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Vanadium-Catalyzed Esterification of Carboxylates: Synthesis, X-Ray Crystal Structure and Biological Studies

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Abstract

The potentials of vanadium (III) chloride as catalyst were checked for the esterification of substituted carboxylic acids with methanol. The synthesised precursors and methyl esters formed were characterised by various analytical techniques such as FT-IR, ^{1}H-NMR, ^{13}C-NMR and single crystal analysis. The antimicrobial activities of the carboxylates and their methyl esters were found good against various strains of bacteria (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pasteurella multocida*) and fungi (*Alternaria alternata, Ganoderma lucidum* and *Penicillium notatum*). All these esters were tested for their activity against the alkaline phosphatase (ALP) and Methyl 4-oxo-4-(2,4,6-trichlorophenylamino)but-2-enoate **(compound 1)** was found to be potent inhibitor while Methyl 4-(3,4-dichlorophenylamino)-4-oxobut-2-enoate **(compound 2)** showed intercalative mode of binding with DNA.

Keywords: *Vanadium, Coordination Modes, Vibrational Spectroscopy, Antimicrobial Activities, Bacteria, Fungi*

1. Introduction

The synthesis of carboxylic esters is one of the most important fields in organic chemistry to obtain beneficial compounds. However, most esterification procedures require rather punitive situations such as the presence of strong acids, bases, time consuming or expensive catalysts. Moreover, such reactions frequently proceed only at higher temperatures. Studies revealed that esters derived from cinnamic and ricinoleic acids showed enhanced antimicrobial activeties as compared to their parent compounds (Narasimhan et al, 2004).

The membrane-disruptive and antimicrobial activities of esters are well recognized and are often active against a broad range of bacteria and certain viruses (Hugo & Ayliffe, 1992). Esters are being used extensively in various fields like preservatives in pharmaceuticals, cosmetics, foods, and industrial products. Similarly the alkyl esters of aliphatic as well as aromatic acids showed enhanced antimicrobial properties against various strains of *staphylococci, colon bacilli, aerobic bacilli, Gram-positive* and *Gram-negative* bacteria and fungi as compared to the respective acids (Aalto et al, 1953 and Grishchuk et al, 2007).

Vanadium complexes can catalyse the oxidation of ethylbenzene to acetophenone, epoxidation of styrene and oxidetive bromination of salicylaldehyde (Maurya et al, 2005). Vanadium(III) chloride plays the equivalent role as that of $AlCl_3$ in the esterification of carboxylic moieties (Karade et al, 2004). Many oxides, alkoxides, acetylacetonate and co-ordination compounds of vanadium have been reported as effective catalysts in hydrogenation and dehydrogenateon/oxidation catalysis of organic and inorganic substrates

(Sigel & Sigel, 1995). They also act as mediators in redox reactions driven by nicotinamide adenine dinucleotide phosphate (NADPH) (Vyskočil et al, 1980). Selective oxidation of alkenes and cyclohexene by molecular oxygen was also carried out by using oxovanadium(IV) tetradentate Schiff base complexes as catalysts (Boghaei & Mohebi, 2002 and Mohebbi et al, 2005). The complexes of bis(maltolato) oxovanadium supported on silica gel have been employed for the oxidation of linear and cyclic alkanes in batch reactors (Mishra et al, 2007). A number of oxovanadium compounds were curtained as prospective catalysts for the oxidation of alcohols, polymerization of ethylene and ethylene/propylene copolymerization in the presence of a number of organoaluminium co-catalysts (Limberg, 2007 and Da Silva et al, 2011).

Aim of the present work is to check the potentials of vanadium (III) chloride as catalyst for the esterification of 4-(3,4-dichlorophenylamino)-4-oxobut-2-enoic acid (HL^1), 4-oxo-4-(2,4,6-trichlorophenylamino)but-2-enoic acid (HL^2) and 4-(4-bromo-phenylamino)-4-oxobut-2-enoic acid (HL^3) and thereafter, to assess the antifungal and antibacterial activities of the esters formed.

2. Materials and Methods

2.1. Materials

Analytical reagents, such as 3,4-dichloroaniline, 2,4,6-trichloroaniline, 4-bromoaniline, maleic anhydride, vanadium (III) chloride, *p*-nitrophenyl phosphate hexahydrate (*p*-NPP), diethanolamine and magnesium chloride were acquired from Sigma Aldrich (USA) and used without further purification. Sodium salt of Salmon sperm DNA (SS-DNA) was obtained from Acros Organics, Belgium, and used as received. All the solvents purchased from E. Merck (Germany) were dried before use according to the reported procedures (Armarego & Christina, 2003). Human serum (after patient consent) was used as a source of alkaline phosphatase (ALP).

2.2. Characterization of Compounds

Fourier transform infra-red (FT-IR) spectra in the range of 4000-400 cm^{-1} were recorded on a Thermo Nicolet-6700 FT-IR Spectrophotometer. Multinuclear NMR (^1H and ^{13}C) spectra were recorded on a Bruker-300 MHz FT-NMR Spectrometer (Figure 1), using $CDCl_3$ as a solvent [δ ^1H ($CDCl_3$) = 7.25 ppm and δ ^{13}C ($CDCl_3$) = 77 ppm]. Chemical shifts are given in ppm (in Sections 2.6.1-2.6.3) and coupling constants (J) values in Hz. The multiplicities of ^1H NMR signals are mentioned with chemical shifts (s= singlet, d = doublet etc.). The absorption spectra were measured on a Shimadzu 1800 UV-Visible Spectrophotometer. The melting points were determined by using an electrothermal melting point apparatus, Gallenkamp (U.K.). The X-ray diffraction data was collected on a Bruker SMART APEX CCD diffractometer, equipped with a 4 K CCD detector set 60.0 mm from the crystal. The crystals were cooled to 293±2 K for compound **HL^1** and 296±2 for Methyl 4-oxo-4-(2,4,6-trichlorophenylamino)but-2-enoate (compound 1) and Methyl 4-(3,4-dichlorophenylamino)-4-oxobut-2-enoate (compound 2) using the Bruker KRYOFLEX low temperature device and intensity measurements were accomplished using graphite monochromated Mo-Kα radiation from a sealed ceramic diffraction tube (SIEMENS). Generator settings were 50 KV/40 mA. The structures were solved by Patterson Methods (Herbst-Irmer & Sheldrick, 1998) and extension of the model was done by direct methods using the program DIRDIF or SIR2004. Final refinement on F^2 was made by full-matrix least squares technique using SHELXL-97, a modified version of the program PLUTO (preparation of illustrations) and PLATON package.

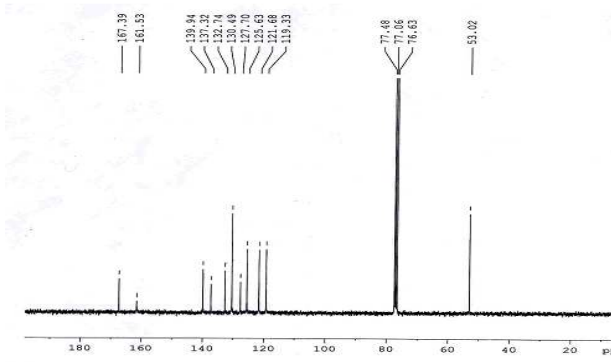


Figure 1. ^{13}C-NMR Spectrum of Compound 1

2.3. Salmon Sperm DNA Binding Studies

Salmon sperm DNA (SS-DNA) (20 mg) was dissolved and stirred for overnight in doubly deionised water (pH = 7.0) and kept at 4 ^oC. The nucleotide to protein ratio (N/P) was obtained from the ratio of absorbance at 260 and 280 nm respectively and was found to be 1.9, indicating that the DNA is appropriately free from protein (Dey et al, 2010). The DNA concentration was determined by absorption spectroscopy using the molar absorption coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm for SS-DNA and was found to be 2.0×10^{-4} M (Sastri et al, 2003). The compound was dissolved in 70% ethanol at a concentration of 2 mM. For UV absorption studies 10 mL solution of known concentration of SS-DNA and compound were prepared by varying the concentration of SS-DNA while keeping the concentration of compound fixed. A blank/reference solution was also prepared without compound. Compound-SS-DNA solutions were endorsed to incubate for 30 minutes at room temperature (25±1 ^oC) before the absorption measurements were made. Absorption spectra were recorded using cuvettes of 1 cm path length at room temperature. The intrinsic binding constant "K" was calculated by using the Benesi-Hildebrand Equation given below (Shujha et al, 2010).

$$A_o/(A-A_o) = \varepsilon_G/(\varepsilon_{H-G}-\varepsilon_G) + \varepsilon_G/(\varepsilon_{H-G}-\varepsilon_G) \times 1/K[DNA]$$

Where:

K = *Binding constant*
A_o = *Absorbance of the drug*
A = *Absorbance of the drug-SS-DNA adduct*
ε_G = *Absorption coefficient of the drug*
ε_{H-G} = *Absorption coefficient of the drug-SS-DNA adduct*

The binding constants was obtained from the intercept-to-slope ratios of $A_o/(A-A_o)$ *vs*. 1/[DNA] plots. The change in Gibb's free energy (ΔG) was calculated by using following equation:

$$\Delta G = -RT \ln K$$

Where:

R = *General gas constant, 8.314 J* $K^{-1}mol^{-1}$
T = *Temperature, K*
K = *Binding constant*

2.4. Alkaline Phosphatase Inhibition

For the preparation of assay the method used was similar to as reported earlier with slight modifications (Malik et al, 2011). Working substrate was made by mixing four parts of reagent A [Diethanolamine (pH 9.8) 2 mol/dm^3 and magnesium chloride 0.5 mmol/dm^3] and one part of reagent B [*p*-nitrophenyl phosphate 50 mmol/dm^3]. Substrate was incubated for five minutes at 25 ^oC. In a cuvette 2 mL of the substrate was taken and 40 µL of human serum having the activity of 165 IU/L was added into it. After incubation of 1 minute, the absorbance was recorded to confirm the activity of enzyme. The hydrolyzed *p*-NPP will be of yellow colour due to the formation of *p*-nitrophenol according to the following reaction that absorbs at 405 nm.

$$p\text{-Nitrophenyl phosphate} + Mg^{+2} + H_2O \xrightarrow{ALP} p\text{-Nitrophenol} + Pi$$

Where:

"Pi"= inorganic phosphate

Various amounts of esters were, then, added periodically from the 25 M stock solution to the above essay, incubated for 3 minutes and absorbance was recorded again after 1, 2, 3, 4 and 5 minutes. The average of these five values was used to calculate the percentage inhibition.

2.5. Antimicrobial Activities

The fungicidal and bactericidal activities of carboxylates and their respective esters against various bacteria and fungi were carried out in two steps.

Step 1. Growth Medium, Culture & Inoculum Preparation

The bacterial strains (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pasteurella multocida*) were cultured overnight at 37 ^oC in nutrient agar. The pure bacterial cultures obtained were maintained in the medium in slants and petri plates. For inoculums preparation, 13 g of nutrient broth was suspended in one litre of distilled water, mixed homogenously and autoclaved for 15 minutes at 121 ^oC. Then 10 µL of pure culture of a bacterial strain was added to 100 mL of freshly prepared nutrient broth medium and shacked (140 rpm) at 37 ^oC for 24 hours. The prepared inocula were stored at 4 ^oC. The inocula with 1×10^8 spores/mL were used for activity measurement (Jab-een et al, 2012). The fungal strains (*Alternaria alternata*, *Ganoderma lucidum* and *Penicillium notatum*) were cultured overnight at 28 ^oC using potato dextrose agar. The pure cultures were maintained in sabouraud dextrose agar (SDA) medium in slants and petri plates, which were pre-sterilised in hot air oven at 180 ^oC for 3 hours. These cultured slants were incubated at 28 ^oC for 3-4 days for the multiplication of fungal strains.

Step 2. Antimicrobial Assay by Disc Diffusion Method

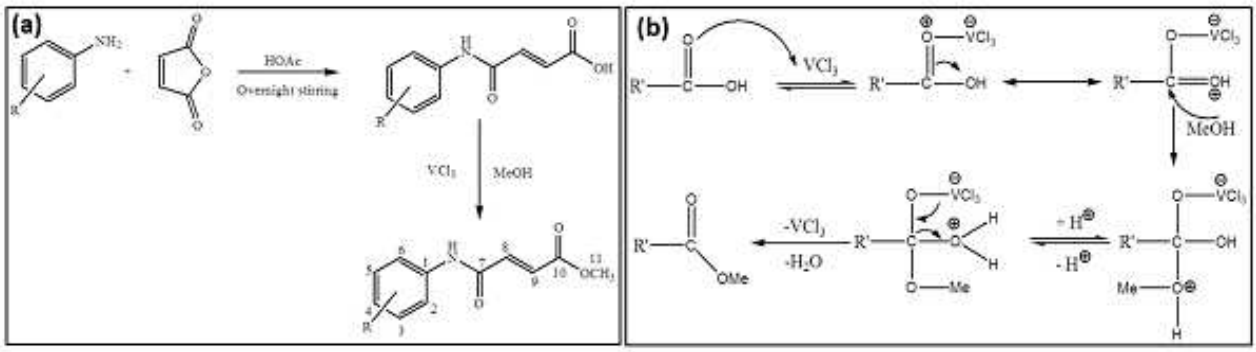
Antimicrobial activities were determined by means of disc diffusion method (Standards, 2006). Nutrient agar 2.8 g (for antibacterial activities) or potato dextrose agar 3.9 g (for antifungal activities) was suspended in 100 mL distilled water and was sterilized by autoclaving at 121 ^oC for 15 minutes. It was then mixed well with 100 µL inoculums and was poured in sterilized petri plates. Finally small filter paper discs (9 mm) each soaked with 100 µL of a specific solution were laid flat on growth medium. The petri plates were then incubated at 37 ^oC for 24 hours or 28 ^oC for 48 hours for the growth of bacteria or fungi respectively. Clear zones of inhibition formed around the discs were measured in millimetres using a zone reader (Huynh et al, 1996 and Bhalodia & Shukla, 2011). The zone of inhibition was calculated by using the following equation:

$$\%\,\text{Growth inhibition} = 100 - \left(\frac{\text{Linear growth in test sample (mm)}}{\text{Linear growth in control (mm)}} \times 100\right)$$

2.6. Synthesis

Substituted carboxylic acids (**HL^1-HL^3**) and their corresponding methyl esters (**1-3**) were prepared by condensation reaction and crystallized in methanol. The generalized chemical reactions and the proposed reaction mechanism are given in **Scheme 1**. All the synthesized compounds were characterised by various types of physical and spectroscopic techniques. Purity of the products was confirmed by their sharp melting points. The proposed structures of compounds were verified by FT-IR, multinuclear NMR (^1H and ^{13}C) studies and single crystal analysis.

2.6.1. Synthesis of HL^1 and Compound (1)



Scheme 1. (a) General Method for the Synthesis of Compounds (b) Proposed Reaction Mechanism

R = 3,4-Chloro, 2,4,6-Chloro, 4-Bromo

HL[1] was synthesized by the condensation of 3,4-dichloroaniline with maleic anhydride. To a solution of maleic anhydride (1 mmol) in acetic acid (HOAc) (300 mL) was added a solution of 3,4-dichloroaniline (1 mmol) in acetic acid (150 mL) and the mixture was stirred at room temperature overnight. The light brown precipitates formed were filtered, washed with cold distilled H_2O (200 mL) and air dried. Crystals suitable for crystallographic study were grown from methanolic solution by slow evaporation at room temperature. Compound (**1**) was obtained as green crystals by refluxing **HL[1]** (3 mmol) with vanadium trichloride (1 mmol) in methanol for 4 to 5 hours.

Compound (1): Yield: 70%; **m.p.** 112 ^{o}C; **Mol. Wt.:** 274.09; **IR(cm^{-1}):** 1707 (-C=O stretching of –$COOCH_3$ group), 1677 (-C=O stretching of -NH-CO-CH- group), 1256 (-C-O stretching of –$COOCH_3$ group), 3303 (-NH stretching of aromatic secondary amine); **^{1}H-NMR (300MHz, $CDCl_3$ δ/ppm):** 7.91 (1H, s, aromatic), 7.38 (1H, d, *J* = 8.7 Hz, aromatic), 7.49 (1H, d, *J* = 8.7 Hz, aromatic), 6.43 (1H, d, *J* = 13.2 Hz, CO-C<u>H</u>=CH), 6.26 (1H, d, *J*= 13.2 Hz, CO-CH=C<u>H</u>), 3.88 (3H, s, -COOC<u>H</u>$_3$), 11.18 (1H, s,-N<u>H</u>-CO); **^{13}C-NMR (75MHz, $CDCl_3$, δ/ppm):** 139.9 (C_1), 121.7 (C_2), 132.7 (C_3), 125.6 (C_4), 127.7 (C_5), 119.3 (C_6), 167.4 (-NH-<u>C</u>O), 137.3 (CO-<u>C</u>H=CH), 130.5 (CO-CH=<u>C</u>H), 161.5 (-<u>C</u>OOCH$_3$), 53.0 (-COO<u>C</u>H$_3$) (Figure 1).

2.6.2. Synthesis of HL[2] and Compound (2)

HL[2] was synthesized with the same procedure as **HL[1]**, but instead of 3,4-dicholoroaniline, 2,4,6-trichloroaniline was used. Brown colored crystals of compound (**2**) were obtained by refluxing **HL[2]** with vanadium trichloride using methanol as a solvent for 4 to 5 hours.

Compound (2): Yield: 72%, **m.p.** 113 ^{o}C; **Mol. Wt.:** 308.53, **IR (cm^{-1}):**1720 (-C=O stretching of –$COOCH_3$ group), 1660 (-C=O stretching of -NH-CO-CH- group), 1217 (-C-O stretching of –$COOCH_3$ group), 3205 (-NH stretching of aromatic secondary amine); **^{1}H-NMR (300 MHz, $CDCl_3$ δ/ppm):** 7.90 (2H, s, aromatic), 6.47 (1H, d, *J* = 13.2 Hz, CO-C<u>H</u>=CH), 6.26 (1H, d, *J* = 13.2 Hz, CO-CH=C<u>H</u>), 3.89 (3H, s, -COOC<u>H</u>$_3$), 11.25 (1H, s, -N<u>H</u>-CO); **^{13}C-NMR (75MHz, $CDCl_3$ δ/ppm):** 126.4 (C_1), 134.1 (C_2 and C_6), 128.5 (C_3 and C_5), 131.0 (C_4), 167.6 (-NH-<u>C</u>O), 138.6 (CO-<u>C</u>H=CH), 133.5 (CO-CH=<u>C</u>H), 161.6 (-<u>C</u>OOCH$_3$), 52.9 (-COO<u>C</u>H$_3$).

2.6.3. Synthesis of HL[3] and Compound (3)

HL[3] was also prepared by adapting the above procedure, by taking 4-bromoaniline and maleic anhydride as starting materials. Light green crystals of compound (**3**) were obtained by refluxing **HL[3]** with vanadium trichloride for 4-5 hours using methanol as a solvent.

Compound (3): Yield: 73%, **m.p.** 95 ^{o}C; **Mol. Wt.:** 283.10, **IR (cm^{-1}):** 1722 (-C=O stretching of -$COOCH_3$ group), 1657 (-C=O stretching of -NH-CO-CH- group), 1214 (-C-O stretching of –$COOCH_3$ group), 3241 (-NH stretching of aromatic secondary amine); **^{1}H-NMR (300 MHz, $CDCl_3$ δ/ppm):** 7.46 (2H, d, *J* = 8.4 Hz, aromatic), 7.59 (2H, d, *J* = 8.4 Hz, aromatic), 6.44 (1H, d,*J* = 13.2 Hz, CO-C<u>H</u>=CH), 6.25 (1H, d, *J* = 13.2 Hz, CO-CH=C<u>H</u>), 3.87 (3H, s, -COOC<u>H</u>$_3$), 11.04 (1H, s, -N<u>H</u>-CO); **^{13}C-NMR (75MHz, $CDCl_3$ δ/ppm):** 140.3 (C_1), 125.2 (C_2 and C_6), 132.0 (C_3 and C_5), 121.6 (C_4), 167.4 (-NH-<u>C</u>O), 136.9 (CO-<u>C</u>H=CH), 132.7 (CO-CH=<u>C</u>H), 161.5 (-<u>C</u>OOCH$_3$), 52.9 (-COO<u>C</u>H$_3$).

3. Results and Discussion

3.1. Crystal Structures of HL[1], Compound (1) and (2)

Crystal data and structure refinements of carboxylic acid **HL[1]** and methyl esters (**1**) & (**2**) are given in Table 1, whereas the selected bond lengths, bond angles and details of hydrogen bonding are listed in Table 2. Figures 2 shows the ORTEP diagrams along with atomic numbering scheme of the compounds. The observed bond lengths and bo-

nd angles in carboxylic acid **HL[1]** and methyl esters (**1)** and (**2)** are almost similar to those reported in literature for other structurally related compounds (Shahid et al, 2003 and Munawar et al, 2010).

is also intramolecular hydrogen bonding between the amide hydrogen and carbonyl oxygen of the ester group (N1—H1--O3). In compound (**2)** there is only one kind of intramolecular hydrogen bonding between the amide hydrogen and carbonyl oxygen (N1—H1--O1).

Table 1. Crystal and Structure Refinements Data for Compounds HL[1], (1) and (2)

Compounds	HL[1]	Compound (1)	Compound (2)
Chemical formula	$C_{10}H_7Cl_2NO_3$	$C_{11}H_9Cl_2NO_3$	$C_{11}H_8Cl_3NO_3$
Formula weight	260.07	274.09	308.53
T / K	293(2)	296(2)	296(2)
Wavelength / Å	1.5418	0.71073	0.71073
Crystal system	Triclinic	Monoclinic	Monoclinic
Space group	$P\ \bar{1}$	P 2_1/c	C 2/c
a / Å	7.1885 (3)	6.8375(3)	28.3957(12)
b / Å	11.6364(5)	17.654(8)	4.6349(2)
c / Å	13.1264(7)	10.5182(5)	19.7123(11)
α / ^{o}	85.180(4)	90	90
β / ^{o}	75.13	97.387(2)	94.019(2)
γ / ^{o}	81.22	90	90
V / Å^3	1047.63(8)	1224.26(10)	2587.98(19)
Z	4	4	8
Absorption coefficient, mm^{-1}	5.524	0.524	0.706
F(000)	528	560	1248
Crystal size, mm	0.5 × 0.3 × 0.3	0.34 × 0.25 × 0.22	0.28 × 0.22 × 0.18
θ range for data collection, ^{o}	3.49 to 62.65	2.28 to 25.25	2.07 to 25.25
Reflections collected	11738	2204	2345
Independent reflections	3312	1808	1842
Goodness-of-fit on F^2	1.031	1.063	1.096
Final R indices [I > 2σ(I)]	R_1 = 0.0318, wR_2 = 0.0899	R_1 = 0.0425; wR_2 = 0.0780	R_1 = 0.0540, wR_2 = 0.0951
R indices (all data)	R_1 = 0.0345, wR_2 = 0.0927	R_1 = 0.0323, wR_2 = 0.0724	R_1 = 0.0371, wR_2 = 0.0782
Data/restrains/parameters	3312/0/289	2204/0/155	2345/0/164
Calculated Density, mg/cm^3	1.649	1.487	1.584

It is interesting to note that in synthesized compounds; the Cl attached to benzene ring lie in the plane of benzene ring to which it is attached. Asymmetric unit of **HL[1]** contains two molecules, while (**1)** and (**2)** exist as monomers. The ORTEP diagram of **HL[1]** (Figure 2) shows that the carboxyl group adopts an antiplanar conformation. There is intermolecular hydrogen bonding between the amino and carboxylic group (N1—H1--O6 and N2—H2--O2) as well as intramolecular hydrogen bonding between carboxylic hydrogen and carbonyl oxygen (O3—H3--O1 and O5—H5--O4). In compound (**1)** intramolecular C—H--O interact-ions, between the benzene ring and the amide group (C2—H2--O1 and C6—H6--O1), generates a S(6) ring motif as well as interaction between vinyl hydrogen and oxygen of the amide group (C9—H9--O1) also generates S(5) ring motif. There

3.3. FT-IR Spectroscopy

Fourier transform infrared spectra of the examined esters show strong signals in the range of 1707-1722 cm^{-1} which are allocated to υ(C=O) and appearance of sharp bands around 1657-1677 cm^{-1} and 1463-1486 cm^{-1} are attributed to the asymmetric and symmetric vibrations of υ(COO), respectively. While υ(COO) bending vibrations are observed in the range of 1434-1448 cm^{-1}. Presence of peaks in the region of 3241-3303 cm^{-1} are due to the υ(NH) stretching vibrations. Aliphatic and aromatic (CH) groups show stretching vibrations around 2951 and 3183 cm^{-1}, respectively. The CH_3 bending vibrations are seen at 1398-1400 cm^{-1}.

Some weak bands due to υ(-CH=CH-) are also visible in the range of 640 to 650 cm^{-1}.

Table 2. Selected Bond Lengths, Bond Angles and Hydrogen Bonding for Compounds HL[1], (1) and (2)

Compound HL[1]			
Bond Lengths (Å)		**Bond Angles (°)**	
Cl2-C11	1.726(2)	O3-C10-C9	120.87(18)
N2-C17	1.339(3)	C17-N2-C14	128.47(16)
N2-C14	1.413(2)	C10-O3-H3	109.5
O3-C10	1.299(3)	C15-C14-N2	116.85(17)
O6-C20	1.212(3)	C7-C8-H8	115.8
O1-C7	1.240(2)	O2-C10-C9	118.67(19)
Compound (1)			
Cl1-C3	1.727(2)	C10-O2-C11	116.39(14)
O2-C10	1.316(2)	C1-N1-C7	128.30(14)
N1-C1	1.402(2)	C1-N1-H1	116.00
C2-H2	0.9300	N1-C1-C6	117.75(14)
O1-C7	1.222(19)	Cl1-C3-C2	117.79(15)
Compound (2)			
Cl1-C2	1.724(3)	C10-O2-C11	115.8(3)
Cl2-C4	1.737(3)	C1-N1-C7	121.32(19)
O1-C7	1.219(3)	C7-N1-H1	119.00
O2-10	1.332(4)	N1-C1-C2	120.0(2)
O2-C11	1.443(4)	N1-C1-C6	122.9(2)
Hydrogen bonding HL[1]			
D-H...A	**d(D-H)**	**d(H...A)**	**d(D...A)**	**<(DHA)**
N1-H1...O6	0.86	2.03	2.867(2)	164.7
N2-H2...O2	0.86	2.03	2.872(2)	166.3
Hydrogen bonding (1)			
N1-H1---O3	0.86	2.11	2.9412(19)	161.00
C2-H2---O1	0.93	2.22	2.835(2)	123.00
Hydrogen bonding (2)			
N1-H1...O1	0.86	1.9900	2.780(3)	153

ls of the chemical shifts of diverse kinds of protons and carbons are given in Materials and Methods section. In the case of ^{1}H-NMR spectra, the formation of esters were confirmed by the disappearance of peak of carboxylic protons in their respective regions and appearance of a sharp singlet at 3.87-3.89 ppm due to the methoxy protons (-OCH_3). ^{13}C-NMR spectra showed peak for methoxy carbon (-CH_3) around 53.0 ppm which also supports the formation of the product.

3.4. Drug DNA Interaction

The binding mode of drug with SS-DNA was checked by UV-Visible absorption spectroscopy by the comparison of absorbance and shift in the wavelength of esters with and without SS-DNA. DNA usually shows intercalation, groove binding and electrostatic mode of interaction with the drug. The intercalative mode of binding is referred as hypochromism and bathochromic shift because of stacking interaction between an aromatic chromophore and the nitrogenous base pairs of DNA. The bathochromic shift is caused by the lowering in π-π* transition energy of the complex due to its ordered stacking between the DNA base pairs after intercalation (Sirajuddin et al, 2013).

The binding modes of all the synthesized compounds with SS-DNA were checked and it was observed that only compound (**1**) showed such interactions might be due to the presence of chloro atoms on vicinal carbons of benzene ring. The absorption spectra of compound (**1**) in the absence (i) and presence (ii–viii) of DNA are shown in Figure 3. There exists single band at 260 nm. With the increase in DNA concentration the transition band of compound (**1**) exhibits hypochromism of 6.0, 10, 12.7, 16.7, 19.5, 23 and 28.15% for 10, 19, 27, 35, 42, 48 and 54 µM of DNA respectively. These spectral characteristics show hypochromism which

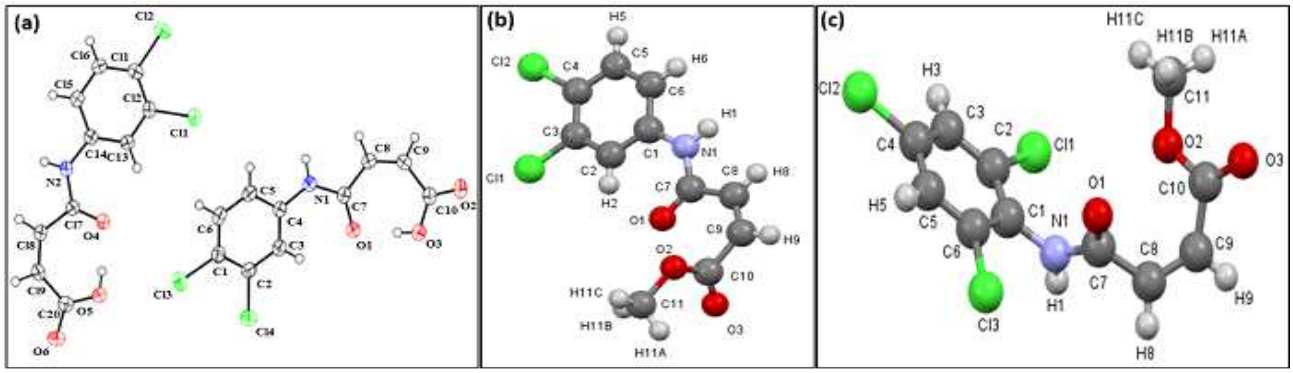


Figure 2. ORTEP Drawing with Atomic Numbering Scheme for (a) HL[1] (b) Compound (1) (c) Compound (2)

3.3. NMR Spectroscopy

Deuterated chloroform ($CDCl_3$) was used to record the ^{1}H and ^{13}C-NMR spectra of the methyl esters **(1-3)**. The detai-

suggests that the compound (**1**) might bind to DNA by an intercalative mode. After intercalating the base pairs of DNA, the π* orbital of the intercalated ligand may couple with π-orbital of base pairs, thus decreasing the π–π* transi-

tion energy. In addition, it shows to some extent interaction with the minor grooves which may be due to hydrogen bonding to bases, typically to N_3 of adenine and O_2 of thymine. Similar findings have been reported by Haq (2002). Same results were obtained by recording the spectrum after 24 hours, which confirms the stability of drug-DNA complex for at least 24 hours.

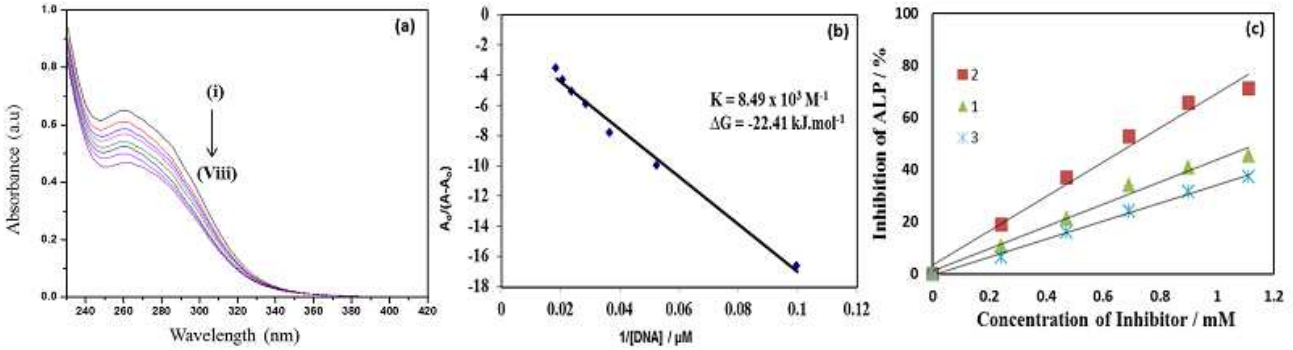


Figure 3. (a) Absorption Spectra of Compound (1) in the Absence (i) and Presence of 10 µM (ii), 19 µM (iii), 27 µM (iv), 35 µM (v), 42 µM (vi), 48 µM (vii) and 54 µM (viii) DNA. The Arrow Direction Indicates Increasing Concentrations of DNA (b) Plot of $A_o/(A-A_o)$ *vs* 1/[DNA] (c) Concentration Dependent Inhibition of Alkaline Phosphatase (ALP) by Esters (1-3)

Binding constant and Gibb's free energy was calculated for compound (**1**) at 260 nm. The binding constant was found to be 8.49×10^3 M^{-1} while the change in Gibb's free energy was -22.41 kJ/mol. Negative value of ΔG shows that the interaction of the drug with DNA is a spontaneous process.

of compounds was increased the activity of enzyme was decreased. Compound (**2**) was observed as the most potent inhibitor among the tested compounds. Maximum enzyme inhibition effect could probably be due to its more electronegative nature, which blocks the active site of ALP, resulting in failure of enzyme molecule to bind with the substrate. Hence, there is decrease in absorption due to less product formation.

3.6. Antimicrobial Activity

Table 3 shows the antimicrobial activity of the compounds.

Table 3. Antibacterial and Antifungal Activities of Carboxylic Acids and their Methyl Esters

Compounds	Antibacterial Activities				Antifungal Activities		
	Average zone of inhibition, mm						
	E. coli	***B. subtilis***	***S. aureus***	***P. multocida***	***A. alternata***	***G. lucidum***	***P. notatum***
Streptomycin	$30^{ab}\pm 0.25$	$30^{ab}\pm 0.22$	$30^{ab}\pm 0.19$	$30^{ab}\pm 0.28$	-	-	-
Fluconazole	-	-	-	-	$38^{a}\pm 0.36$	$41^{a}\pm 0.31$	$35^{bc}\pm 0.33$
HL^1	$20^{bc}\pm 0.15$	$19^{bc}\pm 0.17$	$18^{c}\pm 0.09$	0	$20^{bc}\pm 0.12$	$28^{bc}\pm 0.20$	$44^{ab}\pm 0.31$
1	$31^{ab}\pm 0.28$	$30^{ab}\pm 0.24$	$35^{ab}\pm 0.31$	$32^{ab}\pm 0.29$	$24^{bc}\pm 0.19$	$29^{bc}\pm 0.22$	$48^{ab}\pm 0.30$
HL^2	$16^{c}\pm 0.07$	$16^{bc}\pm 0.08$	$20^{bc}\pm 0.03$	$23^{bc}\pm 0.15$	$21^{bc}\pm 0.17$	$20^{c}\pm 0.13$	$28^{c}\pm 0.17$
2	$38^{a}\pm 0.29$	$34^{a}\pm 0.32$	$39^{a}\pm 0.27$	$34^{a}\pm 0.30$	$23^{bc}\pm 0.16$	$26^{bc}\pm 0.15$	$33^{bc}\pm 0.14$
HL^3	$18^{bc}\pm 0.14$	$15^{c}\pm 0.09$	$24^{bc}\pm 0.17$	$22^{c}\pm 0.16$	$19^{c}\pm 0.11$	$23^{bc}\pm 0.16$	$32^{bc}\pm 0.18$
3	$33^{ab}\pm 0.29$	$34^{a}\pm 0.22$	$39^{a}\pm 0.34$	$33^{ab}\pm 0.25$	$27^{bc}\pm 0.18$	$30^{b}\pm 0.23$	$49^{a}\pm 0.33$

Concentration = 1 mg/mL in DMSO; Standard = Streptomycin (Antibacterial), Fluconazole (Antifungal); 0 = No activity, 5-10 = Activity present, 11-25 = Moderate activity, 26-40 = Strong activity; Antibacterial and antifungal values are mean ± S.D of samples analysed individually in triplicate at $p < 0.1$; Different letters in superscripts indicate significant differences. a = maximum activity, b = intermediate activity, c = minimum activity, ab = activity between maximum and intermediate and bc = activity between intermediate and minimum.

3.5. Enzyme Inhibition Studies

All the synthesized esters were screened for their inhibition against alkaline phosphatase (ALP) and the results are shown in Figure 3c which depicts that the inhibition of the enzyme is concentration dependent. As the concentration

The carboxylic acids and their respective ester derivatives were screened to see their *in-vitro* response against various strains of bacteria (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pasteurella multocida*) and fungi (*Alternaria alternata, Ganoderma lucidum* and *Penicillium notatum*) by disc diffusion method. Streptomycin and Flu-

conazole were used as the positive controls for antibacterial and antifungal screening tests, respectively, while DMSO was used as a negative control. Each paper disc was soaked with sample solution having concentration of 1 mg/mL in DMSO and the zones of inhibition formed in the respective media were measured in millimetres. The data have been summarized in Table 3. The perusal of data reveals that a close relationship exists between structure and activities of the investigated compounds, the esterified products possessed significantly higher activity towards the tested organisms than the respective free carboxylic acid precursors. The potential activities were appreciably enhanced after esterification against bacterial as well as fungal strains. All the alkyl esters exhibited their biological activities even equivalent to or higher than the standard antibacterial and antifungal drugs Streptomycin and Fluconazole, respectively. The enhanced activities may be explained due to the increased lipophilicity on esterification resulting in enhanced permeability and diffusion through cell membranes of microbes thus ultimately leading to increased death rate of the pathogen by esterified product relative to the free carboxylic acid. The difference in the effectiveness of various biocidal agents against different organisms depends upon the permeability through the plasma membrane (Fyles, 2007).

4. Conclusion

Methyl esters of carboxylic acids were synthesised in presence of as catalyst VCl_3. It was believed that Lewis acid "VCl_3" homogeneously catalysed the esterification reaction. Formations of esters were confirmed by their spectrescopic studies like FT-IR, multinuclear NMR and especially XRD. The biological screening of the methyl esters showed enhanced activities against various strains of fungi and bacteria as compared to their respective acids. The esters also have inhibitory effects for ALP. Methyl 4-oxo-4-(2,4,6-trichlorophenylamino)but-2-enoate (compound 1) showed intercalative binding with DNA.

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References

Aalto, T., Firman, M., and Rigler, N. (1953) p-hydroxybenzoic acid esters as preservatives. I. Uses, antibacterial and antifungal studies, properties and determination. **Journal of the American Pharmaceutical Association**, 42(8), pp. 449-457.

Armarego, W.L., and Christina, L.C. (2003) **Purification of Laboratory Chemicals**. 5 ed. Elsevier Science.

Bhalodia, N.R., and Shukla, V. (2011) Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. **Journal of Advanced Pharmaceutical Technology & Research**, 2(2), p. 104.

Boghaei D.M. and Mohebi S. (2002) Synthesis, characterization and study of vanadyl tetradentate Schiff base complexes as catalyst in aerobic selective oxidation of olefins. **Journal of Molecular Catalysis A: Chemical,** 179(1-2), pp. 41-51.

Da Silva, J.A., da Silva, J.J.F., and Pombeiro, A.J. (2011) Oxovanadium complexes in catalytic oxidations. **Coordination Chemistry Reviews**, 255(19-20), pp. 2232-2248.

Dey, S., Sarkar, S., Paul, H., Zangrando, E., and Chattopadhyay, P. (2010) Copper (II) complex with tridentate N donor ligand: synthesis, crystal structure, reactivity and DNA binding study. **Polyhedron**, 29(6), pp. 1583-1587.

Fyles, T.M. (2007) Synthetic ion channels in bilayer membranes. **Chemical Society Reviews**, 36(2), pp. 335-347.

Grishchuk, B., Klimnyuk, S., Simchak, R., Pokryshko, O., Baranovskii, V., and Gorbovoi, P. (2007) Synthesis and antibacterial and antifungal activity of 2-thiocyanato-2-methyl-3-arylpropionic acid allyl esters. **Pharmaceutical Chemistry Journal**, 41(11), pp. 594-595.

Haq, I. (2002) Thermodynamics of drug–DNA interactions. **Archives of Biochemistry and Biophysics**, 403(1), pp. 1-15.

Herbst-Irmer, R., and Sheldrick, G.M. (1998) Refinement of twinned structures with SHELXL97. **Acta Crystallographica Section B**, 54(4), pp. 443-449.

Hugo, A.D.R., and Ayliffe, G.A.J. (1992) **Types of Antimicrobial Agents, Principles and Practice of Disinfection, Preservation and Sterilization**. 2nd ed. Blackwell Scientific Publications, Ltd., Oxford, pp.1-200.

Huynh, Q., Borgmeyer, J., Smith, C., Bell, L., and Shah, D. (1996) Isolation and characterization of a 30 kDa protein with antifungal activity from leaves of Engelmannia pinnatifida. **Biochem. J.**, 316(3), pp. 723-727.

Jabeen, M., Ali, S., Shahzadi, S., Shahid, M., Khan, Q., Sharma, S., and Qanungo, K. (2012) Homobimetallic complexes of ligand having O-and S-donor sites with same and different di-and trialkyl/aryltin (IV) moiety: their synthesis, spectral characterization and biological activities. **Journal of the Iranian Chemical Society**, 9(3), pp. 307-320.

Karade, N., Shirodkar, S., Potrekar, R., and Karade, H. (2004) An exceedingly efficient and chemoselective esteri-

fication with activated alcohols using AlCl3/NaI/CH3CN system. **Synthetic Communications**, 34(3), pp. 391-396.

Limberg, C. (2007) Calixarene-Based Oxovanadium Complexes as Molecular Models for Catalytically Active Surface Species and Homogeneous Catalysts. **European Journal of Inorganic Chemistry**, 2007(21), pp. 3303-3314.

Malik, M.R., Vasylyeva, V., Merz, K., Metzler-Nolte, N., Saleem, M., Ali, S., Isab, A.A., Munawar, K.S., and Ahmad, S. (2011) Synthesis, crystal structures, antimicrobial properties and enzyme inhibition studies of zinc (II) complexes of thiones. **Inorganica Chimica Acta**, 376(1), pp. 207-211.

Maurya, M.R., Sikarwar, S., Joseph, T., Manikandan, P., and Halligudi, S. (2005) Synthesis, characterization and catalytic potentials of polymer anchored copper (II), oxovanadium (IV) and dioxomolybdenum (VI) complexes of 2-(α-hydroxymethyl) benzimidazole. **Reactive and Functional Polymers**, 63(1), pp. 71-83.

Mishra, G.S., da Silva, J.J.F., and Pombeiro, A.J. (2007) Supported bis (maltolato) oxovanadium complexes as catalysts for cyclopentane and cyclooctane oxidations with dioxygen. **Journal of Molecular Catalysis A: Chemical**, 265(1-2), pp. 59-69.

Mohebbi, S., Boghaei, D.M., Sarvestani, A.H., and Salimi, A. (2005) Oxovanadium (IV) complexes as homogeneous catalyst-aerobic epoxidation of olefins. **Applied Catalysis A: General**, 278(2), pp. 263-267.

Munawar, K.S., Ali, S., and Tahir, M.N. (2010) Methyl (2Z)-3-[(4-nitrophenyl) carbamoyl] prop-2-enoate. **Acta Crystallographica Section E: Structure Reports Online**, 67(1), p. o77.

Narasimhan, B., Belsare, D., Pharande, D., Mourya, V., and Dhake, A. (2004) Esters, amides and substituted derivatives of cinnamic acid: synthesis, antimicrobial activity and QSAR investigations. **European Journal of Medicinal Chemistry**, 39(10), pp. 827-834.

Sastri, C., Eswaramoorthy, D., Giribabu, L., and Maiya, B.G. (2003) DNA interactions of new mixed-ligand complexes of cobalt (III) and nickel (II) that incorporate modified phenanthroline ligands. **Journal of Inorganic Biochemistry**, 94(1-2), pp. 138-145.

Shahid, K., Ali, S., Shahzadi, S., Badshah, A., Khan, K.M., and Maharvi, G.M. (2003) Organotin (IV) complexes of aniline derivatives. I. Synthesis, spectral and antibacterial studies of di-and triorganotin (IV) derivatives of 4-bromomaleanilic acid. **Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry**, 33(7), pp. 1221-1235.

Shujha, S., Shah, A., Muhammad, N., Ali, S., Qureshi, R., Khalid, N., and Meetsma, A. (2010) Diorganotin (IV) derivatives of ONO tridentate Schiff base: synthesis, crystal structure, in vitro antimicrobial, anti-leishmanial and DNA binding studies. **European Journal of Medicinal Chemistry**, 45(7), pp. 2902-2911.

Sigel, A., and Sigel, H. (1995) Vanadium and its role for life. In: **Metal Ions in Biological Systems**. vol. 31. CRC Press.

Sirajuddin, M., Ali, S., and Badshah, A. (2013) Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltammetry. **Journal of Photochemistry and Photobiology B: Biology**, 124(1), pp. 1-19.

Standards, N.C.F.C.L. (2006) **Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standards, National Committee for Clinical Laboratory Standards**. CLSI.

Vyskočil, F., Teisinger, J., and Dlouh, H. (1980) A specific enzyme is not necessary for vanadate-induced oxidation of NADH. **Nature**, 286(5772), pp. 516-519.

Supplementary Material

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC for compound **(1),(2)** and **HL^1** are 956771, 956772 and 956773 respectively. Copies of these information may be obtained free of charges from, The Director, CCDC, 12, Union Road, Cambridge CB2 1EZ [Fax: +44 1223 336 033] or deposit@ccdc.cam.ac.uk; or http://www.ccdc.cam.ac.uk