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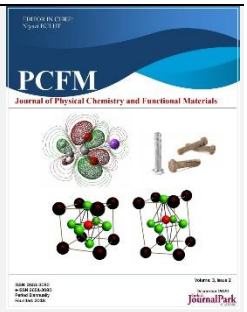

Synthesis, Characterization and Radical Scavenging Activities of 1,3-Disubstituted Benzimidazolium Salts

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ABSTRACT

The reaction of N-substituted benzimidazole and alkyl halides (benzyl bromoacetate and trifluoromethoxybenzyl bromide) gave a series of 1,3-disubstituted benzimidazolium salts. The structure of synthesized compounds was confirmed by various spectroscopic techniques such as ^{1}H, ^{13}C and ^{19}F NMR spectroscopy, FT-IR and elemental analysis. The biological efficiency of these compounds was evaluated by antioxidant activities like ABTS, OH and DPPH radical scavenging activity.

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1. INTRODUCTION

Free radical production and accumulation in the human body creates negative effects leading to oxidative stress [1]. It is essential to inhibit or scavenge free radicals or reactive oxygen species (ROS), which are responsible for oxidation in biological systems, or cause cellular dysfunction and tissue ageing [2-5]. Every year, new therapeutic approaches to the health hazards of oxidative stress become important. Supporting biological systems with antioxidants is one of them. Vitamin C and E, flavonoids, polyphenols etc. are some of the most effective natural exogenous antioxidants [6-9]. Recently, many reports have shown that some new indole and benzimidazole derivatives revealed their antioxidant properties [10-13].

On the other hand, benzimidazole has also proven to be a privileged pharmacophore with various applications such as antimicrobial, anthelmintic, anticancer, antihypertensive, antiviral, antifungal [14-20], cytotoxicity [21] and enzyme inhibition [22, 23] in addition to antioxidant action. Benzimidazole is a heterocyclic molecule widely used as a building block in organic synthesis. Benzimidazoles are formed by the condensation reaction of 1,2-phenylenediamine with carboxaldehyde and carboxylic acids [24]. They are reportedly low toxicity and highly effective against many strains of pathogens. As mentioned above, this heterocyclic ring system has been found in both naturally occurring and synthesized medicinal compounds; and it has been a fundamental building block in the development of many marketed drugs. One of the most important features of such compounds is the ability to adjust the groups attached to the nitrogen atoms to the desired structural motif. Recent studies have indicated that fluorine and ester-containing groups among the structural motifs at the nitrogen atoms of benzimidazole give the molecule specific properties [25-27]. Fluoroalkoxy compounds have attracted the interest of researchers and are increasingly used in the pharmaceutical industry, materials science, and agrochemistry [28, 29]. Pretomanid [30], delamanid [31], riluzole [32], triflumuron [33], thrifluzamide [34], and flurprimidol [35] are trifluoromethoxy (CF_3O) groups containing drugs that are now registered and used in a range of therapies. Widely used in medical, food and cosmetic applications, esters have been reported to have various biological activities, including antimicrobial [36-38], antiviral [39, 40], anti-inflammatory and nematode [41]. An example of a successful pharmaceutical masked with an ester group is

enalapril [42], acetylsalicylic acid (aspirin). The structures of compounds are shown in Figure 1.

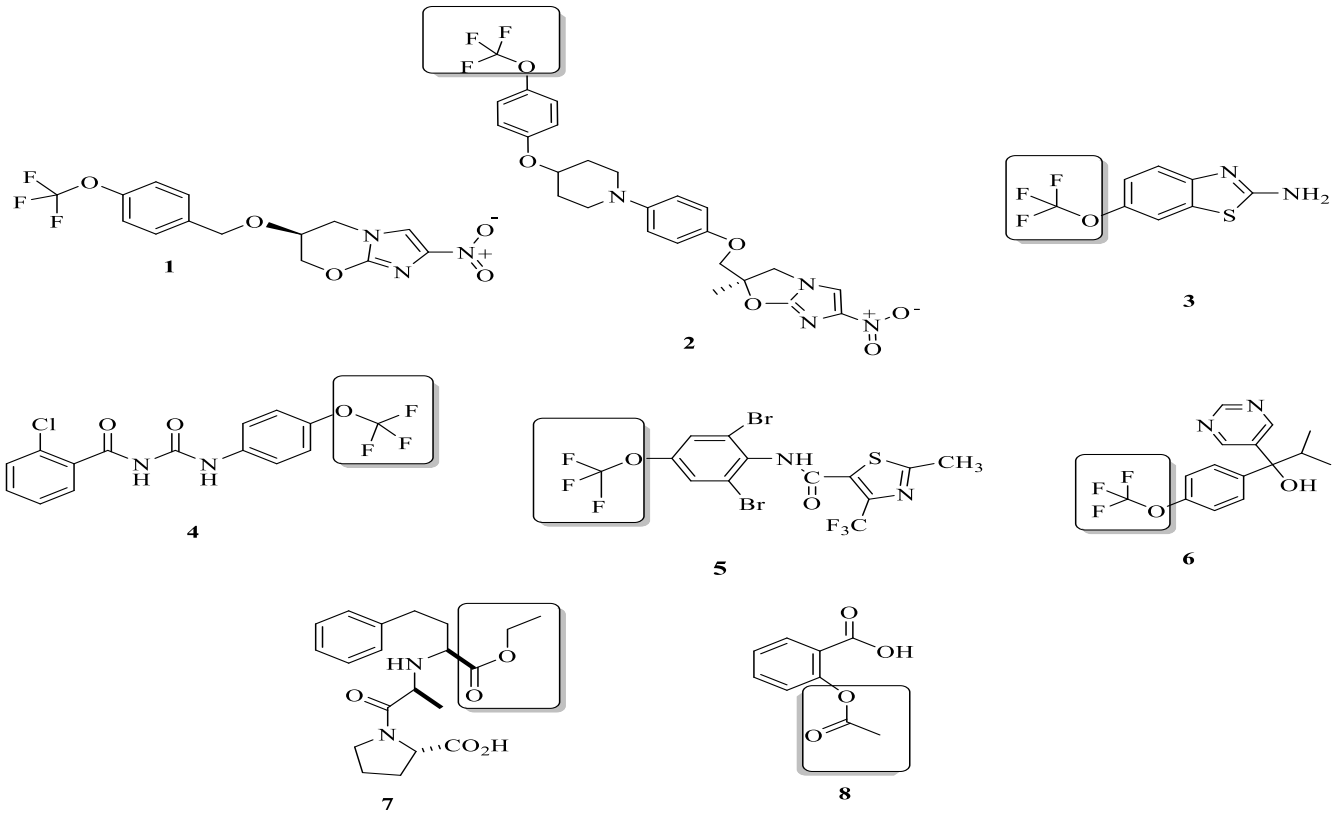


Figure 1 Trifluoromethoxy (CF_3O) and ester grou*p*-containing drugs: Pretomanid (1), Delamanid (2), Riluzole (3), Triflumuron (4), Thrifluzamide (5), Flurprimidol (6), Enalapril (7), Aspirin (8).

Based on the above, we considered it expedient to carry out the synthesis of 1,3-disubstituted benzimidazolium salts containing a trifluoromethoxy benzyl and ester groups. The structure of synthesized benzimidazolium salts was confirmed by various spectroscopic techniques such as ^{1}H, ^{13}C and ^{19}F NMR and FT-IR and elemental analysis. The biological efficiency of these compounds was evaluated by antioxidant activities like ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), OH (hydroxyl) and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity.

2. MATERIAL AND METHOD

The synthesis of 1,3-disubstituted benzimidazolium salts was carried out standard techniques in not inert conditions. All solvents and reagents were obtained through reputable suppliers including Acros, Merck, Isolab, and Sigma-Aldrich Chemical Co. In addition, an Electrothermal-9200 melting point apparatus was used to measure the melting points of all compounds in air using glass capillaries. ^{1}H NMR, ^{13}C NMR and ^{19}F spectra were recorded using a Bruker AC400P FT spectrometer operating at 400 MHz (^{1}H), 100 MHz (^{13}C) in the $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. The Perkin Elmer Spectrum 100 FTIR spectrometer was used to record FT-IR spectrum experiments in the 400-4000 cm^{-1} range. Inonu University Scientific and Technology Center (Malatya, TURKEY) performed the elemental analysis experiment for all compounds. DPPH radical scavenging activity was performed according to the method of Shimada et al. with some modifications.

Synthesis of benzimidazolium salts

The synthesis of 1 and 2 compounds is reported by Gök et al. in 2022 [43].

1-(2,3,5,6-tetramethylbenzyl)-3-(4-trifluoromethoxybenzyl) benzimidazolium bromide (3)

Compound 3 was prepared from the reaction of 1-substituted benzimidazole (1.0 mmol) and 4-trifluoromethoxybenzyl bromide (1.0 mmol) in dimethylformamide (DMF) (5 mL). The mixture was then stirred for 16 h at 80°C. After adding diethyl ether to the solution, the product precipitated as a white solid. This solid was then washed with diethyl ether (2×10 mL). The crude product was recrystallized from ethyl alcohol/diethyl ether (1: 3) at room temperature. Yield: 82%, (0.42 g); m.p.: 278-279 °C; color: white. *ν*(CN): 1560 cm^{-1}. Anal. calc. for $C_{26}H_{26}BrF_3N_2O$: C: 60.12, H: 5.05, N: 5.39. Found: C: 60.18, H: 5.09, N: 5.28. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 2.26 (s, H, $NCH_2C_6H(CH_3)_4$-2,3,5,6), 2.29 (s, H, $NCH_2C_6H(CH_3)_4$-2,3,5,6), 5.81 (s, 2H, $NCH_2C_6H(CH_3)_4$-2,3,5,6), 6.07 (s, 2H, $NCH_2C_6H_4(OCF_3)$), 7.12 (s, 1H, $NCH_2C_6H(CH_3)_4$-2,3,5,6), 7.19 (d, 2H, *J*= 9.0 Hz, $NCH_2C_6H_4(OCF_3)$), 7.38-7.41 (m, 1H, Ar-H), 7.49-7.65 (m, 5H, Ar-H), 11.05 (s, 1H, 2-C*H*). ^{13}C-NMR (100 MHz,

$CDCl_3$), δ (ppm): 16.2 and 20.6 ($NCH_2C_6H(CH_3)_4$-2,3,5,6), 47.7 ($NCH_2C_6H(CH_3)_4$-2,3,5,6), 50.5 ($NCH_2C_6H_4(OCF_3)$), 113.6, 113.8, 121.6, 127.3, 130.1, 131.5, 131.6, 131.8, 133.9, 134.1, 135.4 and 162.5 (Ar-*C*), 149.6 (Ar-*C*-OCF_3), 142.8 (2-*C*H), 127.1 (OCF_3). ^{19}F NMR (376 MHz, $CDCl_3$), δ (ppm): - 57.87 (OCF_3).

1-(4-biphenylbenzyl)-3-(4-trifluoromethoxybenzyl) benzimidazolium bromide (4)

The same procedure used to obtain compound **3** was also used to obtain compound **4.** Yield: 87%, (0.47 g); m.p.: 279-280 °C; color: white. *ν*(CN):1556 cm^{-1}. Anal. calc. for $C_{28}H_{22}BrF_3N_2O$: C: 62.35, H: 4.11, N: 5.19. Found: C: 62.27, H: 4.14, N: 5.13. ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 5.93 (s, 2H, $NCH_2C_6H_4(C_6H_5)$-4), 6.00 (s, 2H, $NCH_2C_6H_4(OCF_3)$), 7.22 (d, 2H, *J*= 6.0 Hz, $NCH_2C_6H_4(OCF_3)$), 7.36-7.46 (m, 3H, Ar-H), 7.51-7.70 (m, 12H, Ar-H), 12.00 (s, 1H, 2-C*H*). ^{13}C-NMR (100 MHz, $CDCl_3$), δ (ppm): 50.7 ($NCH_2C_6H_4(OCF_3)$), 51.5 ($NCH_2C_6H_4(C_6H_5)$-4), 113.6, 113.9, 121.7, 127.2, 127.3, 127.8, 128.1, 128.8, 128.9, 130.2, 131.2, 131.3, 131.4, 139.8 and 142.2 (Ar-*C*), 149.7 (Ar-*C*-OCF_3), 143.5 (2-*C*H), 127.1 (OCF_3). ^{19}F NMR (376 MHz, $CDCl_3$), δ (ppm): - 57.82 (OCF_3).

1,3-bis(benzylacetate)benzimidazolium bromide, (5)

The same procedure used to obtain compound **3** was also used to obtain compound **5.** Yield: 91%, (0.47 g); m.p.: 158-159 °C; color: white. ν(C =O): 1747 cm^{-1}, ν(CN): 1562 cm^{-1}, and ν(C–O): 1183 cm^{-1}. Anal. calc. for $C_{25}H_{23}BrN_2O_4$: C: 60.62, H: 4.68, N: 5.66. Found: C: 60.57, H: 4.71, N: 5.61.^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.14 (s, 4H, $NCH_2COOCH_2C_6H_5$), 5.58 (s, 4H, $NCH_2COOCH_2C_6H_5$), 7.27 (s, 10H, $NCH_2COOCH_2C_6H_5$), 7.52 (s, 4H, NC_6H_4N), 11.17 (s, 1H, 2-C*H*). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm) : 48.3 ($NCH_2COOCH_2C_6H_5$), 68.7 ($NCH_2COOCH_2C_6H_5$), 113.2, 127.6, 128.7, 128.8, 128.9, 131.2 and 134.2 (Ar-*C*); 144.5 (2-*C*H), 165.2 ($NCH_2COOCH_2C_6H_5$).

Antiradical Activity Analyses

Radical scavenging activities of **1-5** compounds were calculated in percentage using the following equation [44,45]:

$$\%\ \text{Radical Destroy Activity} = \frac{A_0 - A_1}{A_0} x100$$

A_0 = Absorbance value of control

A_1 = Absorbance value after compound addition

DPPH• Radical Scavenging

DPPH radical scavenging activity was performed according to the method of Shimada et al. with some modifications [46]. For this, 1 mM solution of DPPH• radical was used. Compound solutions were transferred to test tubes at a concentration of 500 ppm and completed with pure ethanol to make a total volume of 3 mL. Then, 1 mL of stock DPPH• solution was added to each sample tube. All samples were incubated for 30 min at room temperature in the dark. After incubation, absorbance was measured at 517 nm against a blank consisting of ethanol. As a control, 3 mL of ethanol and 1 mL of DPPH• solutions were used.

$ABTS^{\bullet+}$ Radical Scavenging

This involved mixing, 2.45 mM $K_2S_2O_8$ and 7 mM ABTS solutions in a 1:1 ratio and incubating for 16 hours at room temperature and in the dark. The absorbance of the ABTS radical solution prepared in this way was measured at 734 nm and diluted with ethyl alcohol until an absorbance of 2.250±0.02 was obtained. This absorbance was used as control absorbance. Then, 4 mL of this radical solution was added to the test tubes, to which the combined solutions were added and incubated for 2 hours at room temperature and in the dark. At the end of this period, the absorbance of the samples was recorded against the blank consisting of PBS (Phosphate Buffer Solution, pH=7.4) at 734 nm [47].

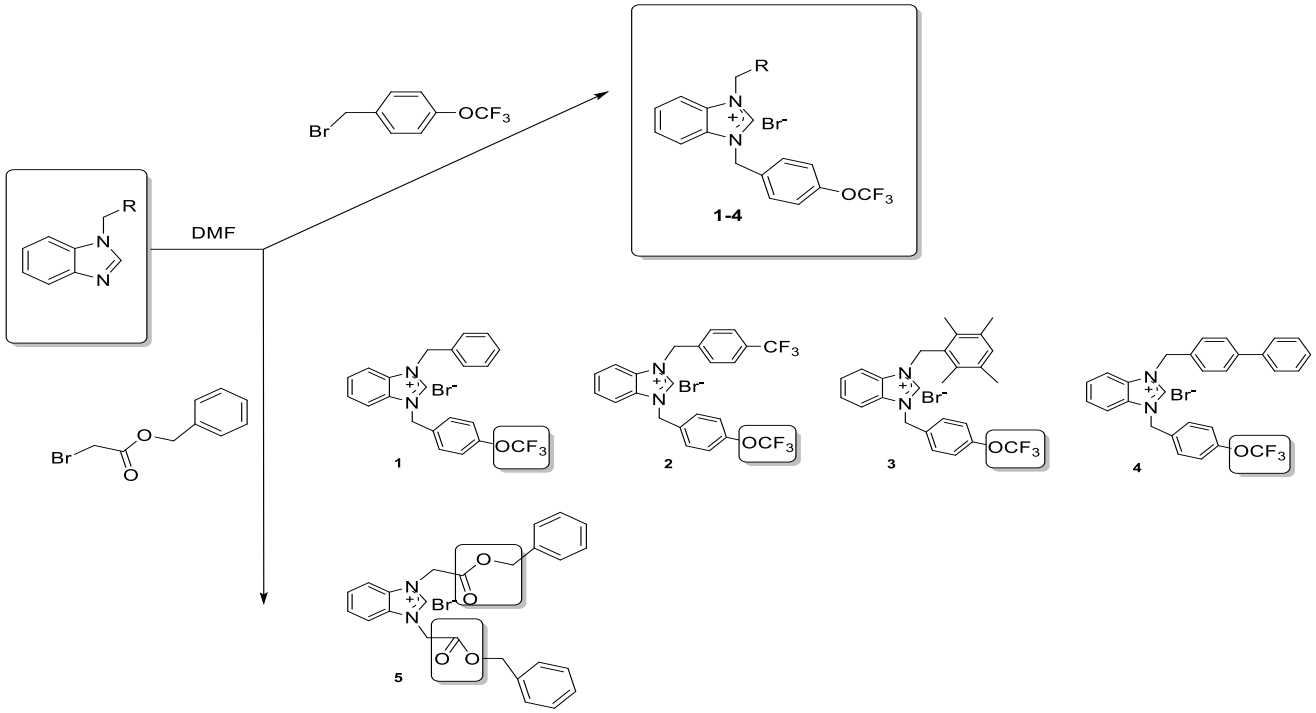
OH• Radical Scavenging

In this method, the OH• radical scavenging activities of the compounds were carried out according to the method of Halliwell et al. [48]. The reaction mixture was composed of the compound solution, 500 µL 3.6 mM deoxyribose, 200 µL 100 µM $FeCl_3$, 200 µL 104 mM EDTA, 100 µL 1 mM H_2O_2 and 100 µL 1 mM ascorbic acid solution and mixed well in a vortex. After waiting for 1 hour in the oven at 37 °C, 1 mL of 2.8% TCA and 1 mL of 1.0% TBA were added to the tubes and the samples were kept in a hot water bath at 50 °C for 30 min. The samples were taken into the butanol phase and their absorbance were recorded in UV at a wavelength of 532 nm.

3. RESULTS AND DISCUSSIONS

3.1. Synthesis

We designed and synthesized a series of benzimidazolium salts containing trifluoromethoxy and ester structural units, as shown in Scheme 1. The synthesis of compounds (**1-5**) was carried out with alkyl halide and N-substituted benzimidazole in DMF (5 mL) was stirred and heated at 80 °C. The alkyl halides were benzyl chloride, *p*-trifluoromethyl benzyl chloride, tetramethyl benzyl chloride, biphenyl benzyl chloride, benzyl bromo acetate and trifluoromethoxy benzyl bromide (Scheme 1). The structures of all compounds were identified by ^{1}H, ^{13}C, ^{19}F-NMR, IR spectroscopic methods and elemental analysis techniques. The NMR and IR spectrum data were presented in support information.



Scheme 1 Synthesis of benzimidazolium salts containing trifluoromethoxy and ester structural units (**1-5**)

^{1}H NMR, ^{13}C NMR, ^{19} F NMR (for **3** and **4**) and IR spectroscopic methods were used to evaluate the structural properties of benzimidazolium salts. The synthesis of compounds 1 and 2 was reported by Gök et al. [43]. The chemical shifts of the characteristic benzimidazolium salt peaks are 11.05, 12.00 and 11.17 ppm, respectively for **3-5**. The chemical shift values of the N-C*H*$_2$($C_6H(CH_3)_4$-2,3,5,6 groups (for **3**), N-C*H*$_2$($C_6H_4(C_6H_5)$-4 (for **4**) of the compounds were found to be 5.81 and 5.93 ppm. At the same time, peaks of the benzylic-C*H*$_2$ containing trifluoromethoxy functional groups (**3 and 4**) were found at 6.07 and 6.00 ppm, respectively. The peaks of the NC*H*$_2$COOC*H*$_2$$C_6H_5$ were observed at 5.58 and 5.14 ppm as singlet peak for compound **5**. The chemical shift values of the 2-C atoms of the benzimidazole rings of the compounds were 142.8, 143.5 and 144.5 ppm, respectively for **3-5**. The *p*-trifluoromethoxy group's carbon peaks (O*C*F$_3$) were detected for compounds **3** and **4** at 127.1 ppm. Also, the carbonyl carbon (C = O) peak belonging to the ester group in compound **5** was observed at 165.2 ppm. The peaks of acetate carbon came at 68.7, 48.3 ppm. In ^{19} F NMR, the *p*-trifluoromethoxy group's fluor peaks (OC*F*$_3$) were detected for compounds **3** and **4** at -57.8 ppm. The peaks of the NMR signals for compounds **3-5** are given in Supporting Information (SI) file Figures S1, S2, S3, S5, S6, S7, S9 and S10.

When the IR spectra of the benzimidazolium salts were evaluated, the C = N stretching in the benzimidazole ring was observed at 1560, 1556 and 1562 cm^{-1}, respectively for **3-5**. The sharp stretching peaks of C=O and C-O were observed at 1747 and 1183 cm^{-1} for the ester group of compound **5**. Finally, a sharp peak at 1152 cm^{-1}and 1163 cm^{-1} belonging to the C–O stretching appears for the compound **3** and **4** containing the trifluoromethoxy group. The data of the IR signals for compounds **3-5** are given in Supporting Information (SI) file Figures S4, S8 and S11. The calculated values were found to be very close to the found values when the elemental analysis results were evaluated. All spectroscopic data obtained are consistent with the literature [27, 43]. The solubility test for all the synthesized salts were performed with various solvents. Take about 1 mL of solvent in a small test tube, and add about 10 mg of salts. Shake the solution well and after a few minutes the solubility of the salts was observed. Since salts are polar in nature it is mostly soluble in highly polar solvents like dichloromethane, chloroform, methanol, ethanol, DMF and DMSO. However, it was not soluble in non-polar and low polar solvents such as toluene, diethyl ether and n-hexane.

3.2. Antiradical activity of compounds 1-5

The antiradical activity results of **1-5** compounds at a concentration of 500 ppm are shown in Table 1. Accordingly, the standard antioxidant Trolox showed higher radical scavenging activity than 1-5 compounds in ABTS, OH and DPPH radical scavenging activities.

In the ABTS radical scavenging test, the samples are ranked in terms of scavenging activity as follows: Trolox > 2 > 3 > 4 > 1 > 5. In the OH radical scavenging test, the samples are ranked in terms of scavenging activity as follows: Trolox > 5 > 2 > 3 > 1 > 4.In the DPPH radical scavenging test, samples are ranked in terms of scavenging activity as follows: Trolox > 5 = 1 > 4 > 2 > 3.

Table 1 $ABTS^{+\bullet}$, $OH^{\bullet}$, $DPPH^{\bullet}$ radical scavenging activities of **1-5** compounds

Compounds	$ABTS^{+\bullet}$ Scavenging (%) (500 ppm)	$OH^{\bullet}$ Scavenging (%) (500 ppm)	$DPPH^{\bullet}$ Scavenging (%) (500 ppm)
1	32.00 ± 0.68^{d}	78.84 ± 0.90^{b}	39.12 ± 1.36^{b}
2	85.07 ± 1.39^{b}	79.89 ± 1.67^{b}	36.72 ± 1.19^{b}
3	64.18 ± 0.51^{c}	79.40 ± 0.84^{b}	35.99 ± 0.87^{b}
4	39.16 ± 0.56^{d}	78.68 ± 0.56^{b}	37.35 ± 0.48^{b}
5	17.01 ± 1.13^{e}	80.05 ± 1.19^{b}	39.12 ± 1.12^{b}
Trolox	96.85 ± 0.32^{a}	89.59 ± 0.31^{a}	94.29 ± 0.39^{a}

There is no statistical difference between groups with the same letter for each antiradical activity; $p<0.05$

Many studies have shown that benzimidazole compounds have very important antiradical, antioxidant, anticancer, antimicrobial etc. properties [49]. When antiradical studies on the mentioned group of compounds were examined in recent years; Ateş-Alagöz et al. determined new benzimidazole compounds that both indole and tetrahydronaphthalene fragments to the benzimidazole ring [11]. They synthesized destroyed more DPPH radicals than the standard antioxidant BHT. In a similar study, El Ouasif et al. determined new benzimidazole compounds that 2- mercapto benzimidazole derivatives had DPPH radical scavenging activity [50]. In another study, Argirova et al. examined the ABTS and DPPH radical scavenging activity of a new series of 1H-benzimidazol-2-yl hydrazones containing hydroxyphenyl and methoxyphenyl moieties and showed that some compounds had higher activity than the standard antioxidants quercetin and melatonin [51]. Khodja et al. determined that different heterocyclic hydrazone compounds have ABTS and DPPH radical scavenging activities [52]. Shatokhin et al. determined that new N-substituted 3-(benzimidazol-2-yl)-chromones containing 2,6-di-tert-butylphenol fragment they synthesized showed DPPH radical scavenging activity [53]. In a similar study, Prakash et al. suggested that novel bioactive azo compounds fused with benzothiazole compounds showed DPPH radical scavenging activity [54]. When the antiradical activities of the compounds synthesized in the presented study were evaluated, it was determined that compound number **2** showed very high antiradical activity (85.07%) in the ABTS radical scavenging test. All the compounds synthesized in the DPPH radical scavenging test exhibited low antiradical activity (35.99-39.12%). In the OH radical scavenging test, which is one of the most important antiradical activity tests since it is also formed in vivo, all the compounds exhibited high antiradical activity. All the studies mentioned above and our study results show that such compounds have potentially high antiradical activity.

4. CONCLUSION

In conclusion, 1,3-disubstituted benzimidazolium salts containing a trifluoromethoxy benzyl and ester groups were synthesized. The structure of synthesized benzimidazolium salts was confirmed by various spectroscopic techniques such as ^{1}H, ^{13}C and ^{19}F NMR and FT-IR and elemental analysis. The biological efficiency of these compounds was evaluated by antioxidant activities like ABTS, OH and DPPH radical scavenging activity. All the compounds exhibited high antiradical activity. In future studies, 1,3-disubstituted benzimidazolium salts and their metal complexes containing different substituents can be analyzed for antiradical, anticancer and antimicrobial activities.

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Competing interests

The authors declare that they have no competing interests.

Data Availability Statement

Data supporting the results of this study are available in the supplementary material.

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Supporting Information

Synthesis, characterization and radical scavenging activities of 1,3-disubstituted benzimidazolium salts

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^{1}H, ^{13}C, NMR (for 3-5) and ^{19}F-NMR (for 3 and 4) and IR data of the 1,3-disubstituted benzimidazolium salts

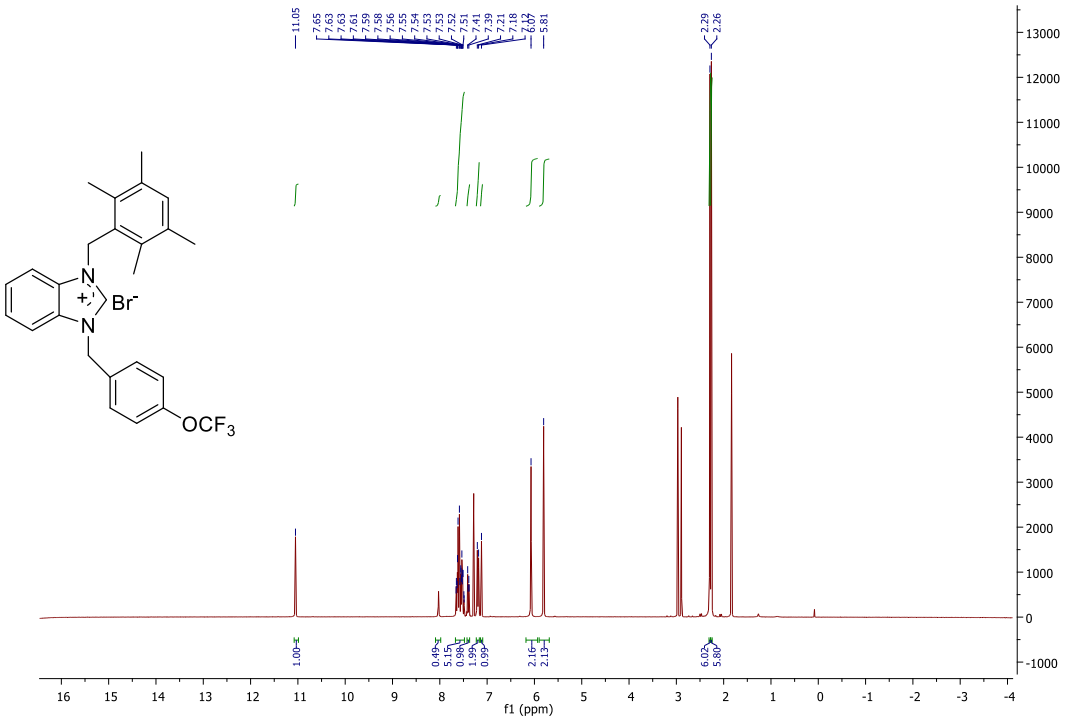

Fig. S1. ^{1}H NMR spectrum of compound **3** ($CDCl_3$)

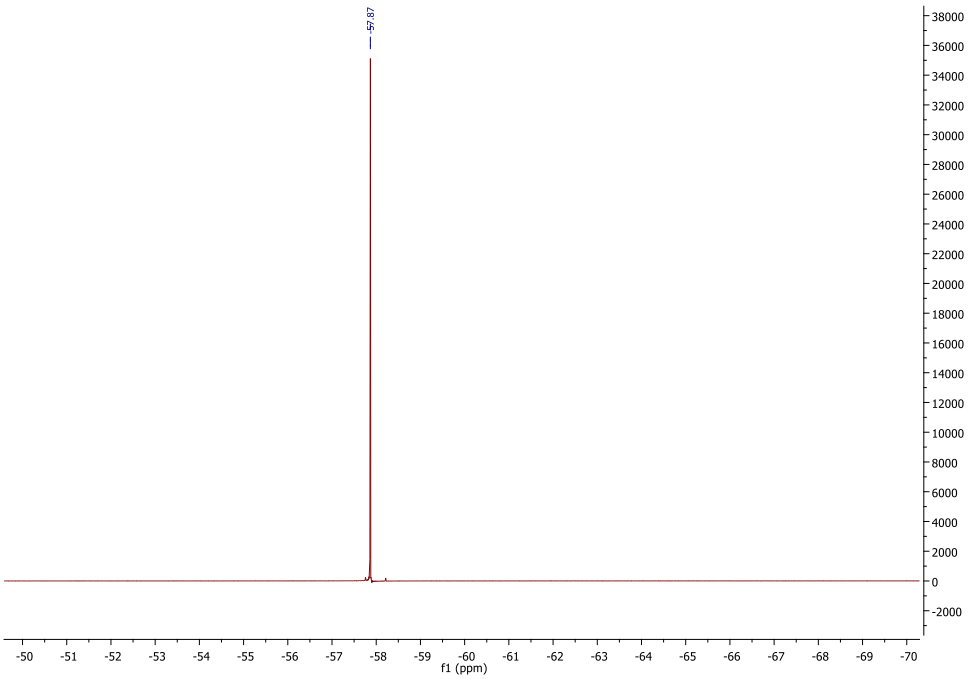

Fig. S2. ^{19}F NMR spectrum of compound **3** ($CDCl_3$)

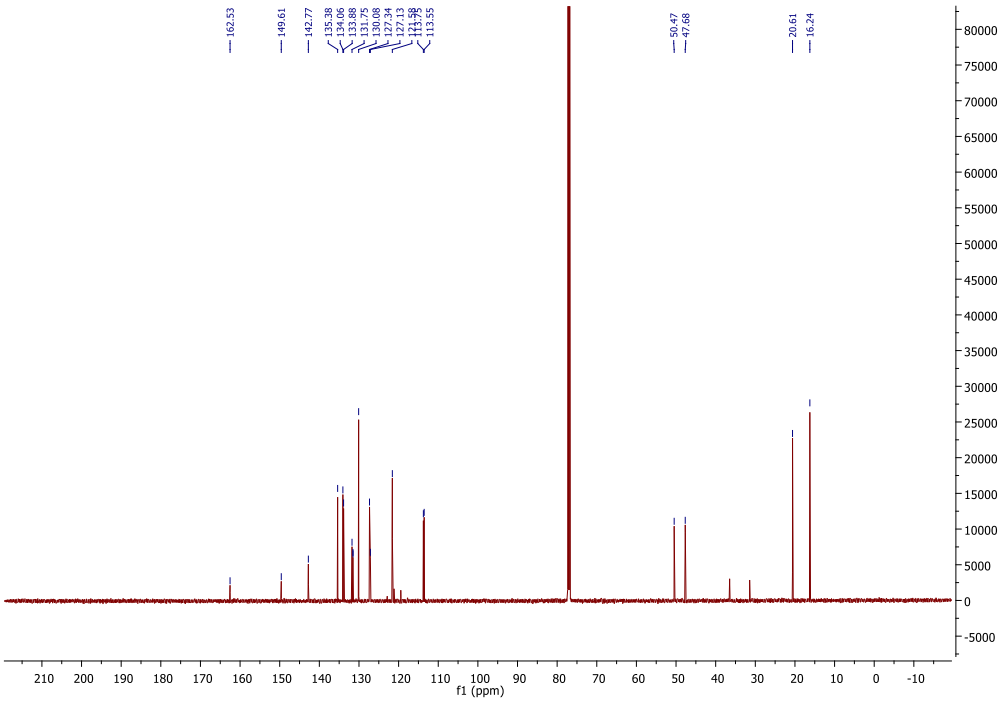

Fig. S3. ^{13}C NMR spectrum of compound **3** ($CDCl_3$)

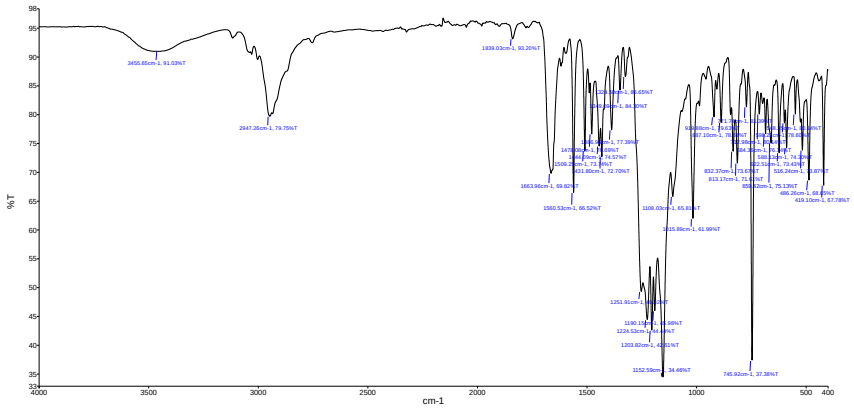

Fig. S4. IR spectrum of compound **3**

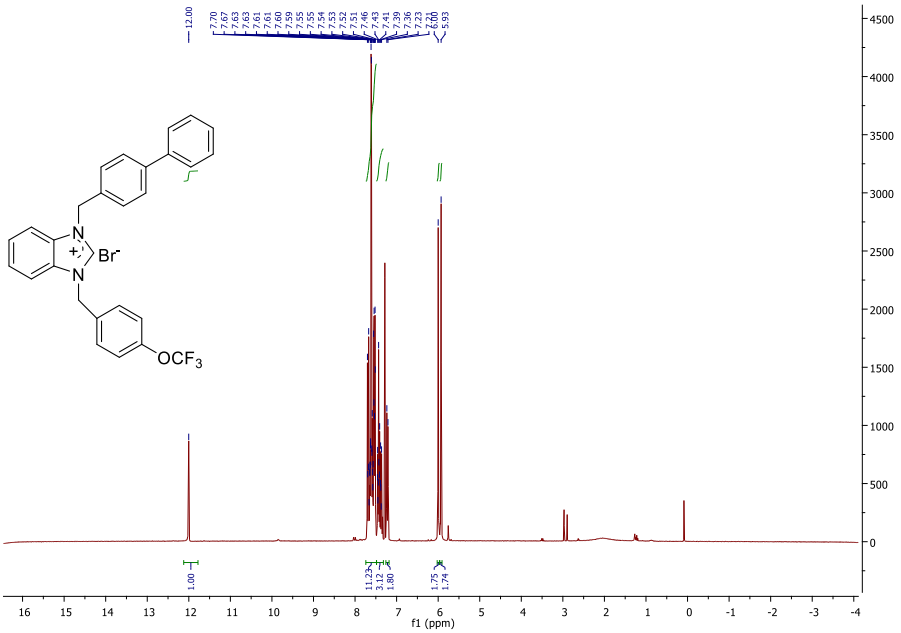

Fig. S5. ^{1}H NMR spectrum of compound **4** ($CDCl_3$)

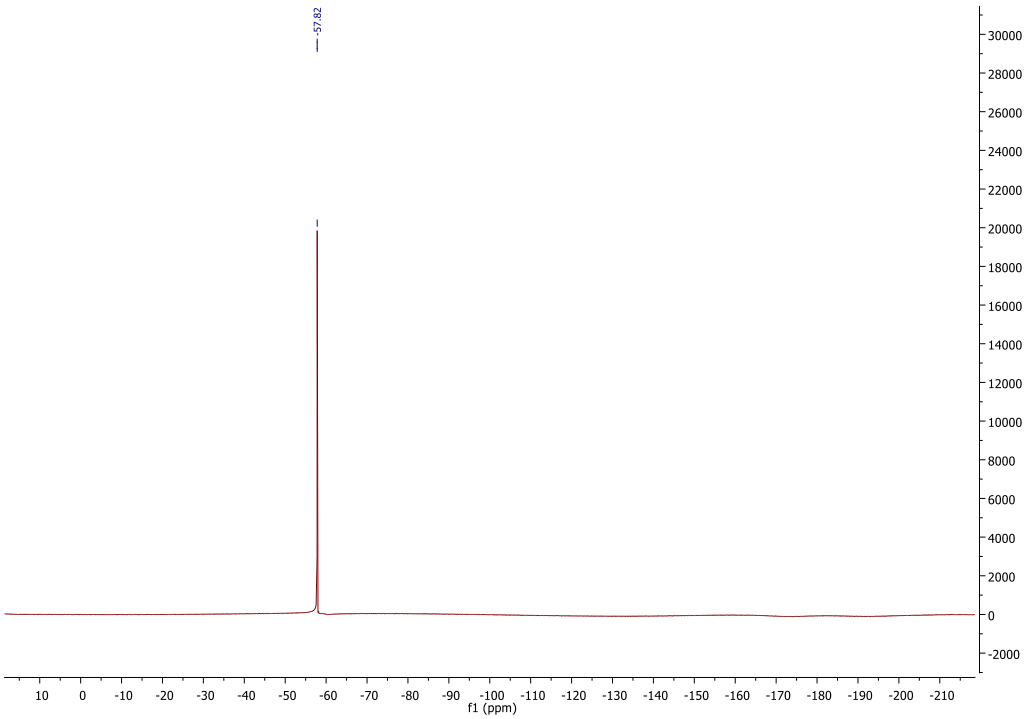

Fig. S6. ^{19}F NMR spectrum of compound **4** ($CDCl_3$)

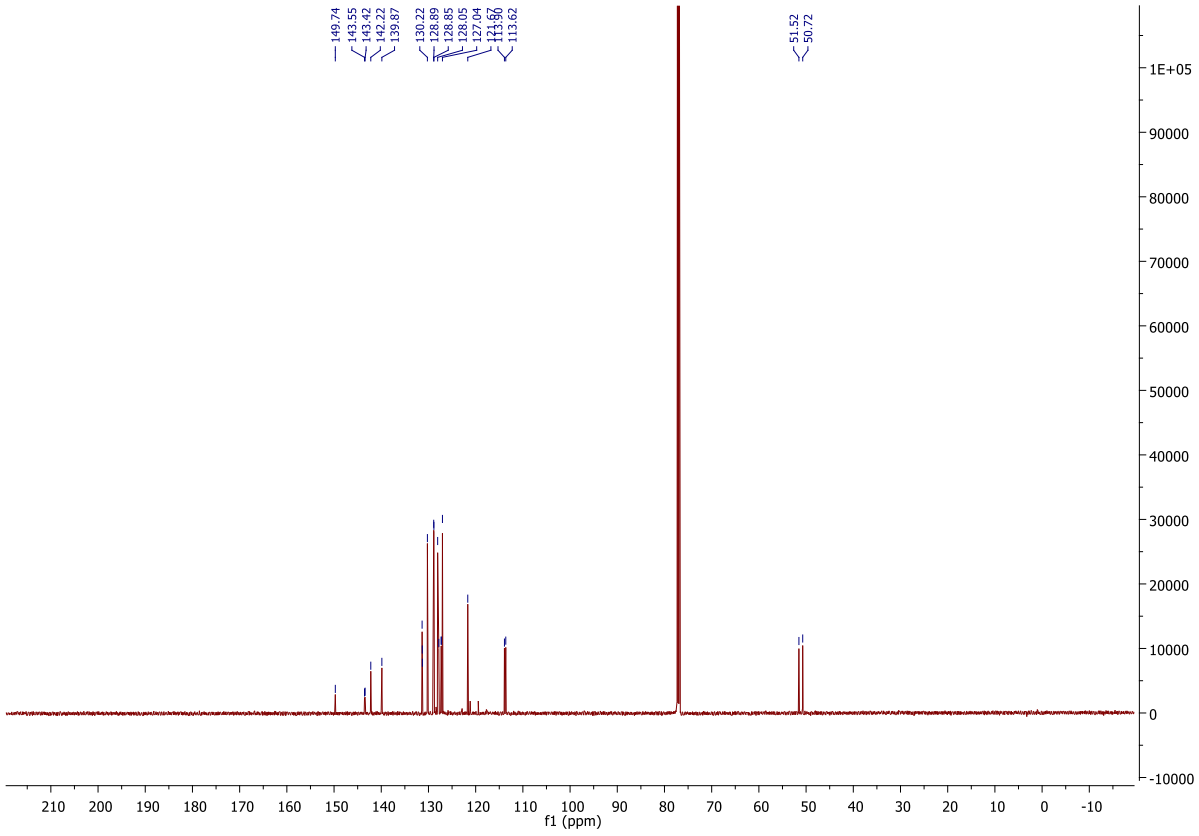

Fig. S7. ^{13}C NMR spectrum of compound **4** ($CDCl_3$)

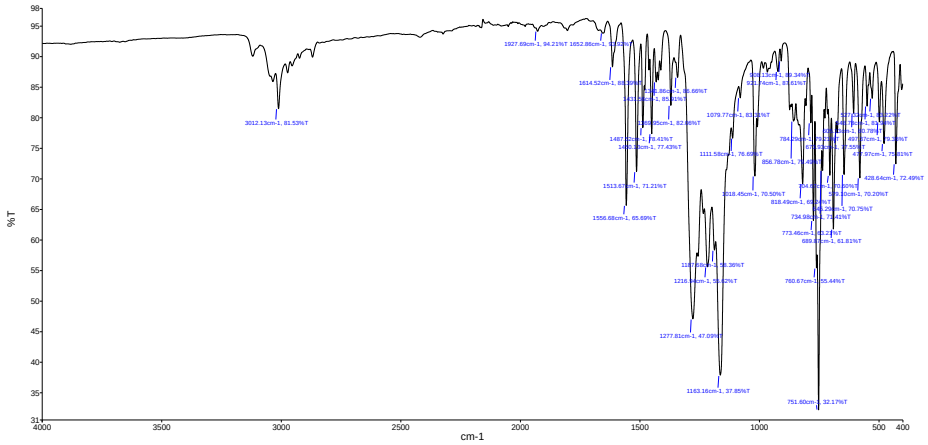

Fig. S8. IR spectrum of compound **4**

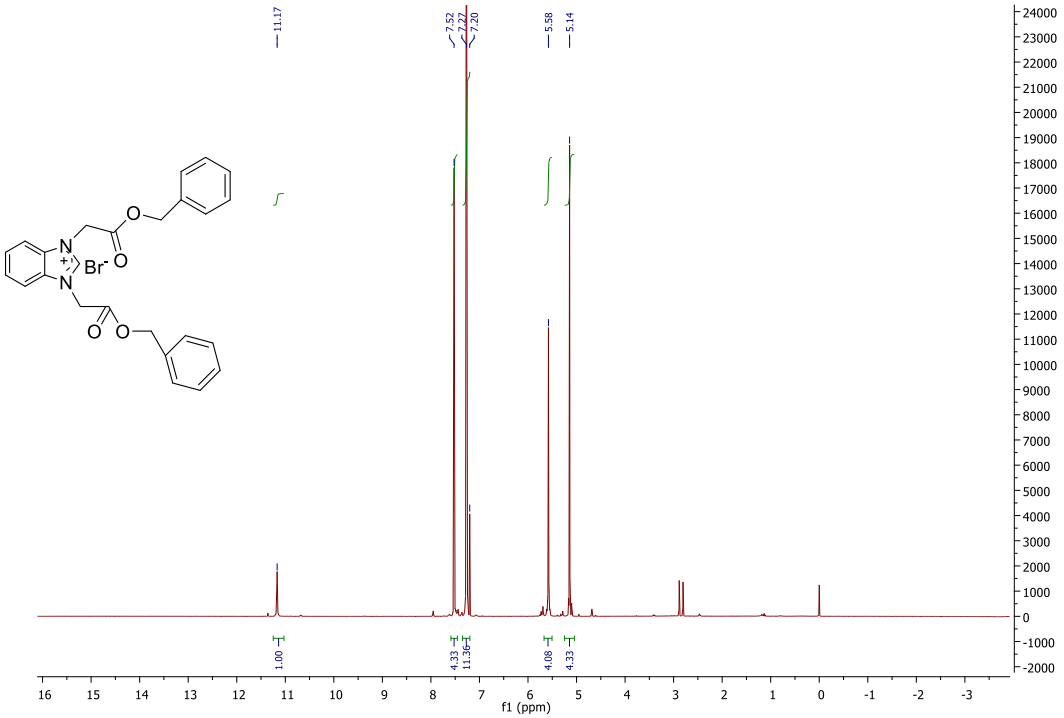

Fig. S9. ^{1}H NMR spectrum of compound **5** ($CDCl_3$)

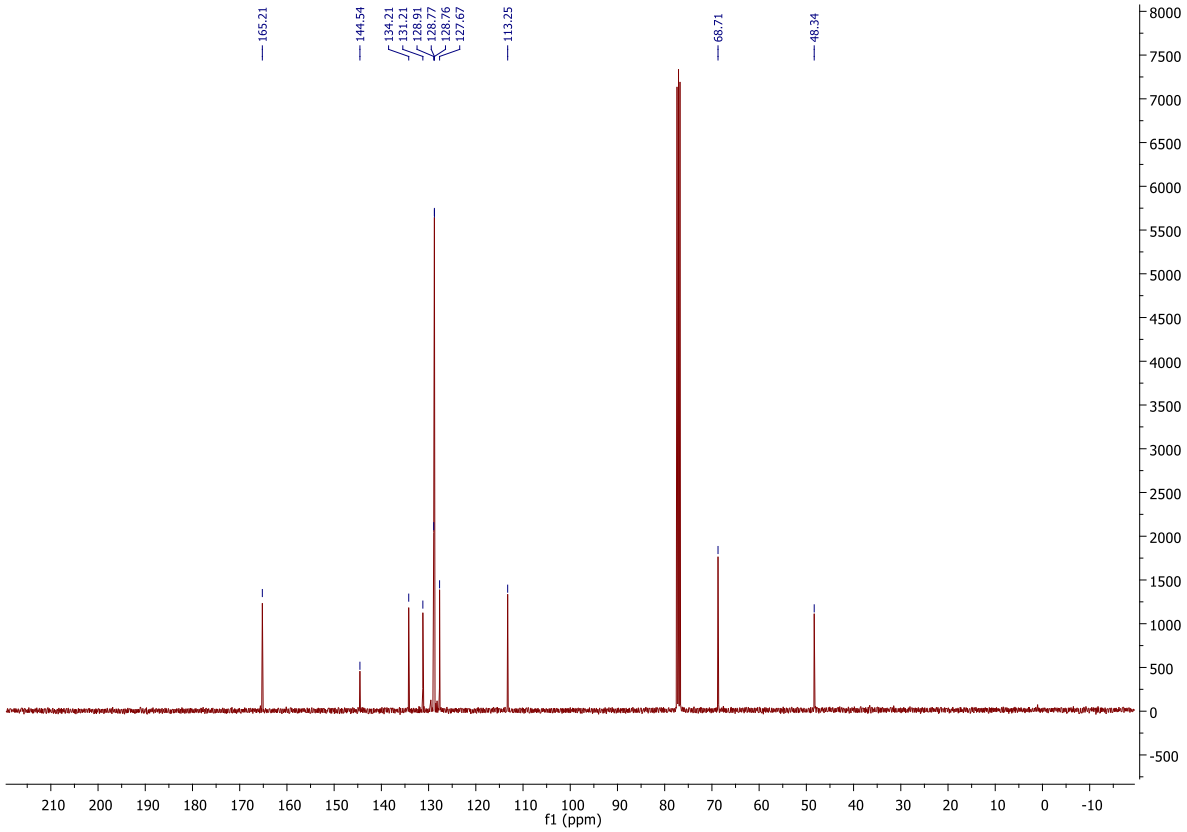

Fig. S10. ^{13}C NMR spectrum of compound **5** ($CDCl_3$)

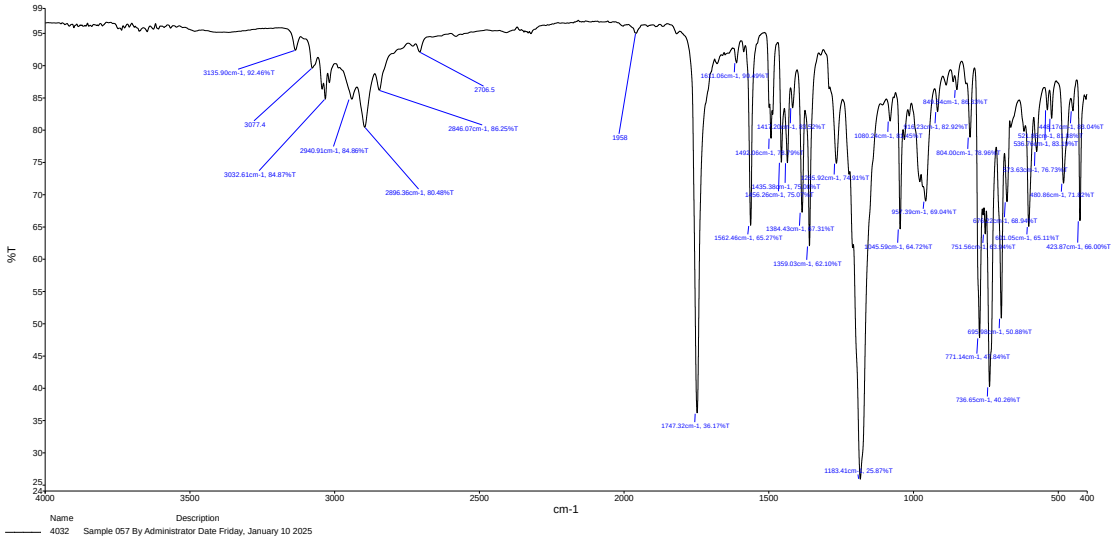

Fig. S11. IR spectrum of compound **5**