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Synthesis, Molecular Docking and Antioxidant Evaluation of Benzylidene Ketone Derivatives

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Abstract

Four benzylidene ketone derivatives were synthesized by reacting 4-nitrobenzaldehyde with several ketone derivatives. The synthesized compounds A, B, C and D had shown antioxidant activity against deoxyribose degradation, while compound C showing the highest activity. The molecular docking simulation indicated that the superior activity of compound C among other compounds can be attributed to the alkyl elongation at ketone chain.

Keywords: Antioxidants, Molecular docking, Deoxyribose degradation, Benzylidene ketone derivatives.

1. Introduction

Oxidative stress maybe defined as the imbalance between the production of free radicals and the ability of the organism to neutralize its action by antioxidant systems (Pisoschi and Pop, 2015). Free radicals are unstable atoms, molecules or ions with unpaired electrons that are chemically reactive with other molecules (Carocho and Ferreira, 2013) such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS). Studies have shown that free radicals are associated with many chronic health problems: such as Parkinson, Alzheimer, cancer, cardiovascular and inflammatory diseases (López-Alarcón and Denicola, 2013, Maulik *et al*., 2013, Toda, 2011). Thus, to prevent such serious medical problems, the elaboration of novel synthetic compounds is significant in the field of modern drug design and discovery.

Free radical formation is mainly controlled naturally by various beneficial compounds known as antioxidants. The terminology describing the actions of antioxidants is unfortunately not completely clear because there are various types of antioxidants (Carocho and Ferreira, 2013). Halliwell defined antioxidants as ‘‘any substance that delays, prevents or removes oxidative damage to a target molecule’’(Halliwell, 2011), while Khlebnikov defined them as ‘‘any substance that directly scavenges ROS or indirectly acts to up-regulate antioxidant defenses or inhibit ROS production’’(Khlebnikov *et al*., 2007).

The screening studies for antioxidant properties of medicinal and edible plants have been performed progressively over the last decades in the hope of finding an effective therapy for numerous modern diseases and also postponing aging symptoms (Halliwell, 2008).

Literature has proved that Curcumin, the active constituent of Curcuma species plant (Masuda *et al*., 1992) has a wide range of physiological activity. Such activities include but are not limited to: antioxidant, anti-inflammatory, anticancer, chemopreventive, antibacterial, antifungal, antiparasitic, antiviral and antihistaminic activities (Kocaadam and Şanlier, 2017).

The modification of the curcumin molecule structure has been studied due to its instability toward light, pH, temperature, and its poor pharmacokinetic profiles. It was intended to enhance the stability and absorption of curcumin when administered orally. Sardijman *et al*. have synthesized many curcumin analogues, one of them was 2,5-*bis*(4'-*hydroxy*-3'-methoxybenzylidene) cyclopentanone (pentagamavunone-0) which were benzylidene ketone derivative (Sardjiman *et al*., 1997). Those compounds have been examined as antioxidant (Reksohadiprodjo, 2004), anti-inflammatory (Masuda *et al*., 1992), and antitumor (Youssef *et al*., 2004).

Deoxyribose degradation will produce malonaldehyde that is identified by red color of the thiobarbituric acid (TBA) complex. A few of benzylidene ketone derivatives have been synthesized and shown the antioxidant properties by inhibiting deoxyribose degradation (Handayani and Arty, 2008). Previously, our studies reported the use of a molecular docking technique as a valuable tool to quickly study the drug-target intermolecular interactions (. Al-Najjar *et al*., 2017, Shakya *et al*., 2016, Mohseni *et al*., 2016, Muchtaridi *et al*., 2014). Several studies highlighted a specific tyrosine kinase enzyme (Src family tyrosine kinase Hck) with the

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potential of being used as a therapeutic target for antioxidant compounds *(Singh, 2015, Sassi et al*., 2017). In the present study, four benzylidene ketone derivatives that contain a nitro group in an aromatic ring have been synthesized, and evaluated for their antioxidant properties via deoxyribose degradation inhibition mechanism, as well as, studying the intermolecular interactions by AutoDock 4.

2. Materials and Methods

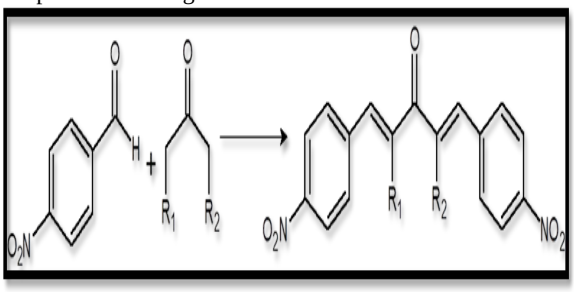
2.1. Chemical Synthesis

4-nitrobenzaldehyde; cyclopentanone; cyclohexanone; acetone; 2-pentanone; 2-heptanone; curcumin (Figure 1); sodium hydroxide; ethanol; chloroform; methanol; ethyl acetate; water; silica F_{254}; dimethyl sulphoxide-d_6; $CDCl_3$; KBr pellet; column C_{18}; 2-deoxyribose; phosphate buffer pH 7.4; hydrogen peroxide; ferrous sulphate; thiobarbituric acid; phosphoric acid. All materials used were analytical grade that were obtained from Sigma Aldrich.

UV_{254} lamp, UV spectrophotometer (Perkin Elmer Lambda 25 UV-VIS spectrophotometer), NMR spectrometer (Bruker Avance NMR 400 MHz); GC-MS (Turbo Mass EI-MS), safety cabinet, and micropipette were used.

2.2. General Synthetic Procedure of Benzylidene Ketone

A 0.066 mole of 4-nitrobenzaldehyde was mixed with several ketone derivatives (0.033 moles) as shown in the reaction scheme 1 (Thirunarayanan and Ananthakrishna Nadar, 2006, Amoozadeh *et al*., 2010). The mixture was stirred homogeneously at 25°C-30°C. 15 mL of ethanol was mixed homogenously with 10 mL of NaOH 10% solution into a separated glass beaker. Half of the second mixture was added to the first mixture, and was then stirred homogenously for fifteen minutes. Thereafter, the remaining second solution was added and stirred gently for thirty minutes. The reaction mixture was kept at room temperature overnight.



Scheme 1: General synthesis route.

The mixture was obtained in an oily form and then dropped wisely using HCl 10 % until the neutral pH was obtained and the crystal started to grow. The mixture was filtered, and the precipitate was rinsed with water and ethanol 70 %. Finally, the crystal was recrystallized using ethanol 95 %; a reddish to deep brown crystal was obtained as a product as in Figure 1.

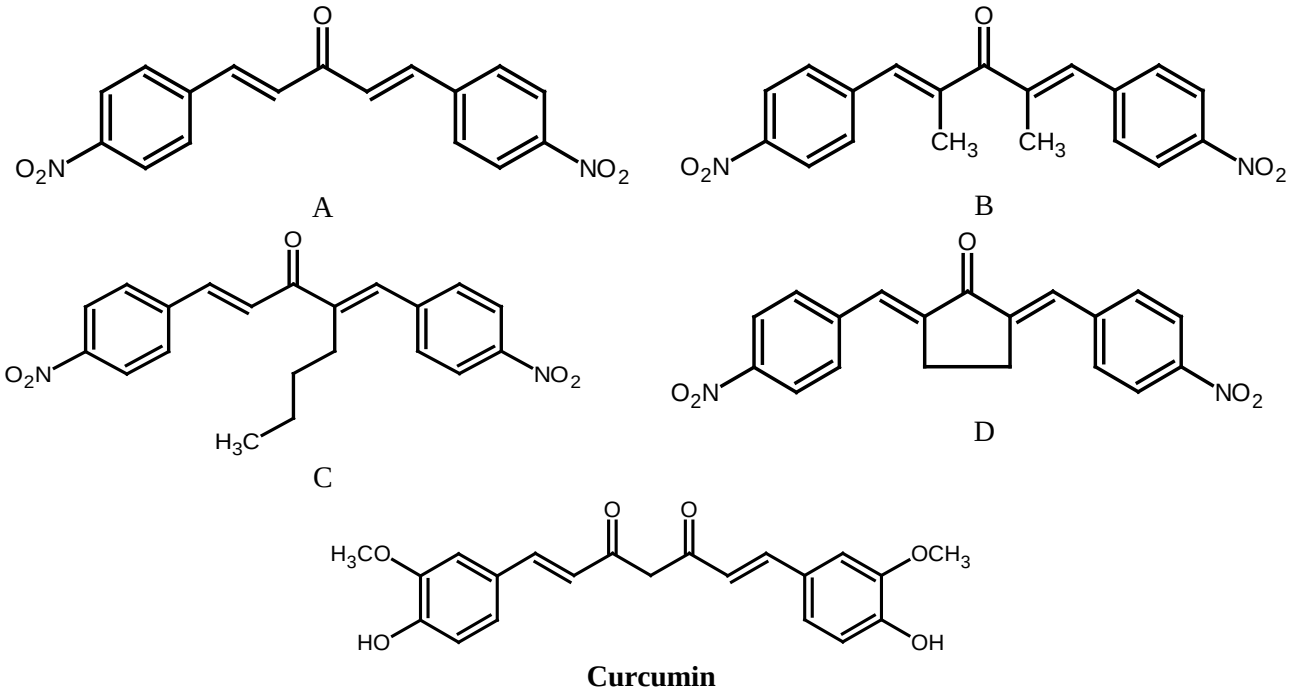


Figure 1. 2D Chemical structures of synthesized compounds and curcumin (positive control).

2.3. Analysis of Physicochemical Properties, Purity and Molecule Structure

The product was examined by thin-layer chromatography for three times using silica gel F_{254} plate with methanol: chloroform (2:8) solvent. The spot was detected by a UV lamp and obtained the R_f. The maximum wavelength was measured by dissolving sufficient amounts of the product in ethanol, and was measured by UV-VIS spectrophotometer. Furthermore, a sample of the product was dissolved in methanol, and was then injected to Gas Chromatography-Mass Spectrometer (Gas-MS) instrument. The condition of GC-MS was listed as follows: Oven: Initial temp 50°C for two minutes, ramp 10°C/min to 250°C, hold eight minutes, Inj = 250°C, Volume = 2 µL, Split = 10:1, Carrier Gas = He, Solvent Delay = 2.00 min,

Transfer Temp= 20^0C, Source Temp = 250^0C, Scan: 28 to 500Da, Column 30,0m x 320µm, TR WAX MS.

Finally, the chemical structures of the synthesized products were confirmed by NMR by dissolving a sufficient amount of the product in DMSO-d_6.

1,5-bis (4-nitrophenyl)penta-1,4-dien-3-one (A)

Brown powder; Yield 52 %; R_f 0.82 (MeOH : water = 8:2); λ_{max} 301 nm; ^{1}NMR: δ 6.88 (2H, d, *J* = 16.6 Hz), 7.84 (4H, ddd, *J* = 8.7, 2.3, 0.5 Hz), 7.93 (2H, d, *J* = 16.6 Hz), 8.08 (4H, ddd, J = 8.7, 1.9, 0.5 Hz).

2,4-Dimethyl-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one (B)

Brown powder; Yield 62 %; R_f 0.82 (MeOH : water = 8:2); λ_{max} 285 nm; ^{1}NMR: δ 1.86 (6H, s), 7.80-7.87 (6H, ddd, 7.84 (ddd, *J* = 8.7, 2.3, 0.5 Hz), 7.83 (s)), 8.07 (4H, ddd, *J* = 8.7, 2.1, 0.5 Hz)

1-(4-nitrophenyl)-4-[(4 nitrophenyl) methylidene] oct-1-en-3-one (C)

Reddish Brown powder; Yield 44 %; R_f 0.85 (MeOH : water = 8:2); λ_{max} 275 nm; ^{1}NMR: δ 0.88 (3H, t, *J* = 6.5 Hz), 1.31 (2H, h, *J* = 6.5 Hz), 1.64 (2H, tt, *J* = 7.1, 6.5 Hz), 2.51 (2H, t, *J*=7.1 Hz), 7.00 (1H, d, *J* = 16.5 Hz), 7.80-7.87 (4H, 7.83 (ddd, *J* = 8.7, 2.3, 0.5 Hz), 7.84 (ddd, *J* = 8.7, 2.2, 0.5 Hz)), 7.89-7.98 (2H, 7.89(s), 7.94 (d, *J*= 16.5 Hz)), 8.04-8.11 (4H, 8.07 (ddd, *J* = 8.7, 2.1, 0.5 Hz), 8.08 (ddd, *J* = 8.7, 1.9, 0.5 Hz)

2,5-bis[(4-nitrophenyl)methylidene]cyclopentan-1-one (D)

Dark Brown powder; Yield 66 %; R_f 0.80 (MeOH : water = 8:2); λ_{max} 334 nm; ^{1}NMR: δ 2.89 (4H, ddd, *J* = 12.3, 8.1, 4.1 Hz), 7.80-7.87 (6H, 7.83 (ddd, *J* = 8.7, 2.3, 0.5 Hz), 7.82 (s)), 8.07 (4H, ddd, *J* = 8.7, 1.9, 0.5 Hz).

2.4. 2.4 Molecular Modeling

The following software packages were utilized in the present research:

- ChemSketch ACD labs release 12.01 (www.acdlabs.com) (Advanced Chemistry Development, 2016).
- Autodock 4.2, Scripps Research Institute (http://autodock.scripps.com) (Morris *et al.*, 2009).

Molecular Docking: molecular docking simulations can be used to study and understand the intermolecular interactions between the synthesized compounds and the molecular target. Molecular docking simulation was performed on the X-ray crystal structures of protein tyrosine kinase (PDB code: 2HCK) (Sicheri *et al*., 1997) utilizing AutoDock 4.2 software (Morris *et al*., 2009). The protein crystal structure was initially prepared by merging all of the non-polar hydrogens and removing water molecules. Both Gasteiger and Kollman united atom charges were added to ligands and enzyme, respectively. The ADDSOL utility embedded in Autodock 4.2 was used to assign the atomic solvation parameters. The grid calculation was performed using Autogrid4 program, in which a box dimension of 22.5 Å and grid spacing of 0.375 Å parameters were set. The Lamarckian genetic algorithm (LGA) was used as a global optimizer and energy minimization for docking simulation.

2.5. Evaluation of Antioxidant Activities

2.5.1. Preparation of Sample Solution

A sufficient amount of the synthesized compounds was dissolved in DMSO to create a concentration series, i.e. 50 ppm, 100 ppm, 250 ppm, 500 ppm, and 1000 ppm. Moreover, curcumin was used as a positive control with the same series concentration.

2.5.2. Antioxidant Assay Using Deoxyribose Degradation Method.

This method is based on the determination of malondialdehyde (MDA), a degradation product of 2-deoxyribose, by the measurement of the condensation product with thiobarbituric acid (TBA). Typical reactions for the blank solution were started by the addition of 0.5 mM $FeSO_4$ to solutions (0.5 mL final volume) containing 5 mM 2-deoxyribose, 10 mM phosphate buffer (pH 7.2) and 2 mM H_2O_2. Reactions were carried out for ten minutes at room temperature (24–25 °C) and were stopped by the addition of 0.5 mL 4 % phosphoric acid v/v followed by the addition of 0.5 ml 1%TBA in 50 mM NaOH solution. After boiling for fifteen minutes, solutions were allowed to cool down to room temperature, and the absorbance was measured at 530.4 nm. The same procedure was repeated by adding a concentration series of the sample after the addition of H_2O_2.

The percentage inhibition was calculated by the following equation:

$$I(\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

The IC_{50} value represented the concentration of the compounds that caused 50 % inhibition calculated by using linear regression between the concentration of samples and its % inhibitions.

3. Results and Discussions

Dichloro- diphenyl- phenylhydrazine (DPPH) method is commonly used to determine the antioxidant property based on the free radical scavenging by polyhydroxyl compounds. Because of its resonance effect along the conjugated double bond once it was triggered by free radicals, it would be reactive to scavenge them and form the stable product. The free radical reaction mechanism was presented in figure 2 (Josephy and Mannervik, 2006).

The hydroxyl radical came from iron (II) and hydrogen peroxide, thus it will oxidize polyhydroxy of deoxyribose producing malonaldehyde. The oxidized product can be detected by thiobarbituric acid which is used to carry out the colorimetric reaction. A Pink solution was obtained due to the conjugated double bond elongation of two molecules of TBA and one molecule of malonaldehyde via Claissen Schimdt condensation under acid condition.

Benzylidene ketone had the long conjugated double bond, therefore it would be an inhibitor analogue in the oxidation process of D-ribose by perhydroxy radical (Figure 2) (Josephy and Mannervik, 2006). The absorbance of the pink color was measured using a visible spectrophotometer at the optimum wavelength (530.40 nm).

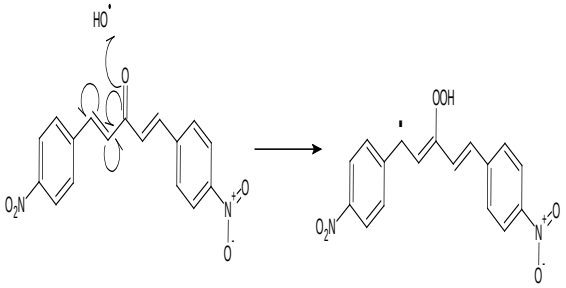


Figure 2. Proposed Mechanism of Hydroxyl Radical Scavenging of Benzylidene Ketone.

The antioxidant activity was calculated as the percentage of the decreasing product absorbance that could prevent the degradation of the 2-deoxyribose compared to the blank. Thus, the more intense the pink color was, the less active the understudy compound was. Using Linear Regression analysis, the IC_{50} of each compound could be determined by extrapolating to the correlation between concentration versus inhibiton percentage (% I). The IC_{50} of four benzylidene ketone derivatives, as well as curcumin are presented in Table 1, in which compound C showed better antioxidant activity than curcumin as displayed lower IC_{50} values.

Table 1. The IC_{50} of synthetic products against curcumin as positive control.

Compounds	IC_{50} (ppm)
A	511.3
B	723.8
C	140.6
D	636.2
Curcumin	527.5

The molecular docking studies have been carried out to evaluate the binding affinity of benzylidene ketone derivatives with protein tyrosine kinases (PDB code: 2HCK). The intermolecular interactions of the actively-docked conformations are identified with all amino acids within 5 Å of the active site. Table 2 shows the docking scores and the estimated free energy of binding (FEB). All the compounds showed comparable energy values with better binding affinity for curcumin. All the compounds bound similarly in the active site as shown in figure 3. As discussed earlier, the compound that showed better antioxidant activity was compound C, thus it will be beneficial to compare the intermolecular interactions with curcumin. Curcumin showed to perform hydrogen bond interactions with Asp404, Lys295, Met341 and Ser345, as shown in figure 4. On the other hand, compound C performed four hydrogen bond interaction with Gln277, Lys295, Met341 and Ser345, as well as hydrophobic interaction with Leu273 and Leu393 as shown in figure 5.

Table 2. Docked energy and FEB for compounds A, B, C, D and Curcumin.

Compound	Docked Energy (Kcal/mole)	Estimated FEB (Kcal/mole)
A	-7.28	-7.53
B	-7.63	-7.86
C	-7.25	-7.84
D	-7.62	-7.79
Curcumin	-8.56	-8.61

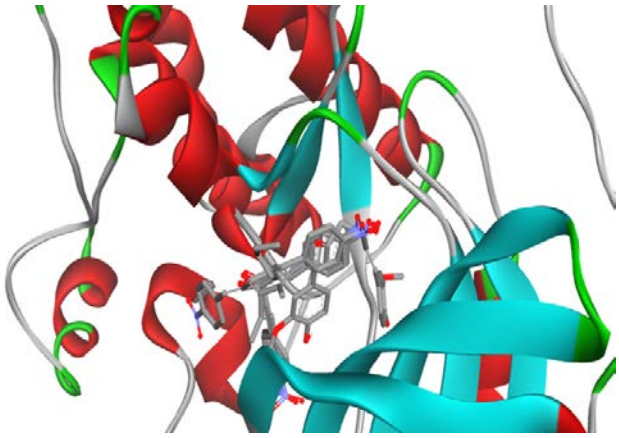
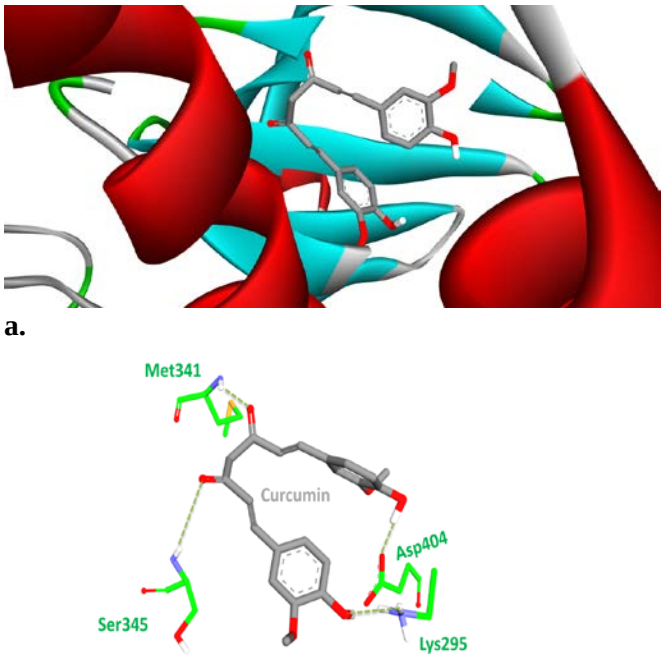


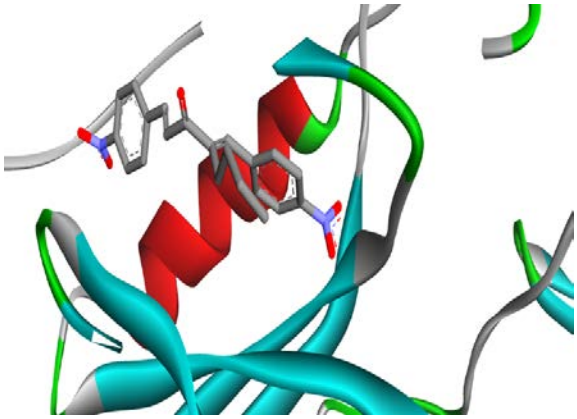
Figure 3. Solid ribbon representation of 2HCK showing the docked compounds (A, B, C, D and curcumin) in the active site.



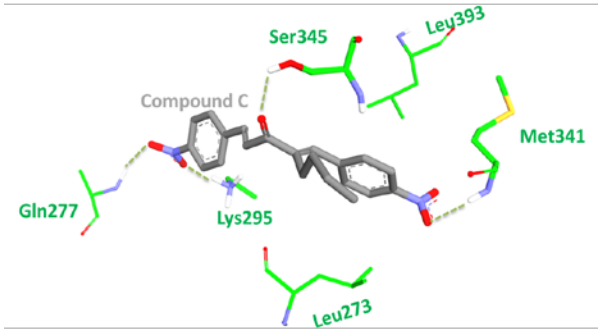
a.

b.

Figure 4. Docked curcumin pose. a. solid ribbon representation of 2HCK with curcumin docked pose. b. stick representation of active site residues that form hydrogen bond interactions (green dashed line) with curcumin.



a.



b.

Figure 5. Docked compound C pose. a. solid ribbon representation of 2HCK with compound C docked pose. b. stick representation of active site residues that form hydrogen bond interactions (green dashed line) with compound C.

4. Conclusion

A synthesis reaction was carried out to synthesize four benzylidene ketone derivatives. All synthetic products had comparable antioxidant activity against deoxyribose degradation with curcumin as positive control. Compound C showed better antioxidant scavenging activity than other compounds. Molecular docking simulation indicated that the superior activity of compound C among other compounds might be attributed to the alkyl elongation at ketone chain. Finally, biological and docking studies may help scientists to design and develop potent and selective antioxidant compounds.

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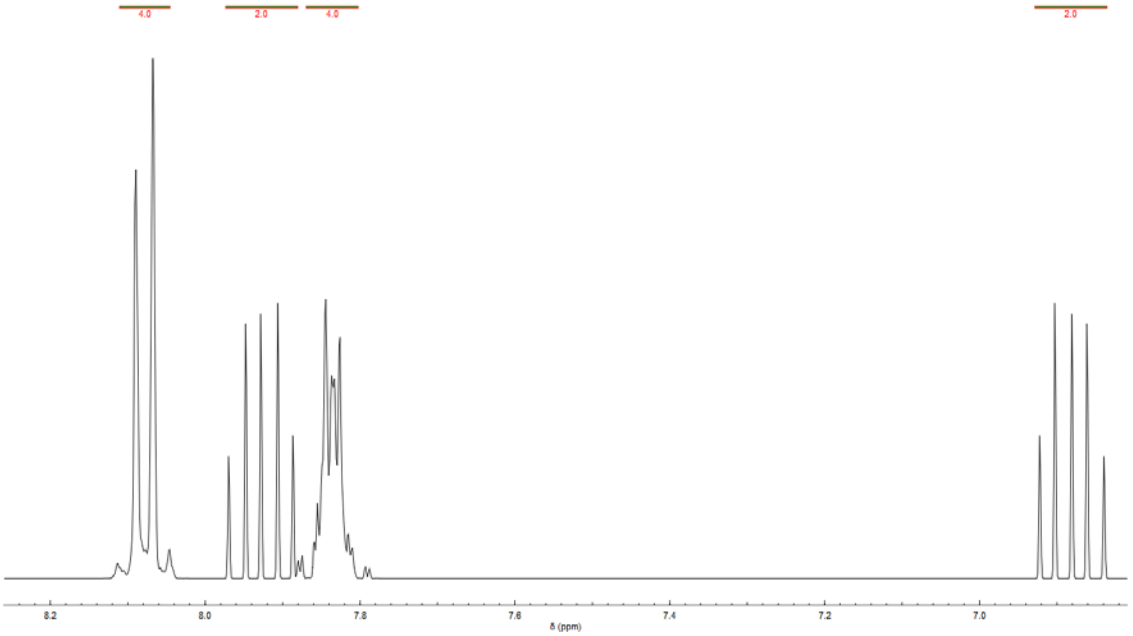
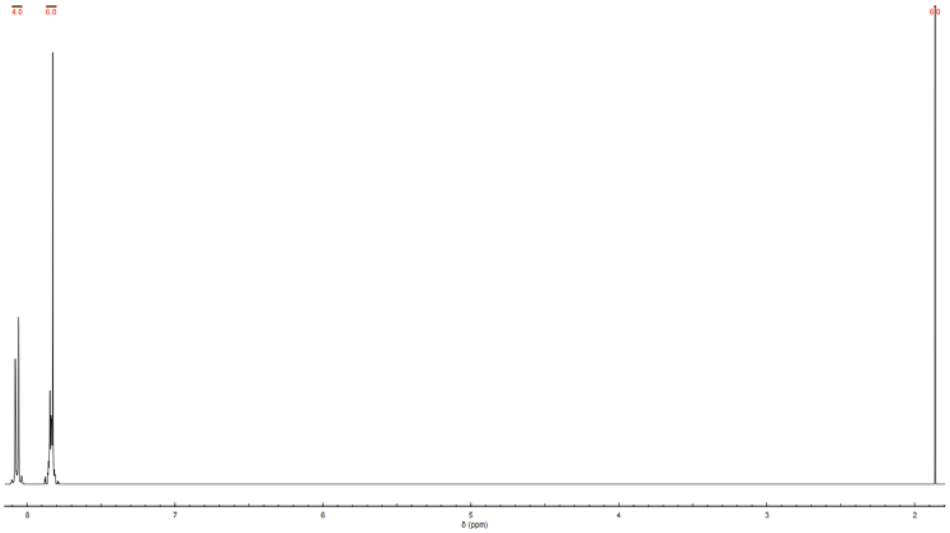
NMR spectra



Compound A



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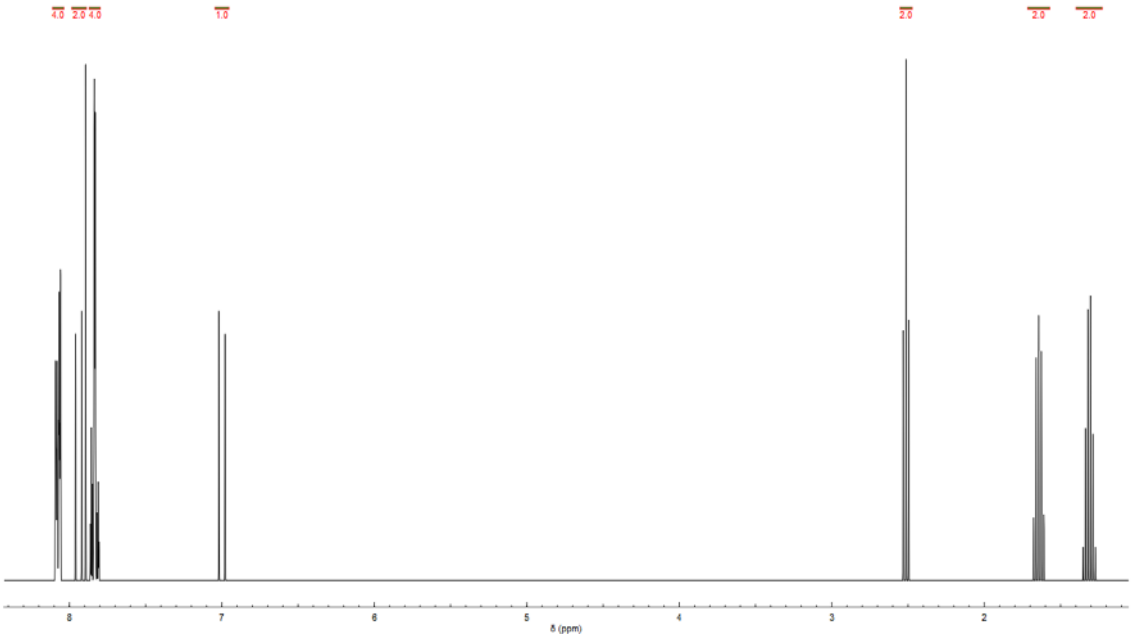
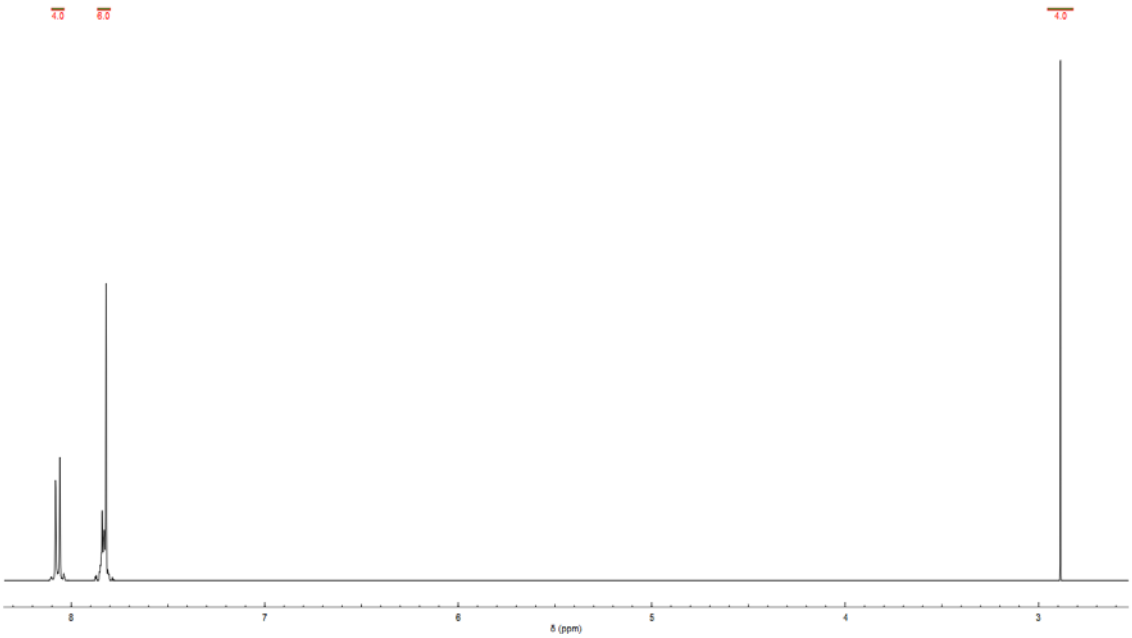


Compound C



Compound D