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Synthesis and Free Radical-scavenging Activities of Di-mannich Bases of Cyclovalone Derivatives

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ABSTRACT

Novel di-Mannich bases of cyclovalone derivatives (1) have been synthesized and evaluated their antioxidant activity using DPPH free radical-scavenger method. The structures of the compounds were confirmed on the basis of FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. The result of antioxidant evaluation showed that di-Mannich derivative of cyclovalone with diethylamine ((2*E*,6*E*)-2,6-bis({3-[(diethylamino)methyl]-4-hydroxy-5-methoxyphenyl} methylidene) cyclohexan-1-one) (2a) exhibited the highest antioxidant activity with IC_{50} = 39.0 µM. Structure-activity relationship study showed that the higher pKa of the Mannich base, the higher activity (the lower IC_{50}) of the compound.

Keywords: Cyclovalone, Mannich base, Synthesis, Antioxidant, Radical scavenger.

INTRODUCTION

Oxidative stress, generating by an imbalance between free radical production and antioxidant defenses, is associated with the damage of various species of molecules including proteins, lipids, and nucleic acids. A role for oxidative stress has been postulated to contribute significantly to the pathogenesis of atherosclerosis, inflammatory conditions, certain cancers, and the process of aging. Besides the complex system of antioxidant metabolites and enzymes that naturally prevent cell damage, the exogenous antioxidant may sometimes be required to keep reactive oxygen species at optimum level [1-3].

Cyclovalone, (2*E*,6*E*)-2,6-bis[(4-hydroxy-3-methoxyphenyl)methylidene]cyclohexan-1-one (1) is a curcumin mono-carbonyl analog in which the pentane-2,4-dione chain of the curcumin is replaced by a cyclohexanone ring. Cyclovalone demonstrated antioxidant activity, antitumor, anti inflammatory, hepatoprotective and cytotoxic activity[4-7]. The structure-activity relationships (SAR) including the antioxidant activity of cyclovalone derivatives have been studied. However, there has

been no report about the effect of aminoalkyl substituents[5,8].

A Mannich reaction is a suitable method to introduce aminoalkyl group into a molecule. In several instances, the Mannich derivatives exhibit better biological activity than the corresponding parent analogs. Moreover, the presence of Mannich side chain increases the solubility and hence the bioavailability of the compounds[2,9-13]. Herein we report the synthesis and antioxidant activity of Mannich bases derivatives of cyclovalone.

MATERIALS AND METHODS

Chemistry

All used chemicals were purchased from Merck or Aldrich Company and used without further purification. Thin layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates (Merck) and spots were detected under an ultraviolet and visible light. Melting points were determined in the capillary tube using electrothermal digital melting point apparatus (Stuart Scientific). The infrared spectra were recorded with FTIR 8400S Spectrometer (Shimadzu). NMR spectra were recorded on NMR spectrometer (Agilent) at 500 MHz for ^{1}H and 125 MHz for ^{13}C using TMS as an internal standard. High-resolution mass spectra (HRMS) were measured with a Waters LCT Premier XE (ESI-TOF) system in positive or negative mode. The absorption in the determination of free radical-scavenging activity was measured using UV-Vis Spectrophotometer 1601 (Shimadzu).

General synthesis of di-Manncih bases of cyclovalone derivatives (2a-e)

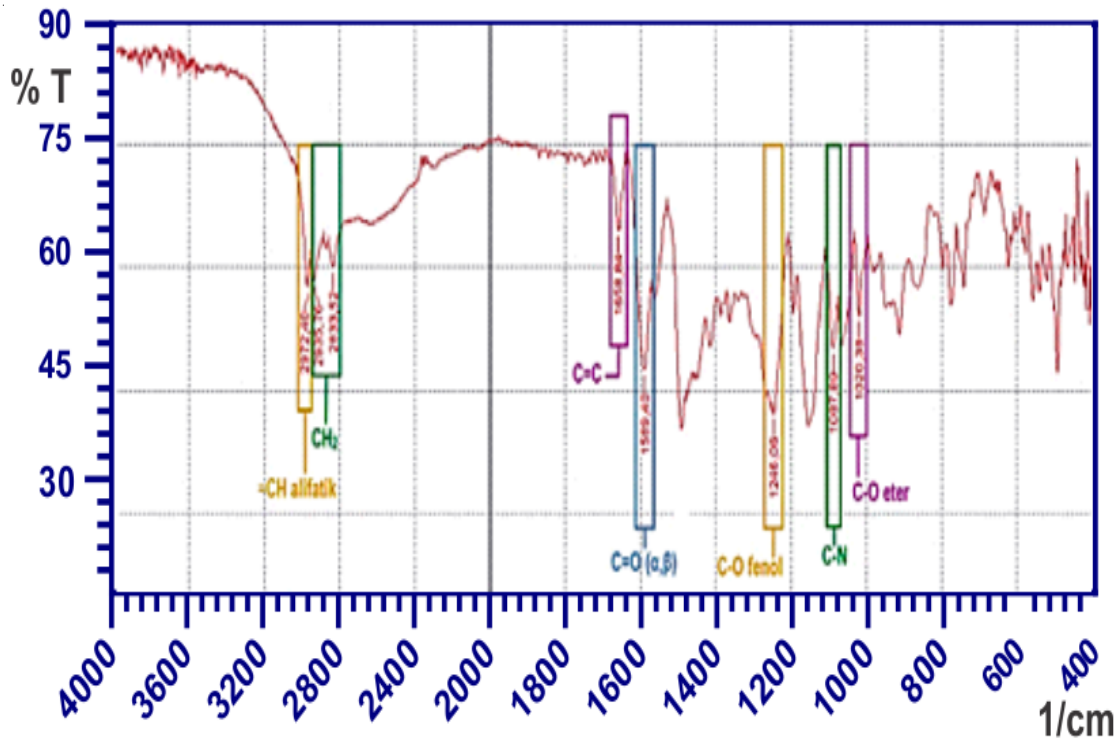
The di-Mannich bases of cyclovalone were synthesized by Mannich reaction of compound **1** according to the method of synthesis of di-Mannich bases of 1,5-bis(4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one reported previously[7], with little modification: The solution of 2 mmol of compound 1 in acetonitrile (50 ml) was added to mixture of 16 mmol paraformaldehyde and 16 mmol of corresponding secondary amine in acetonitrile (50 ml) which previously was heated at 80°C for 10 minutes. The reaction mixture then was refluxed until the disappearance of 1. The completion of the reaction was monitored by TLC for 5-27 h. The reaction solvent was removed *in vacuo* using a rotary evaporator, the crude products were washed with cold acetonitrile and then purified by recrystallization or column chromatography to give compound 2a-e.

(2*E*,6*E*)-2,6-bis({3-[(diethylamino)methyl]-4-hydroxy-5-methoxyphenyl}methylidene) cyclohexan-1-one (2a):

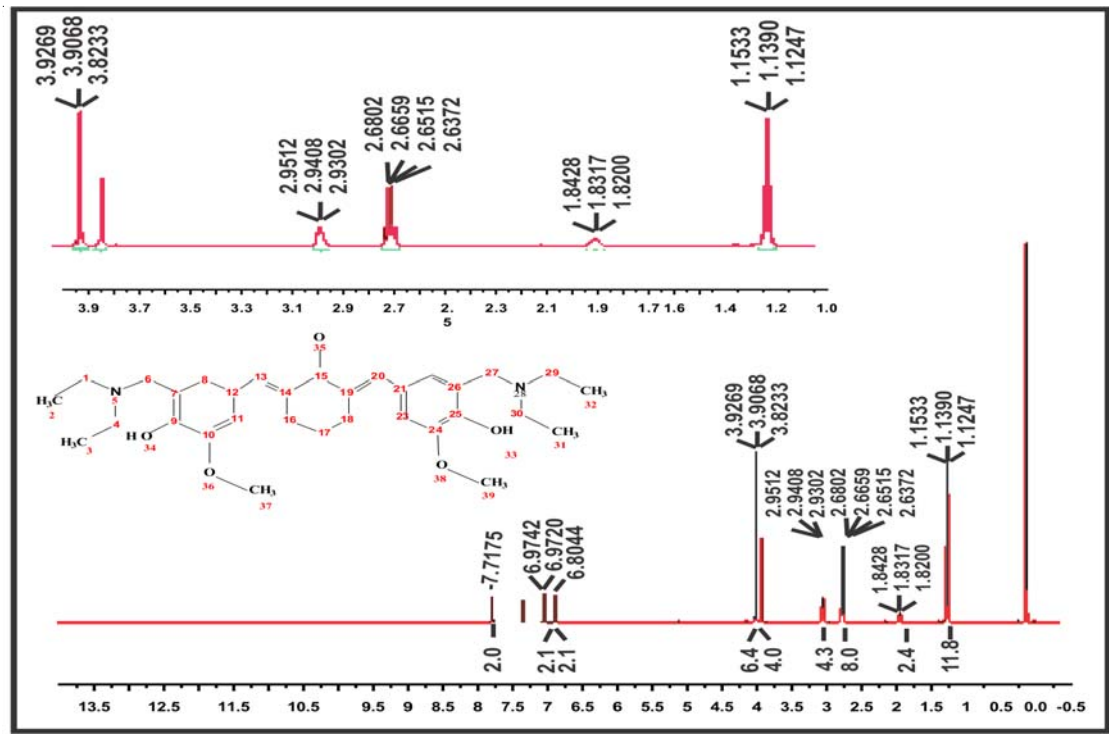
The crude product was purified by recrystallization from ethylacetate-hexane (1:25) gave a yellowish orange crystalline powder at 84.3% yield, m.p. = 58-60°C. IR (KBr), υ_{max}, cm^{-1}: 2972, 2935, 2833, 1639, 1589, 1246 1087, and 1039. ^{1}H-NMR (500 MHz, $CDCl_3$) δ/ppm: 7.71 (2H, s, C=C<u>H</u>-), 6.97 (2H, s, H_{Ar}), 6.80 (2H, s, H_{Ar}), 3.91 (6H, s, OCH_3), 3.82 (4H, s, Ar-<u>C</u>H_2-N-$_{diethylamine}$), 2.94 (4H, t, =C-<u>C</u>H_2-$C_{cyclohexanone}$), 2.66 (8H, q, -CH_3-<u>C</u>H_2-$N_{diethylamine}$), 1.83 (2H, p, C-<u>C</u>H_2-$C_{cyclohexanone}$) and 1.14 (12H, t, CH_2-<u>C</u>H_3 $_{diethylamine}$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ/ppm: 190 (1C, C=$O_{cyclohexanone}$), 149 and 147 (4C, C_{Ar-O}), 133 and 137 (4C, -C=C-), 126, 123, 121, and 113 (8C, C_{Ar-H}), 57 (2C, Ar-<u>C</u>-$N_{diethylamine}$), 56 (2C, Ar-O<u>C</u>H_3), 46 (4C, -N-<u>C</u>H_2 $_{diethylamine}$), 28 (2C, =C-<u>C</u>H_2-$C_{cyclohexanone}$), 23 (1C, C-<u>C</u>H_2-$C_{cyclohexanone}$), and 11 (C-<u>C</u>H_3). HRESIMS (m/z): found 537.3315 ($[M+H]^+$), calculated masses of $C_{32}H_{45}N_2O_5$: 537.3328 (error - 2.4 ppm).

(2*E*,6*E*)-2,6-bis({3-[(dimethylamino)methyl]-4-hydroxy-5-methoxyphenyl}methylidene) cyclohexan-1-one (2b)

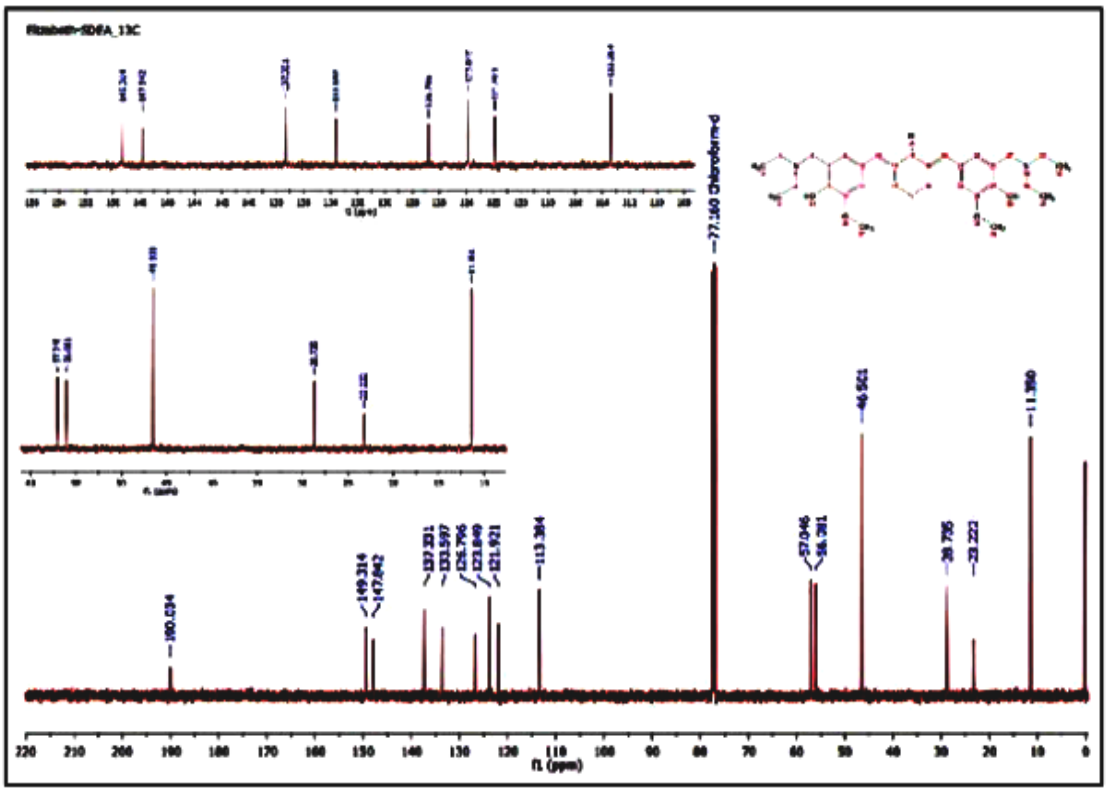
The crude product was purified by column chromatography on silica with mixture of chloroform, methanol and ethanol (10:1:1) as mobile phase gave a brown crystalline powder at 44.3% yield, m.p. = 182-184 °C. IR (KBr), υ_{max}, cm^{-1}: 2993, 2829, 1605, 1558, 1488, 1250 1086, and 1008. ^{1}H-NMR (500 MHz, $CDCl_3$) δ/ppm: 7.70 (2H, s, C=C<u>H</u>-), 6.97 (2H, s, H_{Ar}), 6.79 (2H, s, H_{Ar}), 3.89 (6H, s, OCH_3), 3.68 (4H, s, Ar-<u>C</u>H_2-$N_{dimethylamine}$), 2.93 (4H, t, =C-<u>C</u>H_2-$C_{cyclohexanone}$), 2.35 (12H, s, CH_3-N-$_{dimethylamine}$), and 1.82 (2H, p, C-<u>C</u>H_2-$C_{cyclohexanone}$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ/ppm: 190 (1C, C=$O_{cyclohexanone}$), 149 and 148 (4C, C_{Ar-O}), 134 and 137 (4C, -C=C-), 127, 124, 122, and 114 (8C, C_{Ar}), 63 (2C, Ar-<u>C</u>-$N_{dimethylamine}$), 56 (2C, Ar-O<u>C</u>H_3), 45 (4C, -N-CH_3 $_{dimethylamine}$), 29 (2C, =C-<u>C</u>H_2-$C_{cyclohexanone}$), and 23 (1C, C-<u>C</u>H_2- $C_{cyclohexanone}$). HRESIMS (m/z): found 479.2539 ($[M-H]^-$), calculated masses of $C_{28}H_{35}N_2O_5$: 479.2546 (error -1.5 ppm).



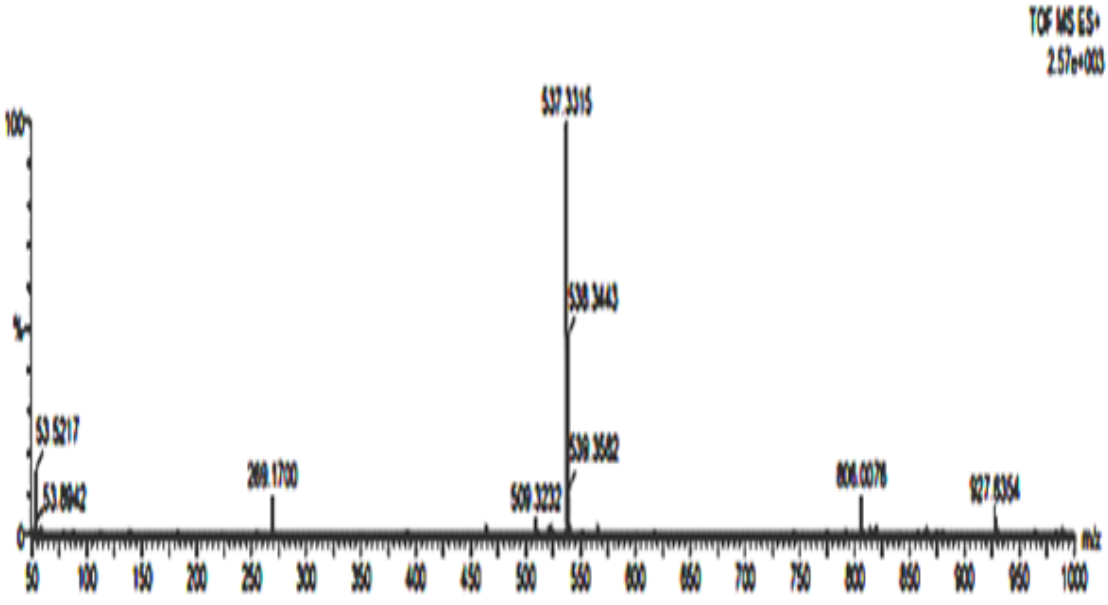
IR Spectrum of compound 2a in KBr



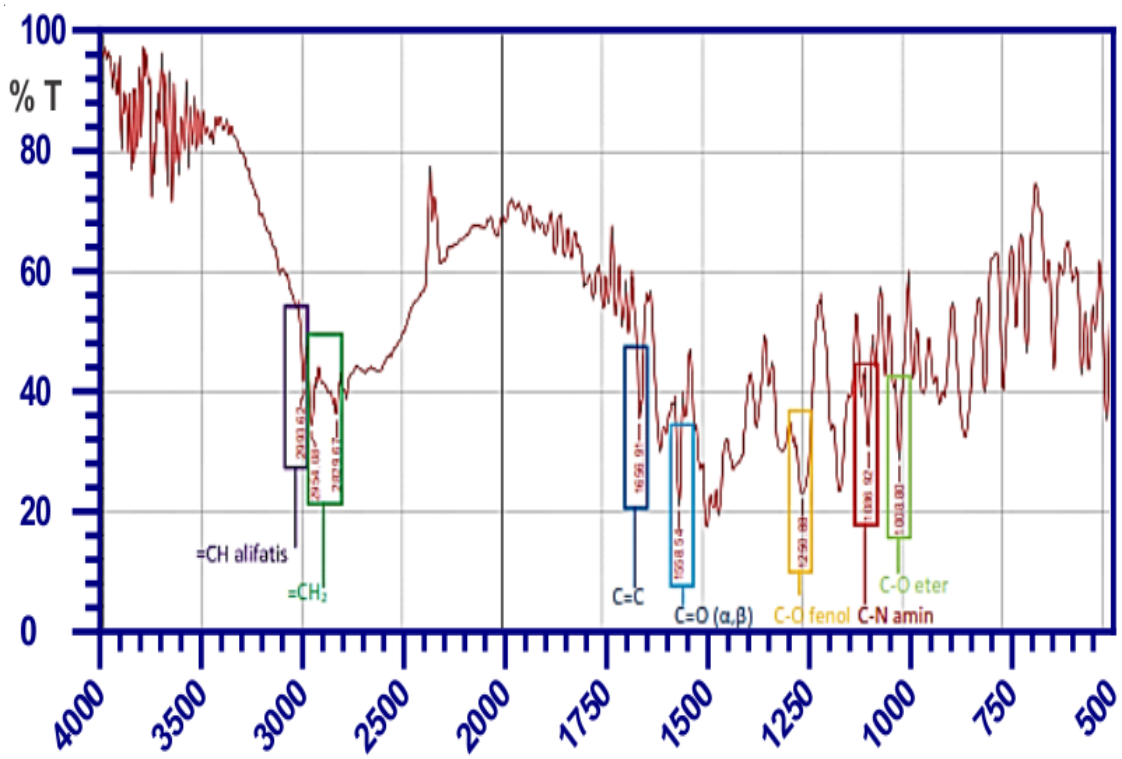
^{1}H NMR Spectrum of compound 2a



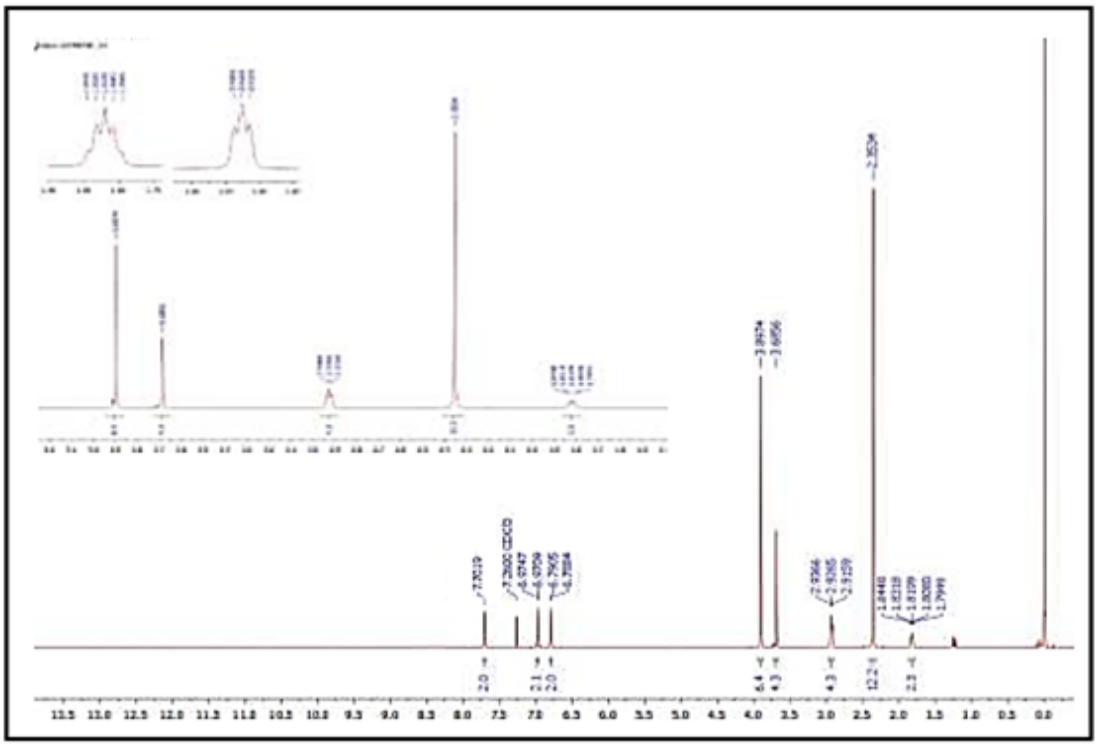
^{13}C NMR Spectrum of compound 2a



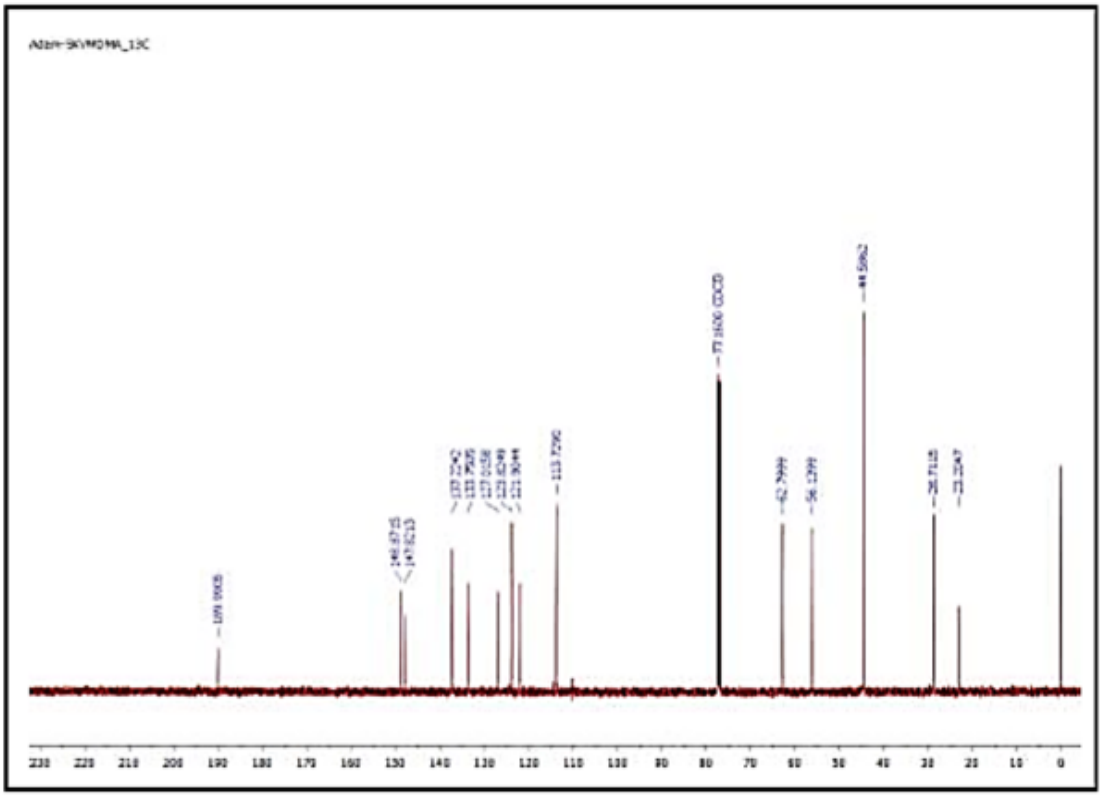
MS Spectrum of compound 2a



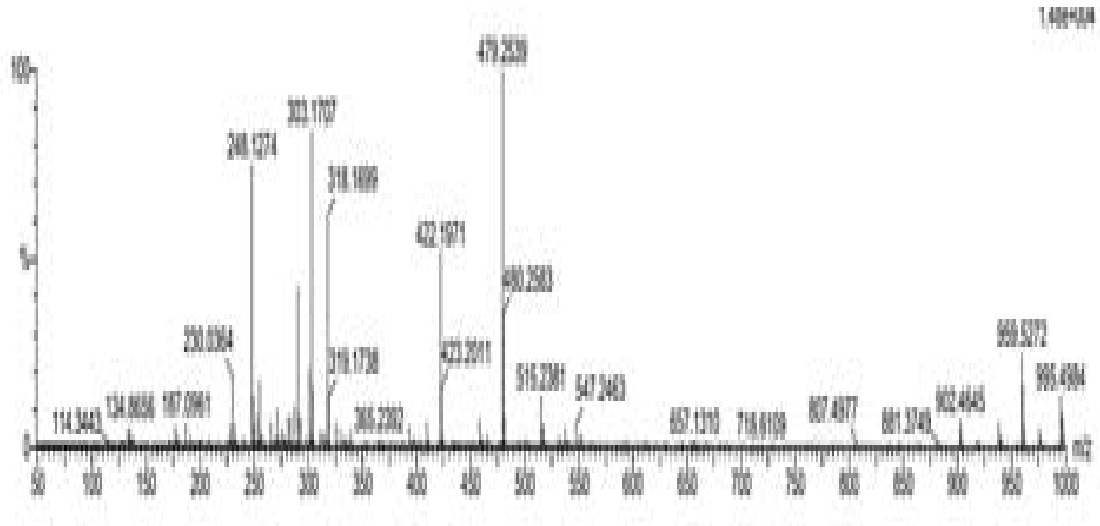
IR Spectrum of compound 2b in KBr



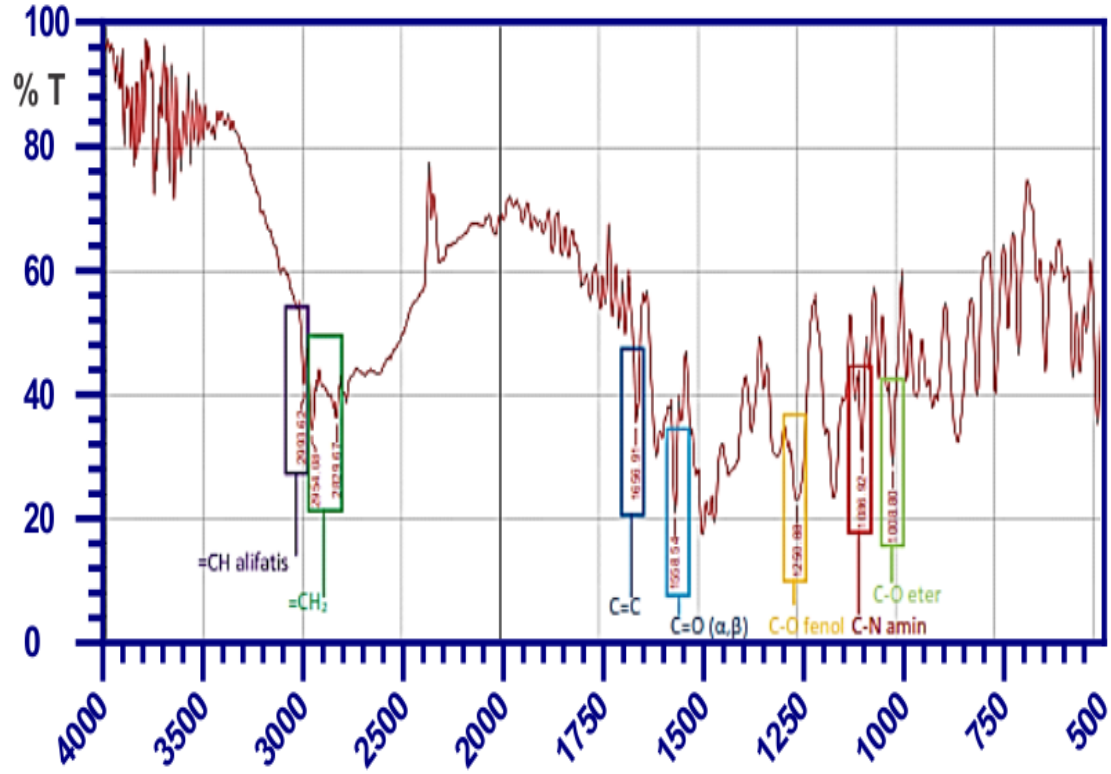
^{1}H NMR Spectrum of compound 2b



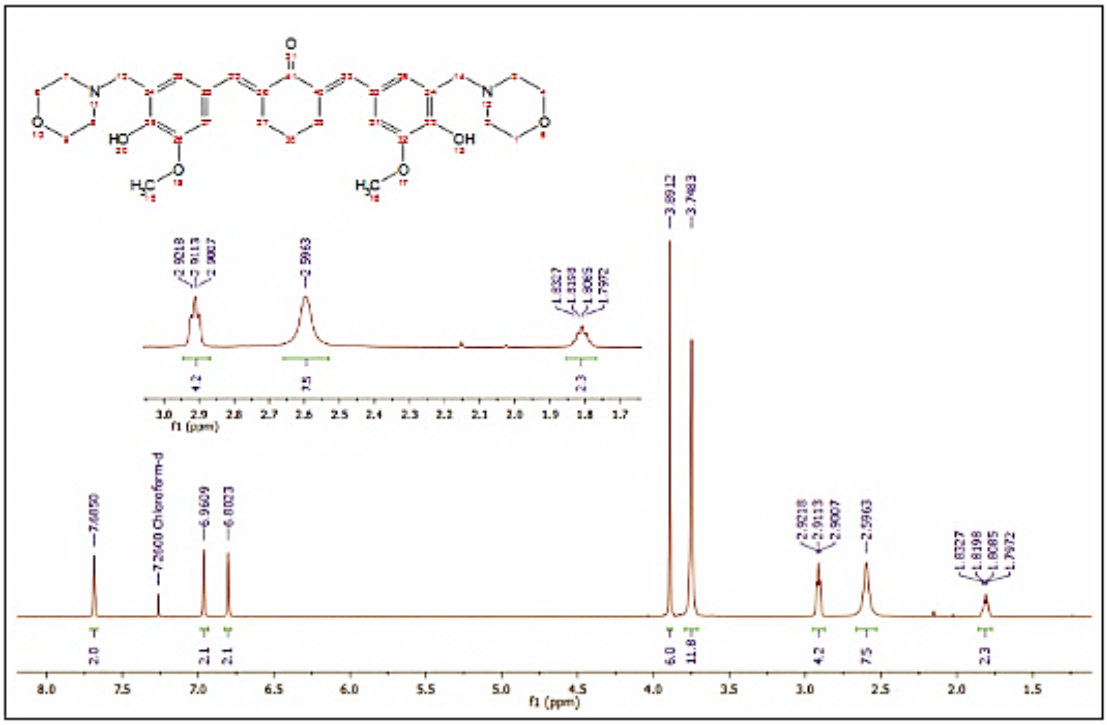
^{13}C NMR Spectrum of compound 2b



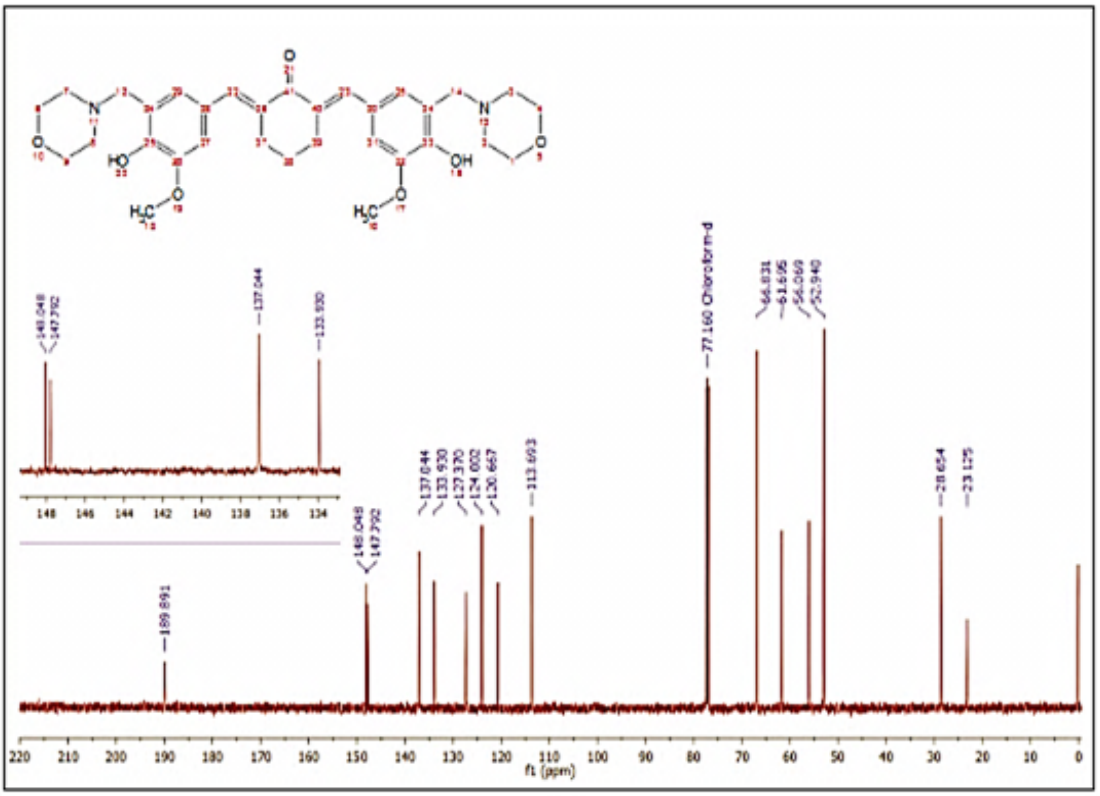
MS Spectrum of compound 2b



IR Spectrum of compound 2c in KBr



^{1}H NMR Spectrum of compound 2c



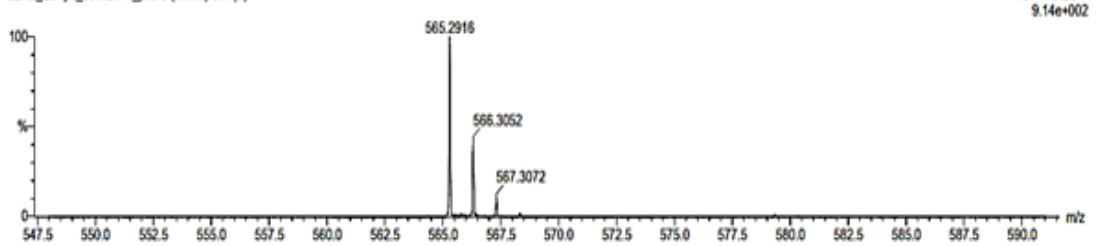
^{13}C NMR Spectrum of compound 2c

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off

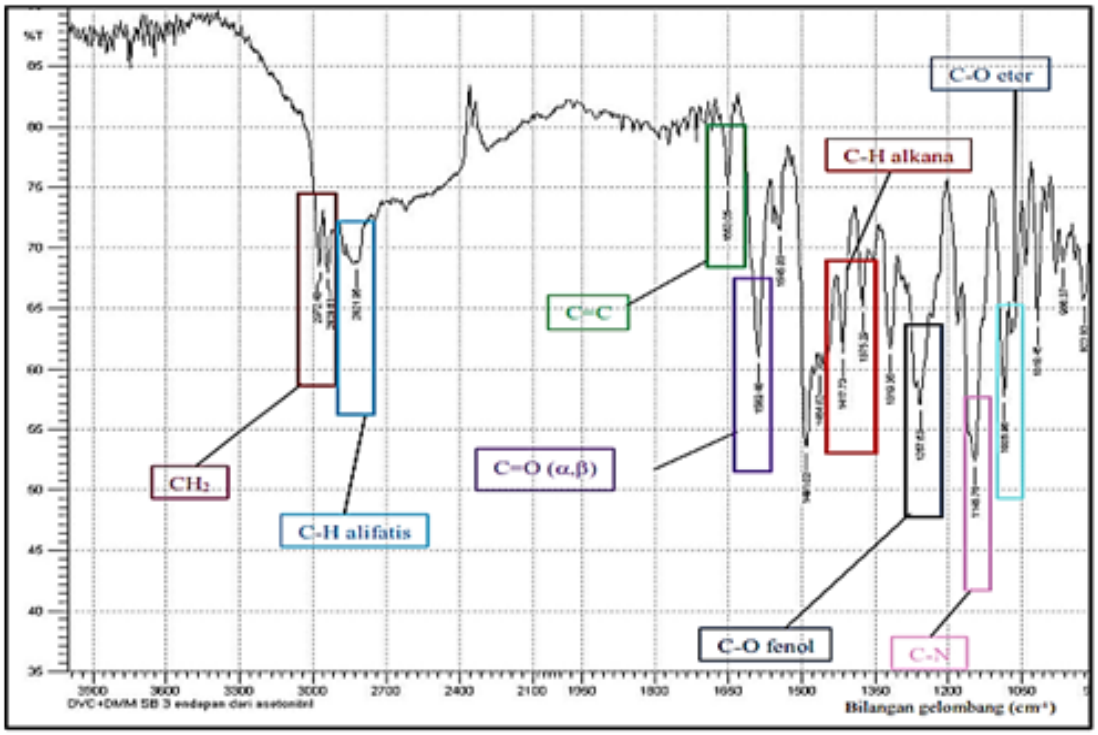
Monoisotopic Mass, Even Electron Ions
3150 formula(e) evaluated with 47 results within limits (up to 5 closest results for each mass)
Elements Used:
C: 0-1000 H: 0-1000 N: 0-500 O: 0-500
ESI- TOF
Sandi_Seny1_565-2914_pos 5 (0.068) Cm (5)

TOF MS ES+
9.14e+002

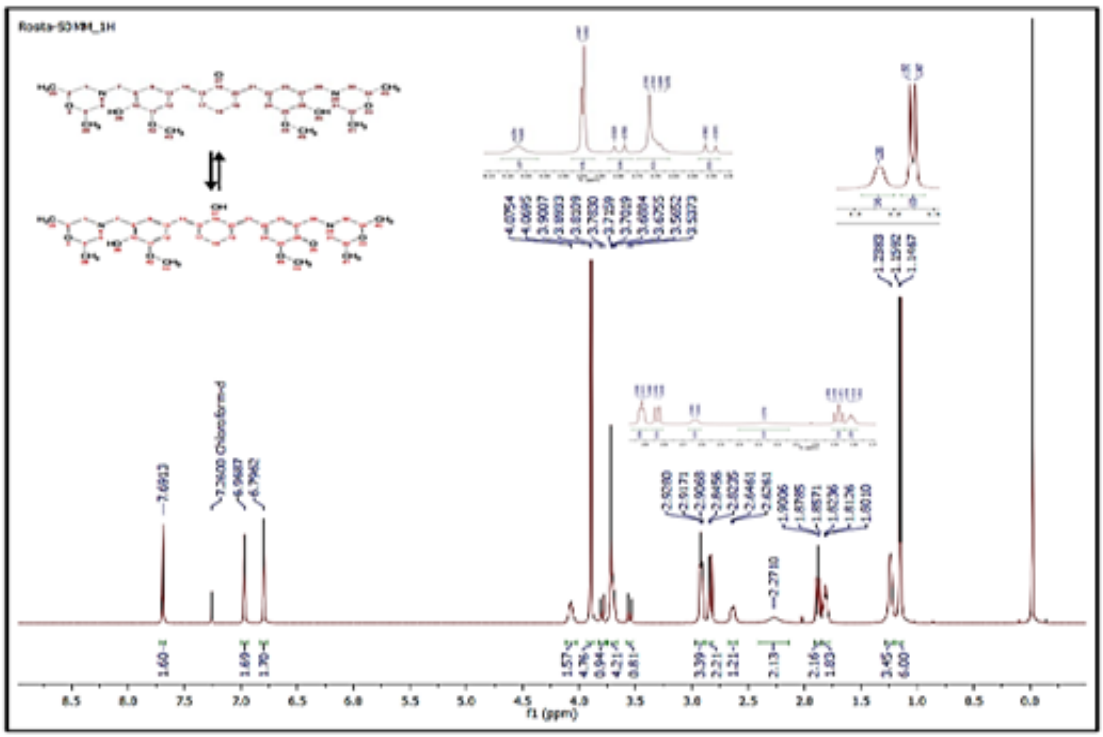


Minimum:				-1.5	
Maximum:		10.0	15.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	Formula
565.2916	565.2914	0.2	0.4	13.5	C32 H41 N2 O7
	565.2919	-0.3	-0.5	6.5	C17 H37 N14 O8
	565.2924	-0.8	-1.4	-0.5	C2 H33 N26 O9
	565.2927	-1.1	-1.9	18.5	C33 H37 N6 O3
	565.2905	1.1	1.9	12.5	C14 H29 N24 O2

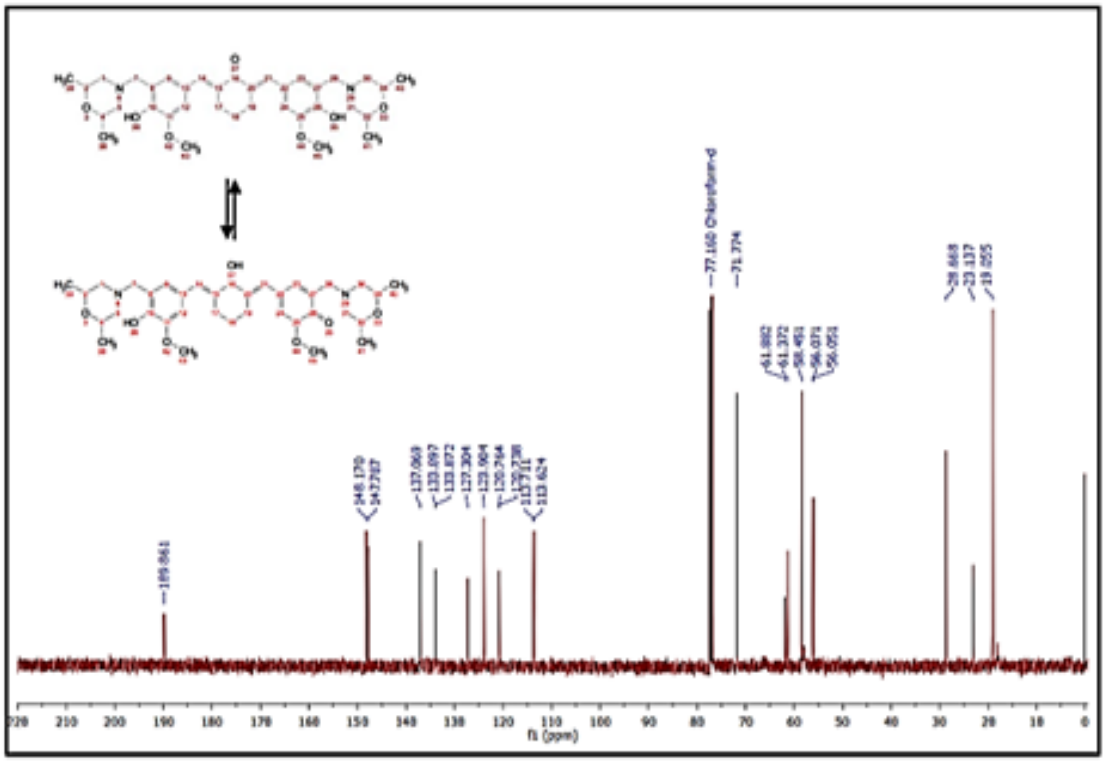
MS Spectrum of compound 2c



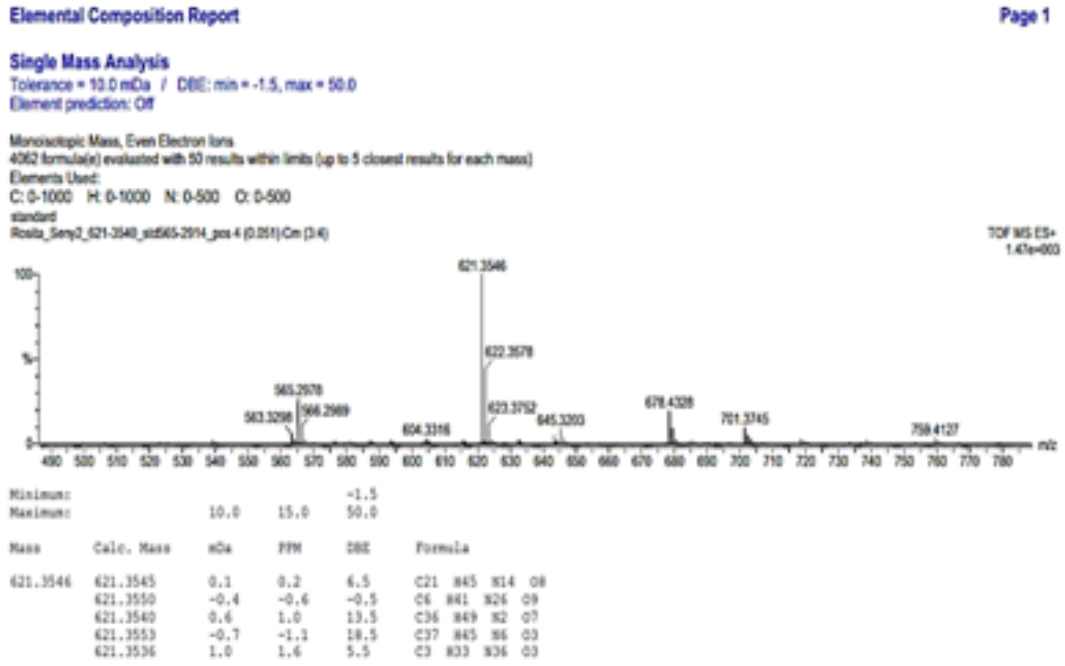
IR Spectrum of compound 2d in KBr



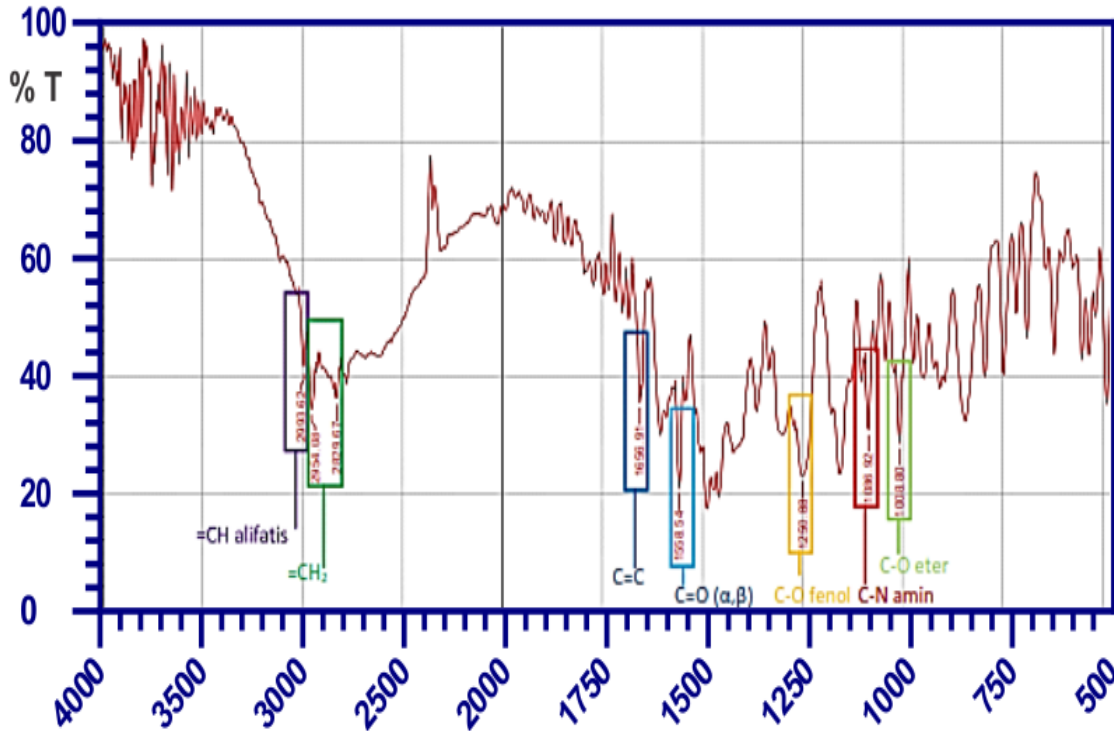
^{1}H NMR Spectrum of compound 2d



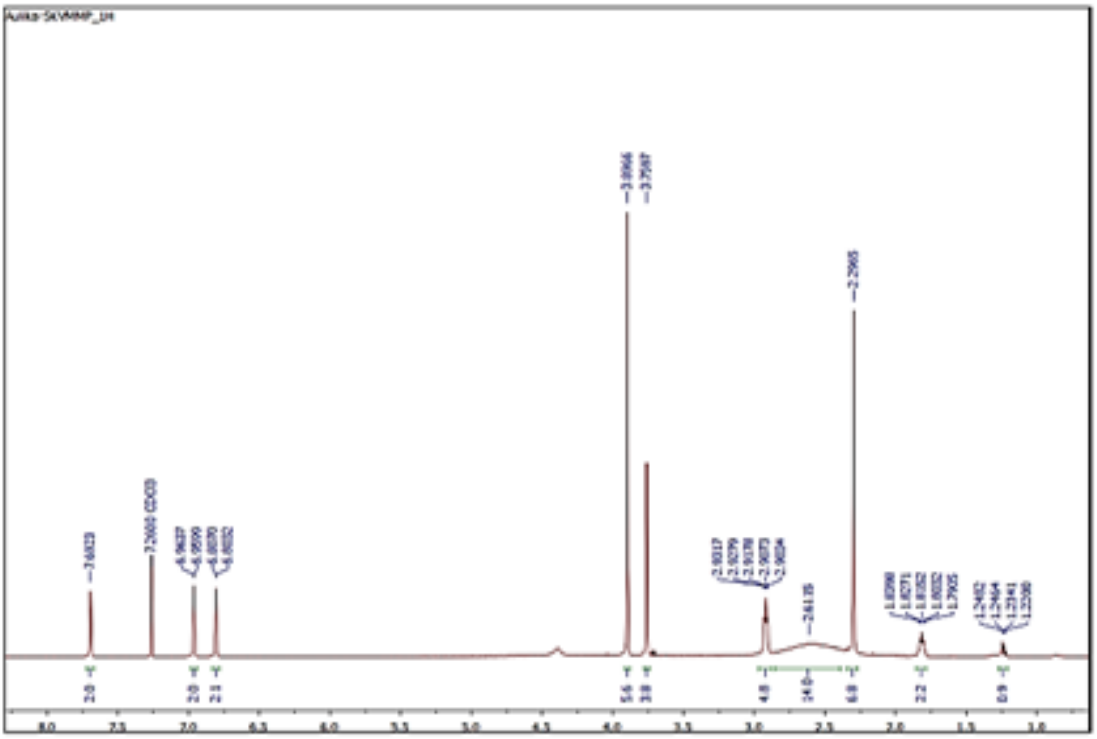
^{13}C NMR Spectrum of compound 2d



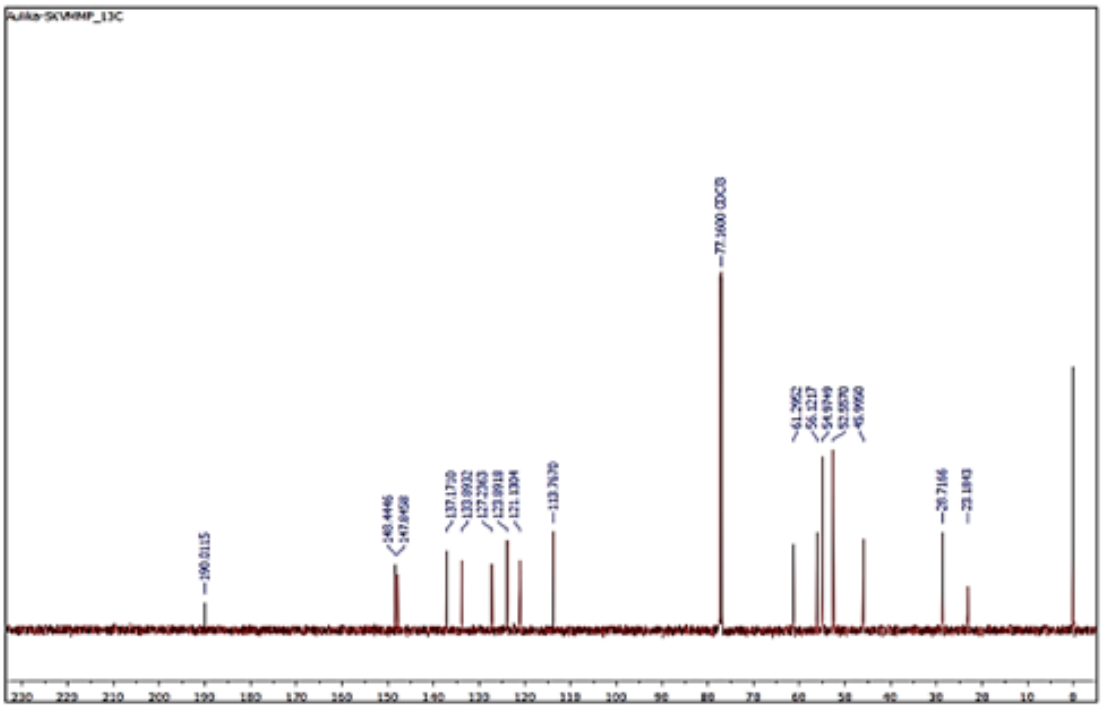
MS Spectrum of compound 2d



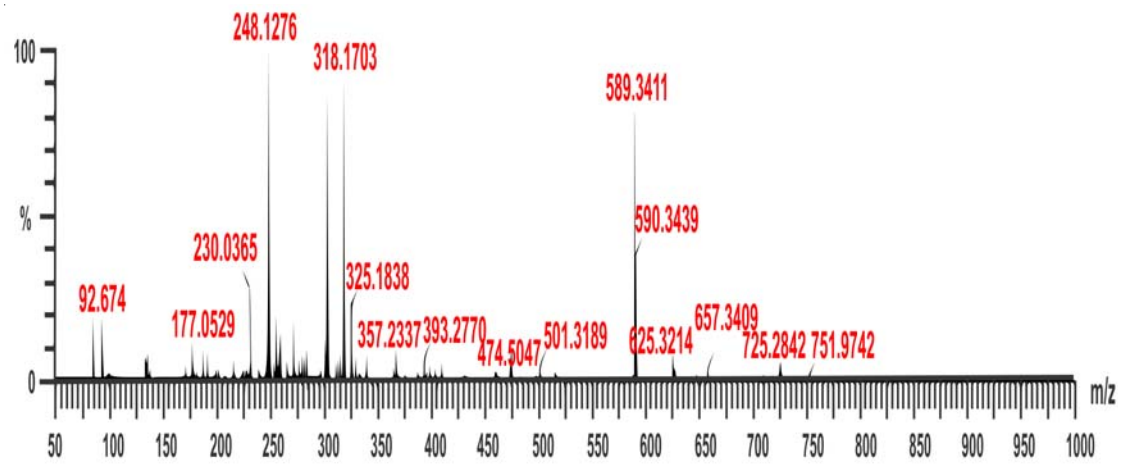
IR Spectrum of compound 2e in KBr



^{1}H NMR Spectrum of compound 2e



^{13}C NMR Spectrum of compound 2e



MS Spectrum of compound 2e

(2*E*,6*E*)-2,6-bis({[4-hydroxy-3-methoxy-5-(morpholin-4-ylmethyl)phenyl]methylidene}) cyclohexan-1-one (2c)

The crude product was purified by recrystallization from ethylacetate-hexane (1:1) gave a yellow crystalline powder at 63**%** yield**,** mp = 172-174°C. IR (KBr) υ_{max}, cm^{-1}: 2933, 2837, 1654, 1591, 1494, 1305, 1259, 1087, and 1001. ^{1}H-NMR (500 MHz, $CDCl_3$) δ/ppm: 7.68 (2H, s, C=C<u>H</u>-), 6.96 (2H, s, H_{Ar}), 6.80 (2H, s, H_{Ar}), 3.89 (6H, s, OCH_3), 3.75 (12 H of 4H, Ar-CH_2-$N_{morpholine}$ and 8H, C-<u>CH_2</u>-$O_{morpholine}$), 2.91 (4H, t, =C-<u>CH_2</u>-$C_{cyclohexanone}$), 2.59 (8H, t, C-<u>CH_2</u>-$N_{morpholine}$), and 1.81 (2H, p, C-<u>CH_2</u>-$C_{cyclohexanone}$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ/ppm: 190 (1C, C=$O_{cyclohexanone}$), 148 and 147 (4C, $C_{Ar\text{-}O}$), 137 and 134 (4C, -C=C-), 127, 124, 121, and 114 (8C, C_{Ar}), 67 (4C, C-O-C), 62 (2C, Ar-C-$N_{morpholine}$), 56 (2C, Ar-O<u>C</u>H_3), 53 (4C, C-N-$C_{morpholine}$), 29 (2C, =C-

$\underline{C}H_2$-$C_{cyclohexanone}$), and 23 (1C, C-$\underline{C}H_2$-$C_{cyclohexanone}$). HRESIMS (m/z): found 565.2916 ($[M+H]^+$), calculated masses of $C_{32}H_{41}N_2O_7$: 565.2914 (error 0.4 ppm).

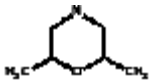
(2*E*,6*E*)-2,6-bis({3-[(2,6-dimethylmorpholin-4-yl)methyl]-4-hydroxy-5-methoxyphenyl}methylidene)cyclohexan-1-one (2d)

The crude product was purified by recrystallization from chloroform-methanol (1:3) gave a pale yellow crystalline powder at 79**%** yield**,** m.p. = 192-194 °C. IR (KBr), υ_{max}, cm^{-1}: 2972, 2939**,** 2821**,** 1589**,**1417, 1375, 1653**,** 1257**,** 1085 and 1145. ^{1}H-NMR (500 MHz, $CDCl_3$) δ/ppm: 7.69 (2H, s, C=CH-), 6.97 (2H, s, H_{Ar}), 6.79 (2H, s, H_{Ar}), 4,07 (2H, br, OH), 3.89 and 3.90 (two peaks of 6H, s, OCH_3 of the two isomers), 3.71 (s, 4H, Ar-CH_2-$N_{morpholine}$), 3,52-3,82 (4H, m, C-$\underline{C}H$(-O-)-$C_{morpholine}$), 2.92 (4H, t, =C-$\underline{C}H_2$-$C_{cyclohexanone}$), 2.83, 2.63, 2.27 and 1.87 (four peaks of 8 H, C-$\underline{C}H_2$-N-$_{morpholine}$ of the two isomers), 1.81 (2H, p, C-$\underline{C}H_2$-$C_{cyclohexanone}$), 1.15 and 1.24 (two peaks of d, 12H, $\underline{C}H$-$\underline{C}H_3$ of the two isomers). ^{13}C-NMR (125 MHz, $CDCl_3$) ä/ppm: 190 (1C, C=$O_{cyclohexanone}$), 148 and 147 (4C, $C_{Ar\text{-}O}$), 137 and 134 (4C, -C=C-), 127, 124, 121, 114 (8C, C_{Ar}), 72 (4C, C-O-C), 62 (2C, Ar-C-$N_{morpholine}$), 59 (4C, C-N-$C_{morpholine}$), 56 (2C, Ar-O$\underline{C}H_3$), 29 (2C, =C-$\underline{C}H_2$-$C_{cyclohexanone}$), 23 (1C, C-$\underline{C}H_2$-$C_{cyclohexanone}$), 19 (4C, $\underline{C}H_3$-$C_{morpholine}$). HRESIMS (m/z): found 621.3546 ($[M+H]^+$), calculated masses of $C_{36}H_{49}N_2O_7$: 621.3540 (error 1.0 ppm).

(2*E*,6*E*)-2,6-bis({4-hydroxy-3-methoxy-5-[(4-methylpiperazin-1-yl)methyl]phenyl}methylidene)cyclohexan-1-one (2e)

The crude product was purified by column chromatography on silica with mixture of chloroform and methanol (1.5:1) as mobile phase gave a brownish orange crystalline powder at 57.0% yield**,** m.p. = 163-165 °C. IR (KBr), υ_{max}, cm^{-1}: 2939**,** 2839, 2797**,** 1657**,** 1586**,** 1500, 1302, 1249**,** 1086 and 1006. ^{1}H-NMR (500 MHz, $CDCl_3$) δ/ppm: 7.69 (2H, s, C=CH-), 6.96 (2H, d, *J=2* Hz, H_{Ar}), 6.80 (2H, d, *J=2* Hz, H_{Ar}), 4.4 (2H, br, OH), 3.89 (6H, s, OCH3), 3.76 (4H, s, Ar-CH_2-$N_{piperazine}$), 2.92 (4H, t, =C-$\underline{C}H_2$-$C_{cyclohexanone}$), 2.61 (16H, N-C-$\underline{C}H_2$-$N_{piperazine}$), 2.29 (6H, s, CH_3-$N_{piperazine}$), 1.81 (2H, p, C-$\underline{C}H_2$-$C_{cyclohexanone}$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ/ppm: 190 (1C, C=$O_{cyclohexanone}$), 148 and 147 (4C, $C_{Ar\text{-}O}$), 137 and 134 (4C, -C=C-); 127, 124; 121, and 114 (8C, C_{Ar}), 61 (2C, Ar-C-$N_{piperazine}$), 56 (2C, Ar-O$\underline{C}H_3$), 55 and 53 (8C, C-N-$C_{piperazine}$), 46 (2C, $\underline{C}H_3$-$N_{piperazine}$), 29 (2C, =C-$\underline{C}H_2$-$C_{cyclohexanone}$); 23 (1C, C-$\underline{C}H_2$-$C_{cyclohexanone}$). HRESIMS (m/z): found 589.3411 ($[M-H]^-$), calculated masses of $C_{34}H_{45}N_4O_5$: 589.3390 (error 3.6 ppm).

Table. 1: Free radical-scavenging activity (FRSA) (IC50) of Mannich bases derivatives of Cyclovalone (2a-e)

No.	R-N-R	Compounds	IC50 (μM)1) (mean ± SD)	Calc. pKa2)
1	-	1	69.91 ± 0.37	9.48 (ArOH)
2	C2H5-N-C2H5	2a	39.53 ± 0.51	8.70 (ArOH) and 8.69 (N)
3	CH3-N-CH3	2b	45.83 ± 0.20	8.70 (ArOH) and 8.13 (N)
4		2c	163.15 ± 1.23.	8.70 (ArOH) and 6.27 (N)
5		2d	127.44 ± 1.06	8.70 (ArOH) and 6.89 (N)
6		2e	95.96 ± 0.76	8.70 (ArOH), 7.44 (N1) and 7.97 (N4)
7	-	Quercetin	21.75 ± 0.02	n.c.3)

1) n = 3; 2) Calculated using MarvinSketch 6.1.0. [16]; 3)n.c.= not calcutated

Free Radical-Scavenging Activity Evaluation

The antioxidant activities of Mannich bases of cyclovalone derivatives (2a-e) and cyclovalone (1) were evaluated by the free radical-scavenging activity of stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the methodology described by Brand-Williams et al. with a little modification[17-18]. Quercetin was used as reference standard. The test or reference compounds were prepared at five different concentrations in methanol. The test or reference solution (0.5 mL) was mixed with 0.5 mL of DPPH radical solution 0.5 mM in methanol and then allowed to stand at room temperature for 30 min. in a dark laboratory condition. The changes in color (from deep violet to light yellow) were measured at 517 nm. The mixture of of methanol (0.5 mL) and of sample solution (0.5 mL) serve as a blank. The control solution was prepared by mixing methanol (0.5 mL) and DPPH radical solution (0.5 mL). The experiment for each test compounds was performed in triplicate. The percent free radical-scavenging activity (% Scavenging) was calculated according to the following equation:

$$\%\ Scavenging = \left[\frac{(A\ control - A\ sample)}{A\ control}\right] x\ 100$$

where A sample is the absorption of DPPH with test or reference compounds and A control is the absorption of DPPH without test compounds. Data obtained was then analyzed using a linear regression equation to determine IC_{50} of free radical-scavenging activity (FRSA) of the compounds.

RESULTS AND DISCUSSION

Chemistry

The title compounds 2a-e were synthesized in two steps by the method summarized in Scheme 1. Vanillin was reacted with cyclohexanone according to the method previously reported to provide cyclovalone (1)[5]. Treatment of **1** with paraformaldehyde and corresponding secondary amine (a-e) in acetonitrile at reflux temperature for 5-27 h (TLC monitoring) afforded the title compounds 2a-e.

The IR spectra of compounds 2a-e appeared CH aliphatic bands at 2,972-2,830 cm^{-1} and showed the disappearance of OH phenolic peak. The bands at 1,246-1,057 cm^{-1} and 1,000-1081 cm^{-1} correspond to C-O phenol, C-O ether, and C-N; while the α,β-carbonyl groups of the cyclovalone are observed as strong bands at 1,639–1,659 cm^{-1} and 1589-1591 cm^{-1}. In ^{1}H-NMR spectra, the protons of two symmetrical aromatic ring remained only four protons appeared at δ 6.9 ppm (2H) and at δ 6.8 ppm (2H) as singlet or doublet with *J=1-2 Hz* indicated that the Mannich base substituted a proton at the ortho position relative to the hydroxyl group of **1**. The data were supported by the disappearance of OH phenolic peak in IR spectra caused by intramolecular hydrogen bond formation between the hydroxyl group and N atom of the Mannich base[14-15]. The structures were further supported by ^{13}C-NMR and MS spectra of the compounds which showed the complete agreement with the assigned molecular structures.

Antioxidant acitivity

The antioxidant activities of the synthesized compounds were evaluated using DPPH free radical-scavenger method because of the suitability of the antioxidant mechanism with the compounds. Furthermore, the DPPH analysis is a fast and an uncomplicated test ensuring the reliable result. The mechanism of antioxidant activity briefly is that the phenolic compounds act as the hydrogen donor to reduce the radical molecules, and then the radical antioxidant will be stabilized by electron delocalization in the aromatic system or coupled with other or the same radical antioxidant to give non-radical molecules[3,8,17-19].

The results of DPPH free radical-scavenging activity of the title compounds are listed in Table 1. All the compounds show free radical-scavenging activity. Compound 2a is the most potent, with IC_{50} = 39.0 µM or about a half of quercetin's antioxidant activity. The activity of compound 2a and 2b are more potent than that of 1, while compound 2e, 2d and 2c are lower than that of **1**. Structure-activity relationship study of the compounds demonstrates the effect of the alkalinity (pKa) of Mannich substituent of the compound on

the antioxidant activity (Figure 1). The higher pKa of the Mannich base compound, the higher activity (the lower IC_{50}) of the compound. In the previous study was reported that the hydroxyl group of the phenolic antioxidant compound is essential for the activity. The small alkyl substituents (e.g. methyl and ethyl) and electron donating groups (e.g. methoxy) at ortho positions relative to the phenol groups enhanced the activity. On the contrary, bulky alkyl substituents (e.g. isopropyl and t-butyl) retarded the activity[5,8].

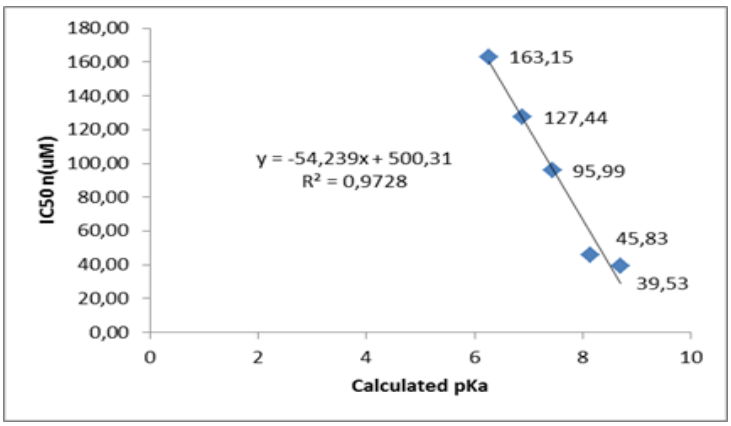


Fig. 1. Relationship between the alkalinity (calculated pKa) and antioxidant activity of the Mannich bases of cyclovalone derivatives

CONCLUSION

A series of di-Mannich bases of cyclovalone derivatives were synthesized and their free radical-scavenging activity evaluated. The data obtained demonstrated the effect of the basicity of Mannich substituent of the compound on the antioxidant activity. The di-Mannich derivative of cyclovalone with diethylamine and dimethylamine (2a and 2b) exhibited higher free radical-scavenging activity than 1.

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