Innovative Discovery Studies of Anti Lungs Cancer Active Drug Substances in Medicinal Chemistry

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1.0 INTRODUCTION

1.1Abstract:

The discovery study of anti cancer active drug substance of (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)] dipentanedioic acid was synthesized and characterized by medicinal chemistry bath way, also this product was obtained via the associated with the synthesis of pemetrexed disodium was performed. The possibility of (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)] dipentanedioic acid forming has been mentioned in literature, **but no study on this structure has been published yet.** This paper describes the development of the synthesis methods and preclinical studies for this compound and discusses their structure elucidation on the basis of NMR experiments and MS data. The identification of anti cancer drug activity of this compound by preclinical test, it should be useful for identify the new anticancer drug in pemetrexed generation.

1.2 Metabolite of Pemetrexed sodium :

The(2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′hexa hydro- 1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1diylcarbonyl amino)] dipentanedioic acid is an antifolate antineoplastic agent that exerts its action by disruptingfolate-dependent metabolic processes essential for cell replication. It acts by inhibiting three enzymes used in purine and pyrimidine synthesis *de novo*—thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide rib nucleotide formyltransferase (GARFT) [1,2]. By inhibiting the formation of precursor purine and pyrimidine nucleotide.

The(2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1diylcarbonylimino)] dipentanedioicacid prevents the formation of DNA and RNA, which are required for the growth and survival of both normal and cancer cells. A pharmaceutical product containing (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)]dipentanedioic Acid as the active ingredient is used for the treatment of malignant pleural mesothelioma (MPM) in combination with cisplatin and as a second line agent for the treatment of advanced or metastatic non-small cell lung cancer (NSCLC).

Currently, the pemetrexed parent drug is used as a single agent or in combination with other chemotherapeutic agents for the treatment of other types of cancer, such as breast cancer, bladder cancer, colorectal carcinoma and cervical cancer. The U.S. Food and Drug Administration (FDA) [6] and the European Medicine Agency (EMA) require complete physicochemical characteristic not only for an active pharmaceutical ingredient but also for its key synthetic intermediates. In addition, the determination of drug substances, including known, especially pharmacopeia durg substances as well as other unknown durg substances, can have a significant impact on the discovery of new drug products.

The health implications of drug substances can be significant because of their potential teratogenic, Mutagenic or carcinogenic effects. Therefore, the International Conference on Harmonization (ICH) sets a high standard for the purity of drug substances. If the dose is less than 2 g/day, impurities over 0.10% are expected to be identified, qualified and controlled. If the dose exceeds 2 g/day, the qualification threshold is lowered to 0.05%. It is therefore essential to control and monitor the drug substances both in the APIs and the finished drug products. It is also a crucial issue in drug development and manufacturing .

This paper describes a study on identification, synthesis and characterization of the drug substances formed during the pemetrexed disodium synthesis.The study will help to understand the formation of (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl]bis(ethylenebenzene4,1.diyl carbonylimino)]dipentanedioicacid synthesis and provide a clue on how to obtain a pure compound.Convergent synthesis of (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5diyl]bis (ethylenebenzen e-4,1-diylcarbonylimino)] dipentanedioic acid from key synthetic intermediates.

(Scheme 1) is well documented and involves firstly the preparation of the *p*-toluenesulfonic acid salt [10]. The acid is activated for coupling by reaction with 2-chloro-4,6 di methoxytriazine (CDMT) in presence of *N*-methylmorpholine (NMM) to form an active ester and then reacted with diethylL-glutamate . The product of peptide coupling is isolated as *p*-toluenesulfonate and then saponified to produce a free acid form of the drug substance.

Finally, the pH is adjusted to pH 8and the crystalline disodium salt is isolated as the heptahydrate form (1a·7H2O).However, we have found a new method for the preparation of pemetrexed disodium in an amorphous form which involves the deprotonation of pemetrexed diacid in the presence of sodium methoxide under anhydrous conditions,

The desired anticancer active 2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diyl carbonylimino)] dipentanedioicacid to be derived from pemetrexed disodium salt

1.3.0 Reagents :

1.3.1Acid-Amine coupling Reagent:

In recent years, amide coupling has become the most frequently used reaction in medicinal chemistry. Found as the backbone of proteins, the amide bond is nominally formed by the Condensation of a carboxylic acid and an amine. The most common method for the formation of an amide bond is the condensation of a Carboxylic acid and an amine. Generally, the carboxylic acid needs to be activated to react with the amine while remaining reactive functional groups need to be protected. This process occurs in two steps in either one pot with a direct reaction of the activated carboxylic acid or steps two with the isolation of an activated “trapped” carboxylic acid with a

reaction with an amine. A broadly applicable method for the formation of amide bonds use carbodiimides such as DCC (dicyclohexylcarbodiimide) or DIC (isopropyl carbodiimides), 1.3.2 2-chloro-4,6,-dimethoxy-1,3,5-triazine (CDMT)for activation. Additives are often required to improve the efficiency of the reactions, especially for solid-phase synthesis.

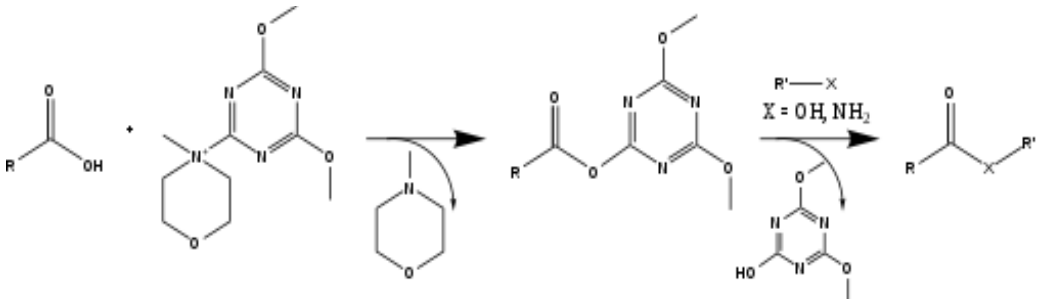
1.3.2 2-Chloro-4,6,-dimethoxy-1,3,5-triazine (CDMT):

DMTMM (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride) is an organic triazine derivative commonly used for activation of carboxylic acids, particularly for amide synthesis. Amide coupling is one of the most common reactions in organic chemistry and DMTMM is one reagent used for that reaction. The mechanism of DMTMM coupling is similar to other common amide coupling reactions involving activated carboxylic acids. Its precursor, 2-chloro-4,6,-dimethoxy-1,3,5-triazine (CDMT), has also been used for amide coupling.

DMTMM has also been used to synthesize other carboxylic functional groups such as esters and anhydrides. DMTMM is usually used in the chloride form but the tetrafluoroborate salt is also commercially available

Reaction mechanism:

Sheme-1



1.3.3 N-Methylmorpholine :

N-Methylmorpholine is the organic compound with the formula $O(CH_2CH_2)_2NCH_3$. It is a colorless liquid. It is a cyclic tertiary amine. It is used as a base catalyst for generation of polyurethanes and other reactions. It is produced by the reaction of methylamine and diethylene glycol as well as by the hydrogenolysis of N-formylmorpholine. It is the precursor to **N-methylmorpholine N-oxide**, a commercially important oxidant.

2.0 SCOPE AND OBJECTIVES

A physicochemical characterization of the (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl] bis (ethylenebenzene-4,1-diylcarbonylimino)]dipentanedioicacid associated With the synthesis of pemetrexed disodium was performed. The possibility of pemetrexed. (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d] pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)]dipentanedioicacid forming has been mentioned in literature, but no study on their structure has been published yet. This paper describes the development of the synthesis methods for these compounds and discusses their structure elucidation on the basis of 1HNMR experiments and MS data.

The identification of this (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl] bis (ethylene benzene-4,1-diylcarbonylimino)]dipentanedioicacid should be useful for the quality control during the production of the pemetrexed disodium .The study will help to understand the formation of (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H-5,6′-bipyrrolo[2,3-d]pyrimidine-5,5′-diyl]bis(ethylenebenzene-4,1-diylcarbonylimino)] di pentane dioicacid synthesis and provide a clue on how to obtain a pure compound.

Convergent synthesis of (2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′- hexa hydro-1′H,5H-5,6′-bipyrrolo [2,3-d]pyrimidine-5,5′diyl] bis(ethylenebenzene-4,1- diyl carbonylimino)] dipentanedioic Acid. The synthesized and c haracterized anti lungs cancer substance of (2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo [2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)] dipentanedioic acid to be confirmed as a anti cancer activity by clinical trial studies.

Based on our clinical data, we need to check the value of IC_{50}, A drug substance which is having less IC_{50} value (IC_{50} Range 100-300), that durg substance should be more potent, so we need to prove that the(2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro 1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic acid is more potent anti cancer active drug substance.

3.0 EXPERIMENTAL METHODS

3.1 MATERIALS

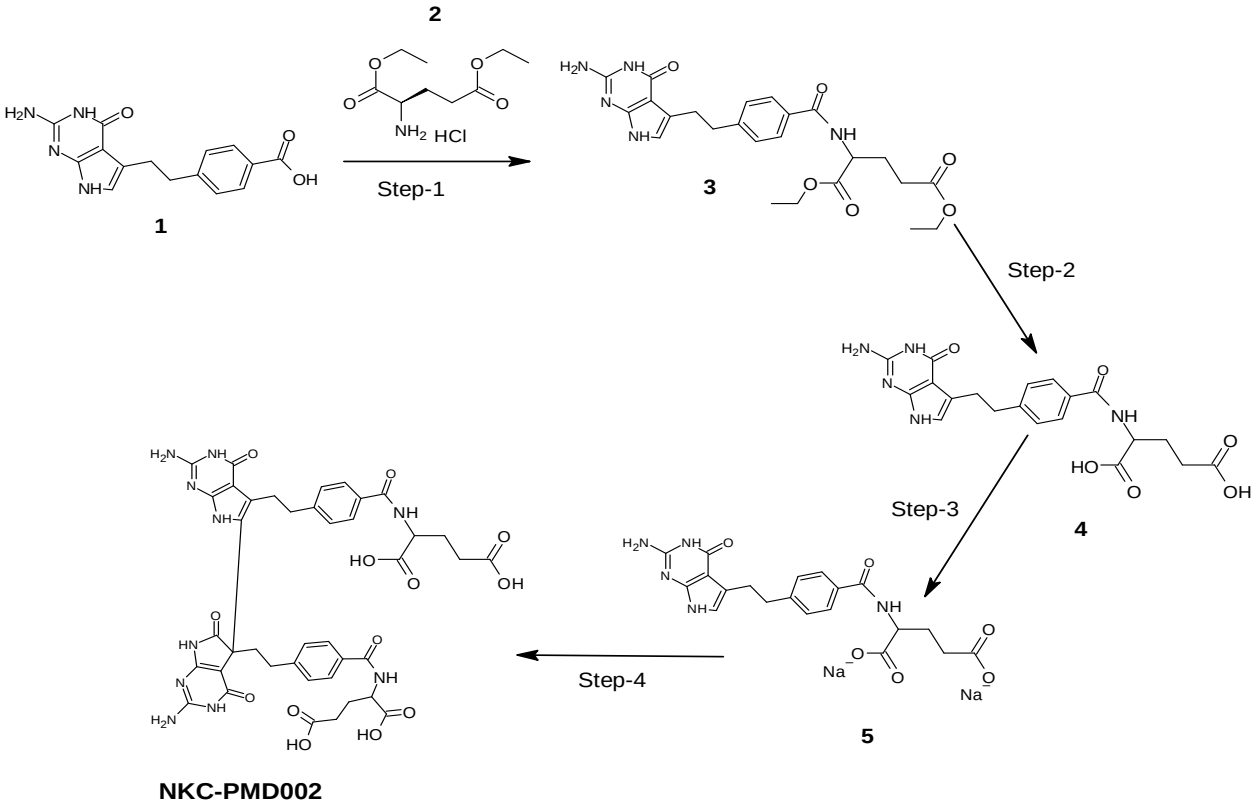
Table.No.1

S.No	Materials Name
1	CDMT
2	NMM
3	DMF
4	p-TSA
5	NaOHaq

6	HClaq
7	NaOMe
8	Ethanol

3.2 Synthetic scheme of anti cancer active drug metabolite of pemetrexed

Scheme-2



3,3 Reaction & Conditions

Step-1 : (a) CDMT, NMM, DMF,RT; (b) 4; (c) p-TSA;

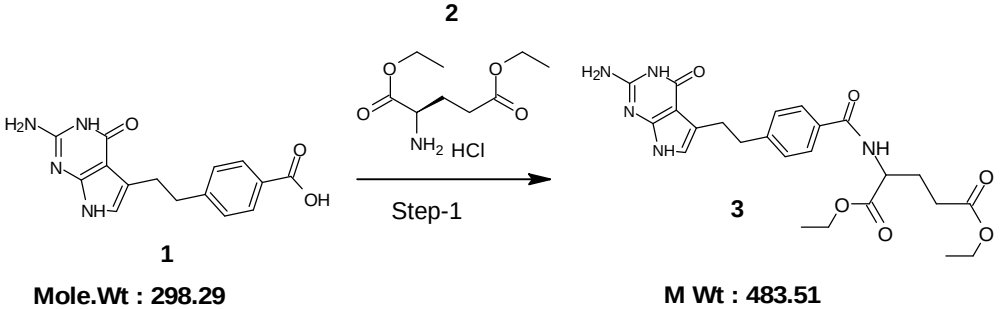
Step-2 : (a) NaOHaq, (b) HClaq;

Step-3 : 1a·7H2O: (a) NaOHaq, (b) HClaq; Or (amorphic form): NaOMe, MeOH).

Step-4 ; NaOH, reflux, 3days

3.4 Step-1: Synthesis of compound (3)

Scheme-3:



Mole.Wt : 298.29 **M Wt : 483.51**

Table.No-2 Calculations

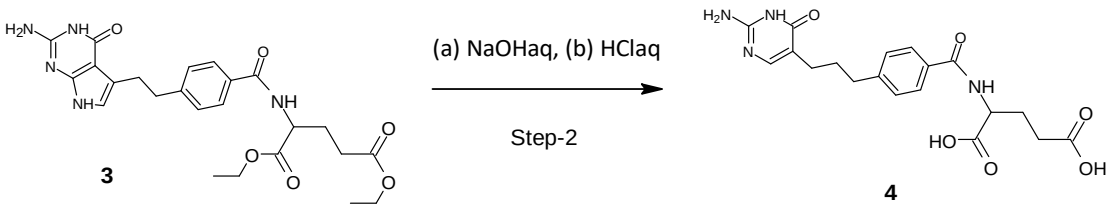
S.No	Material Name	Weight (g/ml)	Mole weight	Millimoles	Equiv/ Vol
1	Compound (1)	1g	298.29	3.351	1
2	Compound (2)	0.788 g	146.14	3.287	0.98
3	NMM (d=0.92)	1.06ml	101.149	9.641	2.9
4	CDM	0.648 g	299.71	3.690	1.1
5	*p*-TSA	1.596	190.22	8.390	2.5
6	DMF	1.65ml	-	-	0.5
7	DCM	9.5ml	-	-	9.5
8	Ethanol	18ml	-	-	18

Experimental procedure:

The N-methylmorpholine (NMM, 1.06 mL, 9.641 mmol) was added to the suspension of Compound **1** (1 g, 3.351 mmol) in DMF (1.65 mL) and CH2Cl2 (9.5 mL), followed by 2-chloro-4,6-dimethoxy-1,3,5-triazine(CDMT, 0.648 g, 3.690 mmol), and the resulting solution was stirred at 38–40 °C for 2 h. To this solution diethyl D-glutamate hydrochloride ***2*** (0.788 g, 3.287 mmol) was added and the resulting mixture was stirred for 2 h. Then water (10 mL) was added and the mixture was stirred for 15 min. The organic layer was separated and the aqueous phase extracted with CH2Cl2 (1 × 7 mL). The organic layers were collected, washed with 1 M NaHCO3aq (1 × 7 mL), and concentrated under reduced pressure to afford oil. EtOH (18 mL) was added to the oil, followed by the solution of the *p*-toluenesulfonic acid monohydrate in EtOH (1.596 g in 18 mL) and the resulting suspension was heated under reflux for 2 h. The mixture was cooled to RT, the crystals of **3** were filtered and washed with EtOH (2 × 60 mL). The wet cake was re slurried in EtOH (40mL), refluxed for 1 h and cooled to *RT*. The crystals were filtered, washed with EtOH (2 × 6 mL) and dried *in vacuo* at 40 °C for 24 h to provide **3** (1.48 g, 66%).

3.5 Step-2 : Synthesis of compound (4)

Scheme-4:



3

Mole weight : 483.51

4

Mole. Weight :402.40

Table.No-3 Calculations

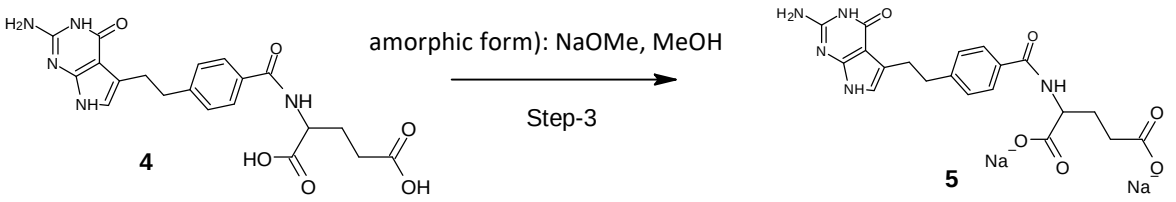
S.No	Material Name	Weight (g/ml)	Mole weight	Millimoles	Equiv/ Vol
1	Compound (3)	1.44 g	483.51	-	1
2	1 M NaOHaq	11.2ml	-	-	-
3	1N HClaq	5.2ml	-	-	-
4	Ethanol	56ml	-	-	-

Experimental procedure:

Compound **3** (1.44 g, 2.198 mmol) was treated with 1 M NaOHaq (11.2 mL), the mixture was stirred at room temperature. After 1 h the reaction mixture was adjusted to pH 8.0 with 1N HClaq and heated to 55–60 °C. EtOH (56 mL) was added to the solution. After cooling to *RT*, the precipitated solid was collected by filtration and washed with EtOH (2 × 8 mL). The wet solid (12.84 g) was dissolved in water (12 mL) and the solution was heated to 55–60 °C. EtOH (50 mL) was added and then the mixture cooled to RT. The solid was filtered, washed with EtOH (2 × 80mL) and dried in vacuo at 35 °C for 48 h to provide the compound (**4**) (0.98 g, 87%).

3.6 Step-3 : Synthesis of compound (5)

Scheme-5:



Mole.Wt : 427.41

Mole : 471.37

Table.No-4 Calculations

S.No	Material Name	Weight (g/ml)	Mole weight	Millimoles	Equiv/ Vol
1	Compound (4)	0.5g	427.41	1.1698	1
2	NaOMe	0.063g	54.02	1.1698	1
3	MeOH	2.5ml	-	-	-
4	Toluene	45ml	-	-	-

Experimental procedure:

The Compound (**4)** (0.5g, 1.1698 mmol) was dissolved in 2.5ml of methanol treated with NaOMe (0.063g, 1.1698 mmol, 1eq), the mixture was stirred at 0ºC temperature. After 1 h the reaction mixture was distilled to remove the methanol completely, azeotrope distillation with toluene (3x15ml) in vacuo at 55 °C and triturated with diethyl ether (3x5ml) and dried with vaccuo at 45ºC , to provide the compound (**5)** (1.2g)

3.7 Step-4 : Synthesis of compound (NKC-PMD002)

Scheme-6:

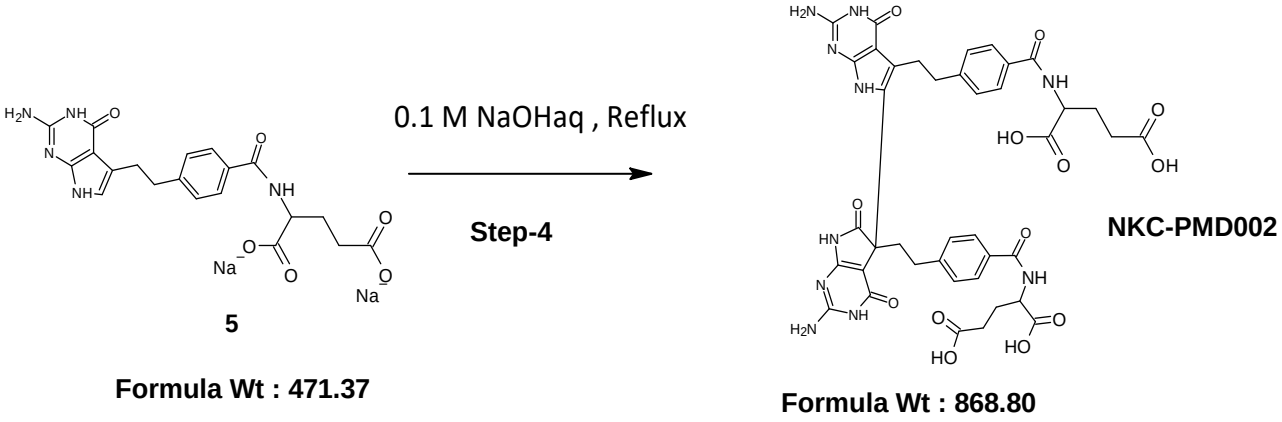


Table.No-5 Calculations

S.No	Material Name	Weight (g/ml)	Mole weight	Millimoles	Equiv/ Vol
1	Compound (5)	1.0g	427.41	1.1698	1
2	1 M NaOHaq	200ml	-	-	-
3	1N HClaq	5.2ml	-	-	-
4	Water	4ml	-	-	-

Experimental procedure:

The Compound (**5**) (1.0 g) was dissolved in 0.1 M NaOHaq (200 mL) and heated under reflux for 3 days (TLC control). Then the mixture was cooled and evaporated under reduced pressure to get crude diastereoisomeric mixture as brown oil. The residue was dissolved in water (1 mL) and the pH was adjusted to 2–3 with 1 M HClaq. The suspension was filtered, and then the solid was washed with H2O (2 × 2 mL) and dried at 40 °C to obtained the mixture Diastereomers The obtained mixture was purified by Preparative chromatography (EtOH-MeOH-AcOEt-4%NH3 aq, 40:30:10:12, *v*/*v*) to got the pure product of **NKC-PMD002** (205 mg, 10%). The purified product of **NKC-PMD002** was confirmed by Mass,**^{1}HNMR**, **HPLC**.

4.0 RESULT AND DISCUSSIONS

4.1 Solubility:

Table.No-6

S.No	Compound name	Solvents
1	(2S,2′S)-2,2′-[[2,2′-Diamino-4,4′,6-trioxo-1,4,4′,6,7,7′-hexahydro-1′H,5H5,6′bipyrrolo [2,3d] pyrimidine 5,5′diyl] bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic Acid	DMSO,Water/ Methanol/ EA/ AcOH)

The above-purified products of compound (**NKC-PMD002)** were characterized by proton NMR, Mass Spectroscopy, HPLC and RT / RRT. The synthesized compounds are dissolved in MeOH,Acetonitrile, DMF and DMSO. Then HPLC was performed in neutral medium (Acetonitrile and water) or Basic medium (Ammonium hydroxide and Ammonium chloride) or Acidic medium (TFA/Water) based on component nature.

The purity of the compound was confirmed by the HPLC technique. Peaks were observed at particular RT, it varies based on compound polarity and the wavelength also varies based on UV activity at a maximum of the compounds will appear at 215- 254nm wavelength. The desired product purity was confirmed by HPLC. Nuclear Magnetic resonance spectroscopy is widely used to determine the structure of organic molecules in solution and study molecular physics and crystals as well as non-crystalline materials. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI). The proton NMR of the above components was done by Bucker-400 MHz, the Compounds were dissolved in DMSO-d6 or CDCl3.

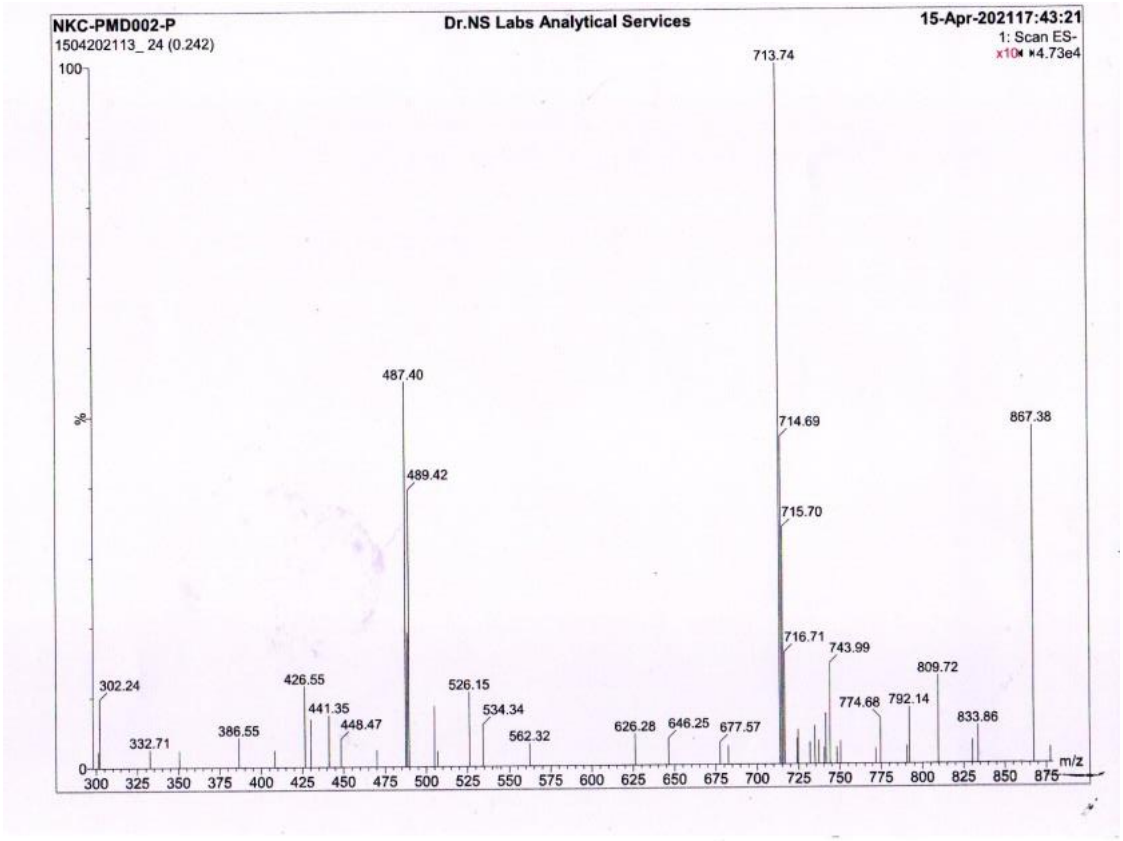
4.2Characterizationof(2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic acid

Mass spectroscopy:

The(2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic acid was dissolved in MeOH the Mass Spectroscopy was done in a Neutral medium using Acetonitrile and water the molecular weight was confirmed by Mass spectroscopic technique.

Molecular weight (g/mole =868.81). The desired product mass was identified in negative mode (M-1) m/z =867.21.

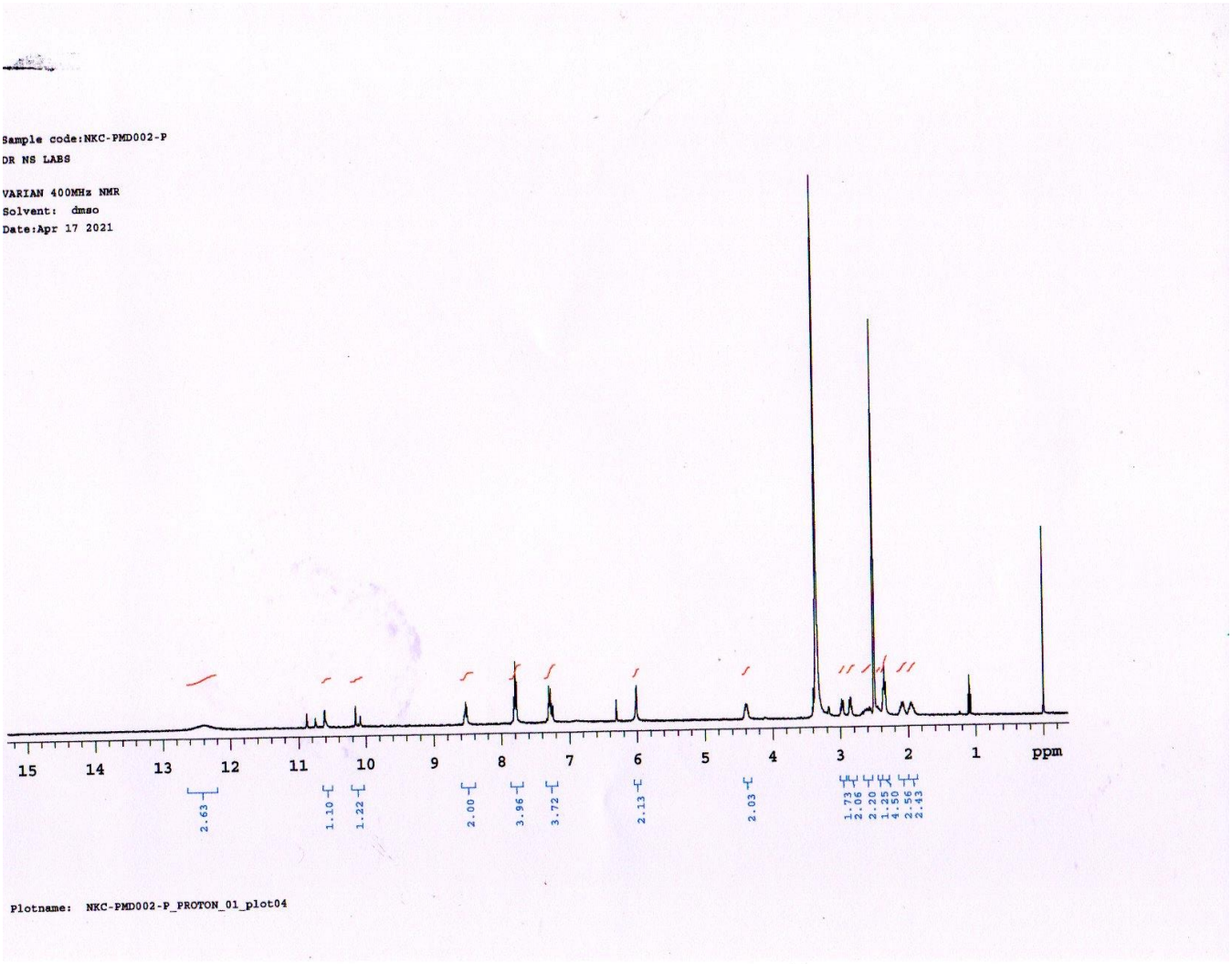
(NKC-**PMD002-P, Mass, Figure.No.1**)



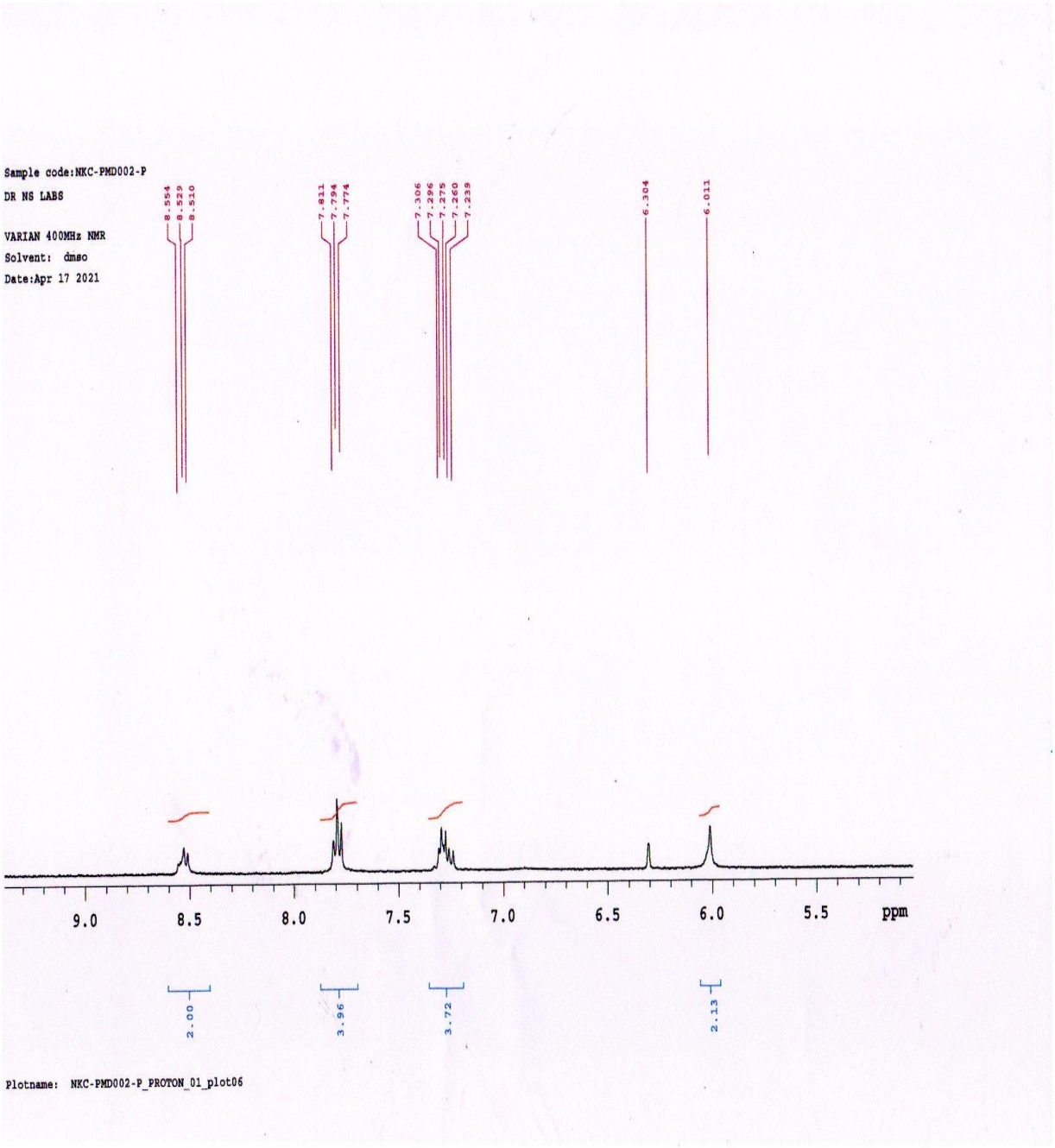
Proton NMR:

(2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic acid was done in the DMSO-d_6 solvent at 400MHz.

(NKC-PMD002-P,1HNMR-DMSO-d6,Page-1,Figure.No.2)



(NKC-PMD002-P, 1HNMR-DMSO-d6, Page-2, Figure.No.3)



(NKC-PMD002-P, 1HNMR-DMSO-d6, Page-3, Figure.No.4)

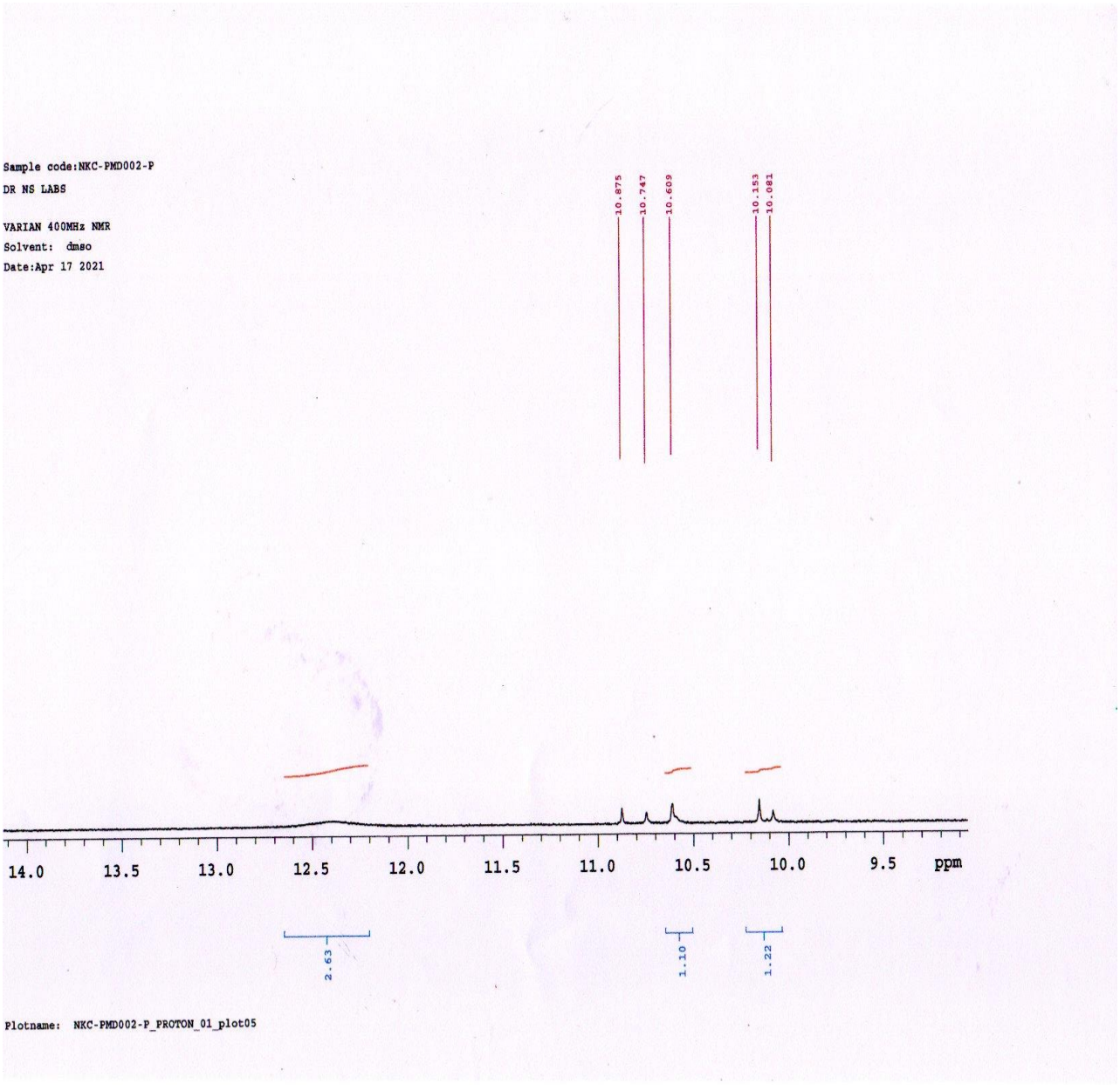


Table.No-7 (^{1}HNMR of NKC-PMD002-P)

S.No	Chemical shift value	Protons
	12.39	3H, ov, total for 21, 21′, 22 and22′ –CO2H
	10.87	1H, s, N9′-H
	10.74	1H, s, N9′-H
	10.60	1H, bs, probably N1′-H
	8.52	2H, 2 × d, ov, N17-H and N17′-H),
	7.80	4H, m, H14 and H14
	7.30	2H, d, *J* = 8.0 Hz, H13′
	7.26	2H, d, *J* = 8.0 Hz, H13
	6.90	2H,bs, probably NH2 at C2
	6.02	2H, NH2 at C2
	4.41	2H, m, H18 and H18
	2.72–2.54	6H, H11,H10 and H10′
	2.45	2H, H11′
	2.40–2.32	4H, H20 and H20
	2.10 and 1.97	4H, H19 and H19′);

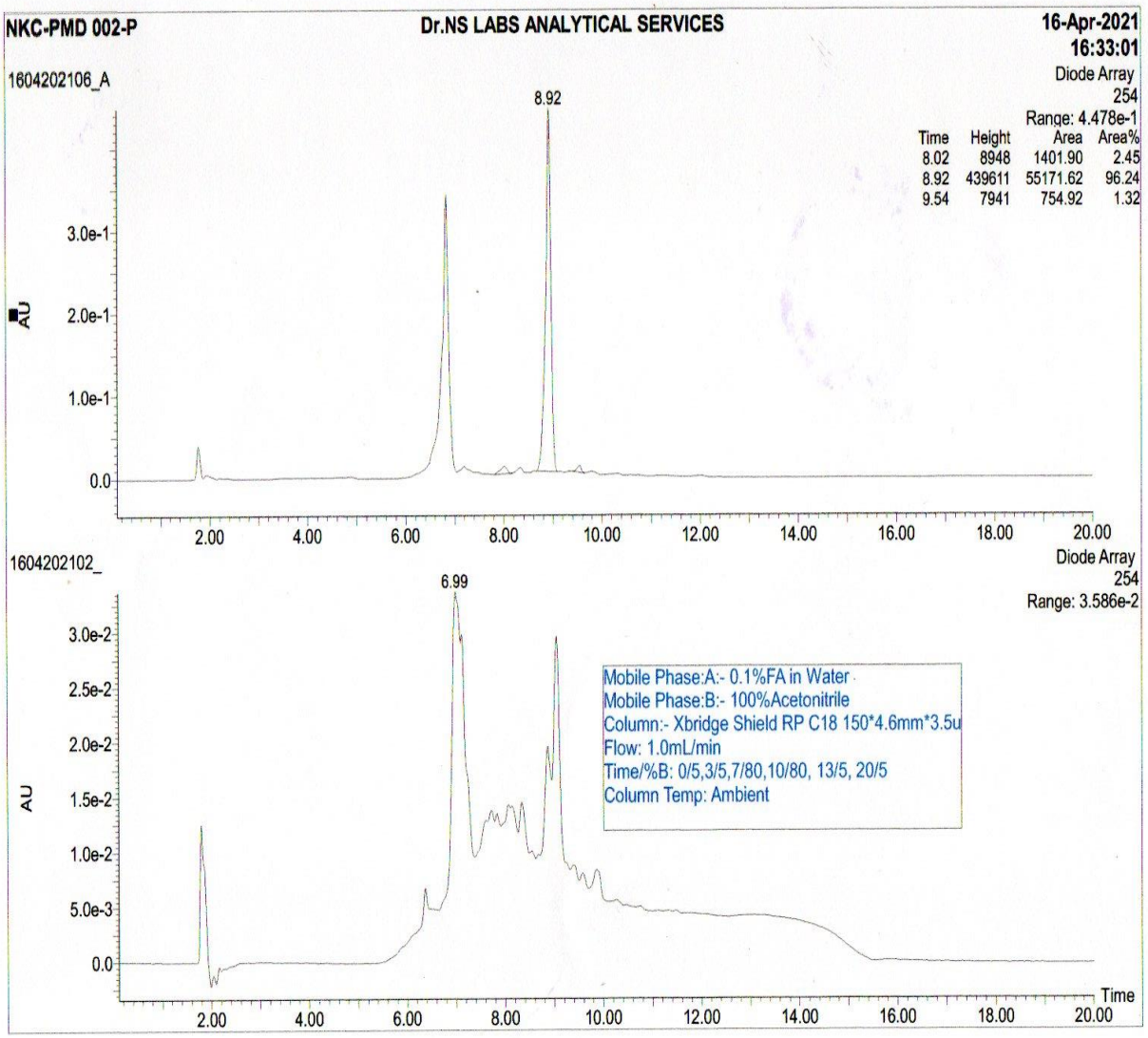
HPLC Report:

(2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic acid was conformed the purity using HPLC Mobile Phase: A:- 0.1%FA in Water ,Mobile Phase:B:- 100% Aceto nitrile ,Column:- Xbridge Shield RP C18 150*4.6mm*3.5u,Flow: 1.0mL/min,Time/%B: 0/5,3/5,7/80,10/80, 13/5, 20/5.Column Temp: Ambient.

The desired peak was observed at RT = 8.92 at 254nm wavelength.

The purity of the product = 96.24%.

NKC-PMD002-P, HPLC, Figure.No.5



4.3 Comparison with Parent Molecules

- (2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)] dipentanedioic acid (**NKC-PMD002**) has more potent drug than parent durg of pemetrexed.

- The disasteromer form of (2S,2′S)2,2′ [[2,2′Diamino 4,4′,6trioxo 1,4, 4′ ,6,7, 7′ hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic acid (**NKC-PMD002**) , one of the pyrrole ring double bond was absent and is became a five member cyclic amide.

- The desired product(**NKC-PMD002**) has another diastereomer also, which was isolated and confirmed by Mass,1HNMR,HPLC

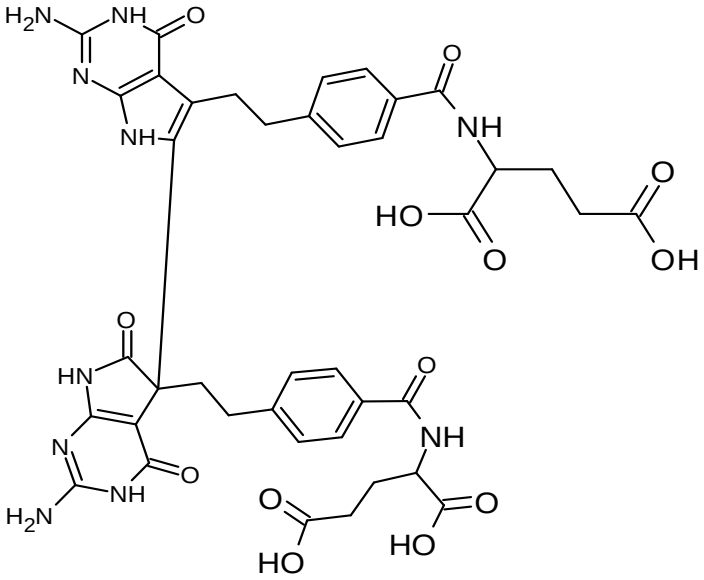
5.0 PRECLINICAL STUDIES

The above synthesized and characterized anti lungs cancer active drugs substance (2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic Acid was confirmed the anti cancer activity by clinical trial studies. Based on our clinical data, we observed the IC_{50} Value of our desired product is 159, A drug substance which is having less IC_{50} value (IC_{50} Range 120-300), that durg substance should be more potent, so the desired compound is more potent anti cancer active drug substance.

(2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic Acid has been to be tested in the MTT assay for the cell growth inhibition property in human lung carcinoma cell line, A549..

Name Of the drug substances:
(2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedioic acid
Molecular weight: 868.81
Molecular formulae: $C_{40}H_{40}N_{10}O_{13}$
CLogP Value: -2.13+/- 1.21

Structure of drug substance:



NKC-PMD002

5.1 STUDY DETAILS

Study Title	:	To evaluate the test item's growth inhibitory property using A549, a human lung carcinoma cell line.
Test Items Name	:	NKC-PMD002
Test Item Concentration:		1000, 300, 100, 30, 10, 3.0, 1.0 and 0.3 µg/ml
Cell line	:	A549 (Human lung carcinoma)

5.2 SUMMARY

The test item has been tested in the MTT assay for the cell growth inhibition property in human lung carcinoma cell line, A549. The results revealed that the compounds showing cytotoxic effect at higher concentration tested. The calculated IC_{50} value is 159 µg/ml on A549 cell line.

5.3 OBJECTIVE OF PRECLINICAL STUDIES

The purpose of this study was to assess the growth inhibitory effect of test item on the human lung carcinoma, A549.

5.4 MATERIALS AND METHODS OF PRECLINICAL STUDIES

1. CO_2 incubator- Sanyo, Japan
2. Multimode micro plate reader- BioTek, USA
3. Refrigerated centrifuge- Remi, India
4. Cell: A549 cell line - NCCS Pune
5. MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) from Sigma
6. Fetal bovine serum from Genetix Biotech, India

7. Trypsin from SRL Chemicals
8. Penicillin/Streptomycin from Sigma
9. DMEM medium from Genetix Biotech, India
10. DMSO from SRL chemicals.

RESULTS

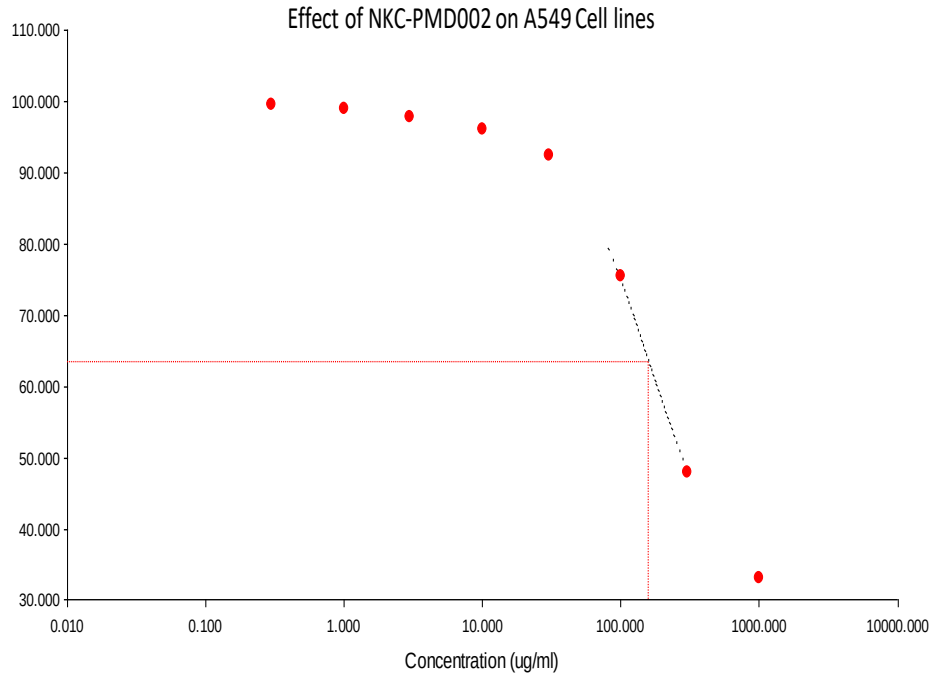
CELL GROWTH INHIBITION PROPERTY

The test item was tested against MCF-7 cell line. The test item concentrations ranging from 1000, 300, 100, 30, 10, 3, 1 and 0.3 µg/ml in semi logarithmic range used to assess the growth inhibition properties of the test compound. Each concentration was performed in quadruplicate and cumulative variation were maintained less than 20% between the data points.

The test compounds is showing cytotoxic effect on the higher concentration tested. The IC_{50} value for the test items showing at 159 µg/ml on the tested cell line. Results and raw data have been illustrated in the following table and graph.

Raw Data Absorbance values at 570nm and percentage growth inhibition

(Figure.No.7)



CONCLUSIONS

- The pemetrexed sodium is available drugs in the market; its metabolites are unknown drug substances in the market and our aim to identify the best anti cancer active drug molecules than parent drug molecules.

- The logP value of a compound, which is the logarithm of its partition coefficient between n-octanol and water log ($c_{octanol}/c_{water}$), is a well established measure of the compound's hydrophilicity.

- Low hydrophilicities and therefore high logP values cause poor absorption or permeation. It has been shown for compounds to have a reasonable propability of being well absorbt their logP value must not be greater than 5.0.

- The cLog P value, (2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′ hexahydro1′H,5H56′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipenta nedioic acid is --2.13+/- 1.21 , it shows that metabolite has better active.

- The cLogP value of (2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′ hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipenta ne dioic acid was exactly matching with the parent molecule, it shows that the metabolite has better drug active.

- Synthesis and characterization of (2S,2′S)2,2′[[2,2′Diamino 4,4′, 6 trioxo 1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimi no)]dipentanedioic acid metabolites very much useful to pharmaceutical research,

- This metabolite is well synthesized and characterized by required analysis like Mass, NMR and HPLC…etc.

- The clinical study very much useful to confirm the better anti cancer active metabolite(2S,2′S)2,2′[[2,2′Diamino4,4′,6trioxo1,4,4′,6,7,7′hexahydro1′H,5H5,6′bipyrrolo[2,3d]pyrimidine 5,5′diyl]bis(ethylenebenzene4,1diylcarbonylimino)]dipentanedic acid.

- The clinical data's shows that the desired molecules have the IC_{50} value showing at 159 µg/ml on the tested, it shows that the desired compound have better anti cancer activity.

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