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Synthesis of Pyrazolo Linked Pyridine Carboxylic Acids and Evaluation of their Liquid Crystal and Biological Studies

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Abstract

The present work deals with the synthesis and characterization of the pyrazole substituted pyridine carboxylate and carboxylic acid. The synthesized compounds are key intermediates for the synthesis of Apixaban. Apixaban is an anti-thromboembolic drug and also used as an anti-coagulating agent. Iodine substituted piperidine is the precursor material, it is treated with the chloroform and lactam to give substituted pyridine. The substituted pyridine is reaction with morpholine and the morpholine substituted compound is treated with an hydrazono acetate to give the piperidine carboxylate and limited hydrolysis to give pyridine carboxylic acids. The intermediate carboxylates and carboxylic acids are characterized for their biological and liquid crystal studies. The synthesized compounds have not showing any biological activity towards antibacterial and anti fungal assay. Further the final compounds showing very good activity towards the liquid crystalline studies. Carboxylic acid showing the very good liquid crystalline Properties. The synthesized compounds are analysed by ^{1}H-NMR, ^{13}C-NMR and LC-MS. The liquid crystal studies are characterized by Polarising optical microscopy and Differential scanning calorimetry.

Keywords: Piperidines; Pyridines; Liquid crystals; Biological studies

Introduction

The present work explores the synthesis of key intermediate for the preparation of Apixaban. Apixaban is an anit-thrombotic in market referred as Eliquis. Numerous methods for the synthesis of apixaban have been reported, mainly relying on the use of organic iodide [1-3].

Eliquis or Apixaban is an extremely effective, choosy and vocally available inhibitor of blood coagulation factor Xa (fXa), was designed and developed in a later-stage private clinical trial for the anticipation and cure of thromboembolic diseases by Bristol-Myers Squibb [4,5]. The key intermediate for the preparation of apixaban was synthesized by 4-methoxy aniline by di azotization method followed by Japp-Klingemann reaction [6-8] with 2-chloro ethyl acetate to give pyrazolo pyridine carboxylate. The pyridine carboxylate was treated with Trifluoroacetic acid and Triethyl amine to form tetra hydro carboxylate and further undergo hydrolysis to give pyridine substituted acids.

To study the diverse activity of the synthesized compounds here we are studied both biological and liquid crystal activities. As for the literature survey the synthesized compounds 6 and 7 are majorely exposured for their biological and pharmacological activities. In the present work we are studied both the biological and liquid crystal studies, but the synthesized compounds not shown prominent activity towards the biological studies. The liquid crystal studies are not studied so for this type of moieties, so the present work is novel to liquid crystal studies.

Materials and Methods

The chemical ingredients, vi, 1-(4-iodophenyl)piperidin-2-one, procured from Sigma Aldrich, USA. Chloroform and phosphorous pentachloride received from SRL, India. Morpholin received from Sigma Aldrich, USA. Triethyl amine and trifluoro acetic acid received from SRL India and all are in analytical grade quality and used as received. Ethanol is procured from Changshu Yanguan Chemical, Silica gel (60-120 mesh size) for column chromatography was procured from LOBA chemie India. The proposed structure for the intermediate compounds and that of the final compound are confirmed by the H^1 and ^{13}C NMR spectra obtained using an Agilent (400 MHz) NMR spectrometer, Deuterated chloroform and deuterated dimethyl sulfoxide as a solvent is procured from Sigma Aldrich, USA and Tetra methyl Silane as internal standard. The following notations denoted the peak types in the spectra: singlet(s), doublet(d), and doublet of doublets(dd), triplet(t), quartet(q) and multiplet (m). Infrared spectra (IR) were using Perkin Elmer spectrometer. The H^1 NMR and IR spectra are used for the confirmation of the molecular structure hydrogen bonding and the purity of the sample. Mass spectra (LC-MS) were obtained using water LCMS spectrometer. Elemental analysis was carried out in a Fissions EA 1108 CHN instrument. Differential scanning calorimetry (DSC) thermograms were obtained using Q-20 TA Instruments, USA. Heating rate was 1°C min^{-1}. The LC phases were characterized by their texture studies carried out using an Olympus BH-2 Polarizing Optical Microscope, fitted with Metler FP52 hot stage and a Metler FP% controller, samples were prepared as thin films between a glass slide and a cover slip. Column chromatography was carried out using a silica gel (60-120 mesh) as the stationary phase. Thin layer chromatography (TLC) was carried out on aluminum sheets coated in Merck Kieselgur silica gel 60, eluting with petroleum ether and ethyl acetate.

Experimental

Synthesis of 3,3-dichloro-1-(4-iodophenyl)piperidin-2-one

Phosphorus pentachloride (3.61 g, 0.017 mol) was added to a solution of 1-(4-iodophenyl) piperidin-2-one (15.0 g, 0.04983 mol) and chloroform (75 mL). The reactant mixture was refluxed for about 4-5 hours, cooled to room temperature and then poured into ice-cold water (50 mL) and quenched thoroughly. Extracted with chloroform 3 × 40 mL, gave the tilted compound as a pale yellow solid with yield = 85%.

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mp:153-155°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23-2.76 (m, 2H), 2.89-2.92 (m, 2H), 3.73(t, J= 6 Hz, 2H), 7.04(d, J= 8.8 Hz, 2H), 7.73(d, J= 8.4 Hz, 2H); IR (KBr) v 3092, 2949, 2896, 2855, 1679, 1634, 1479, 1310, 1191, 1001, 789, 523 cm^{-1}; LC-MS 370 (M^{+1}).

Synthesis of 1-(4-iodophenyl)-3-morpholino-5,6-dihydropyridin-2(1H)-one

The compound 2 (5 g, 0.0135 mol) and a suspension of the crude α, α-dichlorolactam (9, 3.5 g, 0.095 mmol) in morpholine (100 mL) was warmed to reflux (110°C) for 4 hrs. The solid morpholine hydrogen chloride salt was precipitated out from the reaction mixture at 60°C. Water (15 mL) was introduced to the reaction mixture at 60°C, and the resulting solution was further cooled down to 5-8°C (ice bath). Water (20 mL) was then added dropwise to the cooled reaction mixture at 5-8°C with constant stirring. The intense yellow to beige solids were precipitated out straight away from the reaction mixture after addition of water. The early addition rate should be well controlled to evade large solid mass generation. The mixture was kept stirring for another 40 minutes at 5-8°C before the solids were collected by filtration. The solids were washed with water (2 × 25 mL) and hexane (25 mL) and dried in vacuum at 40-60°C for 18 hours to constant weight. The simple, preferred product 3 with 85% was obtained as light-yellow crystals, which were found to be essentially pure by HPLC and was directly employed in the following reaction without further purification.

^{1}H-NMR Data; (400 MHz, $CDCl_3$) δ 2.49-2.50 (m, 2H), 2.88-2.90 (m, 4H), 3.77 (t, 6.8 Hz, 2H), 3.82 (t, 4.6 Hz, 4H), 5.66 (t, J= 4.8 Hz, 1 H), 7.10 (d, J= 9.2 Hz, 2H) 7.68 (d, J= 8.8 Hz, 2H)

IR Data; (KBr) v 3058, 2955, 2887, 2837, 1654, 1618, 1481, 1309, 1258, 1214, 1115, 1060, 931 cm^{-1}, LC-MS:385 (M^{+1})- m/z: 384.03 (100.0%), 385.04 (16.5%), 386.04 (1.7%)

Elemental Analysis: Calculated: C, 46.89; H, 4.46; I, 33.03; N, 7.29; O, 8.33 Observed: C, 46.99; H, 4.36; I, 33.23; N, 7.19; O, 8.25.

Synthesis of (Z)-ethyl 2-chloro-2-(2-(4-methoxyphenyl) hydrazono)acetate

p-Anisidine (8 g, 0.065 mol) was dissolved in a solution of concentrated HCl (20 mL) and water (50 mL) and cooled to 0-5°C. To this solution was added dropwise an aqueous solution (H_2O, 30 mL) of sodium nitrite (4.7 g, 0.068 mol) as to maintain the temperature below -5°C. Once the addition was complete, the diazotized product was stirred for 20 min at 0°C followed by the sequential addition of ethyl chloroacetoacetate (11 g, 0.065 mol), ethanol (50 mL), sodium acetate (16 g, 0.198 mol), and water (150 mL). The reaction mixture was progressively warmed to room temperature and stirred for further 2 hrs. At this point the product that precipitated as a black solid was filtered, washed with excess water, and dried in vacuum to give the preferred product with 88%. Yield.

Analytical data:

H^1-NMR Data: ^{1}H NMR ($CDCl_3$) δ: 8.28 (s, 1H), 7.18 (d, J = 9.1 Hz, 2H), 6.90 (d, J = 9.2 Hz, 2H), 4.41(q, J = 7.0 Hz, 2H), 3.80 (s, 3H), 1.42 (t, J=7.3 Hz, 3H) ppm. LC-MS Data: m/z: 255.06 (99.6%), 256.06 (32.0%), 256.06 (12.5%), 258.06 (4.01%), 258.06 (1.3%).

Elemental Analysis: Calculated: C, 51.47; H, 5.10; Cl, 13.81; N, 10.91; O, 18.70 Observed Value: C, 51.50; H, 5.08; Cl, 13.71; N, 10.81; O, 18.50.

Ethyl 6-(4-iodophenyl)-1-(4-methoxyphenyl)-7a-morpholino-7-oxo-3a,4,5,6,7,7a hexahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate

Equivalent stoichiometric quantity of compound **3** (5 g, 0.013 mol) and **4** (3.35 g, 0.013 mol) are refluxed for 12 hours in a toluene (50 ml) as a solvent and triethyl amine as a base. The reaction was monitored by TLC, After the completion of the reaction, the reaction mass was cooled to room temperature and concentrated under reduced pressure and the concentrate was extracted with ether and neutralized by 1N HCl and the organic layer was concentrated under reduced pressure and the recovered crude product was washed with the water and ethyl acetate finally dried and recrystallised with ethanol to get a tilted product **(5)** with 70% yield and finally given for organic analysis.

Analytical data:

mp: 173-175°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (t, J= 7.2 Hz, 3H), 3.32 (t, J= 6.6 Hz, 2H), 3.80 (s, 3H) 4.09 (t, J= 6.6 Hz, 2H), 4.45 (q, J=6.1 Hz, 2H), 6.91 (d, J= 9.2 Hz, 2H) 7.07 (d, J= 8.4 Hz, 2H) 7.46 (d, J= 8.8 Hz, 2H) 7.67 (d, J= 8.4 Hz, 2H); IR (KBr) v 3076, 2970, 2925, 2843, 1713, 1675, 1509, 1249, 1136, 1022, 839 cm^{-1}; MS 518 (M^{+1}).

synthesis of Ethyl 6-(4-iodophenyl)-1-(4-methoxyphenyl)-7-oxo-3a,4,5,6,7,7a-hexahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylate.

The synthesized compound **5** (1.5 g, 0.0024 mol) is treated with trifluoro acetic acid in methanol as a solvent. The reaction mas was refluxed for 18 hours in a sealed tube. The reaction progress was monitored by TLC by ethyl acetate and petroleum ether (n-hexane 7:3 ratio). After the completion of the reaction the reaction mass was concentrated under reduced pressure and extracted with ethyl acetate and water and then washed with sodium bicarbonate. Then the organic layer was separated and concentrated under reduced pressure to give the pale yellow product **(6)** with 75% yield, finally the compound was recrystallised with ethanol and the pale yellow was separated out.

Analytical data:

solid, yellow colour, m.p; 244°C. H^1 NMR ($CDCl_3$, 400 mHz) δ: 7.2 Hz, 3H), t, (J=6.8, 2H), t, (J=6.8, 2H), q, J=7.2, 2H), (m, 2H), (m, 2H), (m, 2H) ^{13}C NMR ($CDCl_3$, 400 mHz), δ: 14.4, 21.4, 50.8, 55.4, 61.2, 90.9, 113.6, 126.8, 126.9, 127.2, 132.4, 132.8, 137.8, 139.0, 141.4, 156.9, 159.9, 162.0, LC-MS: observed mass: 517.9792 [M^{+1}], Calculated mass: 518.05 [M^{+1}].

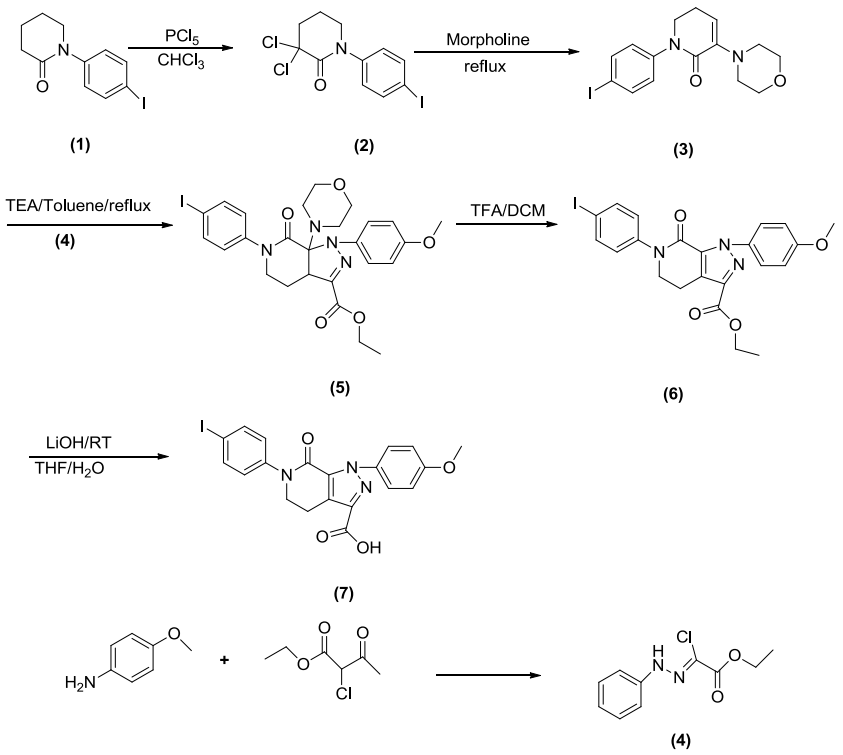
Synthesis of 6-(4-iodophenyl)-1-(4-methoxyphenyl)-7-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxylic acid.

To a weighed quantity of ester intermediate (1 equiv.) in MeOH-H_2O (3:1 ratio), was added NaOH(3 equiv.) and stirred at RT for 10-15 hours. The solvent was removed under reduced pressure and the residue was neutralized/acidified to ph 4 with 1N HCl at 0°C and extracted with ethyl acetate (Filtration can be done if the precipitate was formed) and distilled under reduced pressure to obtain the titled product **7** (Scheme 1).

Analytical data: solid, yellow colour.

^{1}H-NMR ($CDCl_3$, 400 mHz) δ: 7.2 Hz, 3H), t, (J=6.8, 2H), t, (J=6.8, 2H), m, 2H), (m, 2H), (m, 2H) 11.0 (Figure 1).

^{13}C NMR ($CDCl_3$, 400 mHz), δ:14.4, 21.4, 50.8, 55.4, 61.2, 90.9, 113.6, 126.8, 126.9, 127.2, 132.4, 132.8, 137.8, 139.0, 141.4, 156.9, 159.9,



Reaction scheme for the synthesis of compounds 6 and 7

Scheme 1: Reaction scheme for the synthesis of compounds 6 and 7.

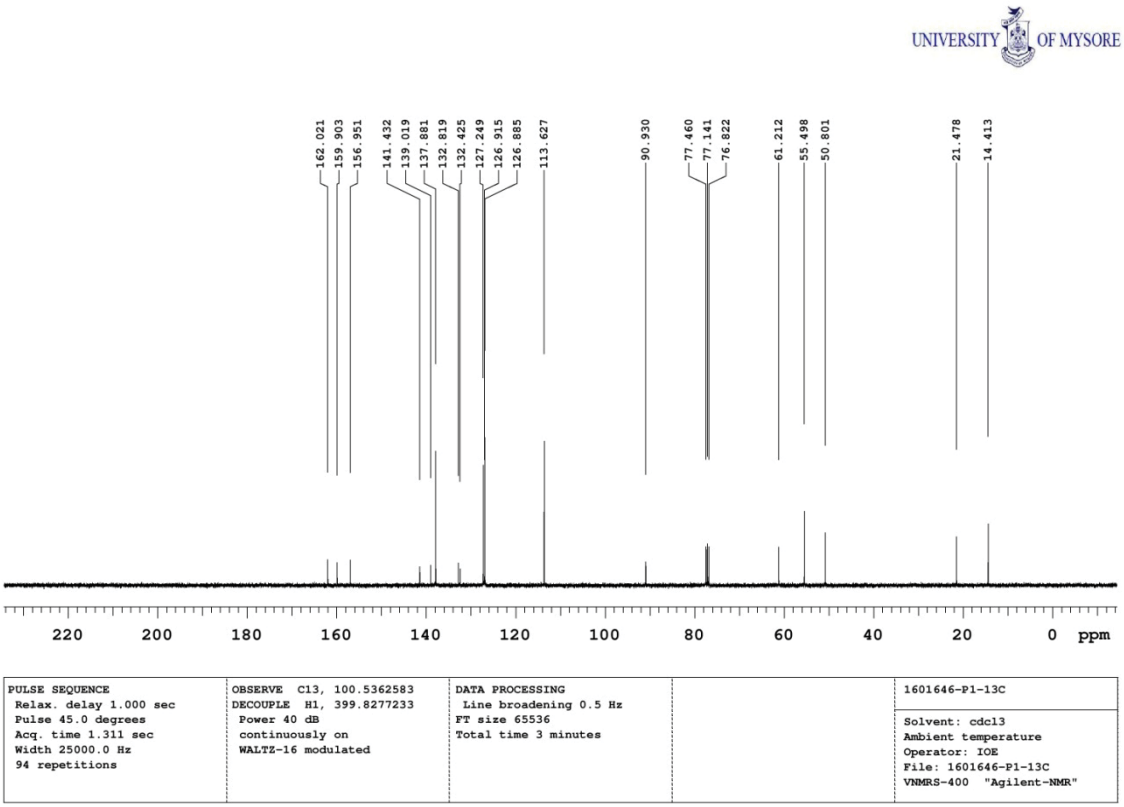


Figure 1: ^{1}H-NMR for the Compound 6.

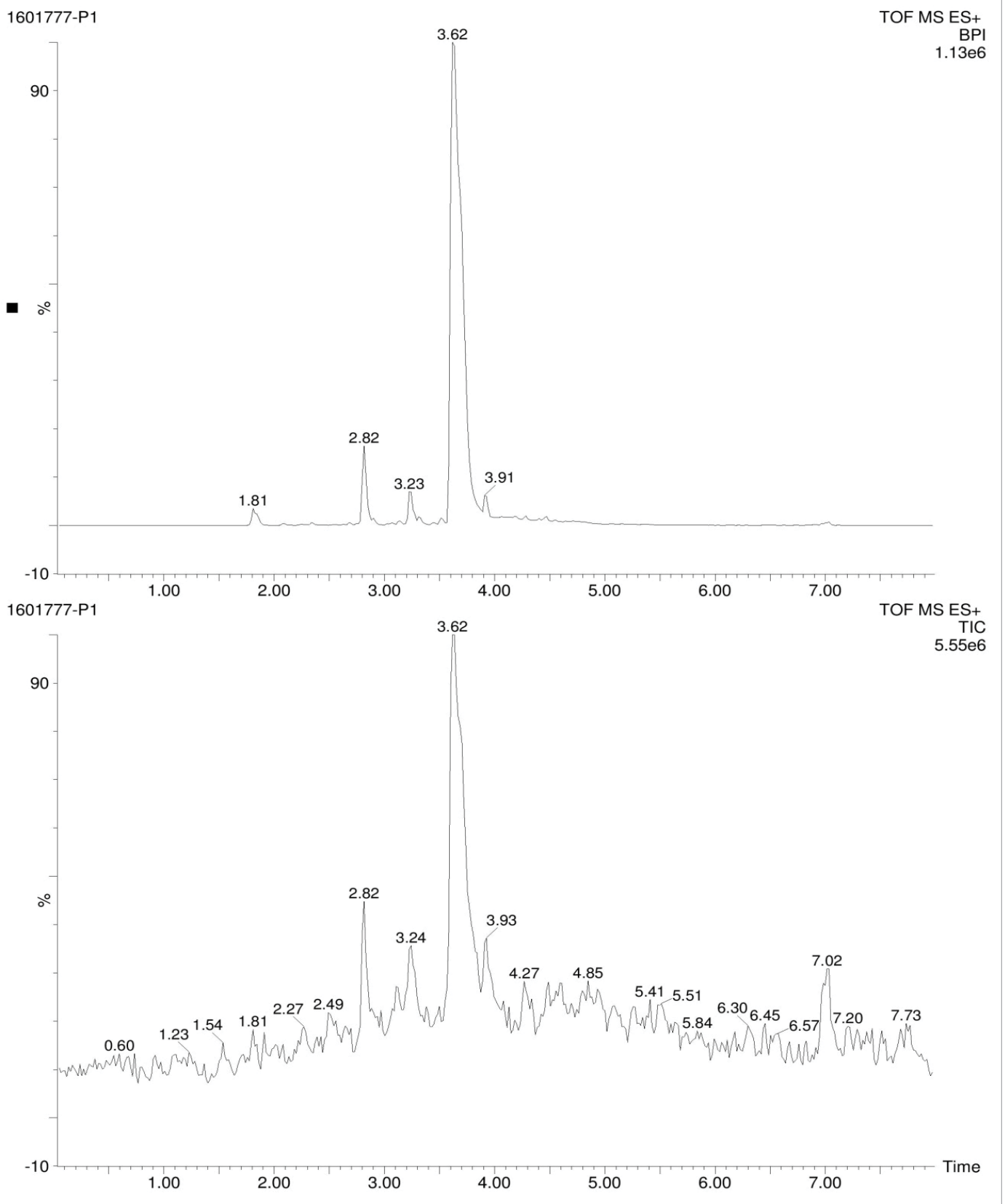


Figure 2: LC-MS for the Compound 6.

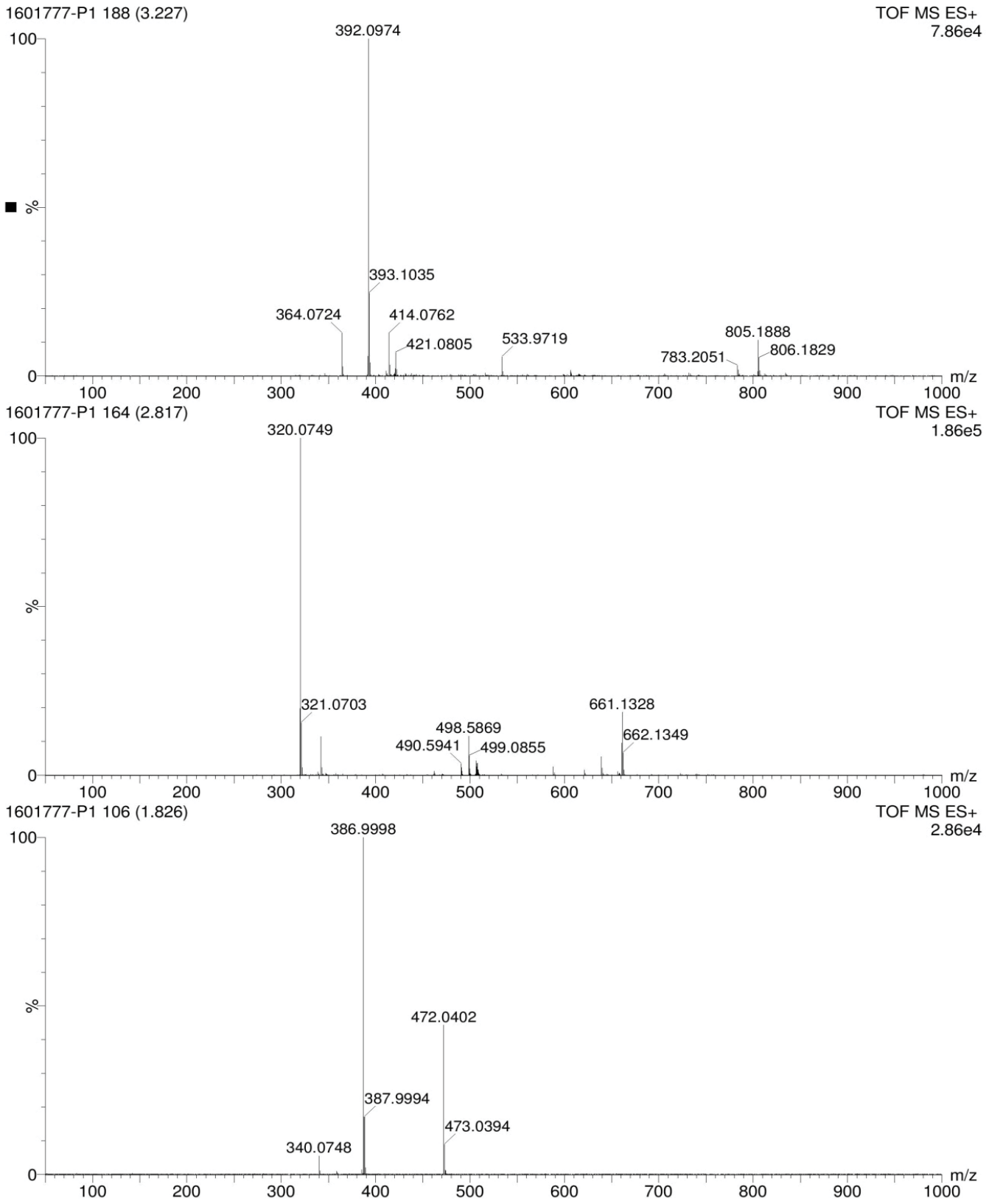


Figure 3: LC-MS for the Compound 6.

Figure 4: Polarising optical microscopic structures for the Compound 6 at 30°C.

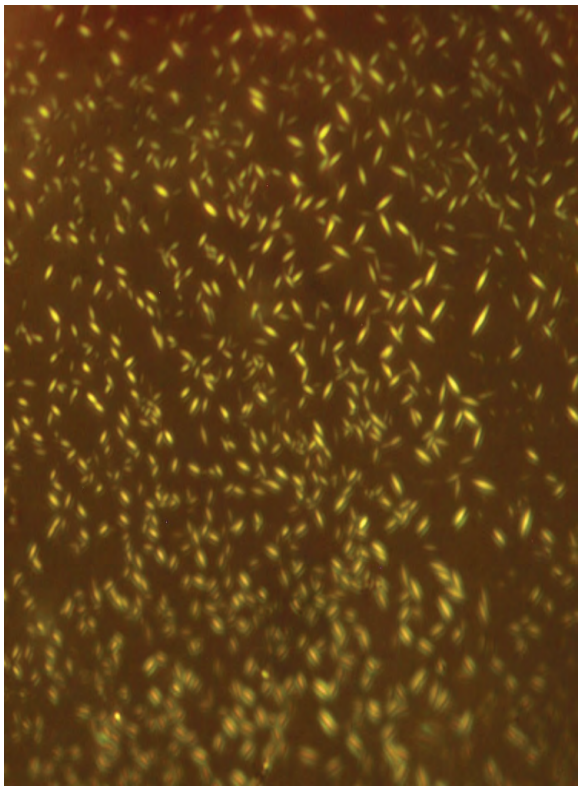


Figure 5: Polarising optical microscopic structures for the Compound 6 at 33°C.

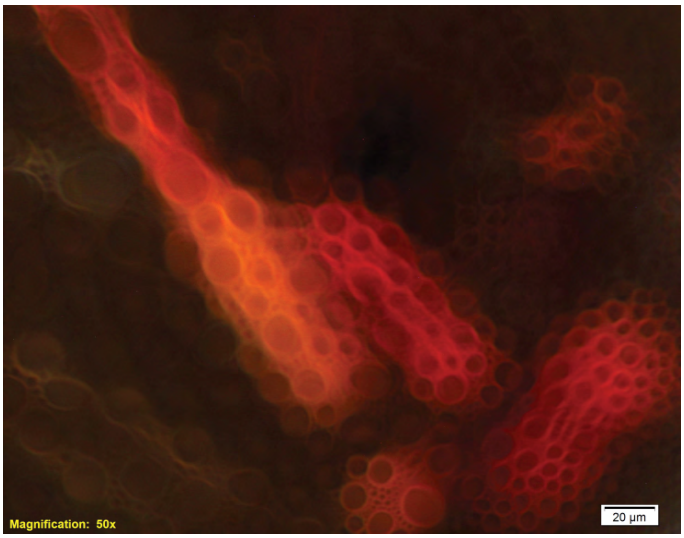


Figure 6: Polarising optical microscopic structures for the Compound 7.

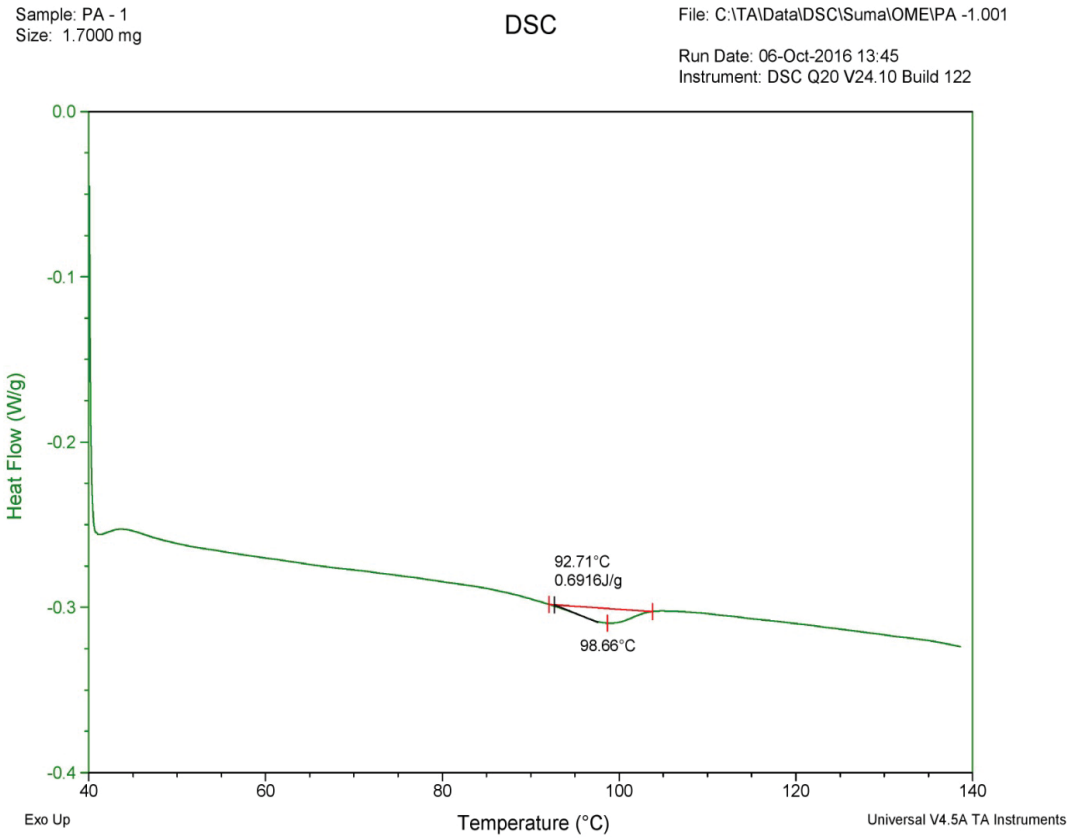


Figure 7: DSC Thermogram for the Compound No. 6.

162.0, LC-MS: observed mass: 517.9792 (Figures 2 and 3), Molecular Weight: 517.32 m/z: 517.05 (100.0%), 518.05 (25.1%), 519.06 (2.8%), 519.05 (1.1%)

Elemental Analysis: C, 51.08; H, 3.90; I, 24.53; N, 8.12; O, 12.37 C, 51.08; H, 3.90; I, 24.53; N, 8.12; O, 12.37.

Results and Discussion

Biological studies

Test pathogens: Human pathogenic bacteria *Escherichia coli* (MTCC 7410), *Staphylococcus aureus* (MTCC 7443), *Bacillus cereus*

Sl No	Test Organism	Test compound	Negative Control	Positive Control	
				Streptomycin	Nystatin
1	*E. coli*	0.0	0.0	35.0	-
2	*S. typhi*	0.0	0.0	30.0	-
3	*S. aureus*	0.0	0.0	28.0	-
4	*B. cereus*	0.0	0.0	33.0	-
5	*P. aurigenosa*	0.0	0.0	29.0	-
6	*B. substillis*	0.0	0.0	28.0	-
7	*C. albicans*	0.0	0.0	-	18.0
8	*M. canis*	0.0	0.0	-	26.0
9	*M. gypseum*	0.0	0.0	-	28.0

Table 1. Antimicrobial activity: Antibacterial and antifungal activity.

S. No.	Compound	Temperature	Texture
Figure 1	6	30°C	Nematic
Figure 2	6	33°C	Smectic- focal conics at 50X
Figure 3	7	25°C	Mielinic Structure

Table 2: Polarising optical microscopic structures for the compound 6 and 7.

(MTCC 121), *Salmonella typhi* (MTCC 733), *Pseudomonas aeruginosa* (MTTC 2453), *Bacillus subtilis* (MTCC 441) and human pathogenic fungi, *Candida albicans* (MTCC 183), *Microsporum gypseum* (MTCC 2830) and *Microsporum canis* (MTCC 2820) obtained from MTCC, Chandigarh, India served as test pathogens (Table 1).

Preparation of inocula: Cultures were grown on Nutrient agar (NA) medium at 37°C for 24 hrs. Morphologically similar colonies were scraped with sterile inoculation loop and mixed with 0.45% sterile saline solution and inoculum turbidity was adjusted to 0.5 McFarland standards spectrophotometrically. Seven day old cultures grown on Sabouraud Dextrose Agar [SDA] media at room temperature were selected. Culture plates were flooded with 0.45% sterile saline solution and inoculum turbidity was adjusted to 0.5 McFarland standards according to CLSI protocol.

Disc diffusion method: Disc diffusion method was performed according to CLSI document. 100 µl of the inoculum was seeded on the plates containing medium (NA for bacteria and SDA for fungi). The plates were allowed to dry for 3-5 minutes. 100 µl of the compound was loaded to the sterile discs of 8 mm diameter and placed on the test plates. The plates were incubated at 37°C for 24 hrs for bacteria and yeasts, 72-96 hrs for filamentous fungi. The diameter of the inhibition zones were measured in mm Discs loaded with respective solvents without extract served as negative control and Streptomycin (for bacteria) and Nystatin (for fungi) served as positive control. All the tests were performed in triplicates.

Results

Antibacterial and antifungal activity of synthesized pyridine was not observed against any of the test pathogens selected at the tested concentration. The compound no.6 and 7 shows the very good mesomorphic behaviours at 28°C and 33°C (Figures 4-6). The compound 6 exhibits the nematic phase at 28°C and exhibits the Mielinic texture at 30°C or some times both the textures of the compound 7 shows smectic phase. The compound no. 7 shows the focal conic structure at 30°C with very good results. The DSC thermogram for the compound 6 (Figure 7) exhibits the endothermic phase at 92.71°C with very small specific heat of 0.6916 J/g and are good agreement between DSC and POM textures observed at different temperatures (Table 2).

Conclusion

The synthesized ester intermediate and the acid cannot exhibit any biological activities for antibacterial and antifungal assay, but they exhibit the very good characteristic towards the liquid crystal activities even at room temperatures Phase transitions during the temperature changes have good agreement between the POM and DSC results.

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