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Synthesis & Characterization of New Schiff Bases Derived From 2-hydroxybenzaldehye & Amino Acids and Their Vanadyl Complexes.

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ABSTRACT

New Schiff bases derived from 2-hydroxybenzaldehye and amino acid(Glycine; D,L-Alanine; D,L-Phenylalanine; D,L-Valine or D,L-Threonine) were prepared via condensation reaction. These Schiff bases were used as ligands to form complexes with Vanadyl ion. Both, the Schiff bases and their complexes were characterized by FT-IR, ^{1}H NMR, mass spectrometry and CHN elemental analysis. The analytical data showed that Schiff bases can act as bidentate ligand using their carboxylate oxygens forming 4-membered ring with the vanadyl ion, or tridentate ligand using the N-isomethine, O-phenolic and O-carboxylate forming 5- and 6-membered stable rings. The low molar conductances of the complexes indicate that they are non-electrolyte and neutral. The complexes were tested by microbial species such as *Staphlococcusaurous,Escherichia coli* and *A.hydrophila*. The results of the tests indicate that these complexes are more or less biological active than their own ligands.

Keywords: Schiff base, amino acid, Vanadyl ion

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INTRODUCTION

Schiff bases are the product of condensation of an amine and carbonyl compounds are important class of ligands that are coordinate to metal ion *via*azomethine nitrogen[1,2]. They have also other hetro-elements like oxygen or sulphur which provides binding sites to the metal ion may form ring structure making the complex more stable and biologically more active in the presence of bio-metal.Metal-Schiff base complexes have important and their metal complexes have a significant important as antibacterial [3,4], anticancer, and antiviral effects [5]. A series of Schiff base metal complexes were synthesized and carried out antitumor experiments, which indicate that aldehyde substituent, was superior to amine substituent in anticancer effect, and salicylaldehyde Schiff bases were superior to other aldehyde Schiff base [6].

A series of ternary complexes of copper with salicylaldehyde amino acid Schiff base as the first ligand and 2,2′-bipyridine or 1,10-phenanthroline as the second ligand show significant antitumor activities in *vitro* [7].

Antibacterial activities of some amino acid Schiff base have been tested against four different microorganisms, show that the activity may be due the carboxyl group [8]. The present study deals withthe synthesis, characterization and biological studies of some salicylaldehydeamino acid Schiff base and their vanadyl complexes.

EXPERIMENTAL

Materials

All chemicals and solvents which employed in the synthesis are of pure –grade and used as received. Glycine, L- Alanine, D-Valine, D,L-Phenylalanine, L-Threonine, Salicylaldehyde and Vanadyl sulfate were obtained from Fluka and Aldrich.

Instrumentation

IR spectra were recorded using Shimadzu FT IR -8300 spectrophotometer in the region 4000-400 cm^{-1} as KBr pellet.^{1}H NMR spectra were recorded on Bruker Avance DX-400 MHz spectrometer. TMS was used as internal standard and referenced to 0.0 ppm. DMSO-d_6 was used as solvent. Mass spectra were scanned by EI-technique at 70 eV using Agilent Technologies 5975C spectrometer. Molar conductances were obtained using Conductometer-Corning model 441 at room temperature. Elemental Analysis (CHN) was performed using CHNS-932 LECO Apparatus.

Synthesis of Schiff Bases

The general procedure for synthesis of Schiff bases includes heating of a mixture of equimolar concentration of salicylaldehyde and amino acid at an appropriate temperature for 30 min with continuous stirring in an oil bath. The resultant product washed with hot ether to yield orange solid crystals.

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Synthesis of Vanadyl -Schiff Base Complexes

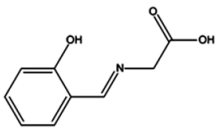
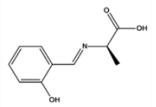
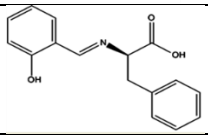
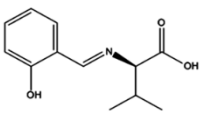
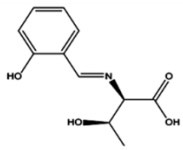
The general procedure for synthesis of complexes includes the preparation of Schiff base solution by mixing of equimolar concentration ofSalicylaldehyde and amino acid in ethanol. Aqueous solution of Sodium hydroxide (2 mole equivalents) was added. The resultant solution was heated at 50 ℃ with continuous stirring for 30 min. A yellow-orange solution was obtained. To this solution, aqueous VanadylSulfate solution (1 mole equivalent) was added drop wise over 15 min, whence a green solution and pale blue precipitate formed. The pale blue solid was filtered out and washed with portions of ethanol, water and acetone and then dried at 80℃. The green solution was concentrated by removing ethanol solvent under reduced pressure. Green crystals obtained, washed several times with absolute methanol and dried at 60℃.

Synthesis of Vanadyl –Salicylidene Threonine Complex

To an equimolar mixture ofSalicylaldehye and D,L-Threonine in 50 mL ethanol,aqueous solution of Sodium hydroxide (2 mole equivalents) was added. The continuous stirring for 30 min. A yellow-orange solution was obtained. To this solution, aqueous VanadylSulfate solution (1 mole equivalent) was added drop wise over 15min, whence a green solution formed. The resultant solution wasconcentrated by removing ethanol solvent under reduced pressure. Green crystals obtained, washed several times with absolute methanol and dried at 60℃.

RESULTS AND DISCUSSION

Table 1: shows the structures formulas of the prepared Schiff bases:

Schiff Base Chemical formula Molecular weight	symbol	Structure
Salicylidene glycine $C_9H_9NO_3$ (179.06)	L1	
Salicylidene alanine $C_{10}H_{11}NO_3$ (193.07)	L2	
Salicylidene phenyl -alanine $C_{16}H_{15}NO_3$ (269.11)	L3	
Salicylidenevaline $C_{12}H_{15}NO_3$ (221.11)	L4	
Salicylidene Threonine $C_{11}H_{13}NO_4$ (223.08)	L5	

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Five new Schiff bases were prepared by condensation reactions of equimolar ratio of salicylaldehyde and corresponding amino acid according to the following equation:

$$o\text{-}OHC_6H_4CHO + NH_2CHRCOOH \rightarrow o\text{-}OHC_6H_4CH{=}NCHRCOOH$$

Salicylaldehyde α-Amino acid α-Schiff base

R= -H , $-CH_3$, $-CH_2-C_6H_5$, $-CH(CH_3)_3$, $-CH(OH)-CH_3$

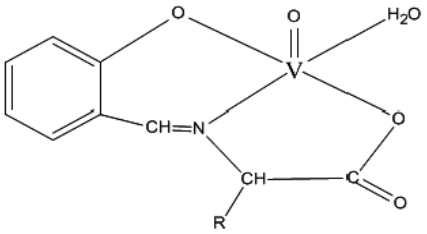
The observed physical properties of these Schiff bases and their CHN elemental analysis are shown in **Table 2**. They are yellow, air stable solids, having sharp melting points.

Table 2: Some physical properties of the prepared Schiff bases and their CHN

Symbo	Melting point (℃)	%C	%H	%N	solubility
L1	Yellow solid 178-180	60.93 (60.33)	4.83 (5.06)	7.45 (7.82)	Soluble in Alcohols, DMF and DMSO
L2	Brown solid 168-170	62.84 (62.17)	5.9 (5.74)	7.61 (7.25)	
L3	Yellow solid 160-162	70.92 (71.36)	5.54 (5.61)	5.54 (5.20)	
L4	Pale yellow 148-150	64.88 (65.14)	7.23 (6.83)	6.56 (6.33)	
L5	Brown solid 166-168	59.32 (59.19)	5.58 (5.87)	6.15 (6.27)	

( )calculated

These Schiff bases were used as ligands towards Vanadyl ion. Two types of complexes were identified after mixing of aqueous solution of VanadylSulphate with alcoholic solution of Schiff base (L1 – L4).The first type was the pale blue complex which precipitated from solution. The low molar conductivities indicate that these complexesare neutral, their physical properties and spectral dataindicate that they may have the following structure (I):



R	Complex
H	VOL1
CH_3	VOL2
$PhCH_2$	VOL3
$CH(CH_3)_2$	VOL4

Structure (I)

In this structure, the Schiff base act as tridentate ligand through its three donor atoms, the imine-nitrogen, the phenolic-oxygen and the carboxylate-oxygen[9]. As Vanadyl ion prefer five coordinate complex, then the presence of coordinate water molecule will be essential to complete this coordination.

The second type was the soluble green complex. The different color complex indicates different coordination, which may occur through the two oxygen atoms of carboxylate group

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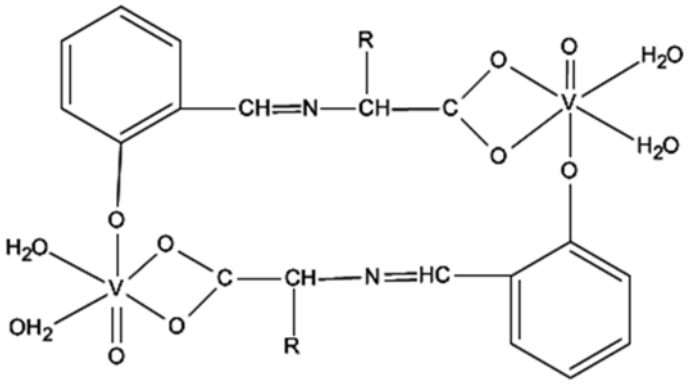
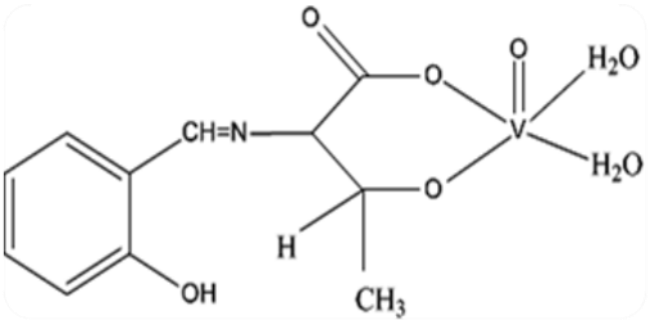
and two water molecules. The low molar conductivities of these complexes may also an indicative of non-electrolyte compounds, but their solubility's in water-ethanol solutions may arise from their ability to form hydrogen bonding. As these complexes were suggested to be neutral, then the charge demands require that these types of complexes should exist as dimeric structures (II).



R	Complex
H	SVOL1
CH_3	SVOL2
$PhCH_2$	SVOL3
$CH(CH_3)_2$	SVOL4

Structure (II)

However, the reaction of SalicylideneThreonine with VanadylSulphate yields only a green solution without any precipitate. This observation indicate that the formed complex is quite similar to those of structure (II) and instead of forming 4-membered chelate ring with the two oxygen atoms of the carboxylate group, a more stable 6-membered chelate ring is highly preferred between the metal ion and the carboxylate oxygen and carboxyl oxygen atom as shown in structure (III).



Structure (III): **SVOL5**

IR Spectra of Schiff Bases

The IR spectra of the prepared Schiff bases (**L1-L5**)(**Fig.1** and **2**) show distinct vibrational frequency $\nu_{CH=N}$ at 1633 $\pm$ 5 for isomethine group [10,11,12], with the disappearance of ν_{NH} for amino group of the amino acid. The IR spectra also show clear $\nu_{COO(asy)}$ and $\nu_{COO(sy)}$ in the range 1606- 1540 cm^{-1} and 1394-1363 cm^{-1}respectively in addition to the strong $\nu_{C=O}$ at 1700±5 cm^{-1}.

On complexation of Schiff bases with Vanadyl ion, $\nu_{CH=N}$ were shifted to lower frequency (**Fig**. **3** and **4**) due to the bonding between the donor nitrogen atom and the metal ion in the complexes. Moreover, the carboxylic and phenolic ν_{OH} disappeared as the oxygen atoms of these

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groups act as donor ligands to theVanadyl ion. New ν_{OH} vibrational frequencies at 3440±10 cm^{-1} due to the coordinated water molecule[13-14]. The IR spectra show also additional important bands in the range 1000-962 cm-1 for $\nu_{V=O}$ [15]. The IR spectral data are summarized in **Table 3**.

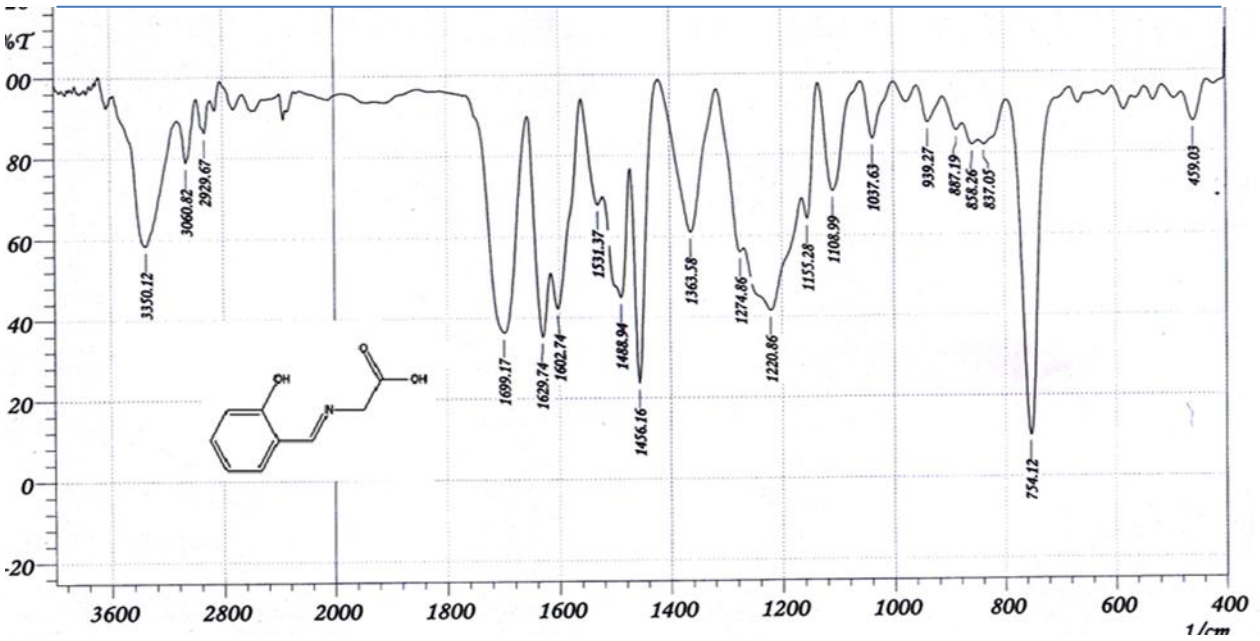

Figure 1: the IR spectrum of Salicylidene glycine

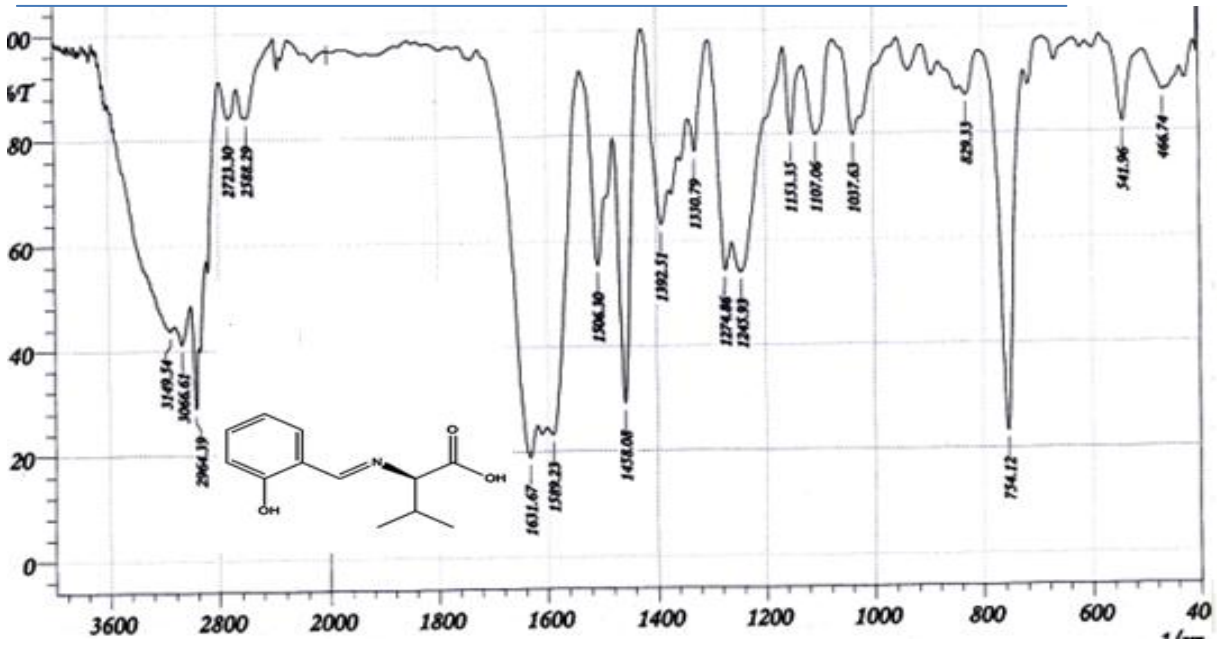

Figure 2: the IR spectrum of SalicylideneValine

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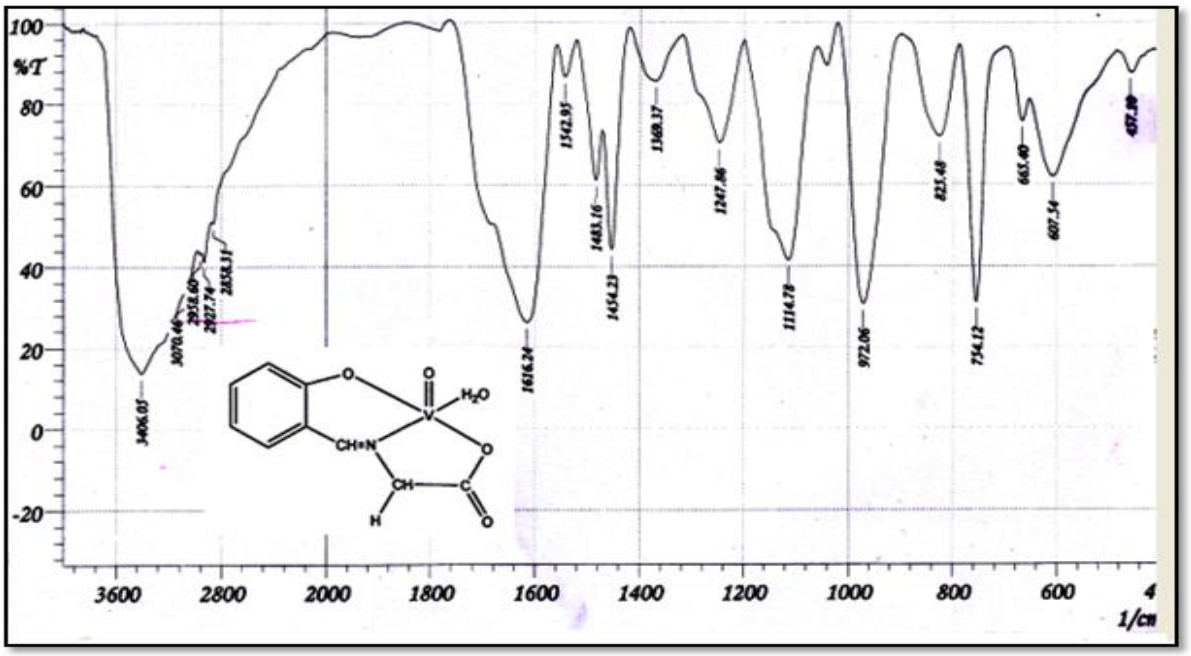

Figure 3: the IR spectrum of VOL1

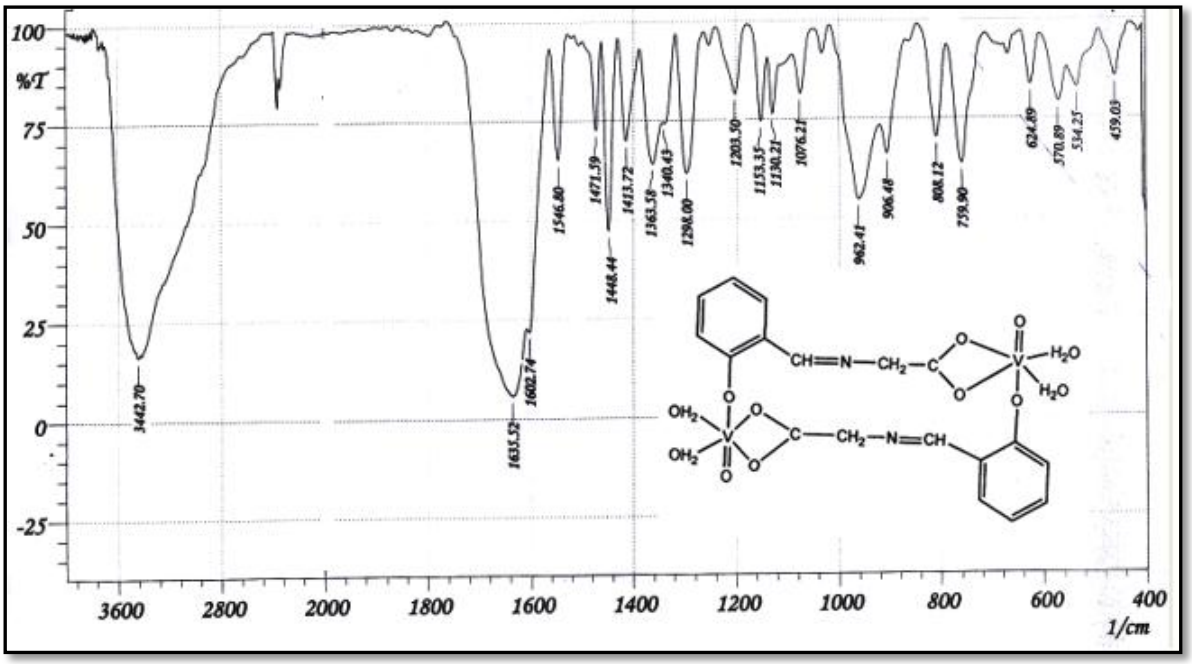

Figure 4: the IR spectrum of SVOL1

Compond	υ OH(COOH)	υOH(Phenolic)	υ (C=O)	υ (CH=N)	υ (υ=O)	υCOO⁻(asy)	υCOO⁻(sy)	υOH (H_2O)
L1	3350.12 br	3350.12 br	1699.17	1629.74	–	1602.74	1363.58	–
VOL1	–	–	1670	1616.24	972.06	1600	1369.37	3406.05
SVOL1	–	–	1635.52	1629	962.41	1602.74	1363.58	3442.70
L2	3600-2800 br	3600-2800 br	–	1631.67	–	1606.59	1394.44	–
VOL2	–	–	–	1637.45	997.13	1600.81	1384.79	_2800 3600
SVOL2	–	–	_1580 1720	1633.59	981.70	1600	1380.94	3448.49
L3	3600-2400	3600-2400	1706.88	1633.59	–	_1540	1382.87	–
VOL3	–	–	_1520 1720	1627.81	985	1600	1360	3431.13
SVOL3	–	–	_1520 1720	1627.81	985.56	_1520 1720	1342.36	3434.98
L4	3600-2800 br	3600-2800 br	-1600 1700	1631.67	–	1589.23	1392	–
VOL4	–	–	_1560 1720	1616.24	1000.99	1593.09	1371.29	_2800 3600
SVOL4	–	–	1666.38	1622.02	979.77	1593	1365.51	3448.49
L5	3600-2800 br	3600-2800 br	1700	1631.67	–	1600	1380.94	–
SVOL5	–	–		1623.95	977.84	1720-1580	1404	421.48

Table 3: the IR spectral data for Schiff bases and their Vanadyl complexes

^{1}H NMR spectra:

The ^{1}H NMR spectra of Schiff base **L4** in DMSO-d_6 solvent at ambient temperature is shown in **Fig. 5**. The spectrum exhibits a signal atδ 8.51ppm may attributed to the isomethine

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proton, asignalatδ 4.73 ppm due to the phenolic-O**H** proton, a signal atδ 3.79 ppm due to HC=NC**H** proton, a signal as doublet atδ 0.95 ppm due to (C**H**$_3$)$_2$CH and signal asmultiplet in the rangeδ 2.22-2.40 ppm due to (CH$_3$)$_2$C**H** proton. The phenyl protons appear as multipetatδ 6.73-7.45 ppm attributed to the hydrogen bonding.

The ^{1}H NMR spectrum of VOL4 (structure I) inin DMSO-d$_6$ solvent at ambient temperature is shown in **Fig. 6**. In comparison with^{1}H NMRspectrum of L4 ligand, the signal atδ 8.65 ppm is attributed to the isomethine proton. However, there are no signals for phenolic-OH and carboxylate protons as these protons were removed upon addition of base [16]. The methyl protons appear as doublet atδ 1.02 ppm and the signal at δ2.50 ppm may attributed to coordinated water protons.

The ^{1}H NMR spectrum of SVOL4 (structure II) in in DMSO-d$_6$ solvent at ambient temperature is shown in **Fig. 7**. The spectrum exhibits similar signals to the ^{1}H NMR spectrum of VOL4. The spectrum is quite clear and its distinguishable signals may arise from its higher solubility in DMSO solvent.

The ^{1}H NMR spectrum of Schiff base **L3** in DMSO-d$_6$ solvent at ambient temperature exhibits similar signals to that for ^{1}H NMR spectrum of L4. The most distinguishable signal in the spectrum is that for methylene protons which attached to the phenyl group of phenyl alanine Schiff base, C$_6$H$_5$C**H**$_2$.

The methylene signals are amenable to analysis as a simple AB system (a projection of the ligand down C-C bond clearly shows the conformational Diastereotopic origin of the nonequivalent of the methylene protons). Two triplets atδ 2.90 and δ3.8 ppm are assigning for H$_\alpha$ and H$_\beta$ for methylene group (**Fig. 8**). The ^{1}H NMR spectral data are listed in **Table 4** .

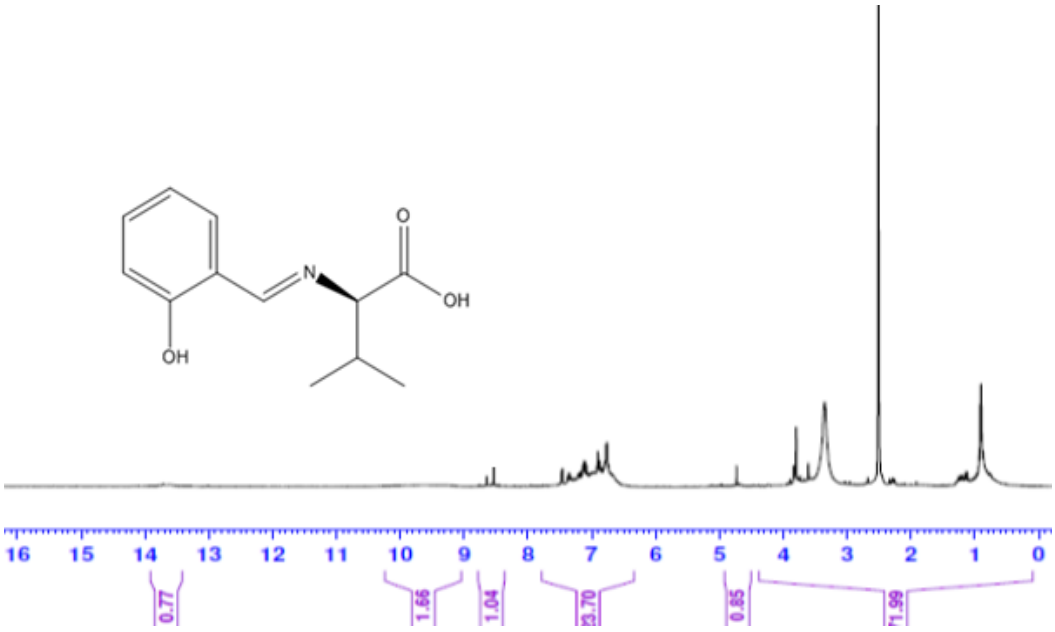

Figure 5: The 1H NMR spectrum of Schiff base L4 in DMSO-d6 solvent at ambient temperature.

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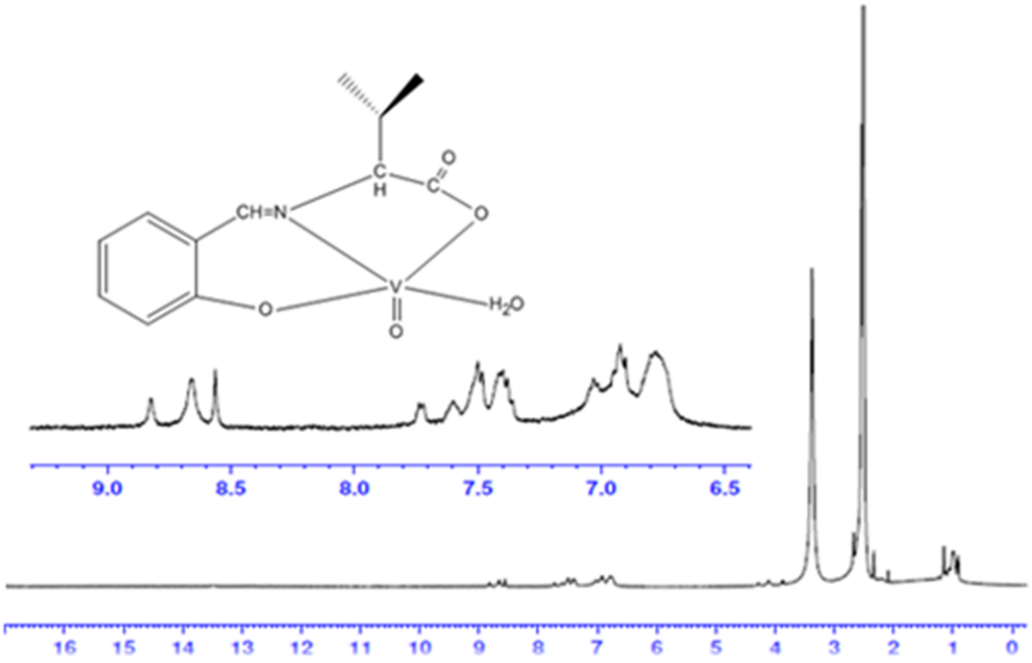

Figure 6: The ^{1}H NMR spectrum of VOL4 in DMSO-d6 solvent at ambient temperature.

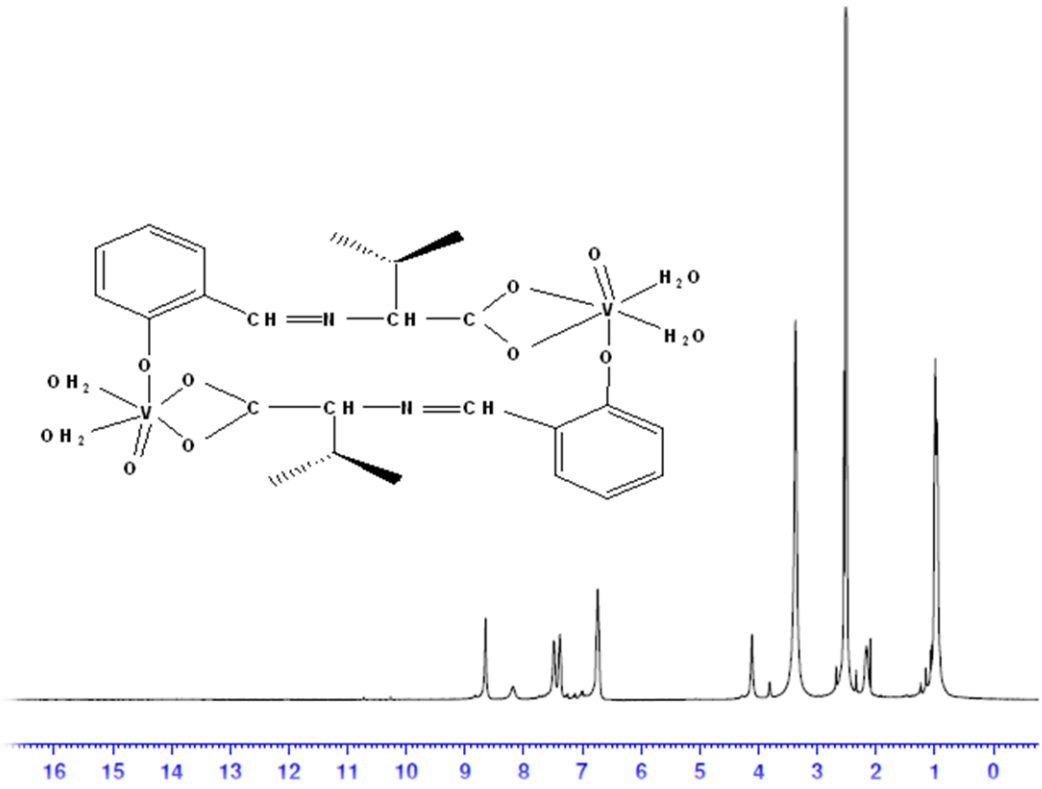

Figure 7: The ^{1}H NMR spectrum of SVOL4 in DMSO-d6 solvent at ambient temperature.

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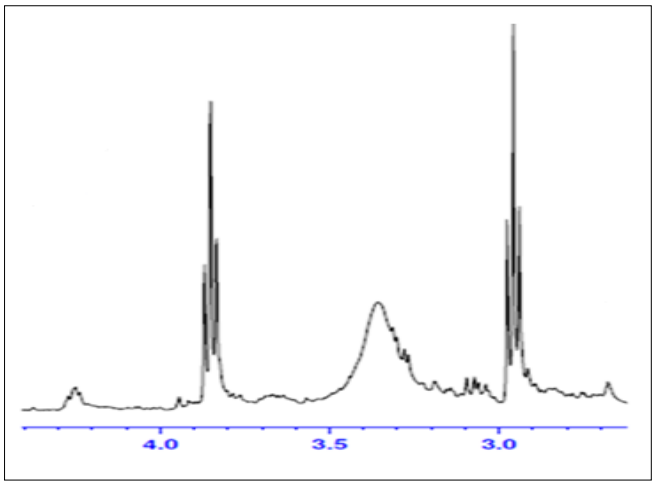

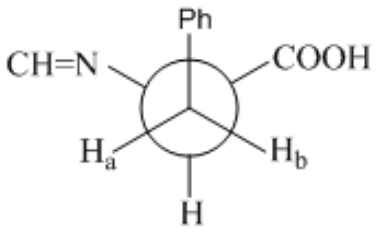

(a) (b)

Figure 8: (a) The ^{1}H NMR spectrum of the methylenic protons of L3 at ambient temperature.

Compound	Chemical shift (ppm)											
	Me	0.95	1.02	0.91	CH_2	H_β	3.8	3.51	3.45	C-H	3.2-3.7(m)	3.7 (m)
	-CH(Me)$_2$	2.22-240(m)	2.22-2.39 (m)	2.15		H_α	2.90	2.95	3.00	CH3 R-OH	1.01 3.08	1.18 –
	H_2O	–	2.5	2.5	–			2.5	2.5	–		2.5
	Aromatic	6.73-7.45(m) 6	6.70-7.55(m)	6.70-7.55 (m)	6.40-7.60(m)			6.35-7.63 (m)	6.55-7.50(m)	6.50-7.52(m)		6.50-7.70(m)
	CH=N-CH	3.79	4.12	4.10	4.70			4.50	4.42	4.00		4.08
	-CH=N	8.51	8.65	8.60	8.50			7.92	7.75	8.40		8.51
	Ph-OH	4.73	–	–	9.3-9.9 (br)			–	–	9.3-10.4		
	COOH	13.5-13.75 (br)	–	–	13.5			–	–	13.7 (br)		–
		L4	VOL4	SVOL4	L3			VOL3	SVOL3	L5		SVOL5

(b) Newman projection of L3 down C-C bond.

Table 4: The ^{1}H NMR spectral data for Schiff bases and their Vanadyl Complexes.

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Mass Spectra

Mass spectrometry has been successfully used to confirm the molecular ion of Schiff bases and investigate the fragment species. The EI-mass spectra of L1 to L3 are shown in **Fig. 9**, **10** and **11**. The Schiff bases showed molecular ion peaks with informative fragment ions such as $[M\text{-}OH]^+$, $[M\text{-}COOH]^+$,given in **Table 5**. The proposed fragmentation keys pathways for **L1**, **L2** and **L3** are shown in **Scheme 1**, **2** and **3** respectively.

Table 5: the mass spectral data for L1-L5 Schiff bases.

Schiff base	*m/z* and % abundances		
	$[M]^+$	$[M\text{-}OH]^+$	$[M\text{-}COOH]^+$
L1	179(2.5)	161 (100)	133 (78)
L2	193(8.3)		148(48)
L3	269(4.3)		225(32)
L4	221(17)		177(36)
L5	227(2.3)		182(42)

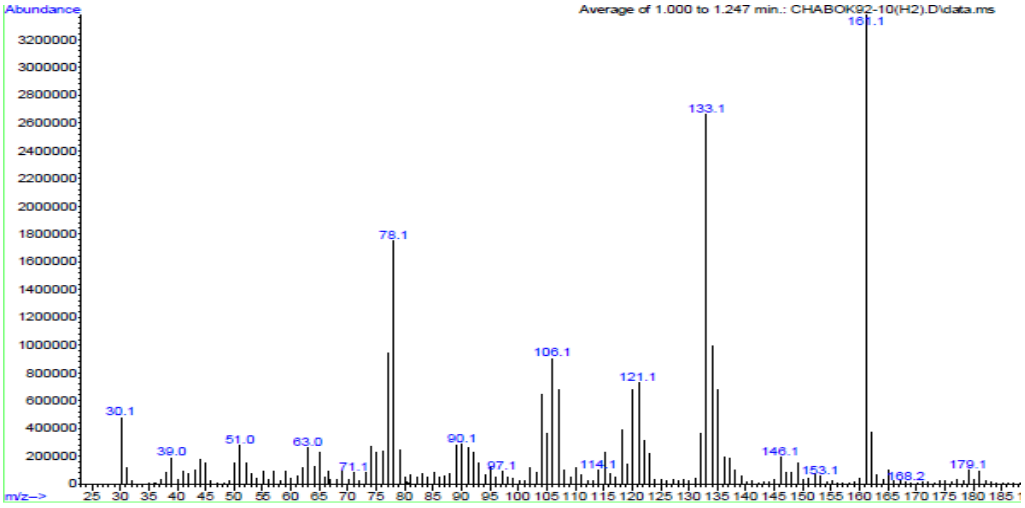

Figure 9: The EI mass spectrum of L1

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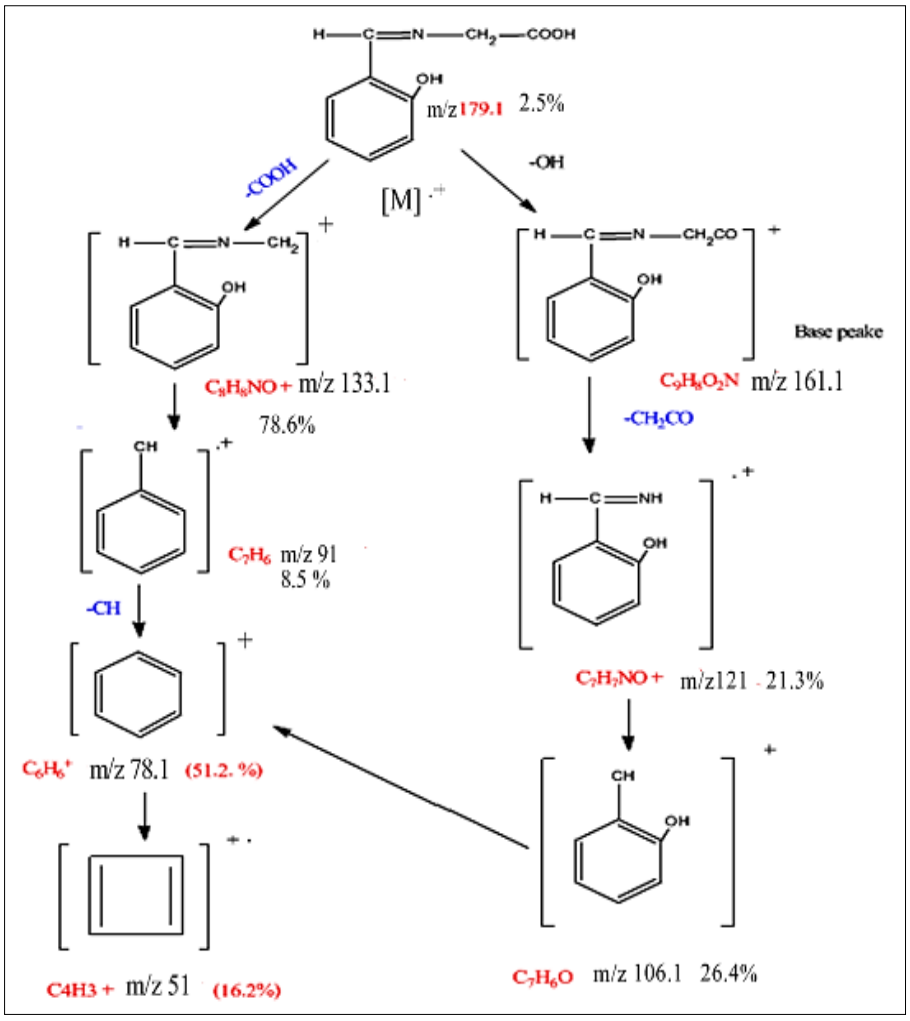

Scheme 1: the proposed key fragmentation pathways for L1

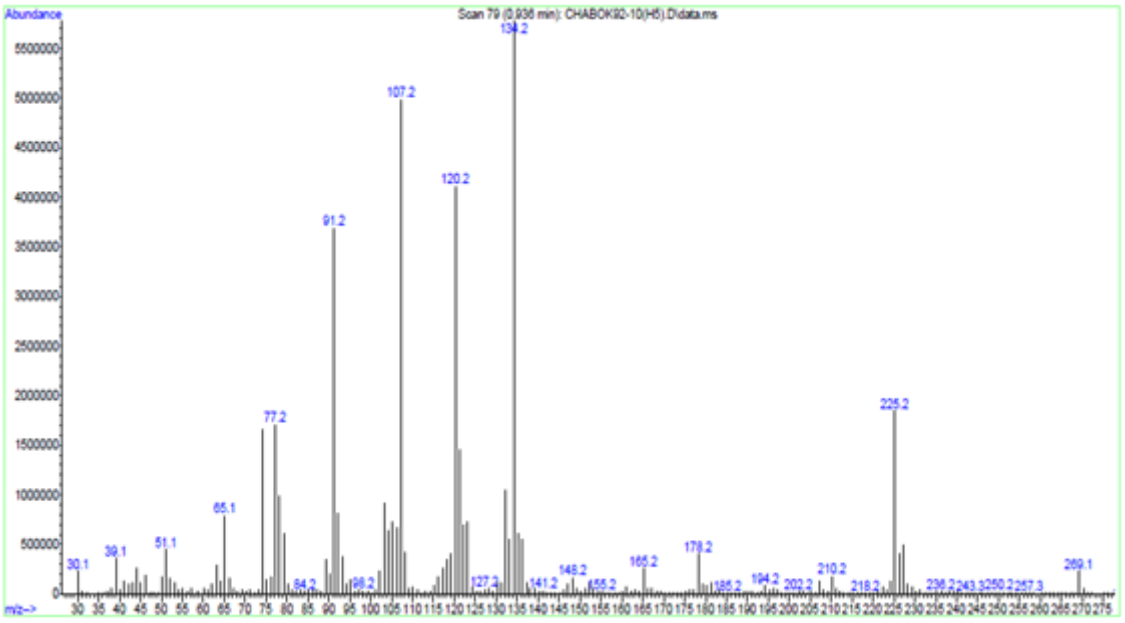

Figure 10: the EI mass spectrum of L2

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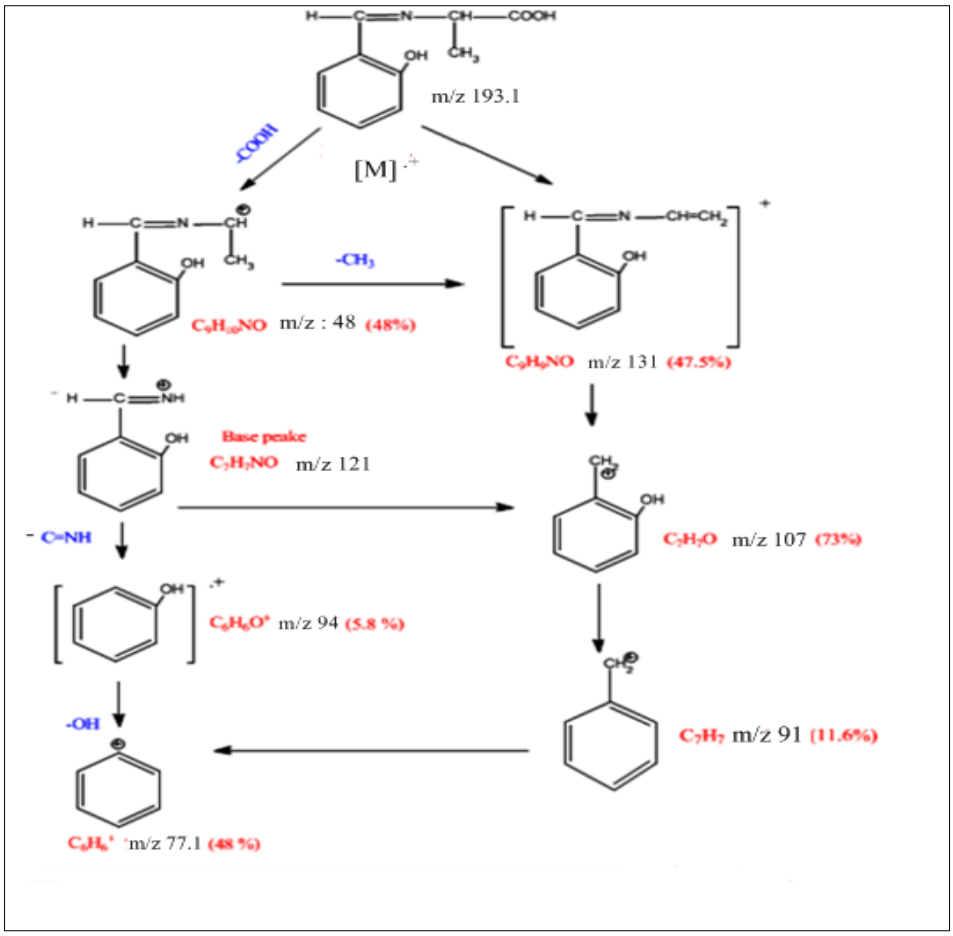

Scheme2: the proposed key fragmentation pathways for L2

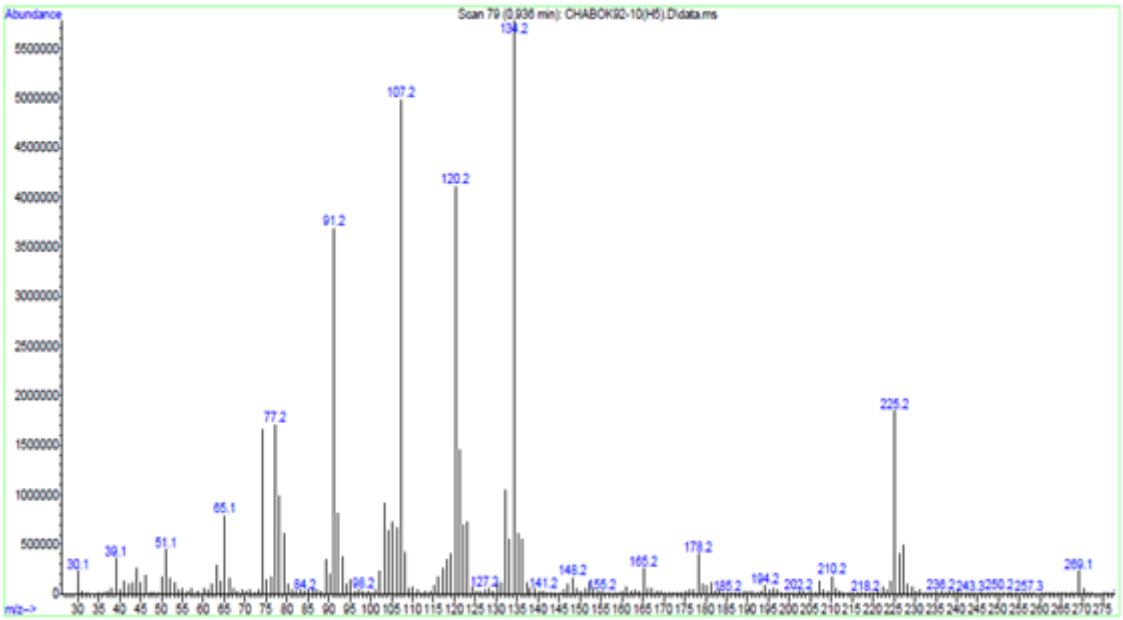

Figure 11: the EI mass spectrum of L3

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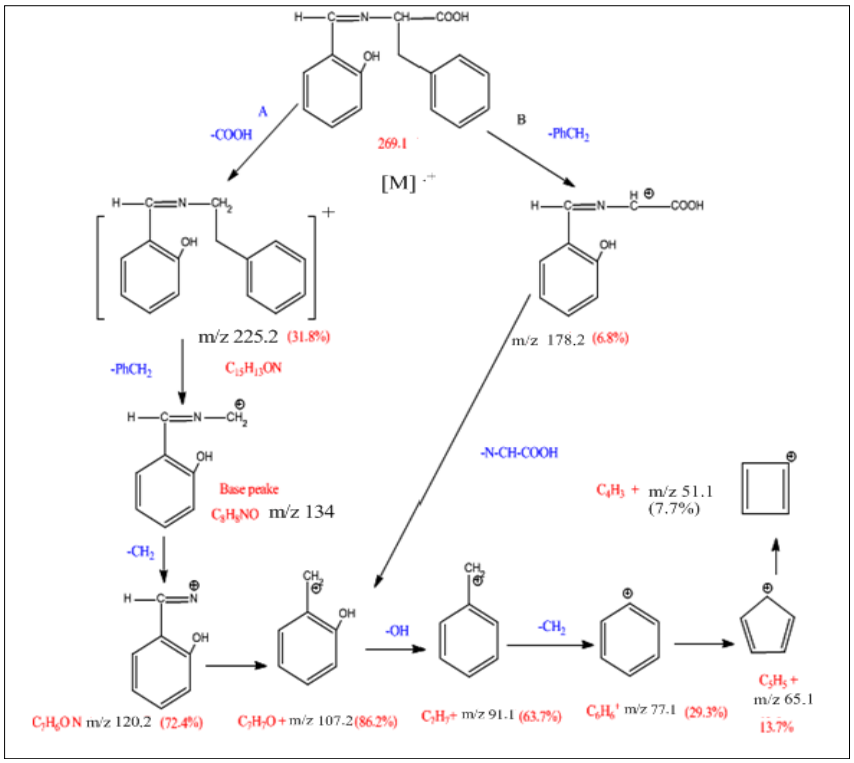

Scheme 3: the proposed key fragmentation pathways for L3

Antibacterial activity

In *vitro* antimicrobial activity of Schiff baes-vanadyl complexes was tested against Gram-positive and Gram-negative*StaphlococcusAureus, A.Hydrophila and E.Coli* bacteria. The agar [17] well- diffusion method was used in these tests in triplicate. The zone of inhibition value of two kinds of complexes VOL and SVOL are shown in Table6.

The results showed that both kinds of complexes gave negative test against Gram-positive bacteria and positive test against Gram-negative bacteria.

Table 6: An antibacterial activity for Schiff base-vanadyl complexes

Bacterial strains	Inhibition zone (mm)			
	VOL4 (1)	VOL3 (2)	VOL1 (3)	VOL2 (4)
S. aureus	0	12	10	0
E. coli	15	10	8	8

Bacterial strains	Inhibition zone (mm)			
	SVOL3 (1)	SVOL4 (2)	SVOL2 (3)	SVOL5 (4)
S. aureus	0	0	0	0
A .hydrophila	10	8	6	4

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