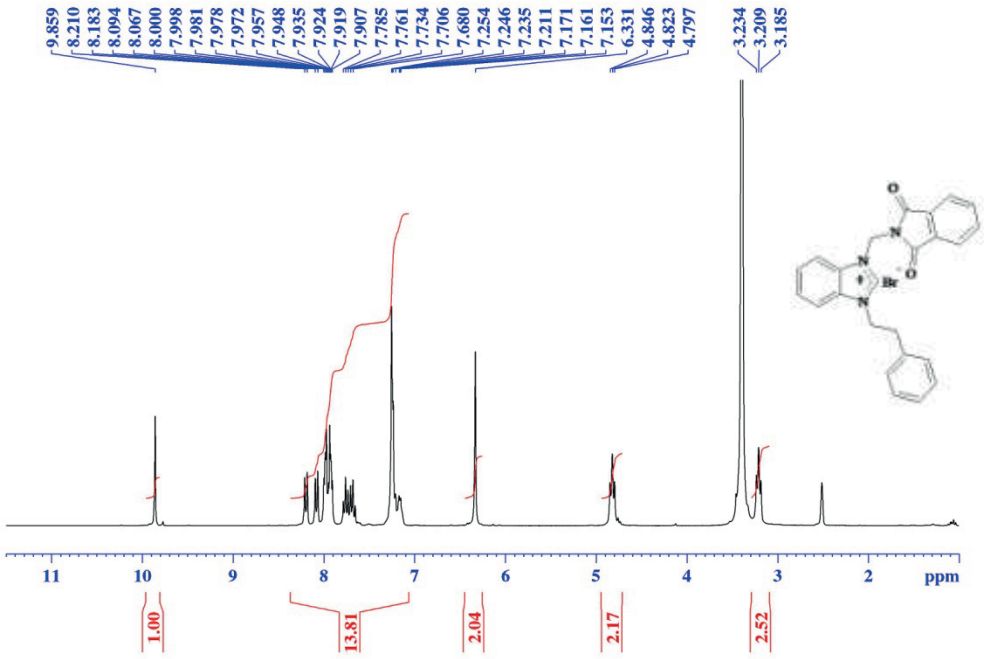

Figure 12. ^{1}H NMR spectrum of 1-phenylethyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**4**).

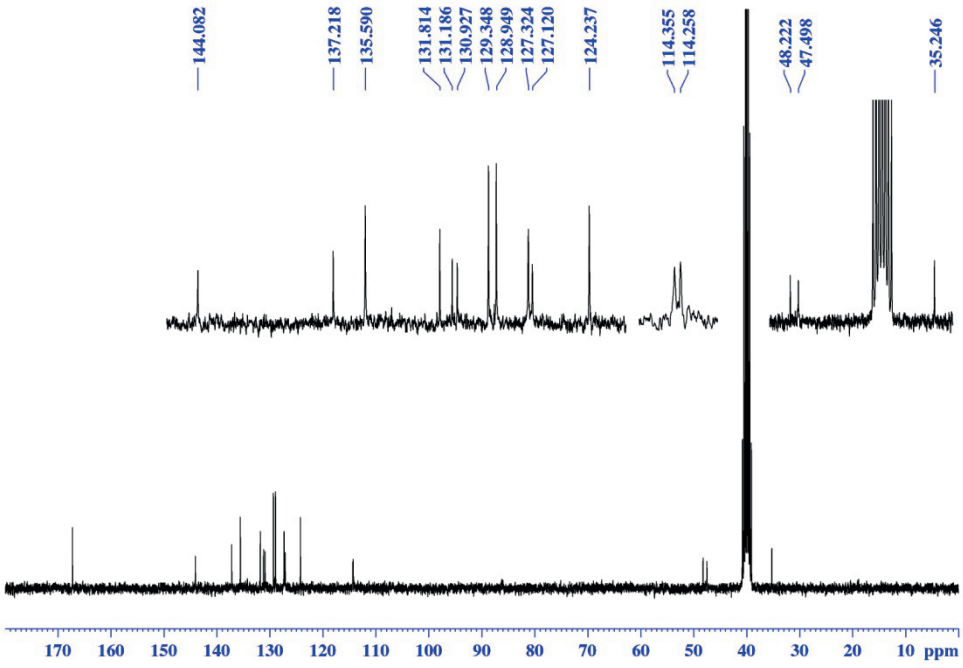

Figure 13. ^{13}C NMR spectrum of 1-phenylethyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**4**).

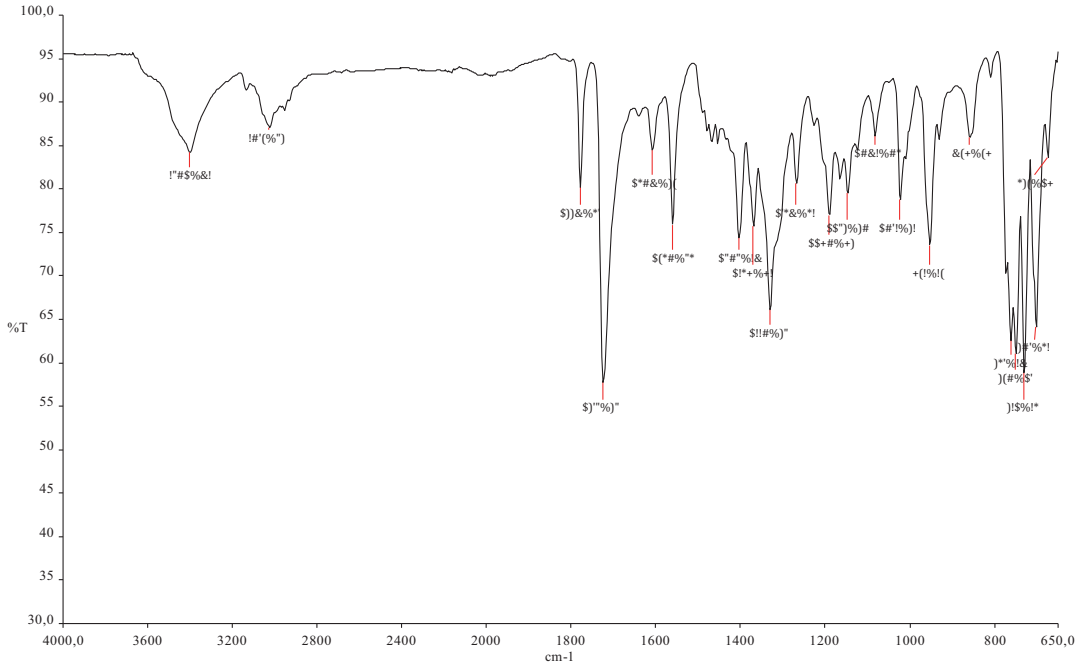

Figure 14. IR spectrum of 1-phenylethyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**4**).

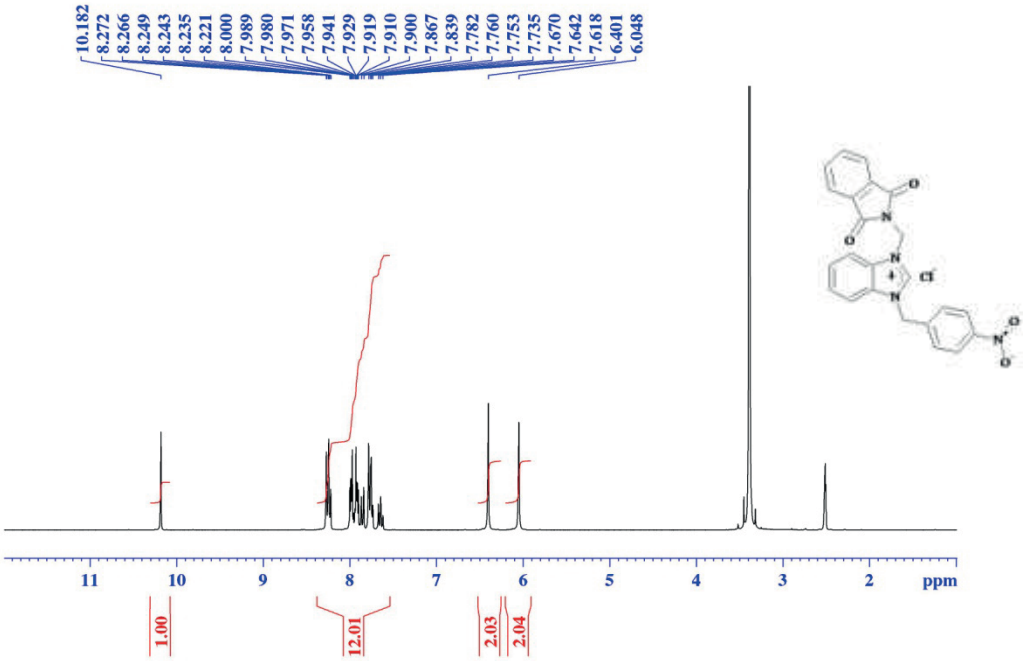

Figure 15. ^{1}H NMR spectrum of 1-(4-nitrobenzyl)-3-(phthalimide-2-yl)methylbenzimidazolium chloride (**5**).

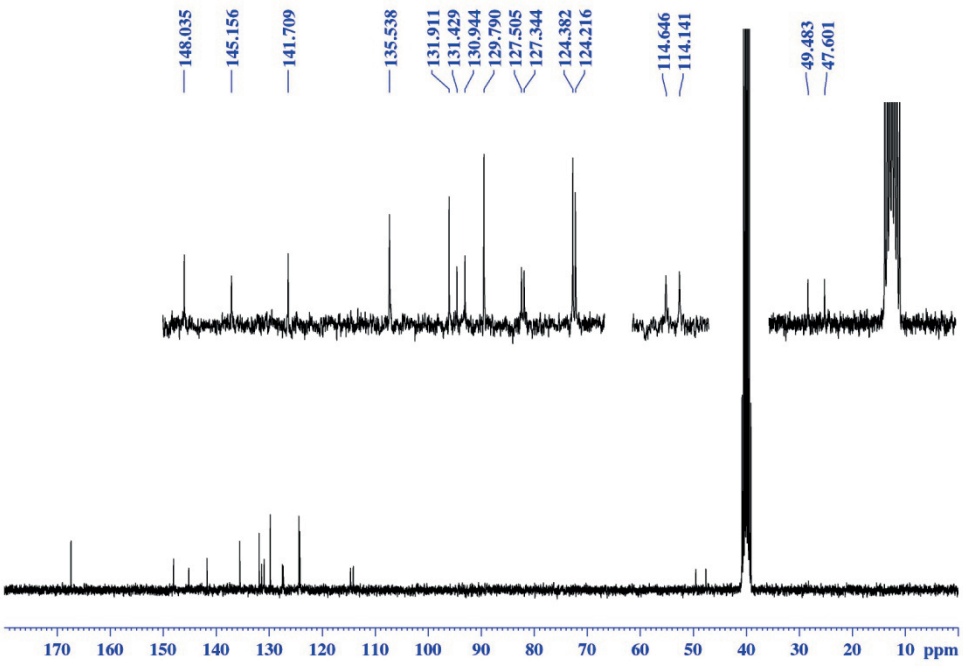

Figure 16. ^{13}C NMR spectrum of 1-(4-nitrobenzyl)-3-(phthalimide-2-yl)methylbenzimidazolium chloride (**5**).

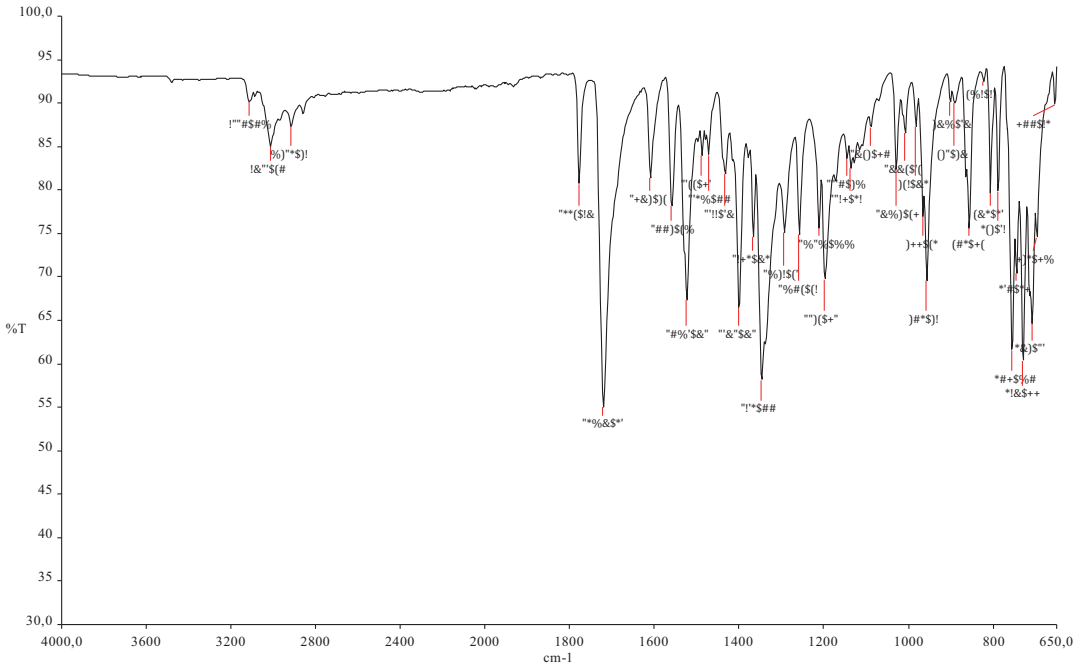

Figure 17. IR spectrum of 1-(4-nitrobenzyl)-3-(phthalimide-2-yl)methylbenzimidazolium chloride (**5**).

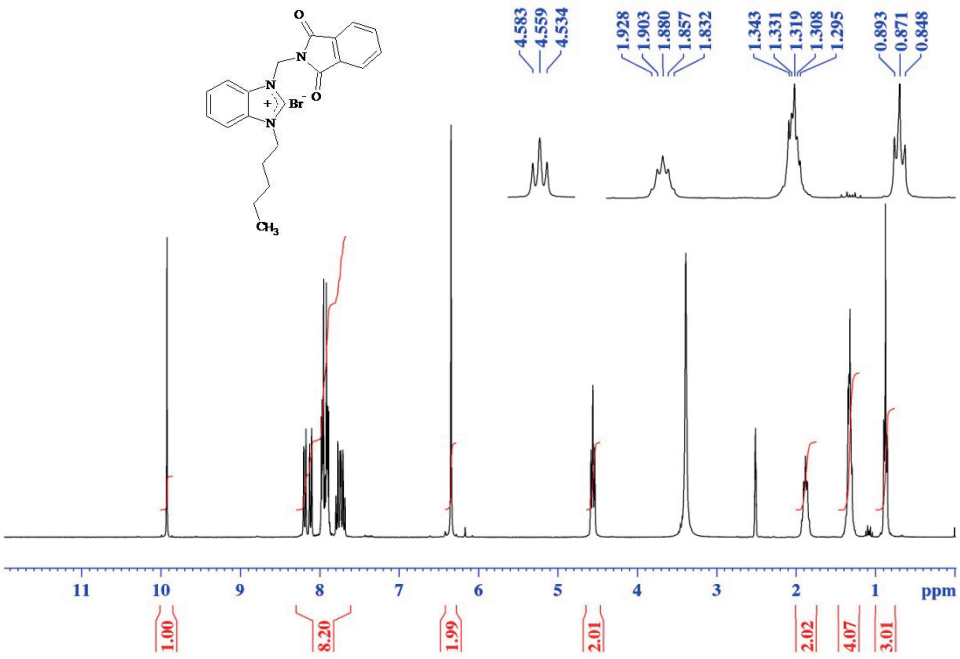

Figure 18. ^{1}H NMR spectrum of 1-penthyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**6**).

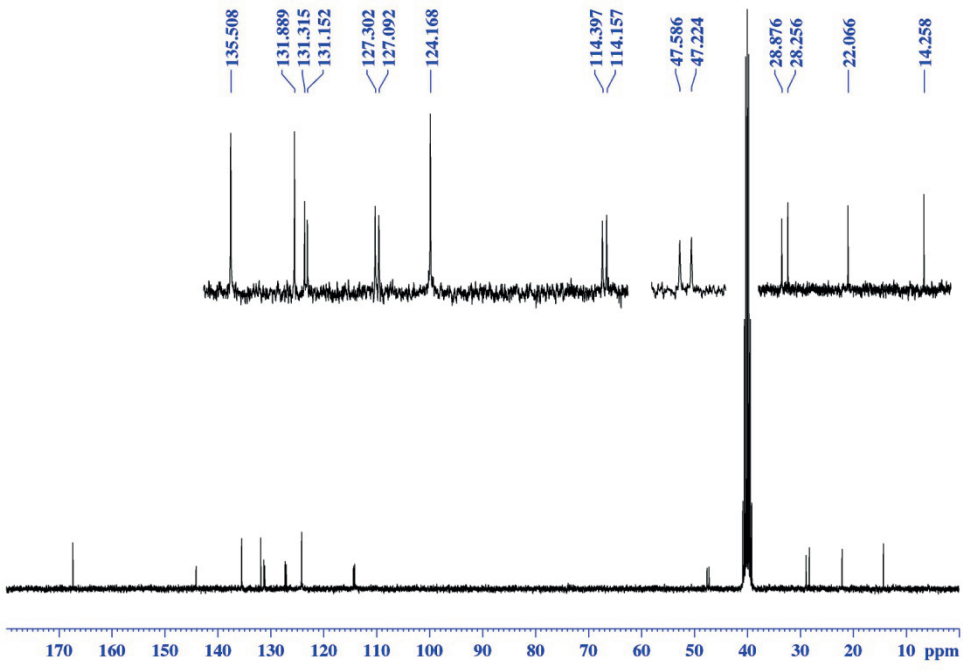

Figure 19. ^{13}C NMR spectrum of 1-penthyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**6**).

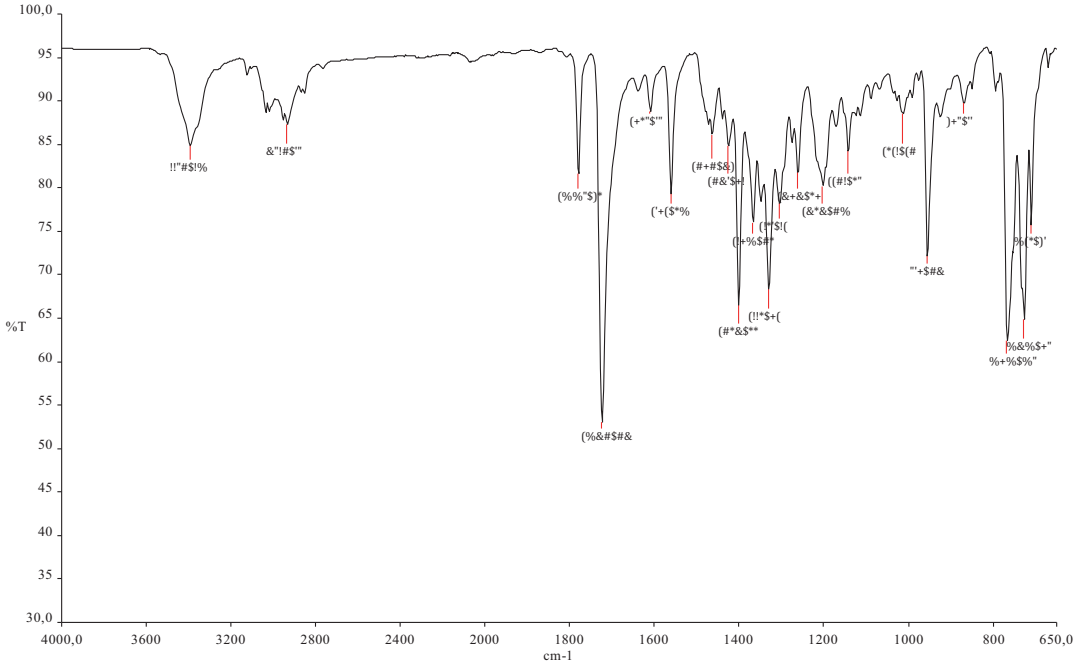

Figure 20. IR spectrum of 1-penthyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**6**).

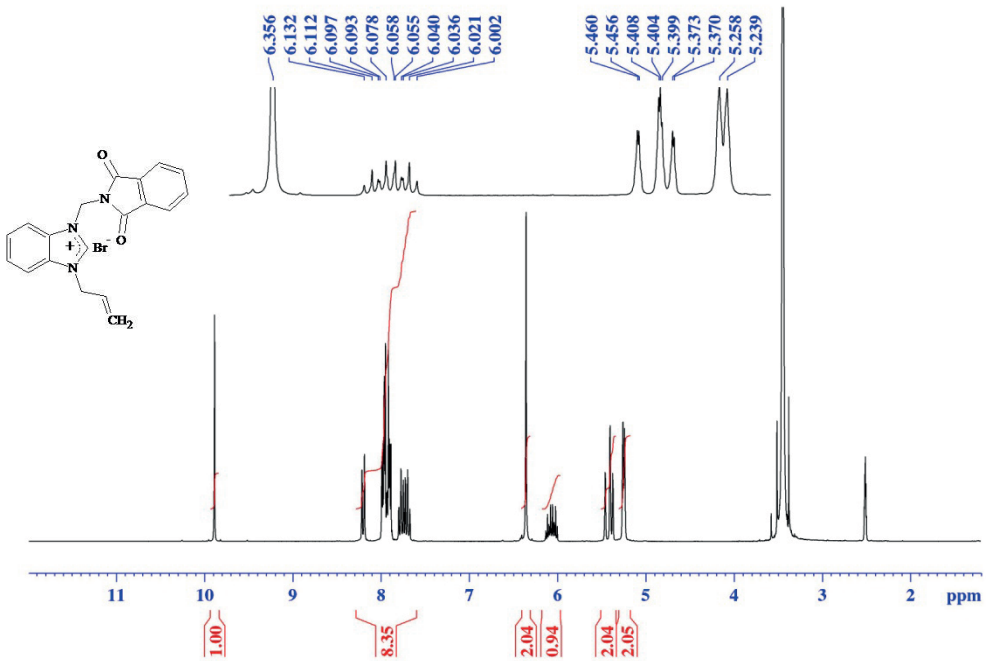

Figure 21. [1]H NMR spectrum of 1-allyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**7**).

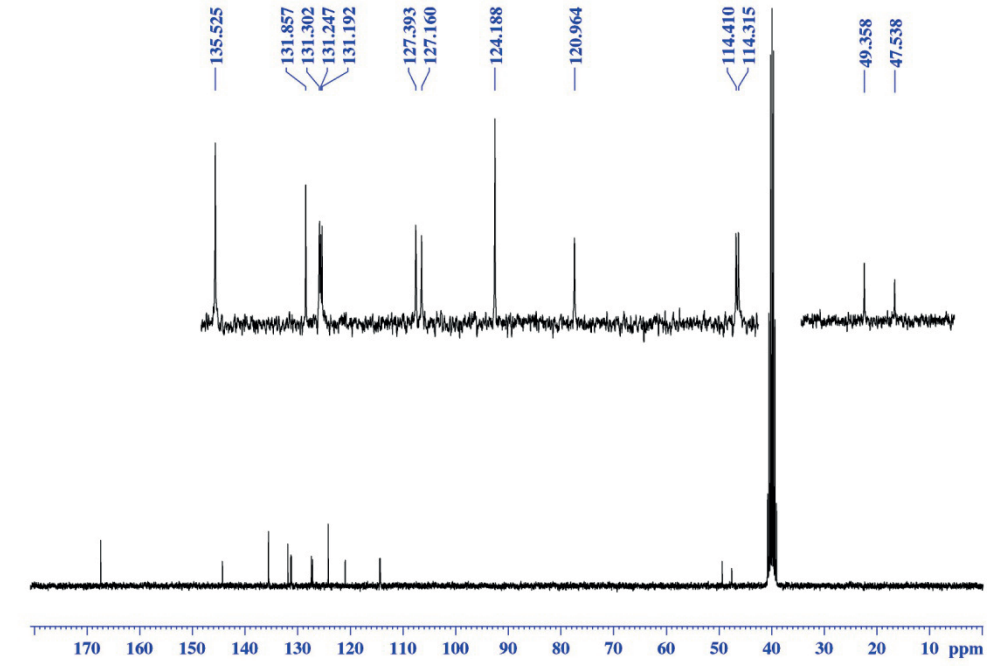

Figure 22. [13]C NMR spectrum of 1-allyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**7**).

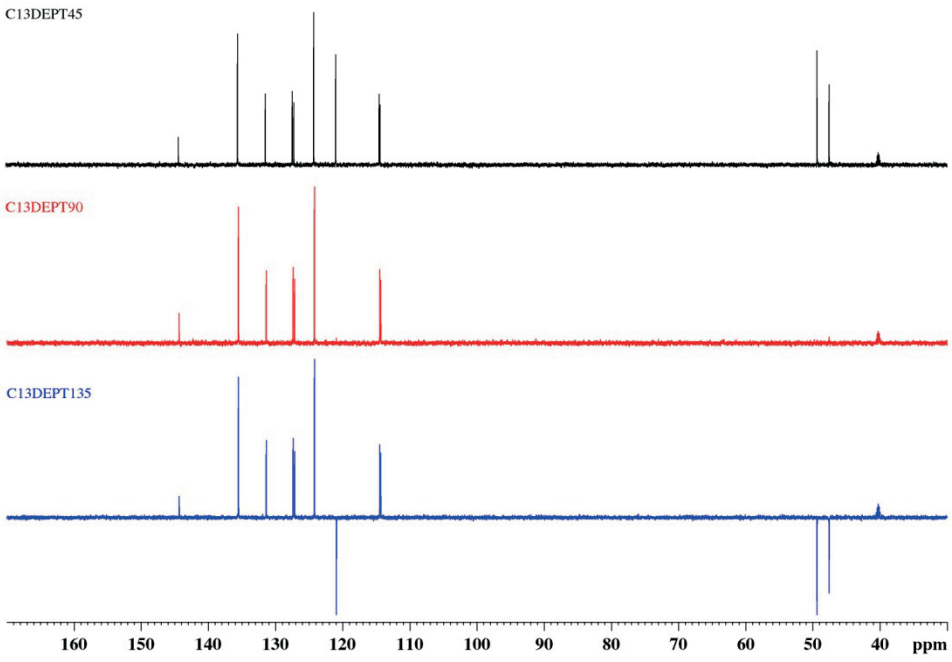

Figure 23. ^{13}C DEPT NMR spectrums of 1-allyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**7**).

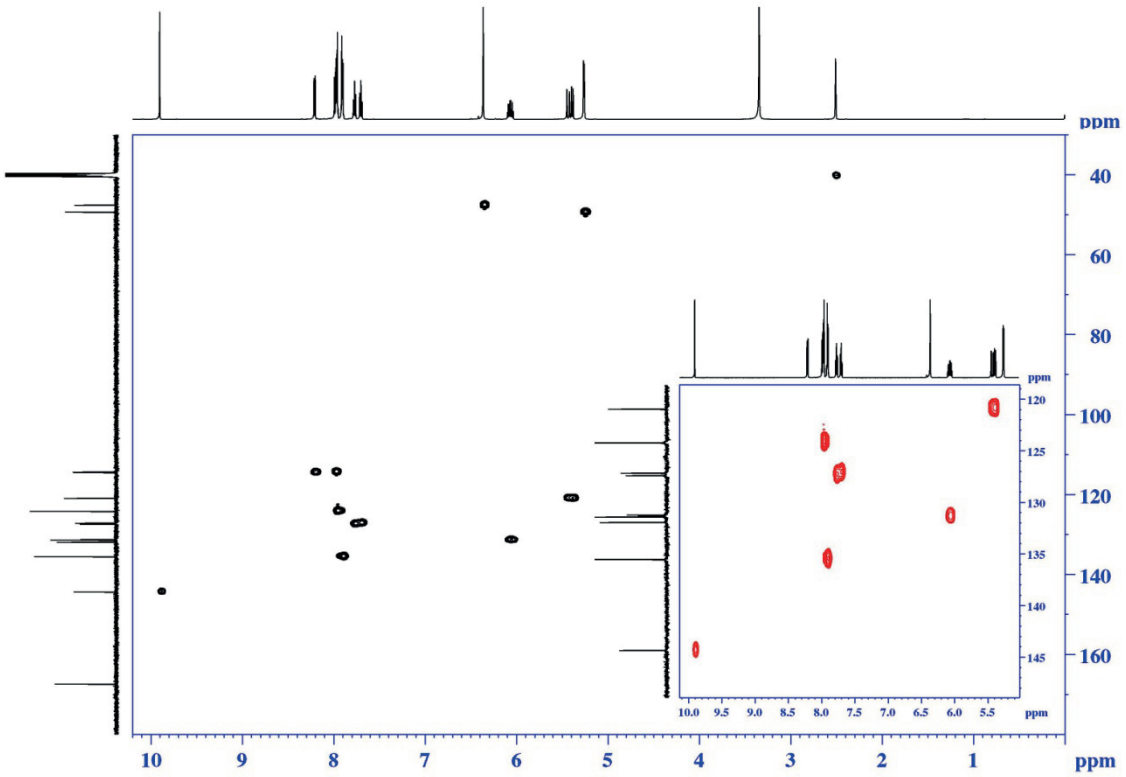

Figure 24. HSQC 2D NMR spectrum of 1-allyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**7**).

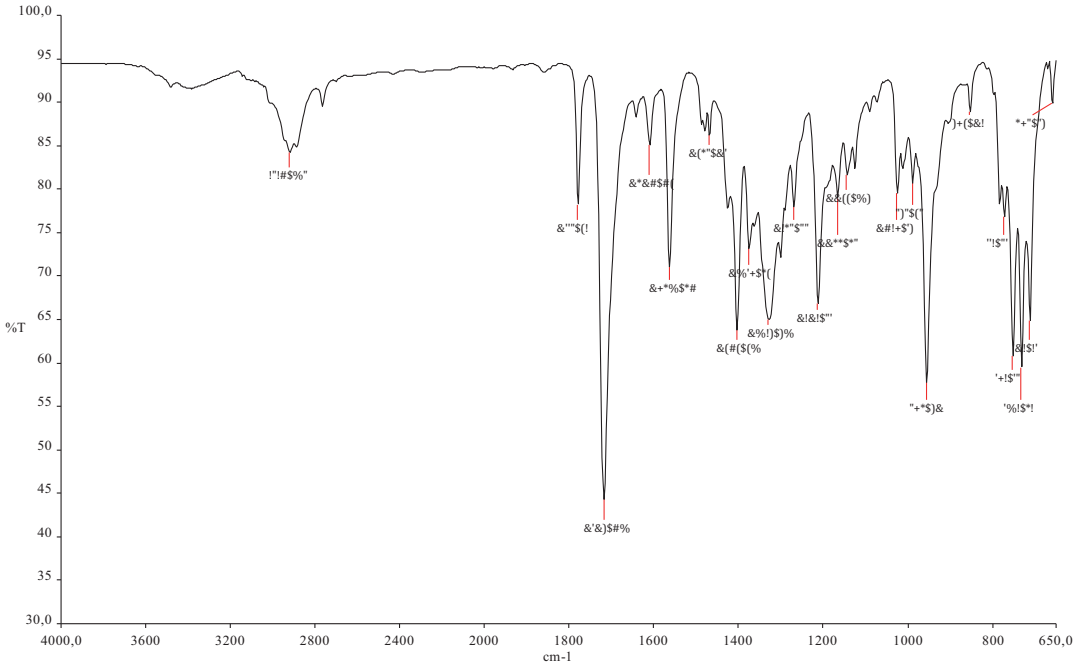

Figure 25. IR spectrum of 1-allyl-3-(phthalimide-2-yl)methylbenzimidazolium bromide (**7**).