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Synthesis, Characterization and Antioxidant Evaluation of New *N*-*A*-Chloroacetylsalicyloyl-*N*-Antipyrine Benzamide

Elham Abdalrahem Bin Selim, Mohammed Hadi Al–Douh*, Faten Abdulaziz Bin Hawiel

Chemistry Department, Faculty of Science, Hadhramout University, Mukalla, Hadhramout, Yemen

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Abstract

The new compound *N*-*α*-chloroacetylsalicyloyl-*N*-antipyrine benzamide was synthesized by a reaction of *N*-benzylidene antipyrine amine and *O*-acetyl salicyloyl chloride. On the other hand, *N*-benzylidene antipyrine amine was obtained by a coupling reaction between benzaldehyde and 4-aminoantipyrine, while *O*-acetyl salicyloyl chloride resulted from acylation reaction of aspirin starting from salicylic acid. All synthesized compounds were characterized by using m.ps., UV-Vis., FTIR, MS, and ^{1}HNMR spectroscopic methods. The method of DPPH was also used as testing to measure the radical scavenging of the new compound. It showed very powerful antioxidant activity with IC_{50} value of 29 ppm. Moreover, its antibacterial activity was tested against three types of bacteria.

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*Corresponding author: m.aldouh@hu.edu.ye

1. Introduction

Benzamide 1 is a benzoic acid 2 derivative. Benzamide and its derivative have been synthesized due to their important properties and applications. They are pharmaceutical drugs such as amino hippuric acid 3 and procainamide 4, which are used in the measurement of renal plasma flow of the kidney [1]. The other benzamide derivatives are useful in the treatment of cardiac arrhythmias [2], anti-convulsant, anti-inflammatory, analgesic, anti-depressant, antitumor, and as potent and selective sigma-1 protein ligands [3-6]. Several studies also showed that *N*-*α*-chlorobenzyl-*N*-hetero benzamides 6 were synthesized to produce six-membered heterocyclic compounds [7-10], Figure 1. On the other hand, 4-aminoantipyrine 7 has important applications, especially as a non-narcotic anti-analgesic, non-steroidal anti-inflammatory, anti-rheumatic drug, peripheral nervous system, and antipyretic drug [11,12]. Some Schiff bases and their metal complexes that derived from 7 were studied and exhibited several properties such as binding and cleavage of DNA, and additional application as antimicrobial activity [13]. Acetyl salicylic acid, an aspirin 11 is a known compound that is an anti-inflammatory, pain and fever pain killer and fever reducer [14]. According to the previous pharmaceutical information, this study reports the synthesis and identification of a new tertiary benzamide compound which is *N*-*α*-chloroacetylsalicyloyl-*N*-antipyrine benzamide 13, figure 1. The antibacterial and antioxidant activities of it were also tested.

2. Experimental Part

2.1. General

The melting points were determined on the Electrothermal Apparatus. The UV–Vis. spectra were recorded with A–580, double beam Spectrophotometer. FTIR spectra in the range 4000–400 cm^{-1} were recorded for the KBr pellets using a Nicolet IS10 FTIR Spectrometer. Mass spectra were carried out on Direct Inlet part to the mass analyzer in Thermo Scientific GC/MS model ISQ, and ^{1}HNMR spectra were recorded on JEOL's spectrometer operating at 500 MHz, using DMSO-d_6 as a solvent and TMS as internal standard.

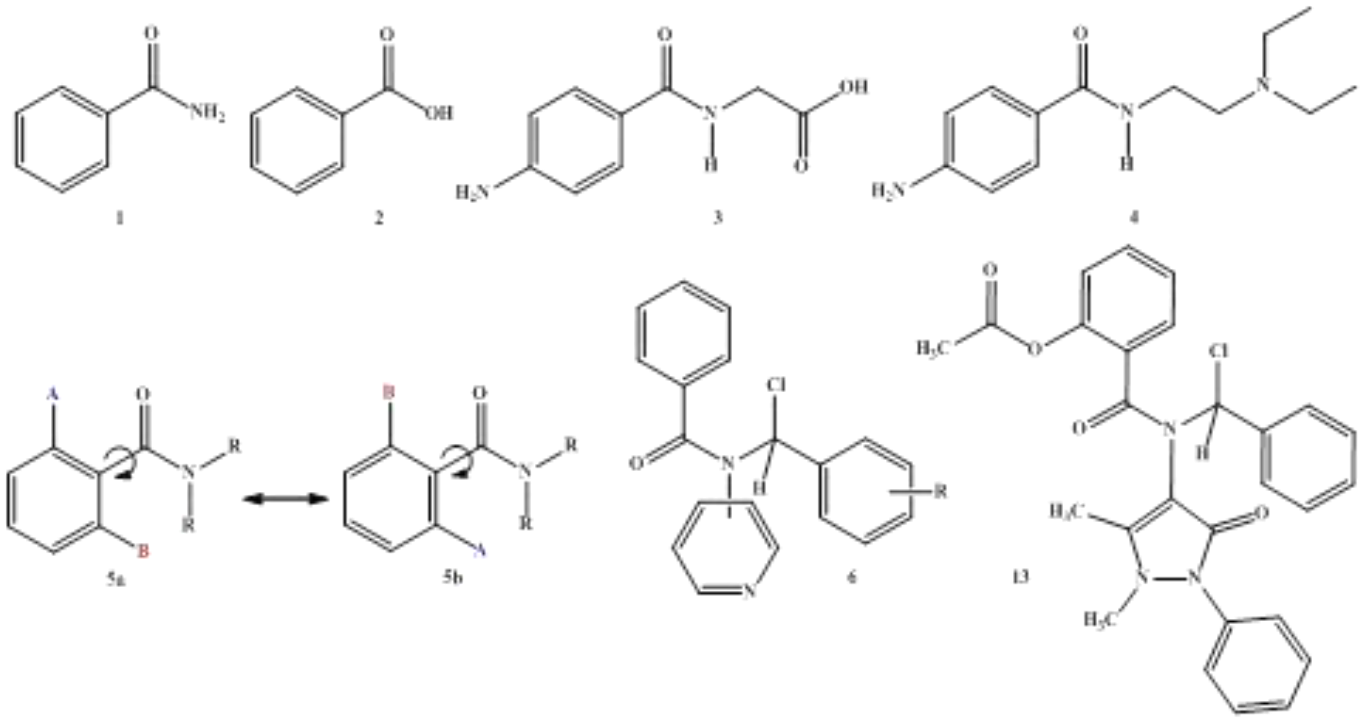


Figure 1. The chemical structures of benzamide 1 and benzoic acid 2, activated benzamides derivatives 3-4, axial chiral benzamides 5, *N*-*α*-chlorobenzyl-*N*-hetero benzamides 6, and the new benzamide 13.

2.2. Synthesis

The 4-(benzylidene amino)-1,2-dihydro-1,5-dimethyl-2-phenylpyrazol-3-one 9 was prepared as described previously [15]. Acetyl salicylic acid 11 was prepared as a common procedure [16,17].

2.2.1. Synthesis of *O*-acetyl salicyloyl chloride 12

In a 100 mL round bottom flask (2.02 g, 0.011 mol) pure acetyl salicylic acid 11, (0.54 g, 0.55 mL, 0.007 mol) pyridine and 10 mL benzene were added. At room temperature, (1.0 mL, 0.014 mol) of thionyl chloride $SOCl_2$ was slowly added for 30 min to the mixture through a condenser connected to the flask. The components were refluxed in a water bath at 70-80°C for 60 min. Then excess thionyl chloride was removed under reduced pressure. A light-yellow liquid was formed of 12 (1.5 g, yield 68%) [18].

2.2.2. Synthesis of *N*-*α*-chloro benzyl-*N*-(1,5-di methyl-3-oxo-2-phenyl-1,2-dihydro-1*H*-pyrazol-4-yl)-2-acetyl-benzamide 13

A (1.8 g, 0.006 mol) of compound 9 was dissolved in 10 mL of benzene and stirred at room temperature. After that, (2 mL, 0.013 mol) of 12 were dissolved in 3 mL of benzene and added slowly for 15 min to the mixture. The reaction was left with stirring for 15 min. Then, the mixture was refluxed in a water bath for 60 min. The red solid product was filtered and dried 13 (m.p. 132°C, 1.07 g, yield 37%).

2.3. Antioxidant activity essay using DPPH method

Different concentrations of 13 (1.0, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mM) in ethanol solvent were prepared. The 3 mL from each concentration was added to a test tube and 1 mL of freshly prepared DPPH (0.4 mM ethanolic solution) was added. The test tubes were shaken and incubated at room temperature for 30 min. in a dark place to complete the reaction. The absorbance of each solution was measured at 1100-190 nm using a spectrophotometer against blank. Percent scavenging of DPPH activity was calculated using the following an equation and compared with Ascorbic acid [19-21] as equation:

$$\%\ inhibition = \frac{(A_o - A_1)}{A_o} \times 100 \qquad \ldots (1)$$

where A_o is the absorbance of DPPH alone, and A1 is the absorbance of DPPH along with different

concentrations of 13. The IC_{50} value was calculated from the graph obtained by using the equation from the slope of the graph [22] as an equation:

$$y = mx + c \quad \ldots(2)$$

3. Results and Discussion

By condensation reaction with benzaldehyde 8, the compound 7 was converted to the 4-(benzylidene amino)-1,2-dihydro-1,5-dimethyl-2-phenylpyrazol-3-one 9 which is characterized by FTIR, ^{1}HNMR and MS spectroscopy. Some physical properties of the compounds are summarized in Table 1. The FTIR spectrum of 9 showed a new absorption at 1650 cm^{-1} indicating imine bond C=N formation, Figure 2.

The comparison of ^{1}HNMR spectra of 7 and 9 reveals the disappearance of the signal at δ 9.5 ppm of benzaldehyde proton, and the appearance of a new signal at δ 8.59-9.60 ppm [23] characteristic to the azomethine group –HC=N in the product, Figure 3. The mass spectrum of compound 9 showed an intense molecular ion peak at *m/z* = 291 corresponding to the molecular formula $C_{18}H_{17}ON_3$, Figure 5a. On the other hand, compound 11 was prepared then its physical properties, FTIR and ^{1}HNMR spectra were compared with the data reported in the literature. Acetyl salicylic acid 11, was treated with thionyl chloride in pyridine and benzene to yield *O*-acetyl salicyloyl chloride 12, Scheme 1.

The FTIR spectrum of product 12 contained absorptions in the range of 698-598 cm^{-1} indicating C-Cl stretching frequency which was not present in the spectrum of the starting material. In all compounds 9, 11, 12, and 13 there are peaks around 3000-3100 cm^{-1} indicated the C-H stretching for unsaturated carbon in benzene rings. Other absorptions at 3000-2700 cm^{-1} correspond to the stretching frequencies of the C-H saturated carbon of methyl groups. In addition to the absence of the broad absorption around 3500 cm^{-1} of the hydroxyl functional group of carboxylic acid. Its total structure was identified by analogy with the spectra of the similar compound, Figure 2a. Compound 9 was dissolved in benzene and treated with compound 12 to afford the new compound 13, Scheme 2.

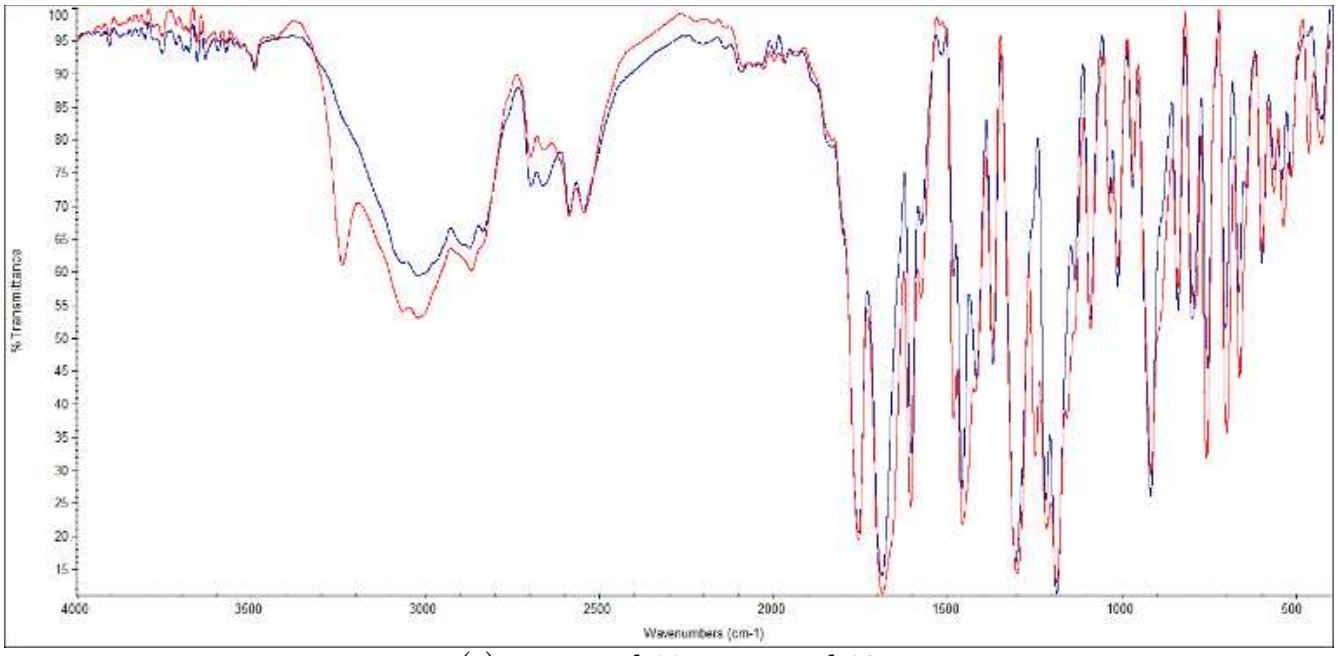
Table 1. Physical Properties of compounds

#	M.F.	M.W. g/mol.	Physical State	Melting point °C	Yield %
9	$C_{18}H_{17}N_3O$	291.35	Yellow crystals	175.8-176	87.2
11	$C_9H_8O_4$	180.16	White crystals	135	70.3
12	$C_9H_7ClO_3$	198.6	Light yellow liquid	–	68
13	$C_{27}H_{24}ClN_3O_4$	489.95	Red solid	132	37

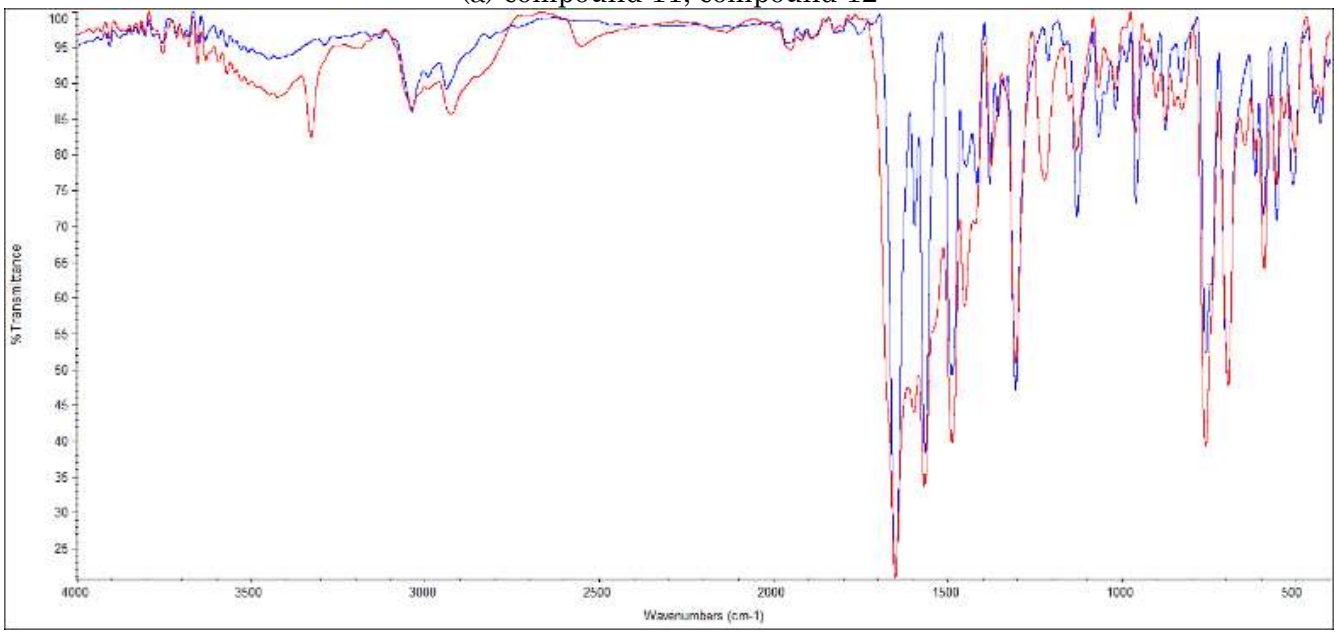
A Fourier transform Infrared FTIR spectrum of compound **13** showed the carbonyl absorption at 1745 cm^{-1} suggesting an ester functional group, and absorption at 1650 cm^{-1} indicated a carbonyl specific amide functional group. The peaks at the range 1595-1489 cm^{-1} confirmed C=C of the aromatic rings. Two absorptions appear around 2923 cm^{-1} and 3038 cm^{-1} representing C-H stretching of saturated alkyl and unsaturated portions in the molecule, Figure 2b. The ^{1}HNMR spectrum of the product **13** lacked the signal of the azomethine proton at δ 8.5 ppm that was assigned to the proton attached to the chlorinated carbon in the starting material **12** and showed a new more deshielding signal at δ 7.33 ppm (proton number one, br *s*).

The ^{1}HNMR spectrum also showed the presence of 14 aromatic protons indicating three benzene rings, one di-substituted and two mono-substituted. The aromatic protons which resonated at δ 7.81 ppm (1H, br *s*), and δ 7.79 ppm (1H, br *s*) were assigned to *H*-5, and *H*-6, respectively. Another set at δ 7.52 ppm (4H, *m*, *H*-3, *H*-4, *H*-2', and *H*-6'). Other sets of aromatic signals appeared at δ 7.44 ppm (3H, *m*, *H*-3", *H*-4", and *H*-5"), and δ 7.35 ppm (5H, *m*, *H*-3', *H*-4', *H*-5', *H*-2", and *H*-6"), Figure 3. The ^{1}HNMR spectrum also showed two signals at δ 2,4 and 3.1 ppm of C-*H* corresponding to two methyl groups, respectively, and the signals in the range of 7.2-7.8 ppm (10H, *m*) due to the aromatic protons, Figure 3. The UV-Vis of compound 13 exhibited three bands, one band appeared at 220 nm that corresponded to $\pi \rightarrow \pi^*$ electronic transitions of benzene rings. Another two bands showed at 275 and 330 nm indicated $\pi \rightarrow \pi^*$ electronic transitions of aromatic rings through the amide bonds, Figure 4. The mass

spectrum of compound 13 contained the M + 2 peak (491.28, due to the presence of isotope ^{37}Cl) which is about a third as large as M+ (*m/z* = 489.42, congaing ^{35}Cl). The mass spectrum showed the peak at *m/z* 447.02 from the loss of an acyl radical. The peak at *m/z* 430.05 corresponds to a loss of acetate radical.



(a) compound 11, compound 12

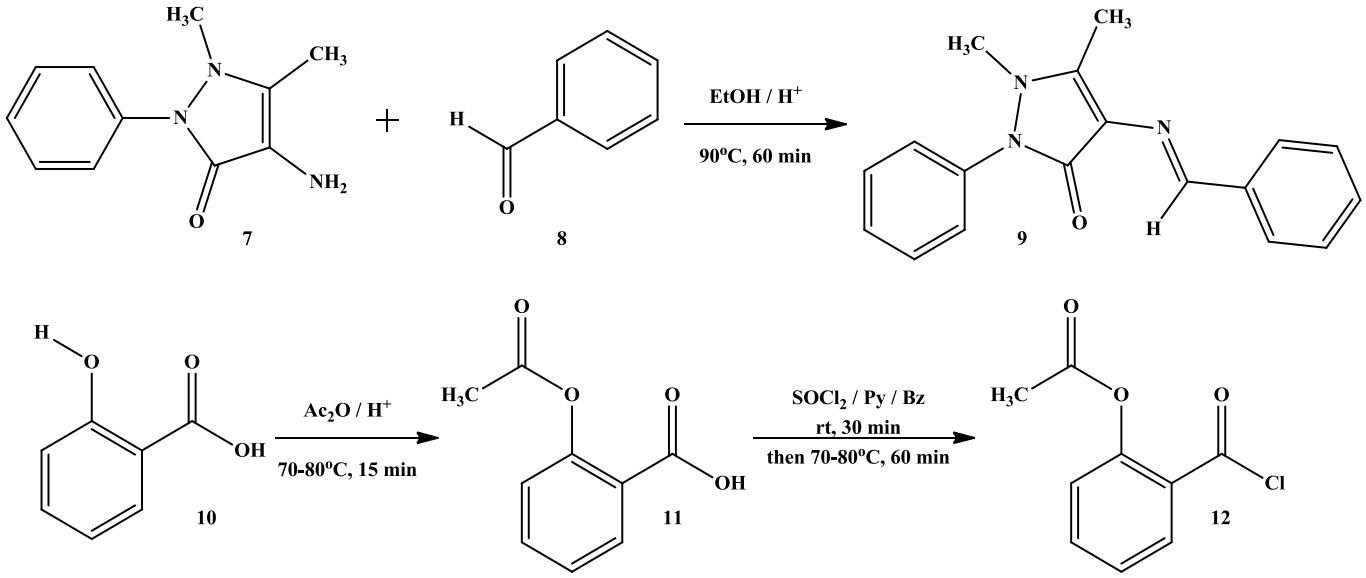


(b) compound 9, compound 13

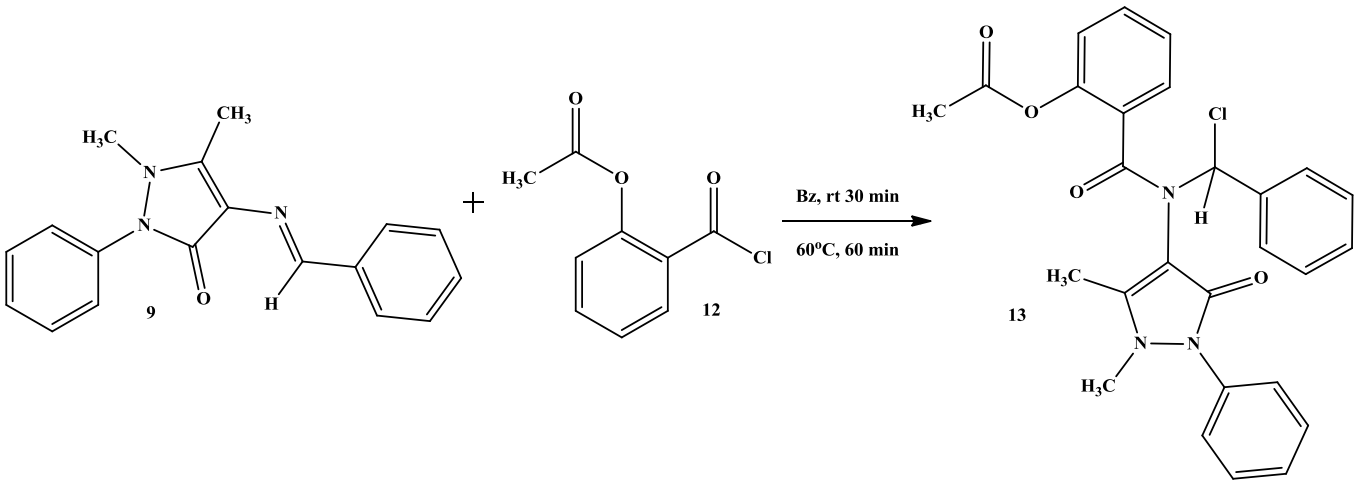
Figure 2. The FTIR spectra of: (a) 11, 12; and (b) 9, 13.

The weak peak at *m/z* 326.76 from loss of mass 163.04 (a $C_9H_7O_3$ radical), Figure 5. The antioxidant data of 13 was tabulated in Table 2 and showed that benzamide 13 was more active than standard ascorbic acid, with an IC_{50} value of 29.4 ppm. According to the IC_{50} value the

substance is classified as a very powerful, strong, or weak antioxidant if the IC_{50} value is less than 50 ppm, 50-100 ppm, or 150-200 ppm, respectively [24]. Based on that benzamide 13 is classified as a very powerful antioxidant substance, Figure 6. The biological activity of 13 was studied using the inhibition method [25]. Three types of bacteria were used. Two types were Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* and another one was Gram-positive *Staphylococcus aureus*. Benzamide 13 did not exhibit any inhibition diameter against any kind of those bacteria.



Scheme 1. The general route to prepare **9** and **12**.



Scheme 2. The route to synthesis **13**.

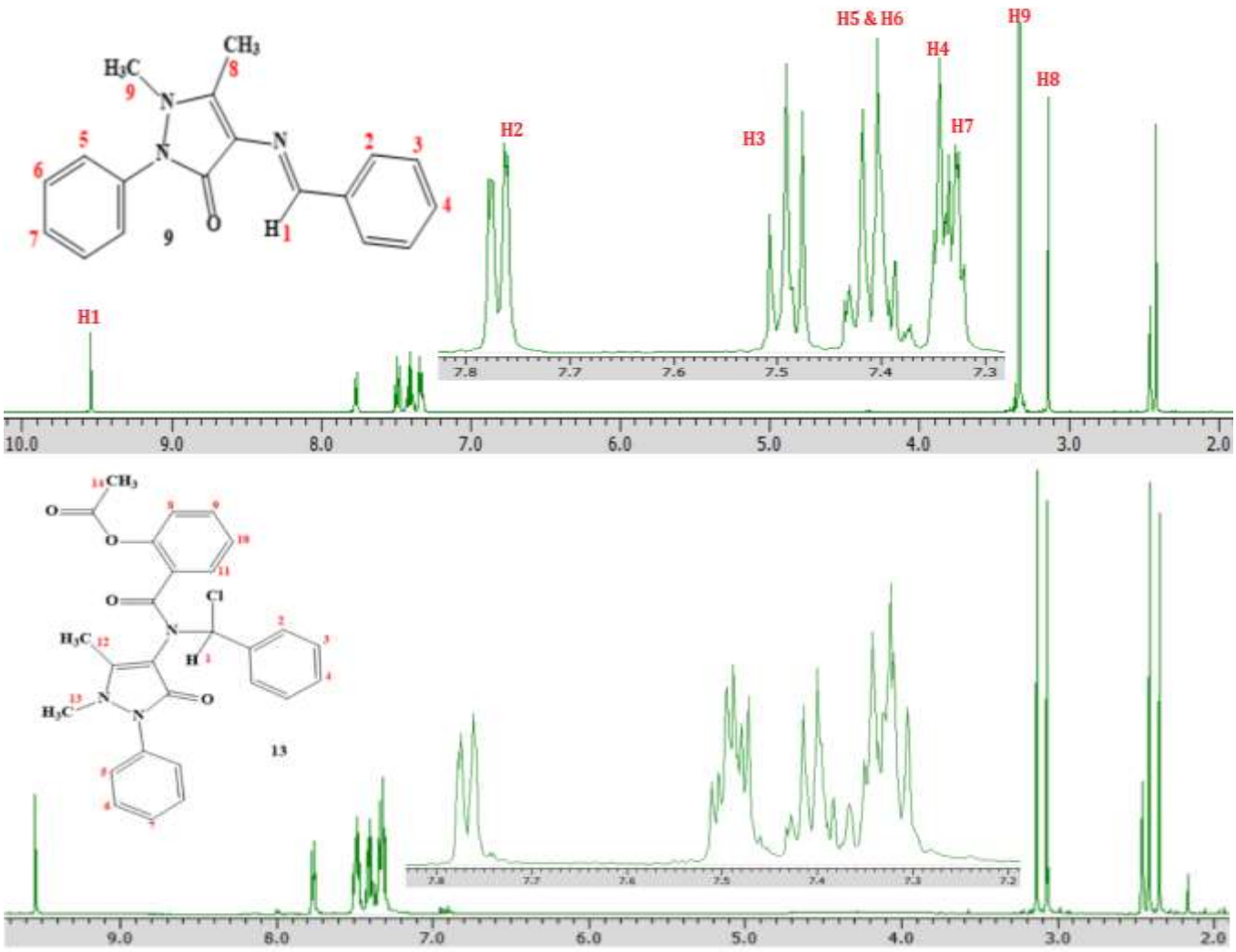


Figure 3. The NMR spectra for compounds 9 and 13.

Table 2. Absorbance, percentage of inhibition and IC_{50} value of compound 13.

Conc. mM	Abs.	% Inh.	IC_{50}
1.0	0.065	94.70	0.06 mM = 29.4 ppm
0.5	0.139	88.66	
0.25	0.252	79.45	
0.125	0.539	56.04	
0.0625	0.618	49.59	
0.03125	0.908	25.94	
Blank	1.226		

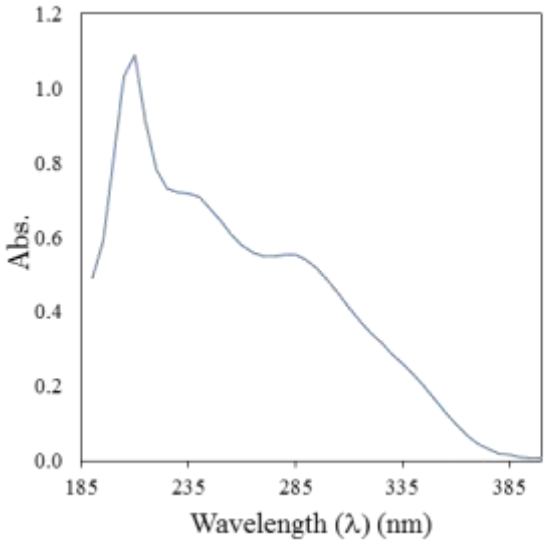


Figure 4. The UV-Vis spectrum of compound **13**.

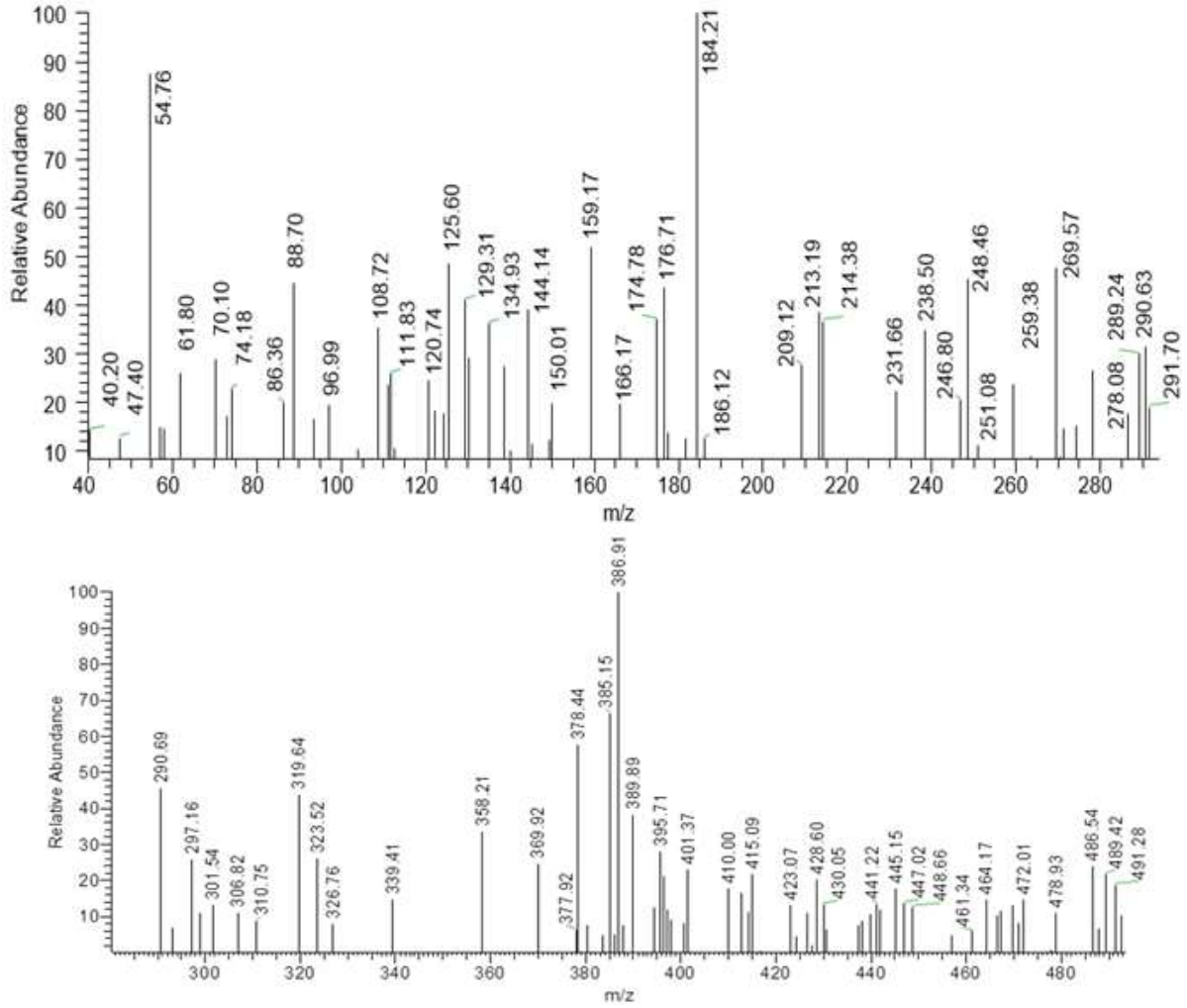


Figure 5. The mass spectra of compounds 9 and 13.

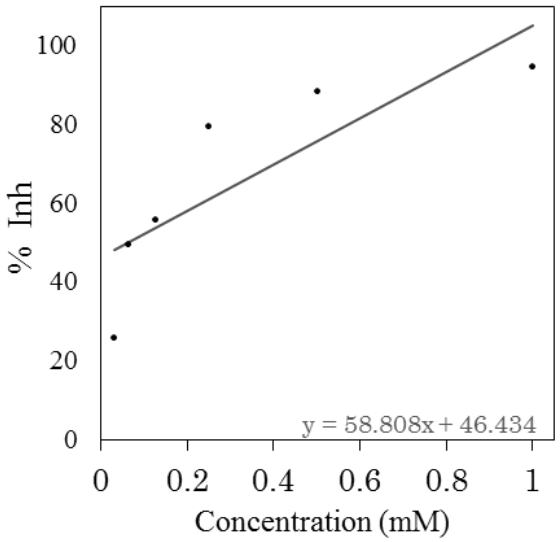


Figure 6. The DPPH scavenging of benzamide 13.

4. Conclusions

In this work, we synthesized a new benzamide compound *via* the reaction of *N*-benzylidene antipyrine amine and *O*-acetyl salicyloyl chloride. The coupling reaction between benzaldehyde and 4-aminoantipyrine produced *N*-benzylidene antipyrine amine, and the *O*-acetyl salicyloyl chloride was obtained during an acylation reaction of aspirin. According to the ^{1}HNMR, MS, UV-Vis, and FTIR spectroscopic data, the structure of a new compound was *N*-*α*-chloroacetylsalicyloyl-*N*-anti-pyrine benzamide. The other prepared compounds were identified also by spectroscopic techniques as well as melting point determination. The DPPH scavenging of *N*-*α*-chloroacetylsalicyloyl-*N*-anti- pyrine benzamide was tested, and it showed very powerful antioxidation with an IC_{50} value of 29 ppm. The new benzamide compound did not pose antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus*.

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