# Synthesis of Calcium bis-(E)-3,5-dihydroxy-7-[4'-(4''-flurophenyl)-2'-cyclopropyl-quinoline-3-yl]-hept-6-enoate a HMG-CoA Reductase Inhibitor via Novel Acetonide Intermediate

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***Abstract*- Calcium bis-(E)-3,5-dihydroxy-7-[4'-(4''-flurophenyl)-2'-cyclopropyl-quinoline-3-yl]-hept-6-enoate 1 a HMG-CoA reductase inhibitor via novel acetonide intermediate 2, were synthesized. The operational simplicity, high purity, eco-friendly conditions and easily scalable process are major benefits of this process. The starting material 3 employed for the preparation were synthesized by known literature method[1].**

***Keywords- Acetonide; HMG-CoA Reductase; Pitavastatin Calcium; Deprotectio; Hydrolysis***

## I. INTRODUCTION

Statin drugs are currently the most therapeutically effective drugs available for reducing the level of Low density lipoprotein (LDL) in the blood stream of a patient at risk for cardiovascular disease. A high LDL in the bloodstream has been linked to the formation of coronary lesions which obstruct the flow of blood and can rupture and promote thrombosis. It is well known that inhibitors against HMG CoA reductase which is rate limiting enzyme for cholesterol biosynthesis[2] have been clinically proved to be potentially useful anti-hyperlipoproteinemic agents and they are considered very effective curative and preventive for coronary artery sclerosis or atherosclerosis[3].

Pitavastatin calcium 1 discovered by Nissan Chemical Industries Limited[4] Japan and developed further by Kowa Pharmaceuticals Tokyo, Japan is a novel member of the medication class of statins. Several methods for the preparation of Pitavastatin calcium are known in Literatures[5-16]. The main highlight of any process in this case would be to maintain the desired stereochemistry in the final product and control the formation of side products in this case the lactone 4. Further the electrochemical behavior associated with the compound i.e reduction or oxidation of functional group in aqueous media will be studied[17-18].

We thus herein report a novel synthetic pathway to prepare calcium bis-(E)-3,5-dihydroxy-7-[4'-(4''-flurophenyl)-2'-cyclopropyl-quinoline-3-yl]-hept-6-enoate 1, pitavastatin calcium via novel acetonide intermediate 2 which not only helps to maintain the desired stereochemistry but also helps to restrict the formation of side products namely lactone 4.

## II. EXPERIMENTAL SECTION

### *A. Materials and Equipments*

All materials were purchased from commercial suppliers and used without further purification. All NMR spectra were recorded on the Avance III Bruker 500 MHz spectrophotometer. IR spectra were recorded in KBr on a SHIMADZU 400-50 infrared spectrophotometer. Mass were recorded on Waters Q-TOF (w) premier spectrometer. Elemental analyses were determined by PERKIN ELMER elemental analysis. The X-ray powder diffractogram were obtained using X'PERT PRO PANalytical Diffractometer. The X-ray generator was operated at 40 mA and 45 kv, using the K-alpha line of copper at 1.54060 $A^o$ as the radiation source. It was scanned in the diffraction range of $4.0^o$ to $30.9^o$ 2Θ at a scan rate of $0.020^o$ 2Θ. Commercially available ethanol and ethyl acetate were used as such without purification.

### *B. Preparation of (3R,5S)-6-[(1E)-2[2-Cycloprop--yl-4-(4-Flurophenyl)-3-Quinoli-nyl]-ethenyl]-2-2-Dimethyl-1,3-Dioxane-4-Acetic Acid Sodium Salt, Compound (2):*

2.0g of 1,3-Dioxane-4-acetic acid,6-[2-[2cyclopr- -opyl-4-(4-fluorophenyl)-3-quinolinyl]ethenyl]-2,2-dimethyl-,1,1dimethyl ethyl ester, [4R[4,6(E)]] was taken in 50 ml ethanol to it, a solution of 1.54 g of sodium hydroxide in 20 ml water was added. The reaction mixture was then maintained at 40- $45^o$ C for 5-6 h. The reaction mass was then filtered through a celite bed which was washed with 10 ml of ethanol. The total filtrate was then concentrated under vacuum at $50^o$C to an oily residue. To this oily residue 50 ml water followed by 20 ml of ethyl acetate was added. The aqueous layer was separated and washed with 20 ml of ethyl acetate. The aqueous layer was separated and concentrated under high vacuum at $50^o$C for 8 h which afforded

1.4 g of the compound 2 as a white crystalline powder.

Melting Point: 92° C; yield 74%; IR $\upsilon_{max}$ (KBr) $cm^{-1}$: 3428, 2994, 1567, 1512, 1489, 1409, 1265, 1212, 1159, 1068, 919, 834, 765, 558. $^{1}$H-NMR (500MHz, CDCl3): δ 0.94-1.02 (m, 1H), 1.06-1.1 (m, 2H), 1.35 -1.50(m, 8H), 2.23(m, 2H), 2.37 (m, 1H), 4.28 (m, 1H), 4.30 (m, 1H), 5.48 (dd, 1H), 6.50 (d, 1H), 7.05-7.26 (m, 6H), 7.48 (m, 1H), 7.86 (d, 1H). $^{13}$C-NMR & DEPT (125.76MHz, CDCl3): δ 9.87(CH2), 10.10(CH2), 15.37(CH3), 19.26(CH3), 29.28(CH2), 35.91(CH2), 44.16(CH-2), 66.76(C-O), 69.27(C-O), 98.61(C), 114.50-(C=C), 114.55(C), 115.67(=CH), 115.72(=CH), 124.89(C=C),125.28(=CH),125.31(=CH),125.73-(=CH) ,128.24(=CH), 128.36(=CH), 131.19(=CH), 131.49(C), 132.79(=CH), 136.96(C), 143.65(C), 146.19(C), 160.60(C), 162.56(C), 178.26(C=O). ESI-MS: Molecular ion m/z (int. %) $[M+H]^+$ 462 (100), 175 (47), 301 (20), 506 (47); EI calcd for $C_{28}H_{28}FNO_4$ $(M+H)^+$ 462.52, found 462.221. Anal. Calc. for $C_{28}H_{27}FNNaO_4$: % of C = 69.49, % of H = 5.58, % of N = 2.80. Found: % of C = 69.46, % of H = 5.53, % of N = 2.80.

*C. Preparation of (3R,5S)-6-[(1E)-2[2-Cyclopropyl-4-(4-flurophenyl)-3-Quinoli-nyl]-ethenyl]-2-2-Dimethyl-1,3-Dioxane-4-Acetic Acid Potassium Salt：*

Replacing sodium hydroxide by potassium hydroxide in the above procedure, 1.37 g of the title compound was prepared as a white crystalline powder.

Melting Point: 105 ° C; yield 70%; IR $\upsilon_{max}$ (KBr) $cm^{-1}$: 3428, 2994, 1567, 1512, 1489, 1409, 1265, 1214, 1159, 1068, 972, 919, 834, 765, 557.

*D. Preparation of Calcium Bis-(E)-3R,5S-Dihydroxy-7-[4'-(4''-Flurophenyl)-2'-Cyclopropyl-Quinoline-3-yl]-Hept-6-Enoate (Pitavastatin Calcium), Compound (1)：*

2.0 g of 1,3-Dioxane-4-acetic acid,6-[2-[2cyclopropyl-4-(4-fluorophenyl)-3-quinolinyl]ethenyl]-2,2-dimethyl-,1,1 dimeth - yl ethyl ester, [4R[4,6(E)]] (3) was taken in 50 ml ethanol to it a solution of 1.54 g of sodium hydroxide in 20 ml water was added. The reaction mixture was then maintained at 40-45° C for 5-6 h. The reaction mass was then filtered through a celite bed which was washed with 10 ml of ethanol. The total filtrate was then concentrated under vacuum at 50°C to an oily residue. To this oily residue 50 ml water followed by 20 ml of ethyl acetate was added. The aqueous layer was separated and washed with 20 ml of ethyl acetate. To the obtained aqueous layer 10ml of aqueous hydrochloric acid followed by 1 gm calcium chloride monohydrate was added at 0-5°C and stirred for 1hour. The precipitated solid was then filtered and washed with excess water and finally dried under high vacuum at 50° C for 18 h which afforded 1.8 g of the compound 1 as a white powder.

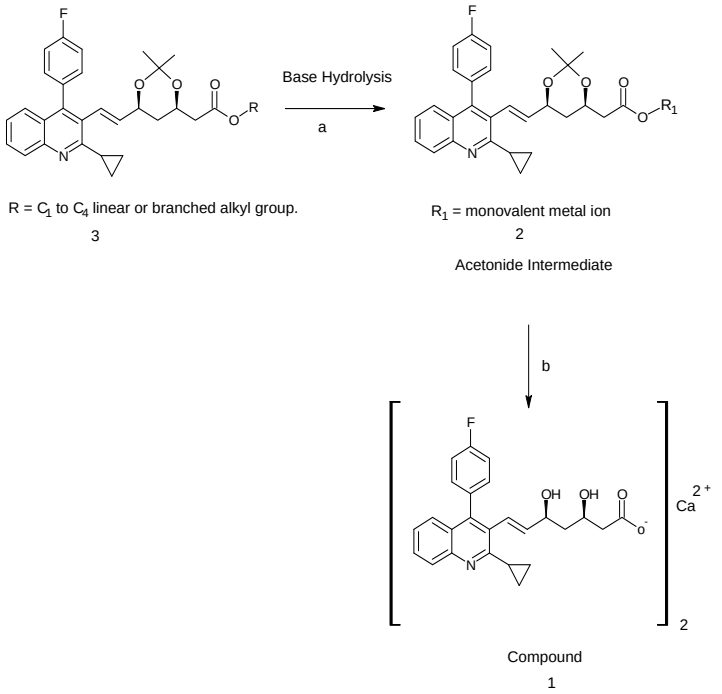
Melting Point: 207° C; yield 50.0%; IR $\upsilon_{max}$ (KBr) $cm^{-1}$: 3366, 2911, 1603, 1567, 1513, 1488, 1416, 1313, 1275, 1221, 1158, 1065,972, 843, 763. $^{1}$H-NMR (500MHz, DMSO-d6): δ 1.01 (m, 2H), 1.09 (m, 1H), 1.19 (m, 2H), 1.41 (m, 1H), 1.98 (dd, 1H), 2.11(d, 1H), 2.50 (m, 2H), 3.66(m, 1H), 4.13 (m, 1H), 4.95 (s, 1H), 5.58 (dd, 1H), 6.49 (d, 1H), 7.35 (m, 6H), 7.59 (m, 1H), 7.83 (d, 1H). $^{13}$C-NMR & DEPT (125.76MHz, DMSO-d6): δ 11.12(CH2), 11.23(CH2), 15.80(CH3), 44.29(CH2), 44.61(CH2), 66.61(C-O), 69.34(C-O), 115.53 (C=C), 115.62(CH), 115.79(CH), 123.59(CH), 126.07(C=C), 128.79(CH), 129.20(CH), 130.07(CH), 132.30(CH), 132.56(CH), 133.51(C), 142.60(C), 144.09(C), 146.37(C), 161.02(C), 163.00(C), 179.13(C=O). ESI-MS: Molecular ion peak $(M+1)^+$ observed at 422 amu. HPLC Purity: 99.20%, (Lactone impurity: 0.09%), any max. individual impurity: 0.32%

## III. RESULTS AND DISCUSSIONS

The synthesis of the Compound 1 as depicted in Scheme 1 was initiated by preparing the key starting material 3 (Fig. 3) by known literature methods[2]. Then synthesis of monovalent metal ion derivatives (acetonide intermediate) 2 (like sodium or potassium) were attempted wherein the starting material 3 as its methyl ester was hydrolyzed with the desired monovalent metal hydroxide (like sodium hydroxide or potassium hydroxide) in a suitable water miscible solvent. The prepared sodium (Fig. 2) and potassium acetonide derivatives 2 were isolated and characterized successfully.

The next efforts were made for converting the so prepared novel acetonide intermediate 2 to Pitavastatin calcium 1 (Fig. 1) wherein first 2 was subjected to acid treatment for deprotection of the acetonide group to free hydroxy acid group which was then treated insitu with calcium source like calcium chloride, calcium acetate or calcium hydroxide most preferably calcium chloride dehydrate to afforded the Compound 1 in 50% yield as in Scheme1. The main highlight of this process is to maintain the desired stereochemistry in the final product and control the formation of side products in this case the lactone 4 (Fig. 4) which was formed during deprotection of ketal group using aqueous hydrochloric acid. Lactone impurity was substantially removed during ethyl acetate washing and it was observed that the prepared Compound 1 was not contaminated with the undesired isomer and lactone impurity.

IR spectrum of Compound 1 exhibited an absorption band at 3466 $cm^{-1}$ accounted for the characteristic -OH stretching vibration. A band at 1603 $cm^{-1}$ was observed, accounted for C=N stretching for aromatic quinoline ring. A characteristic band of substituted olefin was observed at 763 $cm^{-1}$. The $^{1}$H-NMR of Compound 1 showed two double doublets at δ 1.99 and δ 2.12 respectively attributed to single proton of >OC-$H_2$C-COO- group. Two prominent double doublets were observed at δ 5.58 and at δ 6.49 respectively each attributable to single olefinic proton.




Scheme 1. Reagents and conditions: (a) Sodium hydroxide or potassium hydroxide, water, 40-45$^{o}$ C, 5-6 hours, (b) Hydrochloric acid (aq), Calcium chloride , Water, 0-5$^{o}$ C, 60.0 min

## IV. CONCLUSION

In conclusion, we have developed a new and highly efficient neat reaction protocol for Compound 1. The primarily advantage of the present protocol need not require any purification, fairy good yield and eco-friendly. Secondly on the basis of the result obtained, the prepared Compound 1 was not contaminated with the undesired isomer and lactone impurity 4.

## ACKNOWLEDGEMENT

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**FIGURES:**

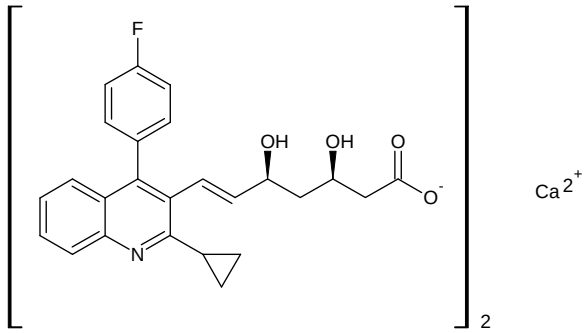



Fig 1: Calcium bis-(E)-3R,5S-dihydroxy-7-[4'-(4''-flurophenyl)-2'-cyclopropyl-quinoline-3-yl]-hept-6-enoate (Pitavastatin calcium).

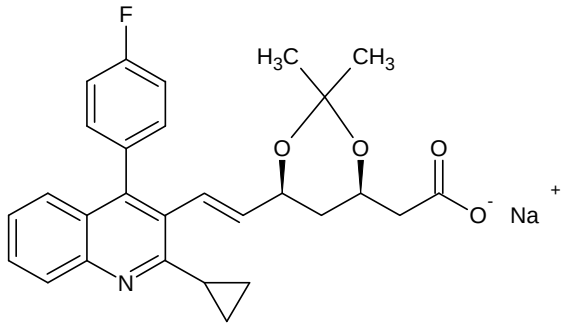
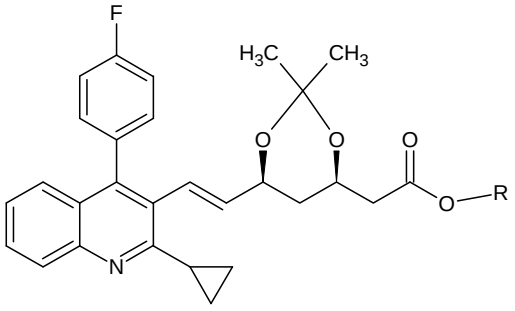



Fig 2: (3R,5S)-6-[(1E)-2[2-cyclopropyl-4-(4-flurophenyl)-3-quinolinyl] ethenyl]-2-2-dimethyl-1,3-dioxane-4-acetic acid sodium salt, (Acetonide Sodium, compound (2)).




R = C 1 to C 4 linear or branched alkyl group.

Fig 3: 1,3-Dioxane-4-acetic acid,6-[2-[2cyclopropyl-4-(4-fluorophenyl)-3-quinolinyl]-ethenyl]-2,2-dimethyl-,1,1dimethyl alkyl ester, [4R[4,6(E)]], compound (3).

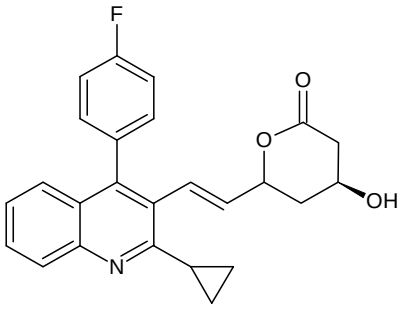



Fig 4: (4R,6S,E)-6-[2-[2-Cyclopropyl-4-(4-fluorophenyl)-quinolin-3-yl] vinyl] tetrahydro-4-hyd-roxypyran-2-one (Lactone impurity 4 ).

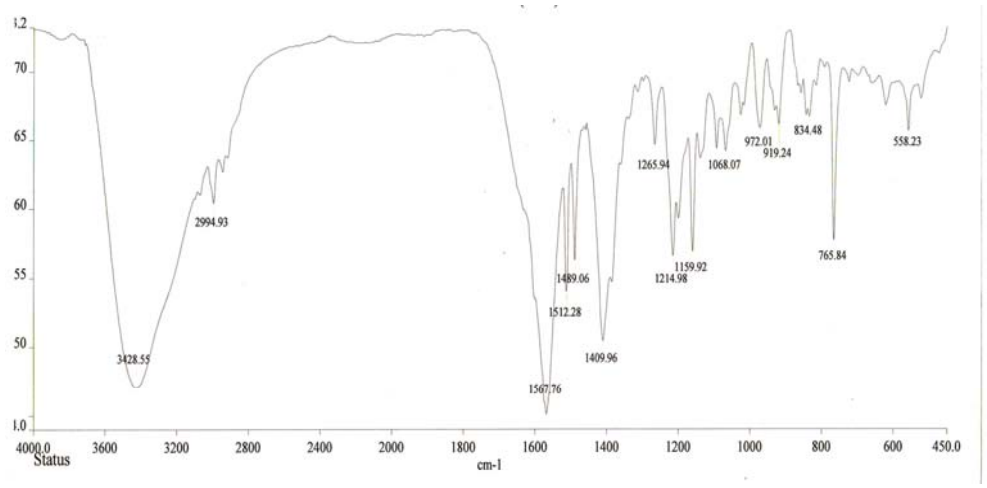



Fig 5: Infra Red (IR) spectrum of compound 2 (Acetonide Sodium)

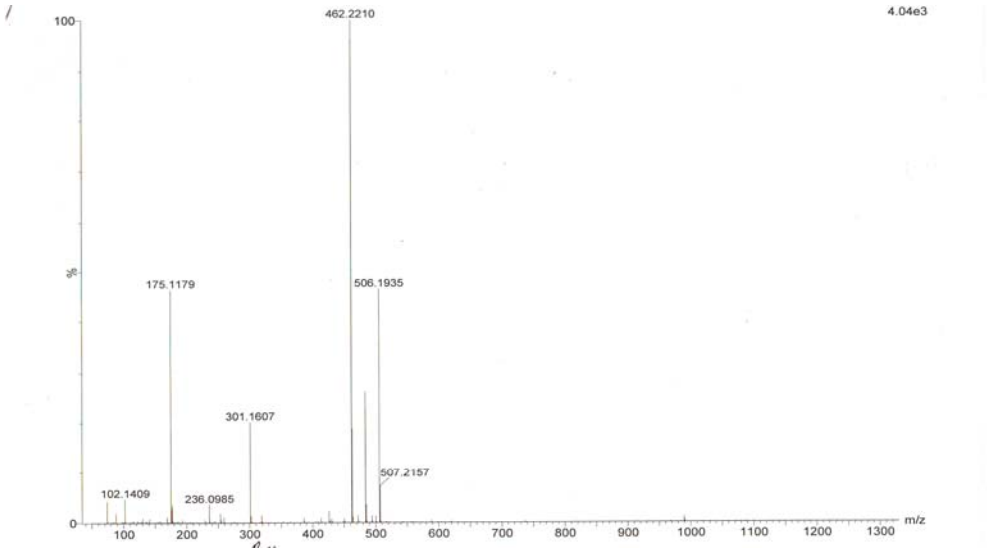



Fig 6: Mass Spectrum of compound 2 (Acetonide Sodium)

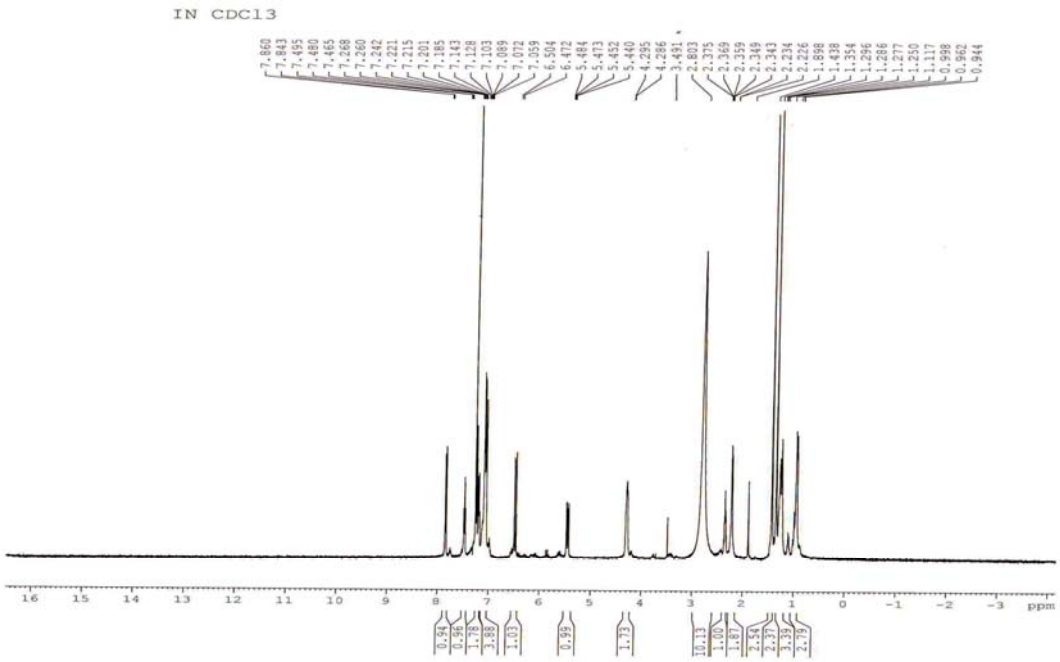



Fig 7: $^{1}$H-Nuclear Magnetic Resonance (NMR) Spectrum of compound 2 (Acetonide Sodium) in $CDCl_3$

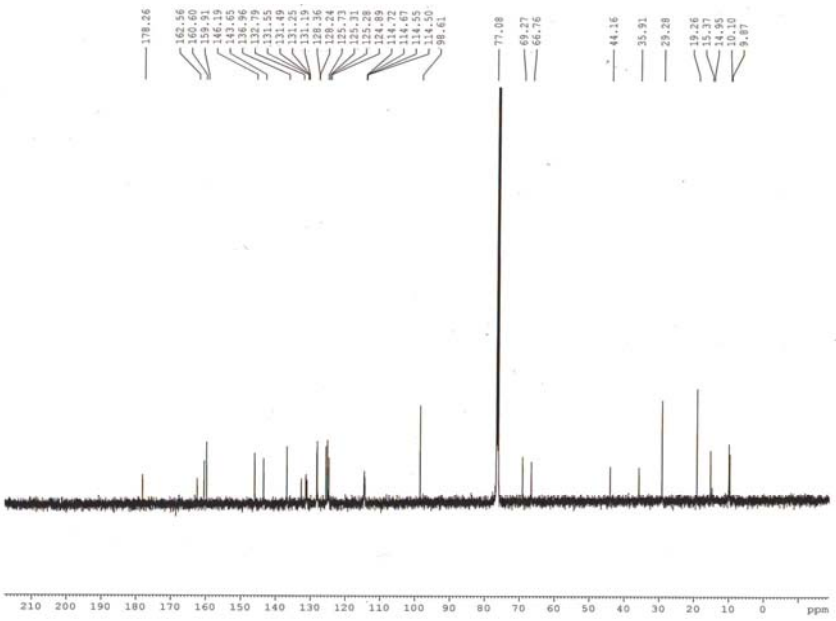
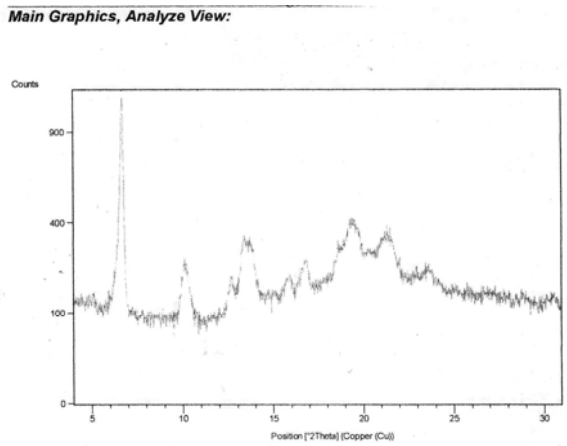



Fig 8: $^{13}$C-Nuclear Magnetic Resonance (NMR) Spectrum of compound 2 (Acetonide Sodium) in $CDCl_3$




**Peak List:**

| Pos. [°2Th.] | Height [cts] | FWHM [°2Th.] | d-spacing [Å] | Rel. Int. [%] |
|---|---|---|---|---|
| 6.6794 | 1009.62 | 0.2180 | 13.22270 | 100.00 |
| 10.1361 | 161.04 | 0.4037 | 8.71979 | 15.95 |
| 13.5158 | 226.06 | 0.7627 | 6.54604 | 22.39 |
| 16.7842 | 82.87 | 0.3518 | 5.27794 | 8.21 |
| 19.3435 | 177.77 | 0.8260 | 4.58503 | 17.61 |

Fig 9: Powder X-Ray diffraction (XRD) pattern of compound 2 (Acetonide Sodium)

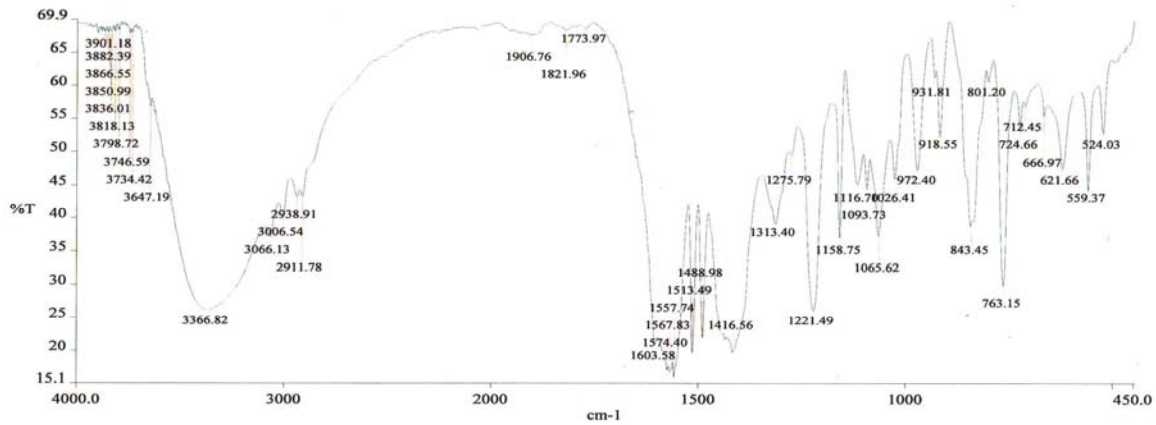



Fig 10: Infra Red (IR) spectrum of compound 1 (Pitavastatin Calcium)

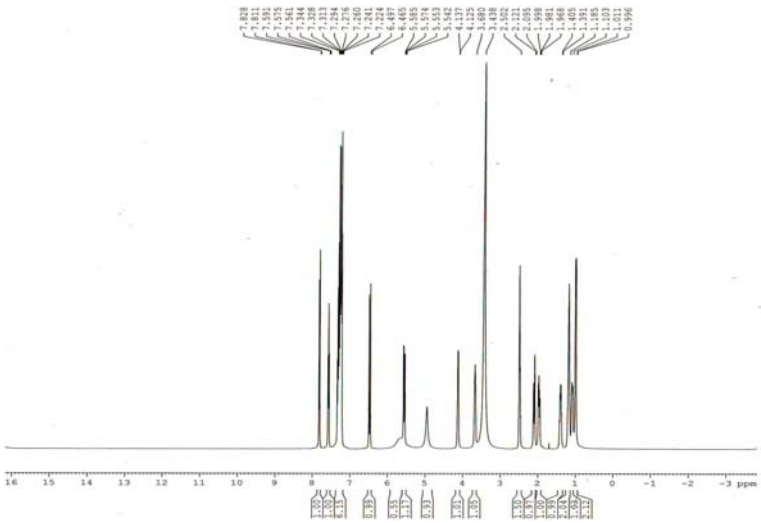


Fig 11: [1]H-Nuclear Magnetic Resonance (NMR) Spectrum of compound 1 (Pitavastatin Calcium) in DMSO [2]

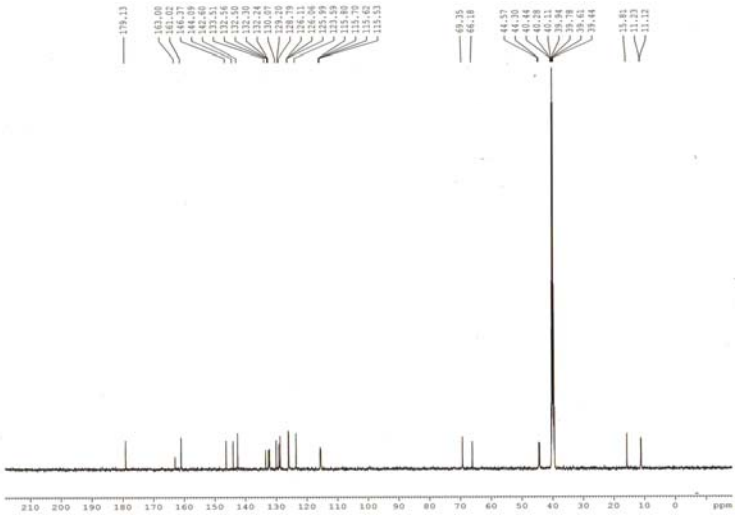


Fig 12: [13]C-Nuclear Magnetic Resonance (NMR) Spectrum of compound 1 (Pitavastatin Calcium) in DMSO

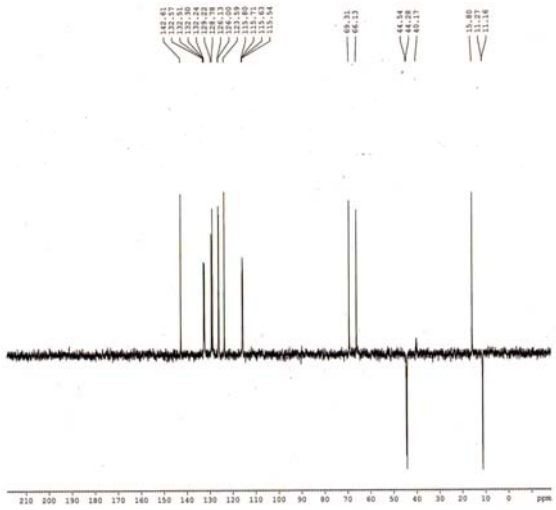


Fig 13: DEPT Nuclear Magnetic Resonance (NMR) Spectrum of compound 1 (Pitavastatin Calcium) in DMSO