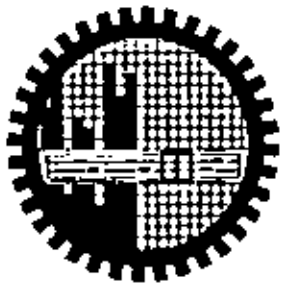
SYNTHESIS OF 5-BROMO-6-AMIDO URACIL FROM OROTIC ACID



M.PHIL THESIS

AUGUST, 2009

A DISSERTATION SUBMITTED IN THE PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PHILOSOPHY IN CHEMISTRY

SUBMITTED BY

MD. FAIAZ AHMED

STUDENT NO- 100603118F

REGISTRATION NO- 100603118

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ORGANIC CHEMISTRY RESEARCH LABORATORY

DEPARTMENT OF CHEMISTRY

BANGLADESH UNIVERSITY OF ENGINEERING & TECHNOLOGY

DHAKA-1000, BANGLADESH.

DEPARTMENT OF CHEMISTRY

BANGLADESH UNIVERSITY OF ENGINEERING & TECHNOLOGY, DHAKA-1000, BANGLADESH.

THESIS ACCEPTANCE LETTER

The thesis titled "**Synthesis of 5-bromo-6-amido uracil from Orotic acid**" submitted by **Md. Faiaz Ahmed**, Roll No. 100603118F, session: October 2006, has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Master of Philosophy in Chemistry on August 2, 2009.

Proposed Board of Examiners

Dr. Md. Wahab Khan — Chairman (Supervisor)
Professor
Department of Chemistry, BUET, Dhaka.

Dr. Md. Wahab Khan — Member (Ex-officio)
Head
Department of Chemistry, BUET, Dhaka.

Dr. A. K. M. Matior Rahman — Member
Professor, Department of Chemistry, BUET, Dhaka.

Dr. Nazrul Islam — Member
Professor, Department of Chemistry, BUET, Dhaka.

Dr. Md. Abdur Rashid — Member (External)
Dean & Professor
Faculty of Pharmacy, University of Dhaka, Dhaka.

DEPARTMENT OF CHEMISTRY
BANGLADESH UNIVERSITY OF ENGINEERING & TECHNOLOGY, DHAKA-1000, BANGLADESH

Student's Declaration

It is hereby declared that this thesis or any part of it has not been submitted elsewhere for the award of any degree or diploma.

Signature of the Candidate

Md. Faiaz Ahmed

Md. Faiaz Ahmed

Date: August 2, 2009.

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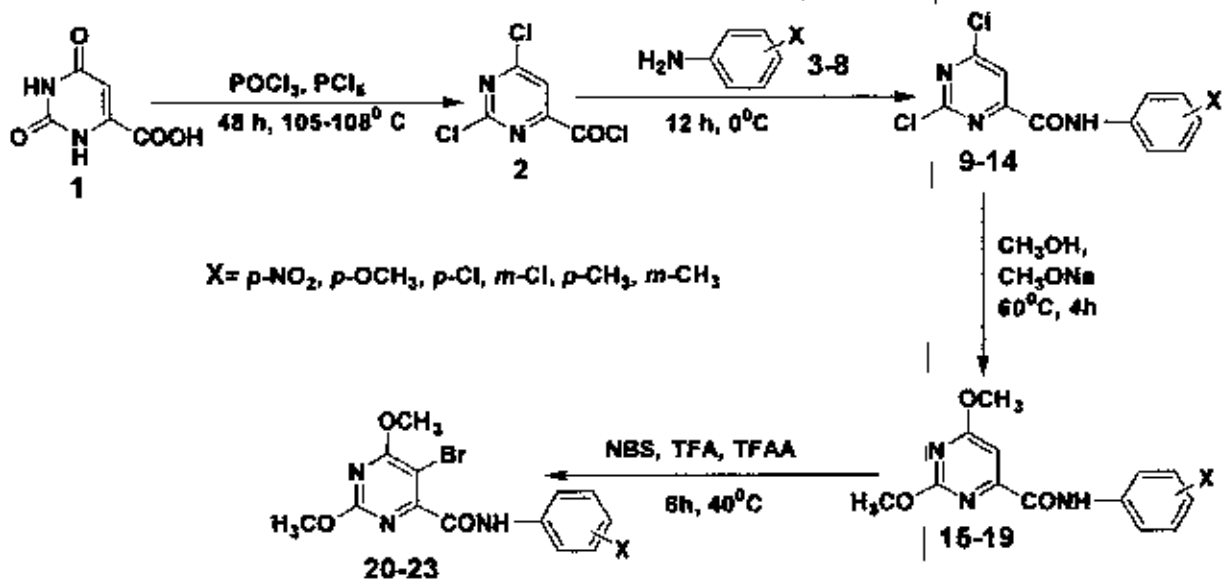
Author

(Md. Faiaz Ahmed)

Thesis Title: "Synthesis of 5-bromo-6-Amido uracil from Orotic acid"

Abstract

In the view of the extensive biological activities of various 5 and 6-substituted uracils and related pyrimidine derivatives, a facile method for the synthesis of a number of 5, 6-disubstituted pyrimidines were developed **(Scheme-1)**. 2, 4-Dichloropyrimidine-6-carbonyl chloride **2** was synthesized by refluxing Orotic acid 1 with phosphorus oxychloride and phosphorus pentachloride. Compound **2** underwent substitute reaction with different substituted aromatic amines to give 2, 4 - Dichloro - 6-substituted phenylamido pyrimidine **9-14**. 2, 4 - dichloro - 6- substituted phenylamido pyrimidines were converted to the corresponding dimethoxy pyrimidine **15-19** on treatment with sodium methoxide in methanol. The bromination reaction was attempted by several methods but only NBS-TFA-TFAA method gave the desired products **20-23**. The structures of the synthesized products were established from their analytical and spectroscopic data.



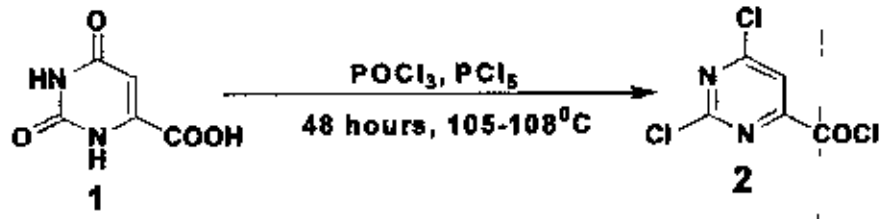
Scheme-1

SUMMARY

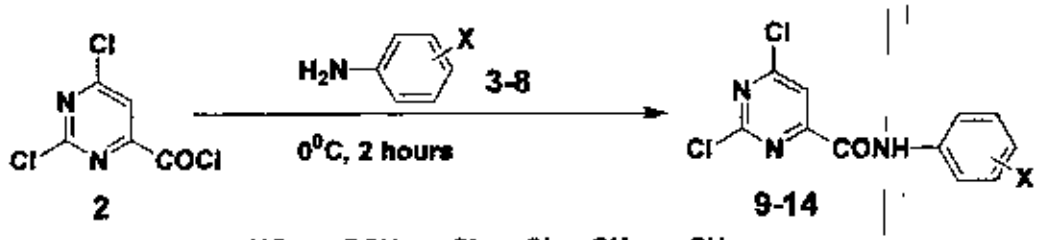
Investigations incorporated in this dissertation entitled "Synthesis of 5-bromo-6-amido uracl from orotic acid" has been presented in four chapters. In chapter 1, background of biologically important and the important synthetic reactions involved in the synthesis are presented. Chapter 2 and Chapter 3 deal with the detailed methodology and experimental procedure for the synthesis of pyrimidine compounds. Chapter 4 deals with the biological test of the synthesized products.

In Chapter 1 the importance and synthesis of 5- and 6-substituted pyrimidine derivatives are presented. 5- and 6-substituted heterocyclic compounds are great interest, because of their occurrence in nature and their outstanding pharmaceutical and medicinal activities. Although various methods have been developed previously for the synthesis of 5 and 6 substituted derivatives, there was no method for the synthesis of 5-bromo-6-amido uracils as per our review.

In Chapter 2, results and discussion for the synthesis of 2, 4-Dichloropyrimidine-6-carbonyl chloride **2**; 2, 4 - Dichloro - 6- substituted phenylamido pyrimidines **9-14**; 2, 4 - Dimethoxy - 6- substituted phenylamido pyrimidines **15-19** and 2, 4 - Dimethoxy – 5-bromo-6-substituted phenylamido pyrimidines **20-23** are described as shown in **Schemes 1-4**. Structures of all of these synthesized compounds have been established on the basis of their UV, IR, ^{1}HNMR, ^{13}CNMR and elementary (C, H, & N) analysis data.

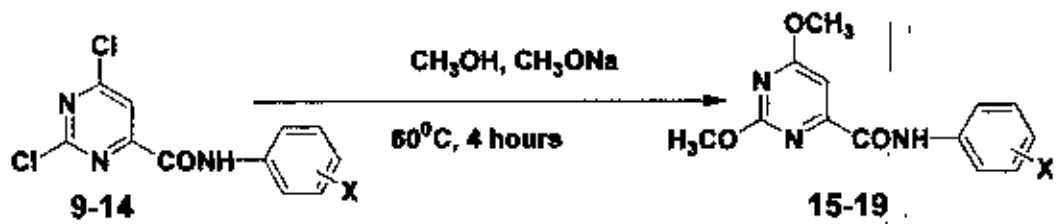


Scheme-1



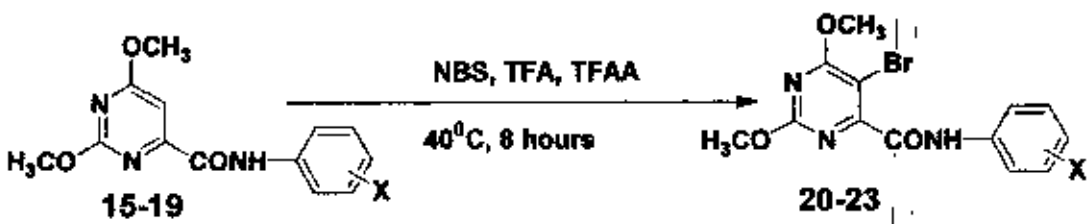
X= *p*-NO_2, *p*-OCH_3, *p*-Cl, *m*-Cl, *p*-CH_3, *m*-CH_3

Scheme-2



X= *p*-NO_2, *p*-OCH_3, *p*-Cl, *m*-Cl, *p*-CH_3, *m*-CH_3

Scheme- 3



X= *p*-NO_2, *p*-OCH_3, *p*-Cl, *m*-Cl, *p*-CH_3, *m*-CH_3

Scheme-4

In chapter 3, all the experimental procedure and analytical data are reported. This chapter also contains references and important spectra of the synthesized compounds.

In Chapter 4, introduction, methodology, results and discussion, references and conclusion of biological test of the synthesized products are presented. Fifteen synthesized pyrimidine analogy have been tested for antimicrobial

activity against five gram-positive and eight gram-negative bacteria as well as three human fungal pathogens. Among the tested compounds, 5-bromopyrimidine derivatives (**20-23**) exhibited relatively greater inhibitory activity against most of the tested organisms.

In Chapter 5 conclusion of the thesis is described.

Prefatory Note

Analytical or laboratory grade solvents and chemicals were used in all experiments and these (Orotic acid, $POCl_3$, PCl_5, TFA, TFAA, NBS, Br_2, Pyridine, etc) were procured from E. Merck, Germany and Fluka, Switzerland. Reagent grade of Chloroform, n-Hexane, Methanol etc were purified by distillation at the boiling point of the respective solvent. Petroleum ether used during this research work had boiling point 40-60°C.

Purification of Reagents and Products

Drying of Methanol:

About 1.25g of clean and dry magnesium turnings and 0.125g of iodine were placed in dry 500 mL round bottle flask containing 30 to 40 mL of reagent grade methanol. The flask was then fitted with a condenser carrying a calcium chloride guard tube on the top. The mixture was warmed until the iodine disappeared; heating was continued until all magnesium was converted into pasty methanolate. About 230 mL of commercial grade methanol was then added to the flask and refluxed the mixture for an additional hour. The resulting mixture was distilled off and the first 10-15 Ml of distillate was discarded. Then the dry methanol was collected into a receiving flask from which it was stored into an air tight bottle.

$$Mg + 2CH_3OH \rightarrow Mg(OCH_3)_2 + H_2$$

$$Mg\ (OCH_3)_2 + H_2O \rightarrow 2CH_3OH + Mg(OH)_2$$

Drying of Synthetic Products:

All organic extracts were dried over anhydrous sodium sulfate (Na_2SO_4) before concentration.

Physical Analysis

Determining of Melting Point:

Melting points were determined on Gallenkamp (England) melting point apparatus and paraffin oil bath.

Infra-red (IR) and Ultra-violet (UV) Spectra:

The Infra-red spectra were recorded on KBr disc or films with a Shimadzu FTIR Spectrophotometer and UV spectra were recorded in dry ethanol with a Shimadzu UV Spectrophotometer at the Department of Chemistry, BUET.

Nuclear Magnetic Resonance (NMR) Spectra:

The NMR Spectroscopy is widely used for the detailed investigation of an unknown compound. With the help of this spectroscopy the structure of unknown compound can determined. ^{1}HNMR (400 MHz) and ^{13}CNMR (100MHz) spectra were recorded in deuterio chloroform ($CDCl_3$) with a Bruker DPX-400 Spectrophotometer (400 MHz) using tetramethylsilane as an internal standard at the Bangladesh Council of Scientific and Industrial Research (BCSIR) laboratory, Dhaka, Bangladesh.

Elementary Analysis:

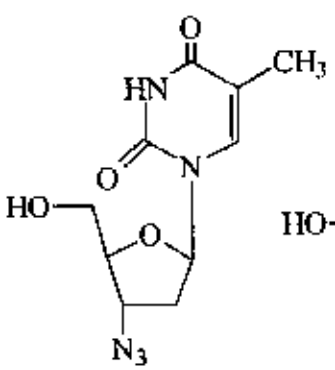
Elemental analyses were performed on a Parkin-Elmer 240C analyser from Nagoa City University, Japan.

Contents

Chapter 1
Introduction

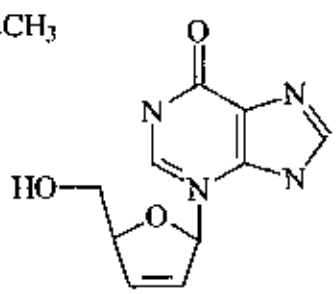
1.1A.

In the past few years the antiviral chemotherapy area has witnessed a remarkable production of antiviral compounds, particularly in the domain of the uracil bases and the nucleoside analogues. The acquired immunodeficiency syndrome (AIDS) was first recognized in 1981 [1] and has since become a major world wide epidemic. After the discovery that human immunodeficiency virus (HIV) is the causative agent of AIDS [2,3a-h,] numerous compounds have been reported to inhibit the application of human immunodeficiency virus (HIV) in vitro [3d,4.], yet only four agents have at this time been formally Licensed (in the USA) for clinical use in the treatment of AIDS[5]. These are zidovudine (3'-azido-2',3'-dideoxythy midine or azidothymidine 1 [AZT]; Retrovir)[6], didanosine(2',3-dideoxyinosine 2 [DD1]; videx)[7], zalcitabine (2',3'- dideoxycytidine 3 [DDC]; Hivid)[8], and stavudine (2',3'-didehydro-2',3'-dideoxy thymidine 4 [D4T]; zerit).



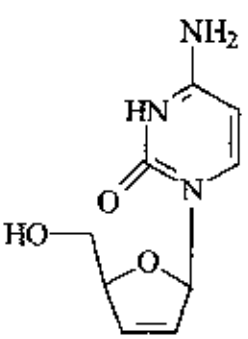
(1)

AZT, 1



(2)

DD1, 2



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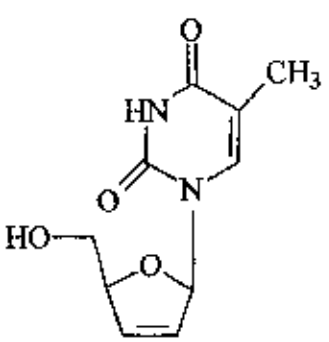
DDC, 3



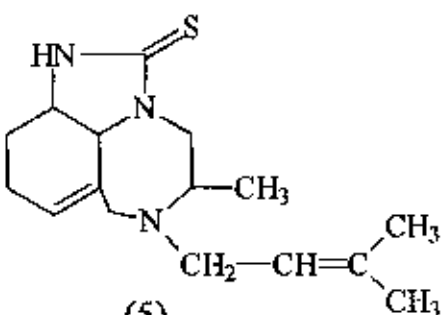
(4)

D4T, 4

The viral enzymes that have critical roles in the life cycle of the human immunodeficiency virus type-1(HIV-1) is the key target in the research for effective drugs useful for AIDS therapy. One such enzyme is reverse

transcriptase (RT) which contains both a DNA polymerase activity that can use either RNA or DNA as a template and a ribo nuclease H acivity [9,10]. A number of inhibitors of HIV-RT have been developed [11,12]. Generally these inhibitors can be divided into two classes:

1. Nucleoside analogues such as AZT **1** and DDC **3**.
2. Non nucleoside RT inhibitors (NNRT1) such as tetrahydroin dazo [4,5-I-jk] [1,4] benzodiazepin - 2 - (1H) – one (TIBO) 5 and 1 - [(2-hyroxyethoxy) methyl] – 6 - (phenylthio) thymine (HEPT) **6**.



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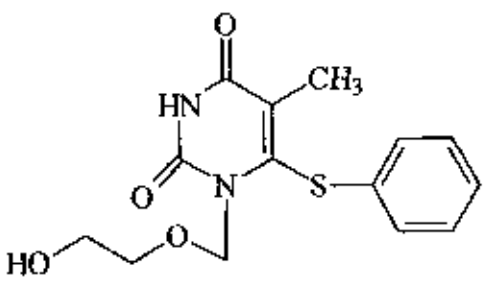


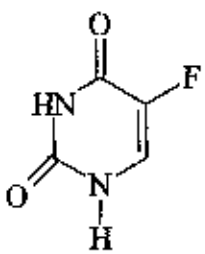
(6)

Here we shall scrutinize the Biological importance of some potent uracils and uridine derivatives substituted at C-5 and C-6 positions very briefly.

1.1B. Biological Evaluation of 5-Substituted Uracil Derivatives and Nucleosides.

5-Flurouracil (5-Fu,7) and 5-fluoro-2′-deoxy uridine (5-Fud R, **8**) developed by Heidelberger[13,14] are used clinically for the treatment of breast colon and rectum Cancer, 5-Fu is known as an anti metabolic and believed to inhibit thymidylate synthase (TC) enzyme.

5-Iodo-2′-deoxyuridine (IDU, **9**) is utilized clinically in the tropical treatment of herpes simplex keradtitis [15], a sighttreatening eye infection. It is also effective against mucocutaneous HSV infection and vaccinia virus (VV).



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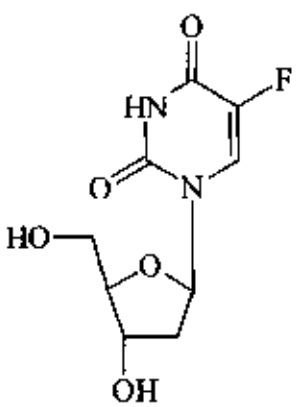

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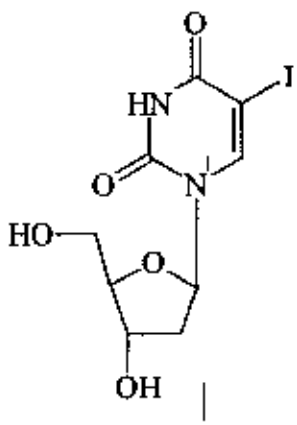


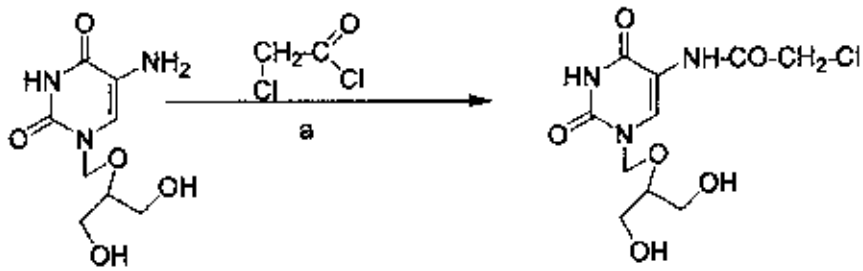
(9)

5-Trifluoromethyl-2′-deoxyuridine (TFT **10**) is an effective inhibitor of HSV[16] and used in the treatment of herpetic Keratitis, TFT, **10**, also exhibited in vitro action against human cytomegalo virus (HCMU) [17].

5-Bromouracil [5-bromo-2, 4 (^{1}H, ^{3}H) pyrimidinedione; 5 BrU] acts as an antimetabolite or base analog, substituting for thymine in DNA and can induce DNA mutation and 5-Bromo-2-deoxy-uridine is used to treat neoplasms.

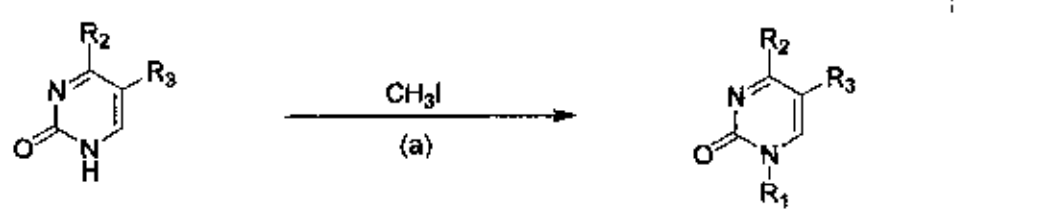
Some modified pyrimidine bases showed very different antiproliferative effects on the investigative panel of cell lines (Table 1 and Fig. 1). Uracil derivatives revealed a much broader range of cell growth inhibitory

activity compaed to cystosines. Compounds **3, 12-17, 24** did not show any or very weak antiproliferative effect. Compounds **4** and **5** showed moderate growth inhibitory activity mostly at the highest tested concentration



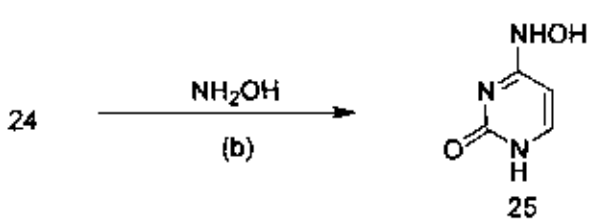
14 **18**

Scheme 3: Synthesis of C^5-chloroacetylated uridine derivatives **18**. Reagents and conditions: (a) $ClCH_2COCl$, $NaOH/H_2O$, 30 min, 0^0C; 90 min, 22^0C; MPLC (RP18, CH_3CN/H_2O=1/4) (**18**: 75%).



19. $R_2 = NH_2$, $R_3 = H$
20. $R_2 = NH_2$, $R_3 = CH_2OH$
23. $R_2 = SH$, $R_3 = H$

21. $R_1 = CH_3$, $R_2 = NH_2$, $R_3 = H$
22. $R_1 = CH_3$, $R_2 = NH_2$, $R_3 = CH_2OH$
24. $R_1 = H$, $R_2 = SCH_3$, $R_3 = H$



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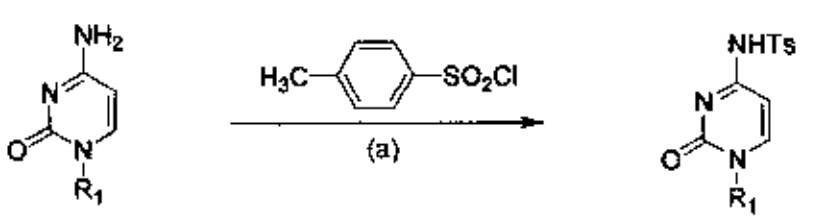
Scheme 4: Synthesis of cytosine derivatives **21, 22, 25** and 4-methylthiouracil **24**. Reagents and conditions: (a) CH_3I, $[(C_4H_9)_4N]OH$, DMF, 2 h, 22^0C; recrystallization from EtOH (**21**: 86%); column

chromatography (SiO_2, CH_2C_{12}/MeOH=1/1), (**22**: 82%); MPLC (RP18, CH_3OH) (**24**: 84%); (b) NH_2OH, EtOH, 5 h, reflux; recrystallizarion from EtOH (**25**: 78%).

On HeLa, MiaPaCa-2 and SW620 cells but not on MCF-7 and H460 cells. On the contrary, compounds 2, 6 and 18 showed rather strong antiproliferative and cytotoxic activity on the tested cell lines, comparable to 5-fluorouracil (5-FU). The most prominent activity was seen on HeLa and Mia-paCa-2 cells by 2 and 6 (Fig. 1), which prompted us to perform additional studies of the impact of 2 and 6 on the cell cycle of HeLa cells. Besides, we additionally tested 2, 6 (Fig. 1), 18 and 5-FU (data do not shown) for their inhibitory activity on human skin keratinocytes HaCaT, as a model of nontumorigenic cell line. Interestingly. Compound 6 strongly inhibited the growth (IC50=9 μM), while 2 and 18 did not (IC50 were > 100 and ≥ 100, respectively). On the other hand 5-FU markedly inhibited the growth of HaCaT cells (IC50=0.4 μM).

In contrast to uracil derivatives, in general cytosine derivatives did not show any growth inhibitory activity (22, 25, and 27). The only exceptions were 1-methylcytosine 21 causing a weak antiproliferative effect exclusively on the HeLa cell line and N1-tosylated cytosine 26, weakly inhibiting the growth of MCF-7 cell line. Further experiments are needed



19. R_1 = H
21. R_1 = CH_3

26. R_1 = H
27. R_1 = CH_3

Scheme 5. Sythesis of tosylated cytosine derivatives **26** and **27**. Reacgents and conditions: (a) TsCl, pyrimidine, 6 h, 220C; column chromatography (SiO_2, CH_2Cl_2/MeOH= 50/1) (**26**: 90%, **27**: 88%).

Table 1: Tumor cell growth inhibition presented as IC_{50} values (μM)

IC_{50}[a] (μM)					
Compound	Cell lines				
	HeLa	MiaPaCa-2	SW620		MCF-7
	H460				
2	4 ± 2	17±3	12±9	15±2	14±1
3	>100	>100	>100	>100	>100
4	50±46	50±47	50±44	>100	>100
5	86±8	80±18	70±25	>100	>100
6	4±3	3±2	70±25	≥100	76±20
12	>100	>100	>100	>100	>100
13	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100
15	30±13	>100	>100	>100	>100
16	>100	>100	>100	>100	>100
17	73±26	>100	>100	>100	>100
18	12±1	18±3	19±3	32±18	24±3
21	12±5	>100	>100	>100	>100
22	>100	>100	>100	>100	>100
24	>100	>100	>100	>100	>100
25	>100	>100	>100	>100	>100
26	>100	>100	>100	27±5	>100
27	>100	>100	>100	>100	>100
5-FU[b]	4±1	10±3	9±2	15±2	2±0.7

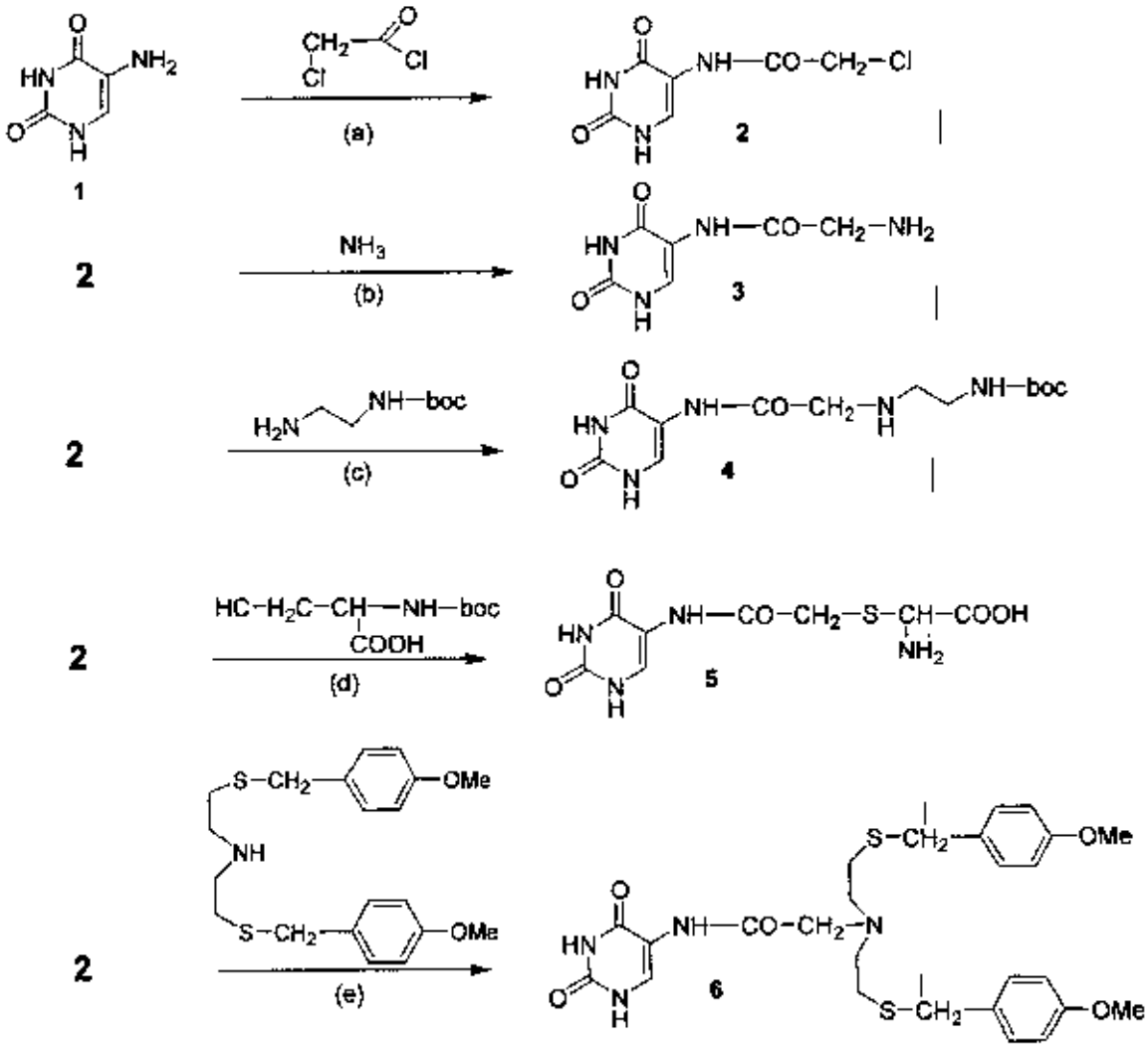
[a]IC_{50}- the concentration that causes 50% growth inhibition.

[b]**5-FU**-5-fluorouracil.

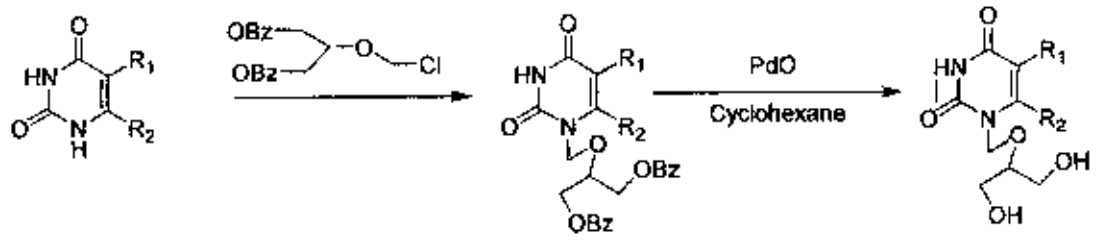
to explain the unique selectivity of 21 and 26 in the cell growth inhibition.

At first, uracil has modified at the C5-position. Starting from 5-aminouracil 1, the corresponding 5-(chloroacetyl) aminouracil 2-already known to show antiviral activity [8]- was synthesized by treatment of chloroacetyl chloride in 1 M sodium hydroxide solution at 0^0C. after acidification with hydrochloric acid the product was obtained in high purity by repetitive crystallization from water. Compound 2 was converted into 5-(aminoacetly)-aminouracil 3 with concentrated ammonia. The product was purified by column chromatography on Sephadex G10 giving 3 in 81% yield. As shown in Scheme 1, other uracil derivatives have been also accessible by conversion of 2. Thus, heating 2 with *N*-boc-ethlenediamine in methanol under pressure at 900C led to 5-(*N*-boc-ethylenediaminoacetyl)aminouracil 4. The reaction product was separated by column chromatography on Sephadex G10. Coupling of 2 with boc-protected cysteine was performed in ethanol and 2 M sodium hydroxide. The protection group was then removed by the treatment with 1 M hydrochloric acid. Purification was carried out by MPLC yielding S-{2-[(2,4-dioxo-1,2,3,4,-tetrahydropyrimidine-5-yl)amino]-2-oxoethyl}-D-cysteine 5. The 5-[bis-(2-p-methoxybenzylthioethyl)amine]acetylaminouracil 6 was synthesized by direct condensation of the persilylated 5-(chloroacetyl)aminouracil 2 with bis-(2-p-methoxybenzythioethyl)amine [9] in acetonitrile followed by column chromatography on silica gel.

Scheme 2 shows the structure of some uridine derivatives recently described as potential substrates of herpes simplex virus type-1 thymidine kinase [10]. These compounds were



also investigated in vitro by screening cellular growth inhibition on several different human tumor cell lines. Briefly, modification of the N^1-position of the uracil molecule was accomplished by direct condensation of the appropriate persilylated base with the chloromethyl ether 1, 3-dibenzyloxy-2-chloro-methoxypropane and tetrabutylammonium iodide as a catalyst. Thus the nucleic bases uracil 7, 5-hydroxyuracil 8, 5-aminouracil 1, 5-iodouracil 9, 6-methyluracil 10 and 5-iodomethyluracil 11 were coupled to 1,3-dibenzyloxy-2-chloro-methoxypropane in dry dimethyl formamide with



7. R_1 = H, R_2 = H
8. R_1 = OH, R_2 = H
1. R_1 = NH_2, R_2 = H
9. R_1 = I, R_2 = H
10. R^1 = H, R_2 = CH_3
11. R_1 = I, R_2 = CH_3

7. R_1 = H, R_2 = H
8. R_1 = OH, R_2 = H
1. R_1 = NH_2, R_2 = H
9. R_1 = I, R_2 = H
10. R^1 = H, R_2 = CH_3
11 R_1 = I, R_2 = CH_3

7. R_1 = H, R_2 = H
8. R_1 = OH, R_2 = H
1. R_1 = NH_2, R_2 = H
9. R_1 = I, R_2 = H
10. R^1 = H, R_2 = CH_3
11. R_1 = I, R_2 = CH_3

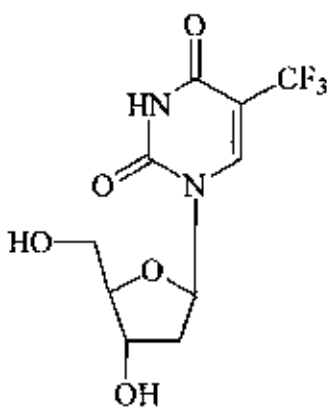
triethylamine yielding the *o*-benzylated precursors. Removal of the benzyl protection groups with palladium oxide in cyclohexene-[1,3-dihydroxy-2-propoxy)methyl]uracil(Amino-acyclur) 14, 5-iodo-1-[(1,3-dihydroxy-2-propoxy)-methyl]uracil (Metacyclur) 16 and 5-iodo-6-methyl-1-[(1,3-dihydroxy-2-propoxy)methyl]uracil (Iodmetacyclur) 17.

Of special inetrest is the chloroacetylated uridine derivative 18 (Scheme 3) which allows a direct comparison of biological activity with the corresponding uracil compound 2. The preparation of 18 was similar to 5-(chloroactyl)aminouracil 2. Amino-acyclur 14 was treated with chloroacetyl chloride in aqueous sodium hydroxide at 0^0C for 90 min. the purification of the reaction product was carried out using reverse phase MPLC.

Furthermore, some cytosine derivatives modified at the N1- and N4-position have been investigated. Methylation of cytosine 19 and 5-hydroxymethylcytosine 20 [11] by the treatment with methyliodide and tetrabutylammonium hydroxide solution in DMF led smoothly to 1-methylcytosine 21 [12] and 1-methyl-5-hydroxymethylcytosine 22. the methylation of 4-thiouracil 23 under the same reaction conditions yielded 4-methylthiouracil 24 [13]. By refluxing 24 with hydroxylamine hydrochloride in dry ethanol followed by treatment with concentrated ammonia, N4-hydroxycytosine 25 [14] was obtained (Scheme 4).

A tosyl group was introduced into both cytosine 19 and 1-methylcytosine 21. Therefore, compounds 19 and 21 were treated with p-toluenesulfonyl chloride in dry pyridine yielding N1-(p-toluenesulfonyl)cytosine 26 [15,16] and 1-methyl-N4- (p-toluenesulfonyl)cytosine 27. Pure products were obtained by column chromatography (Scheme 5)[112].

[E]-5-(2-bromovinyl) - 2′- deoxyuridine (BVDU,**11**) is the powerful inhibitor of HSV-1, VZV and pseudorabies virus,[18].BVDU,**11** is also active against bovid herpes virus type -1(BHV-1), simian varicella virus (SVV) and nuclear poly hedrosis virus (NPV)[18]. Robins et al[19] synthesized various 5-alkynyl -2′-deoxyuridines and observed that 5-ethynyl -2′-deoxyuridine **12** exibited excellent anticancer properties. (ID_{50} = 0.091 μg/ml in L1210 cells) and antiviral properties[20] against HSV-1 (ID_{50} = 0.6μg/ml), HSV 2 (ID_{50}=1.5μg/ ml) in all primary rabbit kidney cells in culture.



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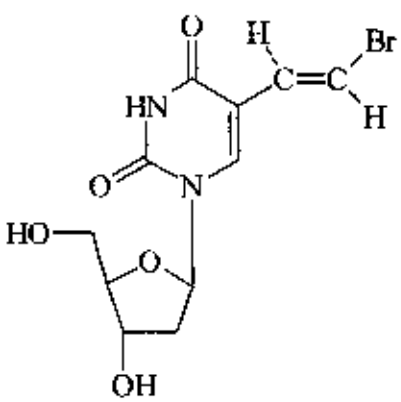

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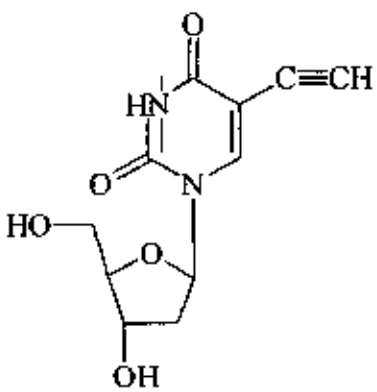
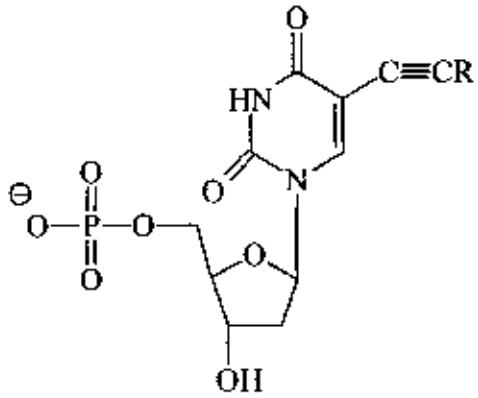


(12)

The 5-alkynyl -2′-deoxyuridines-5′-monophosphates 13 were found to be good inhibitors of T.S enzyme the 5-ethynyl derivative being the most effective [21] (Table-1).

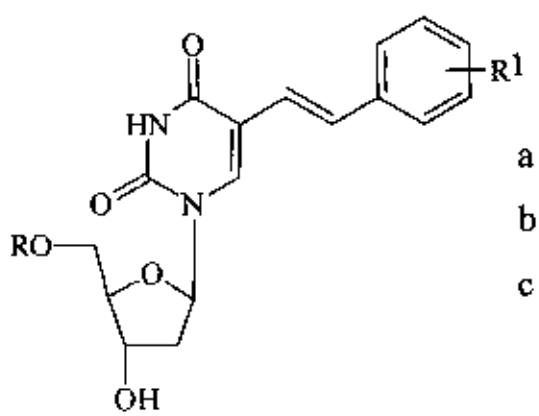


(13)

Table-1: Inhibition of TS enzyme by compound13.

	R	Ki (uM)
13a	H	0.1
13b	CH_2OH	3.0
13c	CH_2CH_2OH	1.9
13d	*n*-Bu	2.6
13e	Ph	2.0

A number of 5-styrul derivatives 14 a-c of d-Urd and d-UMP were synthesized by Bigge et al [22] and evaluated their inhibitory affects on L1210 cell growth [23]. The nucleosides 14 were found to be petent reversible inhibitors of TS enzyme very low value of ki/km ratio ranging from 0.035-0.08.



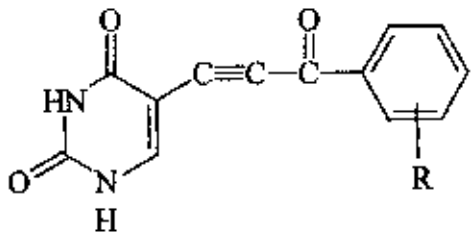
a R = R' = H

b R = H, R' = NO_2 (*m*)

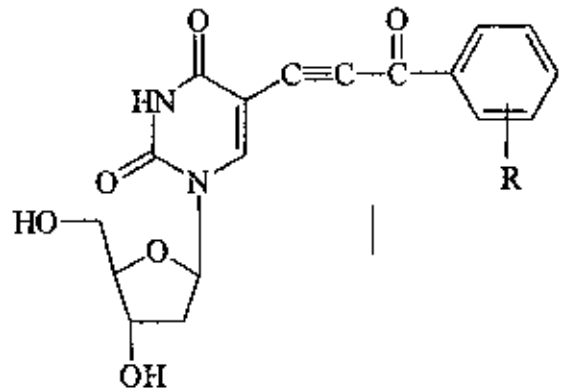
c R = PO_3^{-2}, R` = N_3 (*m*)

(14 a-c)

Kundu *et al*[24] developed a series of 5-acylethynyl uracils and their corresponding 2′-deoxyribonucleosides[25] 16. Compouds 15 displayed excellent anticancer properties in cell culture against CCRF-CEM human lymphoblastoid cells and L12110/0 mouse leukemia cell lines 24, while compounds 16 were comparatively less active.



(15 a-g)

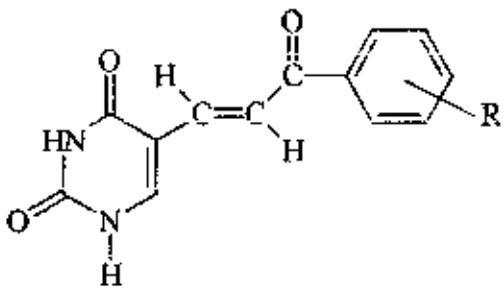


(16)

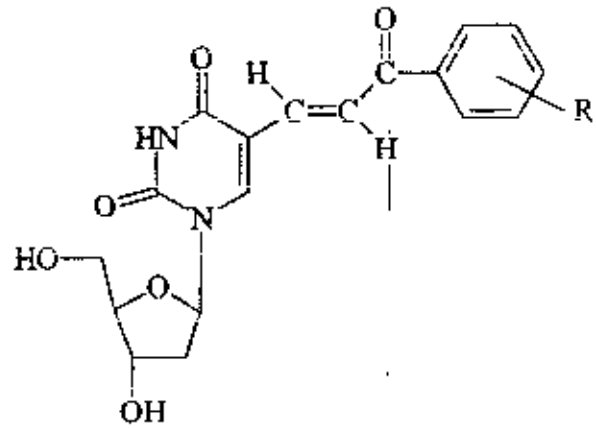
R= H, Me (*p*), OMe (*p*), Cl, Me (*o*), OMe (*o*).

Compounds **15a** and **15 b** were found to be as potent as the parent anticancer drug 5- fluorouracil (5-Fu) against Ehallich ascites carcinoma (EAC) cells in Swiss Albino mice in vitro. Compound **15** were also subjected to TS-inhibition studies and compound **15b** was found to be an effective inhibitor of TS enzyme[24].

Kundu *et al*[26] also synthesized (*E*)-5-(2-acylvinyl) uracils 17 and their corresponding 2′-deoxyribonucleosides[27] **18**.



(17 a-d)



(18 a, b)

R= H, Me (*p*), OMe (*p*), $(OMe)_2$ (3, 4) .

Compound **17** showed moderate anticancer activities when tested in vitro against CCRFCEM human lymphosoblastoid cells, HT-29 colon carcinoma cell and L/1210/0 mouse leukemia cell and also found to inhibit the TS enzyme. Compound **18a** and **18b** were found to be weakly toxic against L1210/0 murine leukemia cells and human T-lymphocyte cells (Molt4/CB CEM) compounds **18** were also tested against various viral cell lines and did not show any appreciable antiviral selectivity against HIV-1 and HIV-2 in CEM cells and other viruses [27].

Although these drugs can extend the life of AIDS patients, non are capable of curing the disease, and serious sides effects are induced. For example, treatment with AZT leads to a suppression of bone–marrow formation which often causes anemia and leucopenia, resulting in the need frequent blood transfusions[28].

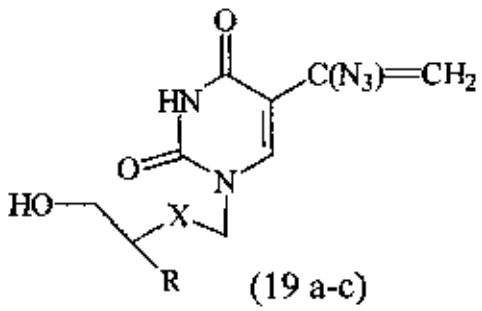
The use of DD1, DDC, D4T is associated with painful sensory-motor peripheral neuropathy, as well as acute pancreatitis [29,30] and hepatotoxicity in some cases. The standard antiviral therapy for initiating treatment of

patient with HIV infection AZT has also a very short half-life in the body, and high doses (250 mg) must be ingested every 4h to maintain a constant level of drug in the body. Long-term treatment of patients with all these drugs has led to emergence of drug-resistant HIV strain[31,32], more importantly. Therefore, the need for other promising AIDS drug candidates having improved selectivity and activity against HIV is extremely urgent[34,35].

A novel class of 5-substituted acyclic pyrimidine 19a-c synthesized by Rakesh Kumar *et al* [36] were found to be exhibit potent and selective in vitro anti HBV activity against duck hepatitis B virus (DHBV) infected primary duck hepatocyte at low concentration (EC_{50} = 0.01-0.1ug/ml range) (19c), the most active anti-DHBV agent, possessing a [4-hydroxy-3-(hydroxymethyl)-1-butyl] substituted at N-1, exhibited an activity [EC_{50} of 0.10-0.0μg/ml] comparable to that of reference compound (-)-β -L-2′3-dideoxy -3′-thiacytidine (3-TC) [EC_{50}=0.01-0.05 μg/ml). In contrast, related 5-[2-C1-azirinyl] uracil analogues (20b,c)[36] were devoid of anti-DHBV activity. The pyrimidine nucleoside (19a-c), 20b,c) exhibited no cytotoxic activity against a panel of 60 human cancer cell lines.

Table -2

In vitro Activity against Hepatitis B virus in primary Duck Hepalocyte Cultures (DHBV) and toxicity stationary and proliferating cell for 5-substituted uracil (19 a-c) and reference compounds.



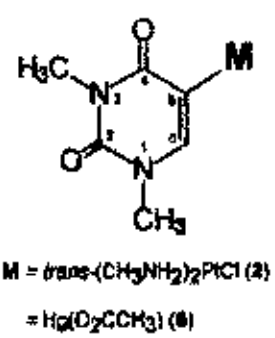
(19 a-c)

No	X	R	% inhibition at 10µg/ml[EC50 ()---g/ml][b]	Toxicity CC_{50}(µg/ml)		Cell proliferation IC(µg/ml)	
			DHBV primary duck hepatocytes	HFF[c]	Vero[e]	HFF[f]	Daudi[g]
19a	0	H	84[0.01-0.1]	>100[d]	>100	>100	>50
19b	0	CH_2OH	86[0.01-0.1]	>100	>200	100	>50
19c	C	CH_2OH	93[0.01-0.05]	>100	>200	>100	>50
3TC	-	-	96[0.01-0.50]	ND	>100	ND	ND

A synthesis of 5-chloro- and 5-fluoro-1-(2'-fluoro-2'-deoxy-beta-D-ribofuranosyl)uracil (4a and 4b) and their 2-14C analogues has been developed. The tissue distribution of these radiolabeled compounds in BDF1 mice bearing Lewis lung tumors has been investigated. Compounds 4a and 4b undergo rapid blood clearance and urinary excretion. Selective retention of radioactivity was observed in tumor tissue, spleen, and intestine and with compound 4b also in the bone. Maximum tumor to blood ratios of 4.2 for the 5-chloro compound 4a and 10.3 for the 5-fluoro compound 4b were observed at 4h. These compounds were resistant to phosphorylytic cleavage and dehalogenation as indicated by the metabolic products observed in the urine and the

absence of radioactivity in the liver. The interaction of 4b with the mouse erythrocyte transporter system was compared with physiological nucleosides in respect to ability to effect zero-trans influx of thymidine. The results show a competitive inhibition between 4b and the natural nucleoside. Evidence is presented for the direct metabolic defluorination of 5-fluorouracil to form uracil[109].

Considerable anti-viral activities found in 5-metal uracil. 1,3-Dimethyluracil (1,3-DimeU) reacts with *trans*-$[(CH_3NH_2)_2Pt(H_2O)_2]^+$ to give *trans*-$[(CH_3NH_2)_2Pt(1,3\text{-DimeU-}C5)(H_2O)]X$ (X = NO_3^-, 1a, ClO_4^-, 1b) and subsequently with NaCl to give *trans*-$(CH_3NH_2)_2Pt(1,3\text{-DimeU-}C5)Cl$ (2) or with NH_3 to yield *trans*-$[(CH_3NH_2)_2Pt(1,3\text{-DimeU-}C5)(NH_3)]ClO_4$ (3). In a similar way, $(dien)Pt^{II}$ forms $[dienPt(1,3\text{-DimeU-}C5)]^+$ (4). Reactions leading to formation of 1 and 4 are slow, taking days. In contrast, $Hg(CH_3COO)_2$ reacts fast with 1,3-DimeU to give (1,3-DimeU-*C5*)$Hg(CH_3COO)$ (5). Both 1-methyluracil (1-MeUH) and uridine (urdH) react with $(dien)Pt^{II}$ initially at N(3) and subsequently with either $(dien)Pt^{II}$ or $Hg(CH_3COO)_2$



M = *trans*-$(CH_3NH_2)_2PtCl$ (2)

= $Hg(O_2CCH_3)$ (6)

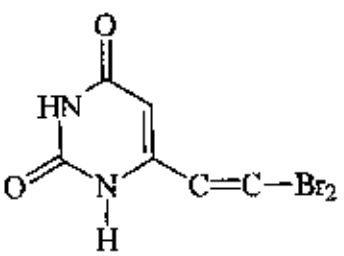
also at C(5) to give the diplatinated species 7 and 9 or the mixed PtHg complex 8. C(5) binding of either Pt^{II} or Hg^{II} is evident from coupling of

uracil-H(6) with either ^{195}Pt or ^{199}Hg nuclei and ^{3}J values of 47–74 Hz (for Pt compounds) and 185–197 Hz (for Hg compounds). J values of Pt compounds are influenced both by the ligands *trans* to the uracil C(5) position and by the number of metal entities bound to a uracil ring. Both 2 and 5 were X-ray structurally characterized. 2: monoclinic system, space group $P2_1/c$, a = 15.736(6) Å, b = 11.481(6) Å, c = 25.655 (10) Å, β = 145.55(3)°, V = 2621.9(28) Å^3, Z = 4. 5: monoclinic system, space group $P2_1/c$, a = 4.905(2) Å, b = 18.451(6) Å, c = 11.801(5) Å, β = 94.47(3)°, V = 1064.77(72) Å^3, Z = 4[110].

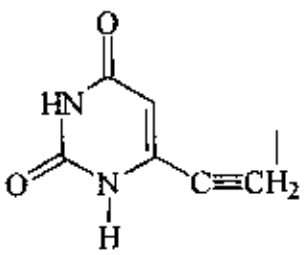
1.1C. Biological evaluation of 6-substituted Pyrimidine and its derivatives:

6-Substituted uracil have got much attention because of their possible use as anticancer and anti-AIDS agents [37,38]. Here we shall discuss the biological activity of some 6-substituted uracil and their nucleosides.

6-(2,2-dibromovinyl) uracil **21** and 6-Ethyl uracil **22** were synthesized and evaluated for their biological properties by Schroeder *et al* [39](compound **21** and **22** showed moderate antitumor activities)

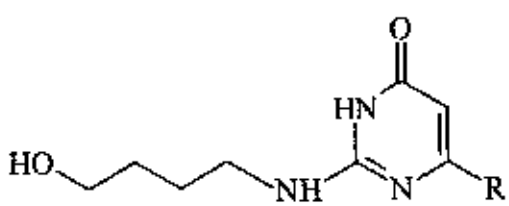


(21)

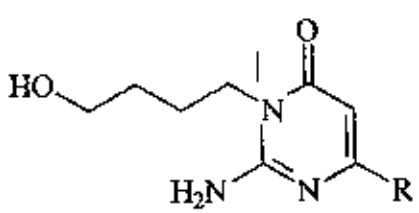


(22)

The solution and solid phase synthesis of substituted 2-(4-hydroxy butyl) amino-4-(3*H*)-pyrimidinones 23 as HIV-1 RT inhibitors was synthesized by Nizi *et al* [40].The evaluated compounds 23a-c and 24a-c in enzyme assays against recombinant HIV-1 RTS from both wild type (Wt) and clinically relevant mutant viruses resistant to T1BO/ neoirapine (L1001, K103N and V106A), using neoirapine as reference drug. The potentiality of 21b to inhibit the recombinant enzymes was found to be as follows: 410(wt), 525 (L1001), 840 (K103N) and 75 (V106A) reported as ki (nm) values.



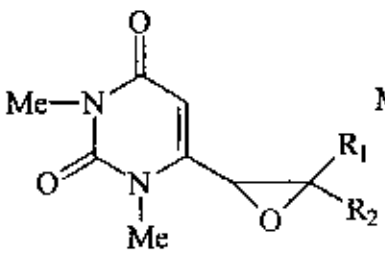
(23 a-c)



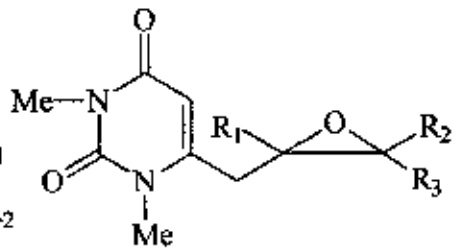
(24 a-c)

23,24 (a-c) R = Me, Br, CH_2Co_2Me.

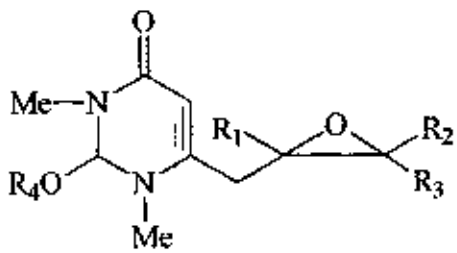
Saladino *et al* [41] synthesized several new 6-oxiranyl uracil 25 a-c, 6-methyloxiranyluracils 26 a-g and pyrimidine derivatives 27 a-d which were found to be a potent and selective antiviral against the parainfluenza 1(sendai) virus replication.



(25 a-c)



(26 a-g)



(27 a-d)

[25a-c] C: R_1 (R_2) = Me (Ph), H (Ph), Ph (H)

26a-g : R1 (R_2) [R_3] = Me (H) [H], Ph (H)[H], t-Bu (H) [H], Me (Me) [H], Me (Me) [H] (CH_2) {$(CH_2)_2$} [H], $(CH)_4$ {$(CH_2)_4$} [H].

27a-d: R_1{R_2}(R_3)[R_4] = $(CH_2)_1$ {$(CH_2)_4$} (H)[n-pr], Ph{H}(H)[n-pr][cyclohesexyl, Me{H}(Me)[cyclohexyl].

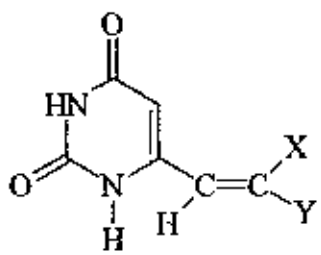
All the compounds have been assayed for antiviral activity on parainflunza 1(sendai) virus replication in Madin Darby cnine kidney cells (MDCK cells) by the measure of the hamagglutinin units (HAV) in the supernatant of the infected cells. The flowing structure-activity relationships could be tentatively reported on the basis of above data;

1) The N, N-dimethyl uracil scaffold very unusual for antiviral compound, along with C-6 substitution on the uracil ring, seems to be an important feature for active compounds.

2) The position, the substitution pattern, and the stereochemistry of the oxirane ring play an important role in modulating both the activity and

the toxic effect of the products. In particular, 6-oxiranyl derivation 25a, and 25b are more active then corresponding 6-methyl oxiranyl derivatives.

6-Vinyl uracils 28 and their corresponding uridines 29 were synthesized by Megati *et al* [42] and 6- vinyluracil 28a was found to be able to inhibit the growth of L1210 in vitro.



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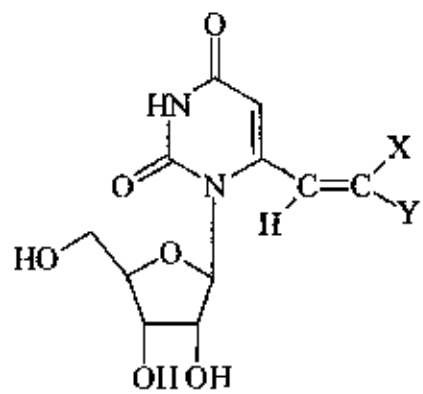


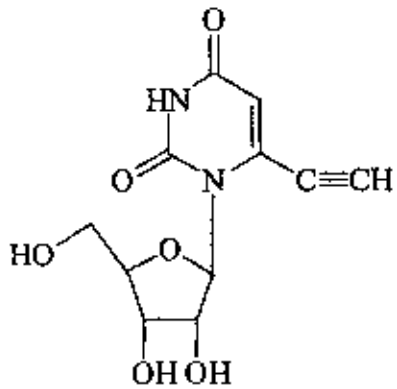
(29)

28a-f: X (Y) = H (H), H (Me), H (COOEt), Br (COOEt), Cl (COOEt);

27 a-d : X(Y)= H(H), H (Me), H(COOEt), Br(COOEt).

6 - (2-Bromo) vinylester **28e** and 6-(2-Chloro) vinylester **28f** of uracil inhibited the cell growth but not 6-vnylester derivative **28d.** In contrast with the free base **28**, the nucleosides **27 a-d** were found to be comparatively inactive.

6- Ethynyl uridine **30** was found to be barren of any cytostatic activity against murine (L1210 and FM 34) cells and human T-lympoblast Molt/4F and MT-4 cells in culture, while its 6-vinyl counterpart **29a** (X= Y= H) showed moderate activity [37].



(30)

A series of 6- substituted uracil acyclonucleoside was recently developed and 1-[(2-hydroxyethoxy)methyl]-6-phenylthiothymine (HEPT,6) and its analogues were found to be an excellent and specific inhibitors of HIV-1 virus type1. The anti-HIV-1 activity and citotoxicity of HEPT 6 were measured and compared with the known drugs like AZT, DDC and DDA which are under activity consideration as anti HIV agent (Table-3).

Table 3: Anti-HIV1 activity of HEPT and Nucleoside analogues in MT-4 cells.

Compound	$EC_{50}(\mu M)^a$	$CC_{50}(\mu M)^b$	SI^c
HEPT	7.0	740	106
AZT	0.016	20	1250
DDC	03	40	133
DDA	6.3	890	141

[a Ec_{50}, effective concentration required to achive 50% protection of MT-4 cells against cytopthic effect of HIV-1. bcc_{50}, cytotoxic concentration required to reduce viability of mockinfected MT-4 cells by 50% . CSI, selectivity index (ratio of CC_{50} / EC_{50})].

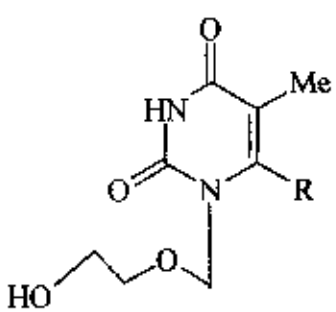
From the table-3 it is observed that HEPT shows inhibitory effect on the cytopathogenicity of HIV-1 (HTLV-111 Bstrain) in MT-4 cells. HEPT

also exhibits comparable effective concentration (EC_{50}) and cytotoxic concentration (CC_{50}) to DDA, but less activity and cytotoxicity than AZT and DDC.

The inhibitor effects of HEPT against other retroviruses were observed the high specificity of HEPT against HIV-1, unlike the common drugs like AZT, DDC or DDA, DNA viruses and other retroviruses including HIV-2 remained unaffected by HEPT[44].

The excellent inhibitory effect of HEPT against HIV 1 encouraged the modifications of HEPT at various position (C-5,C-6, and also in the N-1 acylic chain). Anti HIV-1 activity of HEPT analogues modified at C-6 position of compound **31** is shown in Table-4. It was found that replacement of phenylthio group by simple alkylthio group 31 **b** in HEPT afforded uniform inactivity **31c** showed comparable activity. Replacement of sulfur atom in HEPT by oxygen **31d**, nitrogen **31e** or halogen **31f** atoms gave poor results. On the other compound **31k** had considerable effect against HIV-1.

Also modification at the c-6 modified analogues suggested the necessity of ring structure in the c-6 position for this compound to be effective against HIV-1[45].

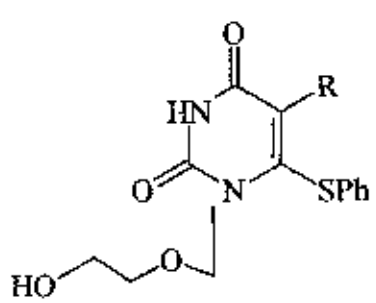


(31)

Table 4

Compound	R	Ec(μM)	CC_{50}(μM)	SI
31a	SPh	7.0	740	106
31b	SMe	>250	>250	~ 1
31c	SC_6H_{11}	8.2	664	81
31d	Oph	85	345	4
31e	NHPhI	>327	327	<1
31f	I	>80	400	>5
31g	C ≡CH	>5.5	5.5	<1
31h	C≡ CP	>14	14	~1
31i	CH =CH_2	>250	250	<1
31j	CH(OH)Ph	>400	400	<1
31k	CH_2Ph	23	352	15.3
31l	CH_2CH_2Pn	>444	444	<1

The modiffcation of HEPT at C-5 position by introducing methyl, iodo, ester, amido, ethynyl or vinyl groups resulted in increasing cytotoxicity with comparable EC_{50} values (Table-5). It was observed that 5-ethyl 32i and 5-isopropyl 30k analogues of HEPT were highly potent and selective inhibitors of HIV-1[46].

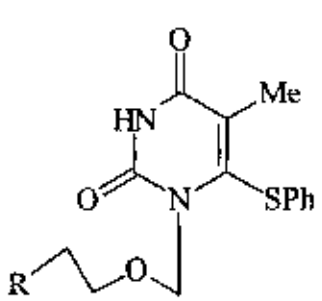


(32)

Table 5: Anti HIV-1 activity of HEPT analogues Modified in c-5 position of 32.

Compound	R	$EC_{50}(\mu M)$	$CC_{50}(\mu M)$	SI
32a	Me	70	740	106
32b	I	3.6	20	5.6
32c	COOMe	6.6	6.6	<1
32d	CONHPh	0.18	18	<1
32e	C ≡CH	>18	18	<1
32f	C ≡ CPh	>3.4	3.4	<1
32g	CH = CH_2	>250	>250	~1
32h	CH_2Ph	>23	23	<1
32i	Et	0.12	400	3300
32j	n-Pr	3.4	244	72
32k	*i* - Pr	0.063	231	3670

The N-1 acyclic side chain of HEPT was also varied and their antiviral activities were evaluated. It was found that O-acylated analogue 33b should comparable anti-HIV-1 activity with increase in cylotoxicity with respect to the parent compound HEPT. The deoxy HEPT analogues 32e-j was found to be more active and among this, the N-1 ethoxy methyl derivatives 33f showed high activity and moderate cell toxicity with selective index[47].

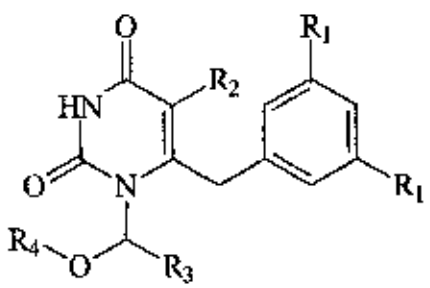


(33)

33 a-j: R= CH_2OH, CH_2OAC, CH_2OMe, CH_2OCHPn, H, Me, Et, CH_2F, CH_2N_3, Pr.

33	R_1	R_2	R_3
a	H	Et	Me
b	H	Et	Et
C	H	i-Pr	Me
d	H	i-Pr	Et
e	Me	Et	Me
F	Me	Et	Et

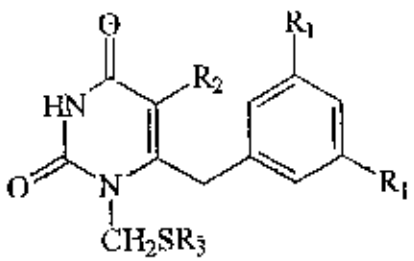
HEPT analogue 6-benzyl-1(ethoxymethyl)-5-isopropyuracil (MKC-442) 34a has been chosen as a candidate for clinical trails with AIDS patients[48] and 6-(3', 5'-dimethylbenzyl)-1(ethoxymethyl)-5 ethyluracil 34e(E-EBU-dM) showed excellent antiviral activitty[49] . Novel 6- 6- benzyluracil analogues of HEPT 34,35,36 including MKC-442 34d and E-EBU-dm 32e were recently synthesized and evaluated[50]. These results are summarized in Table-6 together with these of AZT.



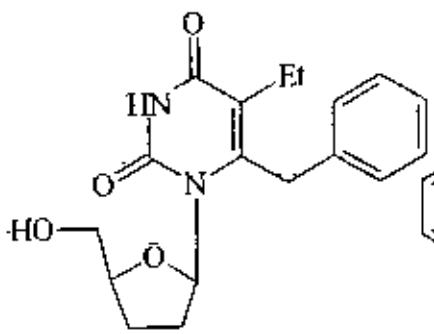
(34 a-g)

34	R_1	R_2	R_3	R_4
a	H	Et	Me	Me

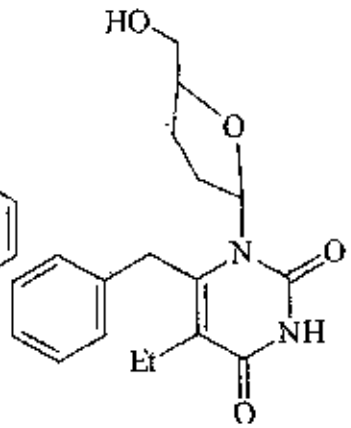
b	H	Et	Me	Et
c	H	Et	Me	$(CH_2)OH$
d	H	i-Pr	H	Et
E	Me	Et	H	Et
f	Me	Et	Me	Me
g	Me	Et	Et	Et



(35 a-f)



(36 a)



(36 b)

Table -6: Antiviral activity of HEPT analogues 34, 35, 36 and AZT against HIV-1 in MT-4 cells.

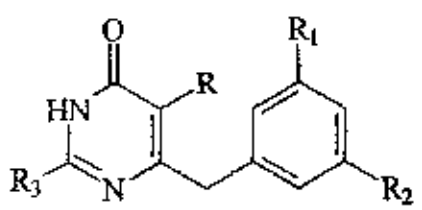
Compound	ED_{50}[a](μM)	CD_{50}[b](μM)	SI[c]
34a	>100	-	-
34b	>100	-	-
34c	>100	-	-
34d	0.005	141	28000
34e	0.004	100	25000
34f	2	100	50
34g	15	130	8.7
35a	0.002	32	16000
35b	0.040	37	925

35c	0.020	37	1850
35d	0.006	37	6200
35e	0.050	52	1.040
35f	0.004	68	17000
36a	37	52	14
36b	0.5	1	1
AZT	0.040	52	1300

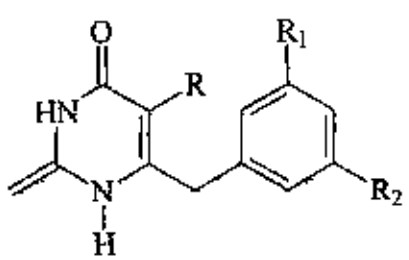
a: Effective dose of compound, achieving 50% inhibition of HIV-1 antigen production in MT -4 cultures.

b: Cytotoxic dose of compound, required to reduce the proliferation of normal uninfected MT-4 cells by 50%.

c: Selectivity index : ratio [CD_{50}/ED_{50}] . It was observed that the analogues 33 with Oxygen replaced with sulfur showed comparable activities and selectivities with those found for MKC-442 34d, and E-EBU-dM, 34e.



(37 a-i)



(38 a-c)

La Colla *et al*[50,51] developed a new class of non-nucleoside reverse transcriptase inhibitors (NNRTTs) viz. 3,4-dihydro -2-ackoxy-6-benzyn-4-oxopyrimidies (DABOs 37). Most of these DABO derivatives 37 were

lacking of cytotoxicity in MT-4 cells, but selectivity inhibited the HIV-1 induced cytotathic effects (Table -7)

Table -7 : Cytotoxicity and Anti-HIV activity of DABOS 37 and 38 in HIV-infected MT4.

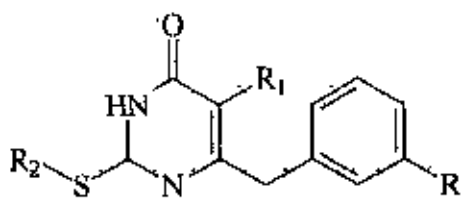
Compound	R	R_1	R_2	R_3	CC50[a]	EC50	SI[b]
37a	H	H	H	Me	>1000	>200	-
37b	H	H	H	Sec-butyl	344	5.5	62
37c	H	H	H	Cyclohexyl	157	9.0	17
37d	H	Me	H	Sec-butyl	>367	3.3	>111
37e	H	Me	H	Cyclohexyl	>335	0.8	418
37f	H	Me	Me	Sec-butyl	>349	2.7	>129
37g	H	Me	Me	Cyclohexyl	>320	1.1	>291
37h	Me	Me	H	Sec-butyl	>350	3.1	>113
37i	Me	Me	Me	Sec-butyl	>333	0.8	>416
38a	H	H	H	H	>1000	200	-
38b	H	Me	H	H	>463	92	5
38c	Me	Me	Me	H	>410	38	>11
HEPT	-	-	-	-	740	7	106
AZT	-	-	-	-	80	0.01	8000

a. CC50: Cytotoxic concentration, concentration required to reduce viability of mock infected MT-4 cells by 50% .

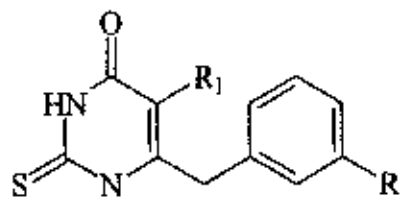
b. SI: Selectivity index (ratio of CC_{50}/EC_{50}).

The size of the alkoxy chain at the C-2 position of the pyrimidine ring appeared to be a determining factor for antiviral activity i.e. the increasing length of the derivatives **37c**, **37e**, **37g** showed better result. Again introduction of methyl group at the 3 –position in the benzyl moiety led to a significant increase in both potency and selectivity **37e**, **37i**.

To develop a more potential and selective compounds, the oxygen atom at C-2 position of pyrimidine ring was replaced by a sulfur atom yielding the thio analogues of DABO(S-DABOs 39)[53]. The antiviral activities of S-DABOs **39** and **40** are summarized in Table 8.



(39 a-g)



(40 a-c)

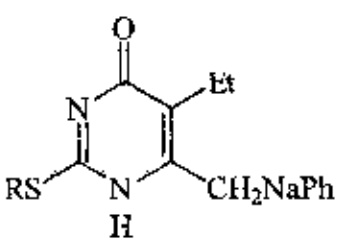
Table-8: Cytotoxicity and anti-HIV-1 activity of S-DABOs 39 and 6-Benzyl-2-thiouracils[40].

Compound	R	R_1	R_2	CC_5	EC_{50}	SI
39a	H	H	Sec-butyl	150	1.2	125
39b	H	H	Cyclohexyl	>330	0.8	>412
39c	H	Me	Sec-butyl	>347	0.6	>578
39d	H	Me	Cyclohexyl	>318	1.5	>212
39e	Me	H	Cyclohex	>333	0.6	>555

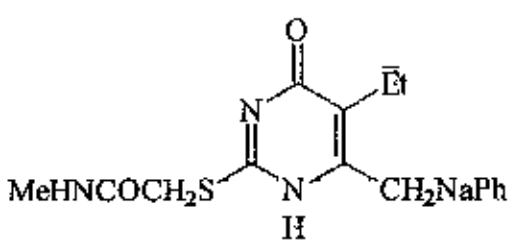
			yl			
39f	Me	H	Cyclohex yl	318	0.6	>530
39g	Me	Me	Cyclohex yl	>318	0.6	>350
40a	Me	H	-	258	>258	<1
40b	H	Me	-	431	>108	<4
40c	Me	Me	-	284	>102	-

Most of the SDABOs 39 were found to be non-toxic in MT4 cells and when assayed in HIV-I infected MT-4 cells, it behaved like the DABOs 37 did. Hence a large alkylthio group like Sec-butyl, cyclopentyl, cyclohexyl at C-2 position of pyrimidine ring and methyl, cyclohexyl at C-2 position of p yrimidine ring and methyl group at the 3' position of the benzyl moiety increased the potency of S-DABO derivatives 39 than that of the DABO derivatives. The 6-benzyl-2-thiouracil derivatives 40 was found inactive as in previous case.

Danel et al[54] generated a number of anti HIV active napthyl analogues 41a-c, 42-24 of HEPT and DABO whose antiviral activities were evaluated.



(41 a-c)



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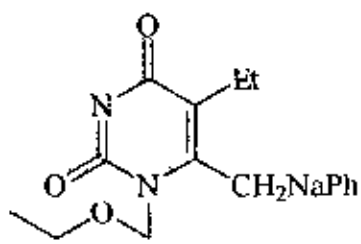
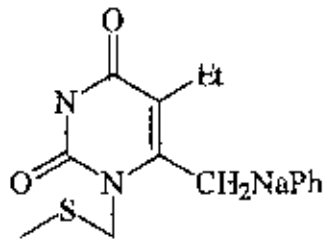


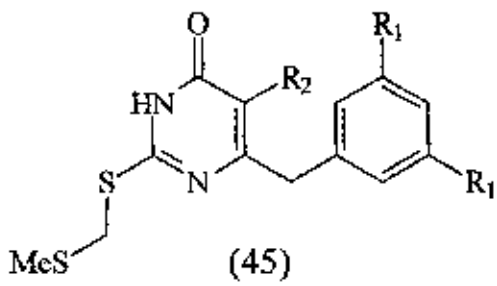
(43)

41	R
a	$MeSCH_2$
b	$Et\ SCH_2$
c	$MeOOCCH_2$



(44)

Vig *et al*[54] synthesized a novel dihydroalkoxybenzyl oxopyrimidine 45 a-d (S-DABO) derivatives targeting the non-nucleoside inhibitor (NNI) binding site of human immuno-deficiency virus (HIV) reverse transcriptase (RT) using a novel computer model for the NNI binding pocket and test for their RT inhibitor activity in cell free assays using purified recombinant HIV RT as well as for their anti HIV activity in HTL VIIIB infected peripheral blood mono nuclear cells [56].

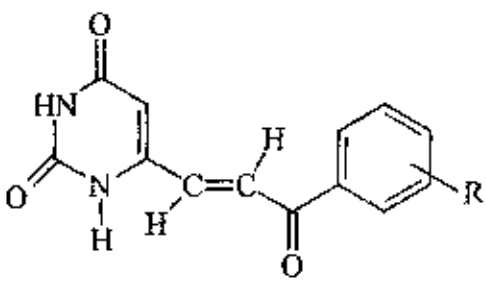


(45)

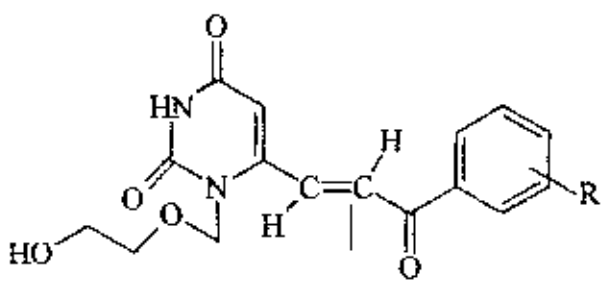
45	R_1	R_2
a	H	Me
b	H	Et
c	H	i-Pr
d	Me	I-Pr

Compound **45** which differ from compound **45c** by the addition of two methyl groups to the 6-benzyl ring providing a better hydrophobic contact with the NNI binding pocket was slightly more potent than compound **45c** in inhibiting recombinant HIVRT. However, compound **45d** failed to inhibit HIV replication in HTL VIIIB-infected cells as effectively as compound **45c**.

Kundu *et. al.*[57] recently developed the palladium catalyzed synthesized of [E]-6-(2-Acyl vinyl)-1-[(e-hydroxyethoxy)methyl]uracil and evaluated their antiviral and cytotoxic activities.



(46 a-c)



(47 a-d)

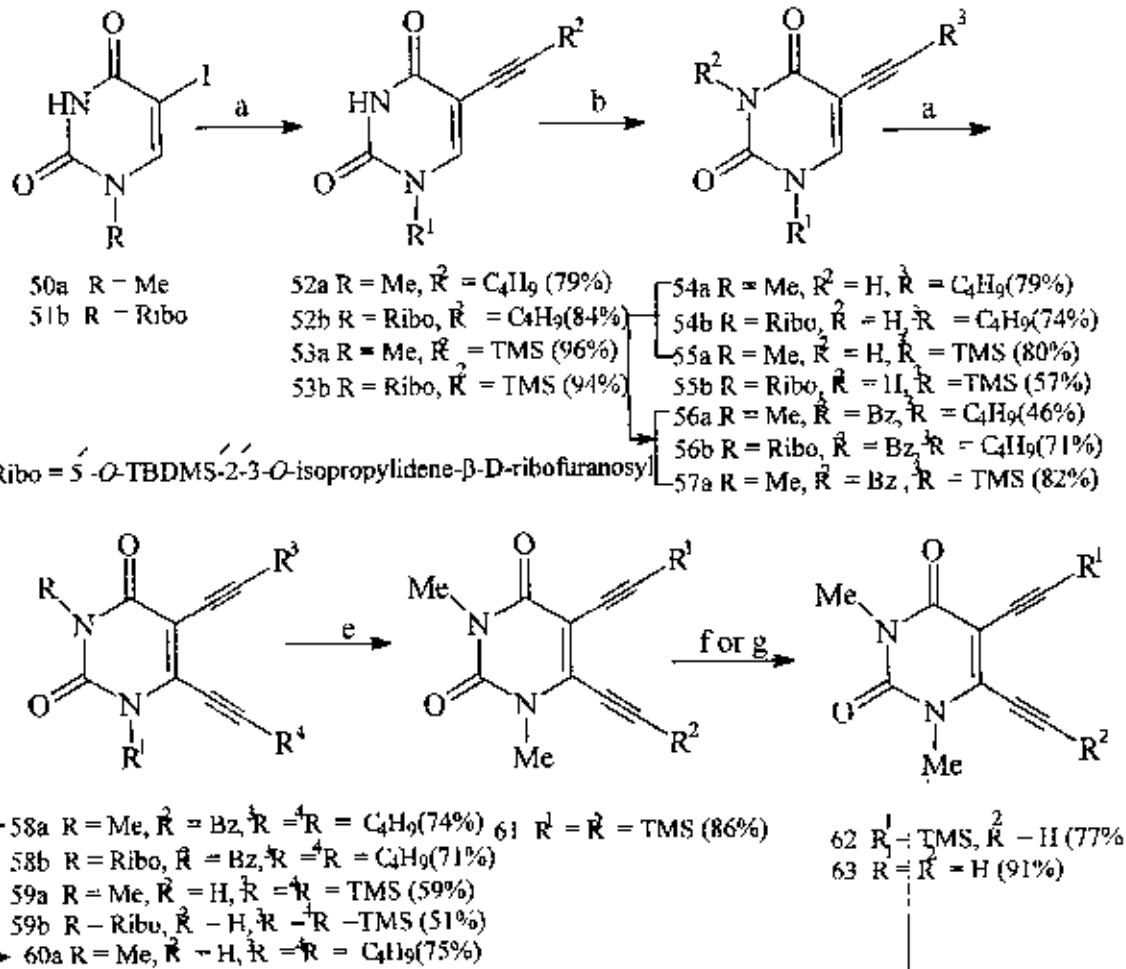
[E]-6-(2-acylvinyl) uracils 46a-c were found to be poorly cytostatic against murine leukemia (L 1210) and murine mammary carcinoma (FM3A)cells. However they were found to be distinctly inhibitory to human T-lymphocyte (Molt 4/C8 and CEM) cells proliferation. Compound 46b was found to be most toxic of three compounds tested.

1.1D. Biological evaluation of 5, 6-disubstituted Pyrimidines and related nucleosides:

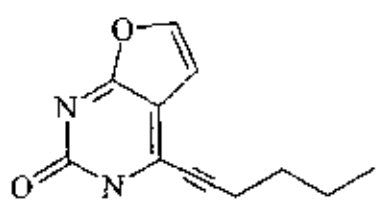
Pyrimidine derivatives are widespread in medicinal and natural products chemistry. A number of commercially important drugs have been incorporated in heterocycle. Specially substituted pyrimidines are valuable intermediates for drug discovery. Particularly, the preparation of 5,6-disubstituted pyrimidines is very difficult as process reported. Several synthetic routes of these compounds are known, some of them are described here. Bergman [59] cycloaromatization reaction is of interest both from a mechanistic point of view and because of its relevance to the mode of action of enediyne antibiotics including the esperamicins, calicheamicins, dynemicins, and kedarcidin. These DNA-cleaving molecules are among the most cytotoxic compounds known[60] and considerable efforts have been focused on the synthesis of analogs with enhanced properties for chemotherapeutic application[61].

Russel and co-workers[62] reported the synthesis and Bergman cycloaromatization of three 5,6-bis (alkyn-1-yl) pyrimidine derivatives. Their synthesis started with 6-chloro-2,4-dimethoxypyrimidine and was not oriented to the preparation of nucleosides. Morris J. Robins et al [63] reported efficient syntheses of 5,6- bis (ethynyl) uracil derivatives and related nucleosides from uracil or uridine. [64a]

The respective 5-iodo -1-methyluracil (50a) or 5'-*O*-(tert-butyldimethy-5) iodo -2',3'-*O*-iso-propylideneuridine (50b) derivatives were readily obtained in 2-3 steps from uracil and uridine (Scheme 1)



Scheme 1. Reagents: (a) $HC_3CR/Pd(0)$, Cu(I). (b) (i) LDA, -78°C; (ii) I2, (c) $BzCl/iPr)_2N$ (d) $NH_3/MeOH$ CH_2N_2/Et_2O, (f) $NH_4F/THFMeoH$[16]



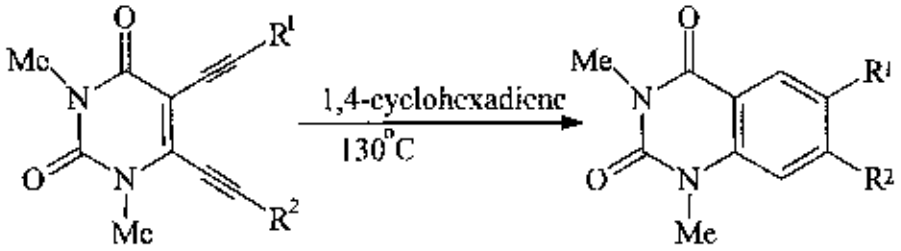
(64)

Sonogashira coupling [64,65] of 50a,b with TMS-ethyne or 1-hexyne proceeded smoothly to give 5-alkynyl derivatives **52a-53b** (79-96%). C-6-lithiation,[66] followed by treatment with iodine gave 5-(alkyn-1-yl)-6-iodo analogs **54a-55b** in good yields. Coupling of **54a** with 1-hexyne afforded minor amounts of 5,6-bis(hexyn-1-yl)derivative 60a, but the major product of this reaction was bicyclic compound **64** . Furano [2,3-*d*

] pyrimidin-2-ones related to **64** are known byproducts of Sonogashira couplings 5-iodouracil subtracts [64,67] and variable amounts (10-15%) of the corresponding bicyclic pyrimidin-2-ones were observed when **52a,b** were prepared from **50a,b**. In the case of 52a,b cyclization was minimized with optimized reaction conditions, but coupling of the 6-iodo derivatives **54a,b** was considerably slower. The longer reaction times invariably gave furano[2,3-*d*] pyrimidin-2-ones as major products. The furan cyclization was circumvented by N^3-benzoylation of **54a,b**. The resulting **56a,b** underwent coupling to give **58a,b** without accompanying addition of O4 to the C5-alkynyl triple bond. Compound **55a** was N^3-benzoylated to give **57a**, but **59a,b** underwent coupling without formation of furano [2,3-*d*]pyrimidin-2-one products. Thus **59a,b** were prepared from **55a** or **55b** by direct coupling with (trimethyl silyl) acetylene. The N^3-benzoyl group was removed standard conditions, and treatment of **58a** with NH_3/MeOH gave **60a** (75%).

Attempts [68] to bis-desilylate **59a** gave intractable mixtures. Remarkably, N^3-methylation over came this problem and **63** was obtained in 91% yield upon treatment of **61** with NH_4F/MeOH. The C6-ethynyl TMS group was selectively cleaved to give **62**(77%) with NH_4F/benzyltriethylammonium chloride (BTAC)/THF.

Enediynes **62** and **63** underwent thermal Bergman cycloaromatization in 1,4-cyclohexadiene at 130°C with half-lives of 8 and 2 h, respectively (sheme 2) compound **61** did not undergo Bergman cyclization under these conditions, and higher temperatures resulted in significant decomposition. Isolated yields of **65** and **66** were approximately 20%, and decomposition of starting material contributed to the modest yields.



62 R^1= TMS, R^2 = H
63 R^1= R^2 = H

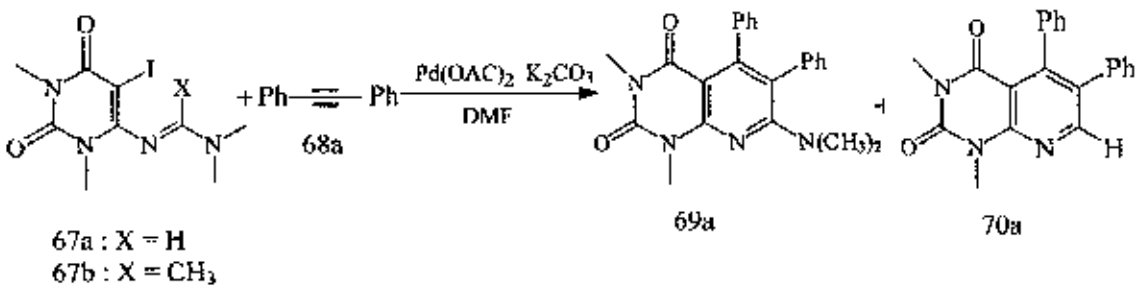
65 R^1= TMS, R^2 = H ($t_{1/2}$ = 8h)
66 R^1= R^2 = H ($t_{1/2}$ = 2h)

Scheme - 2

Activation energies for Bergman cycloaromatiozations have been correlated with the a,b distance between to two terminal alkynyl carbon atoms. Acyclic enedinynes with a,b-distances ›3.5 A were unreactive at 25°C and required heating of effect cyclization. In compounds **61-63**, the a,b distance is calculated to be approximately 4.1 A. Terminally substituted acylic enediynes exhibit increased activation barriers due to unfavorable steric interactions and entropic effects, and the relative reactivates of **61-63** are consistent with these observations. Morris J. Robins et al [63] have prepared 5,6-bis(alkyn-1-yl)-1-methyluracil derivatives and protected nucleosides via successive Sonogashira coupling of 5-and 6-iodo (uracil or uridine) analogs **51a,b** and **56a-56b**. Coupling of the 6-iodo derivatives was sluggish and required longer reaction times resulted in increased formation of furano[2,3-*d*] pyrimidin-2-one byproducts from 5-(hexyn-l-yl) derivatives **54a,b**, but they were not observed with 5-(TMS-ethyn-l-yl) intermediates **53a,b or 55a,b.** The 5,6-bis(ethynl) derivatives underwent Bergaman cycloaromatization at elevated temperatures to give quinazoline-2,4-dine derivatives **65** and **66**. Connection of the two ethynylsubstituents to form fused bicyclic uracil-based enediynes should significantly lower activation barriers to Bergman cycloaromatization.

Recently, Yoon CM and his workers [69] reported an efficient method for the syntheses of pyrido[2,3-*d*]pyrimidines by the reactions of iodouracil, having a formamidine or acetamidine moiety, with various olefins in DMF using a catalytic amount of palladium acetate. [70, 71]

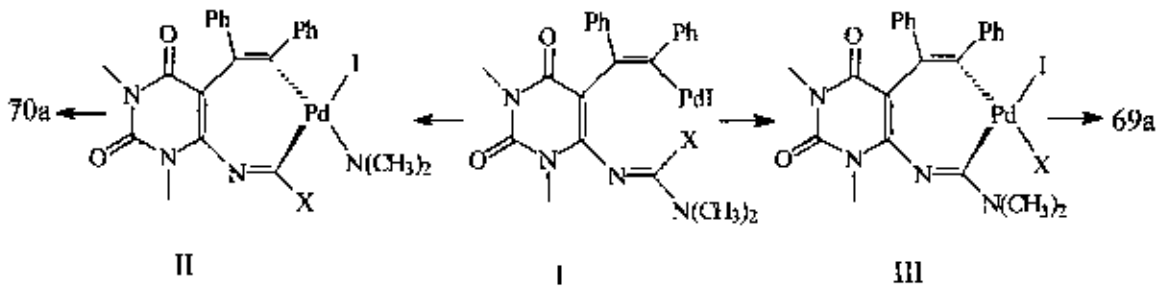
As a continuation of this work, the reactions of iodouracils having a formidine or actamidine moiety **67** with various acetylenes **68** were studied in the presence or absence of lithium chloride, which might play a crucial role in the reaction selectivity (scheme-3).



Scheme - 3

The reaction of iodouracil **67a** with diphenylacetyene **68a** in DMF in the presence of palladium acetate as catalyst and potassium carbonate as base at 120°C gave two pyridopyrimidines **69a** (dehydrogenated one) and **70a** (deaminated one) in 67% and 27% yields, respectively . The selectivity was increased when the same reaction was tried in the presence of 1 equiv. of lithium chloride. Pyridopyrimidine **69a** was obtained in 93% yield and only amount of pyridopyrimidine **70a** was observed by TLC. A similar selectivity observed using 1 eqiv. of bromide instead of 1 equiv. of lithium chloride. However, selectivity was not observed at when tetra-*n*-butylammonium bromide or tera-*n*-butylammonium chloride was used instead of lithium bromide or lithium chloride. On the basis of these experimental results, the selectivity seems to be due to the lithium cation.

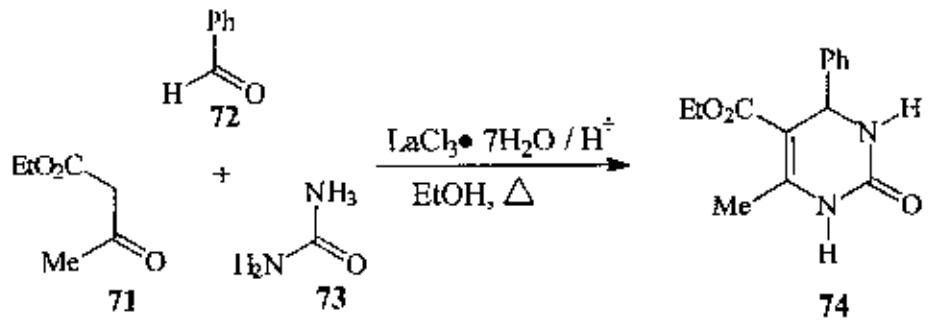
Lithium cations in this reaction might prevent the insertion of palladium of intermediate I (X= H) into the C-N(Mc)$_2$ bond to form intermediate II(X= H), which is necessary for the information of pyridopyridmidine **70a** (scheme 4).



Scheme -4

The reaction of iodouracils having a formamidine moiety **67a** with acetylenes using palladium acetate in DMF in the presence of lithium chloride at 120°C gave pyrido pyrimidines with regioselectivity. The reaction of iodouracil having an acetamidine moiety **67b** with acetylenes without lithium chloride also gave pyrdopyrimidines.

The Biginelli reaction was first reported more than a century ago and recently reviewed [72] and involves the synthesis of 3,4-dihydrooyrimimdin-2(1H)-ones of type **74** by a very simple one-pot condensation reaction of ethyl acetoacetate **71**, benzaldethde **72** and urea **73** in ethanol. However, this one-pot, one-step protocol often provides only low to moderate yields of the target molecules **74** (scheme - 5), in particular when substituted aromatic or aliphatic aldehydes are employed.



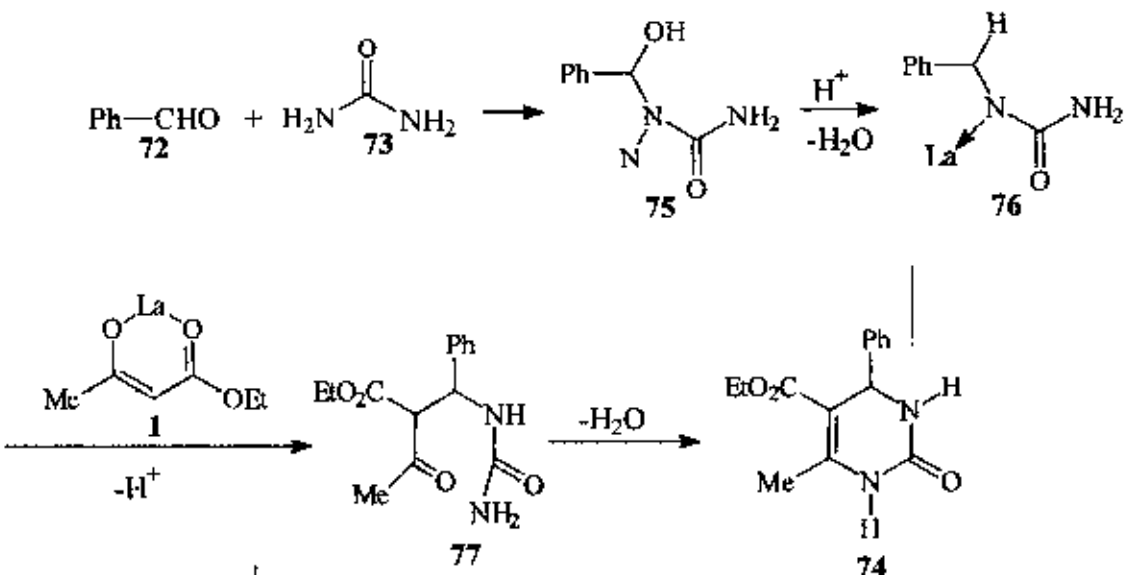
Scheme - 5

In the past decade, dihydropyrimidine derivatives have exhibited important pharmacological properties, e.g. as the integral backbones of several calcium channel blockers, antihypertensive agents, alpha-la-antagonists, and neuropeptide Y (NPY) antagonist. [73] Several improved procures for the preparation of DHPMs (Biginelli compounds) have recently been reported, either by modification of the classical one-pot Biginelli approach itself. [74-78] or by the development of novel, but more complex multistep strategies. [79] In addition, several combinatorial approach towards DHPMs **74** have been advanced using solid-phase or fluorous phase reaction conditions. [80]

Junlu, Yinjuan and coworker[81] have developed a simple and efficient method for the direct preparation of substituted dihydropyrimidinones using lanthanum chloride heptahydrate as a catalyst in good yields from readily available starting materials. This was a novel, one-pot combination that not only preserves the simplicity of Biginelli's one-pot reaction but also consistently produces excellent yields of the dihydropyrimidine -2(1*H*)-ones. In the presence of the $LaCl_3 7H_2O$(5 mmol), the reaction of β-keto ester 1(10mmol), aldehyde **72** (10mmol), and urea or thiourea **73** (15 mmol) was carried out in a one pot condensation employing refluxing EtOH, which had previously been

employed successfully in the Biginelli condensation as solvent. After the reaction was completed, the dihydropyrimidines **74a**-t precipitated from the reaction mixture. Even for aliphatic aldehydes (i.e butyraldehyde and iso- butyraldehyde), which normally showed extremely poor yields in the Biginelli reaction,[82-83] 60% and 56% yields of the corresponding dihydropyrimidin-2(1H)-ones 4j and 4k could be obtained.

Recently, the mechanism of the Biginelli reaction was reinvestigated in detailed by Kappe [84] proposed and established that the first step in this reaction, the acid-catalyzed formation of an acylimine intermediate formed by reaction of the aldehyde with urea, was the key rate-limiting step. Interception of the minimum ion by ethyl acetoacetate produces an open-chain uriden which subsequently cyclized to the dihydropyrimidinones **74.** Because of the **74f** empty orbital in the lanthanum ion, a complex 76 can be formed through a coordinative bond and stabilized by lanthanum. So the proposed mechanism for the lanthanum promoted Biginelli reaction as follows (scheme 6).

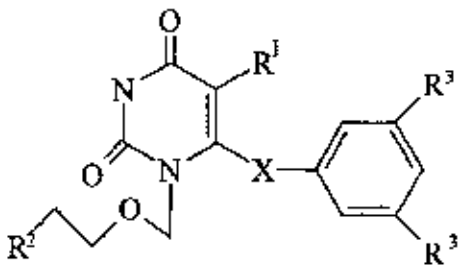


Scheme - 6

Thymidine phosphorylase plays an important role in angiogenesis, which is an attractive target for therapy of cancer and other diseases. In our continuous effort to develop novel inhibitors of thymidine phosphorylase, we have discovered that 6-halouracils substituted at position C5 by certain hydrophobic groups exhibit significant inhibitory activity against this enzyme. The most potent compounds bear a five- or six-membered cyclic substituent containing a pi-electron system at C5 and a chlorine atom attached at C6. 6-Chloro-5-cyclopent-1-en-1-yluracil 7a is the most efficient derivative in this study, with Ki = 0.20 +/- 0.03 microM (Ki/dThdKm = 0.0017) for thymidine phosphorylase expressed in V79 cells and Ki = 0.29 +/- 0.04 microM (Ki/dThdKm = 0.0024) for the enzyme purified from placenta[111].

1.1E. Rationale:

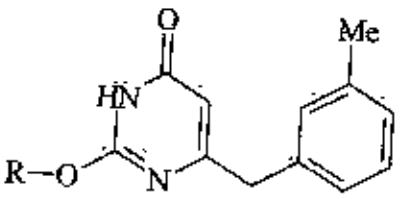
The studies on 6 – substituted uracils have been found limited in literature. The recent pandemic occurrence of AIDS and the discovery of 6-substituted uracils, e.g. 1– (2–hydroxyethoxy methyl) – 6 – (phenylthio) thymine (HEPT), **I**, and related compounds, such as E–EDU, **II**, I–EBU, **III**, E-EBU-dM, **IV**, which acts as specific inhibitors of HIV –1 (human immunodeficiency virus type 1), the causative agent of AIDS, have stimulated interest in 6-substituted uracil derivatives.



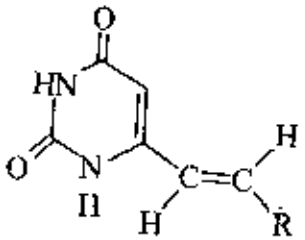
HEPT,	**I**,	X= S,	$R^1 = CH_3$,	$R^2 = OH$,	$R^3 = H$
E-EPU,	**II**,	X= S,	$R^1 = Et$,	$R^2 = H$,	$R^3 = CH_3$
I-EBU,	**III**,	X= CH_2,	$R^1 = i\text{-}Pr$,	$R^2 = R^3 = H$	
E-EBU-dM,	**IV**,	X= CH_2,	$R^1 = Et$,	$R^2 = H$,	$R^3 = CH_3$

Some pyrimidine derivatives, e.g. 3,4-dihydro-2-alkoxy-6-(3`-methylbenzyl) – 4 – oxopyrimidine (DABO), **V**, has shown considerable activities as inhibitors of HIV-1 RT.

The cytotoxic activities of 6-vinyluracil **VI** against L1210 mous leukemia cells have shown. Recently 6- acyvinyl uracils **VII** were found highly cytotoxic against CCRF-CEM human lymthoblastoid cells.

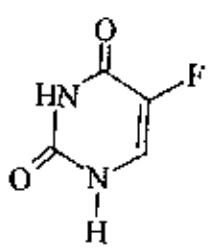


V

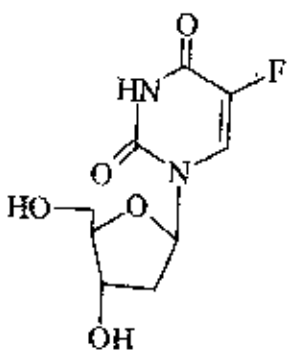


VI. R = H
VII, R = -COAr

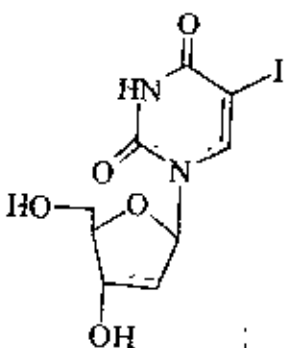
5 = Flurouracil (5-Fu, **VIII**) and 5-fluoro-2'-deoxy uridine (5-Fudr, **IX**) are used clinically for the treatment of breast colon and rectum cancer, 5 = iodo = 2'-deoxyuridine (IDU, **X**) is utilized clinically in the tropical treatment of herpes simplex keradtatis, a sight treatening eye infection. It is also effective against mucocutaneous HSV infection and vaccinia virus (VV).



VIII

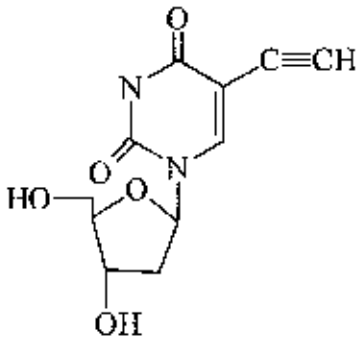


IX



X

5 – Ethynyl – 2' deoxyuridine **XI** exibited excellent anticancer properties (ID_{50} = 0.091 μg/mL in L1210 cells and antiviral properties against HSV-1 in all primary rabbit kidney cells in culture.



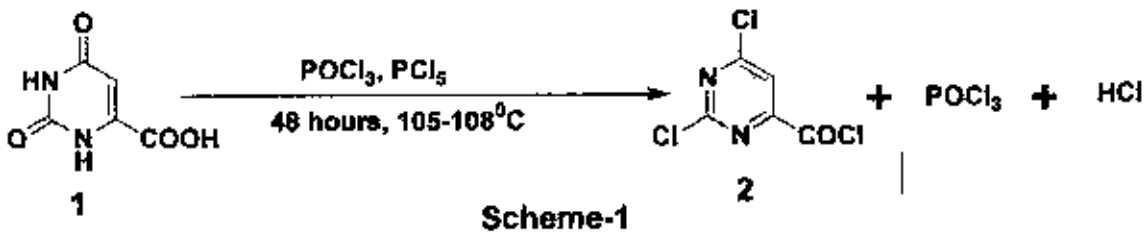
XI

In view of the significant biological activity of various 5 and 6 – substituted uracils and related pyrimidine derivatives we became interested in developing methods for the synthesis of novel 5, 6-disubstituted uracils. In this thesis a facile method for the synthesis of a number of 5, 6- disubstituted uracil derivatives was planned.

Chapter 2

Results & Discussion

2.1. Synthesis of 2, 4- Dichloropyrimidine-6-carbonyl chloride-2 from Uracil-6-carboxylic Acid (Orotic Acid): The 6-substituted pyrimidine was synthesized according to the reaction sequence as shown in the **Scheme 1**. 2, 4- Dichloropyrimidine-6-carbonyl chloride **2** was synthesized according to the procedure of Gershon[85] by heating Orotic acid **1** with phosphorus oxychloride and phosphorus pentachloride.

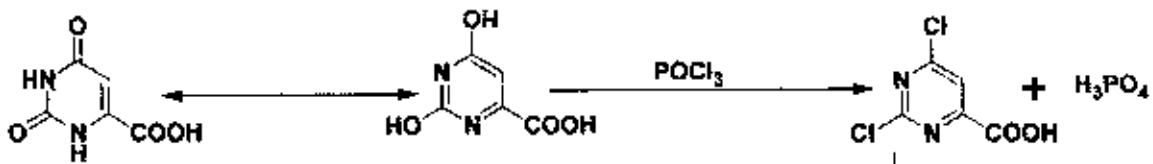


1 2

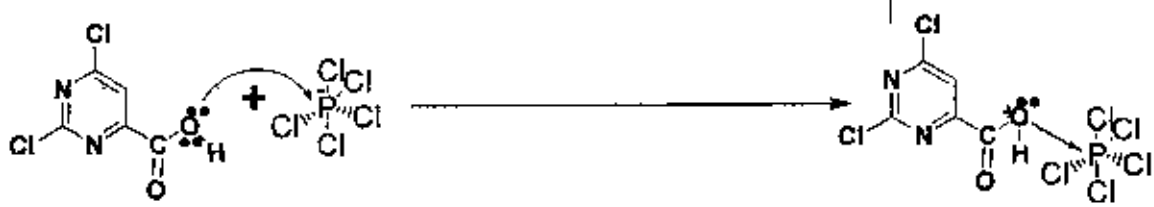
Scheme-1

Mechanism-1: The possible mechanism of the formation of Acyl Chloride from Orotic acid is given below:-

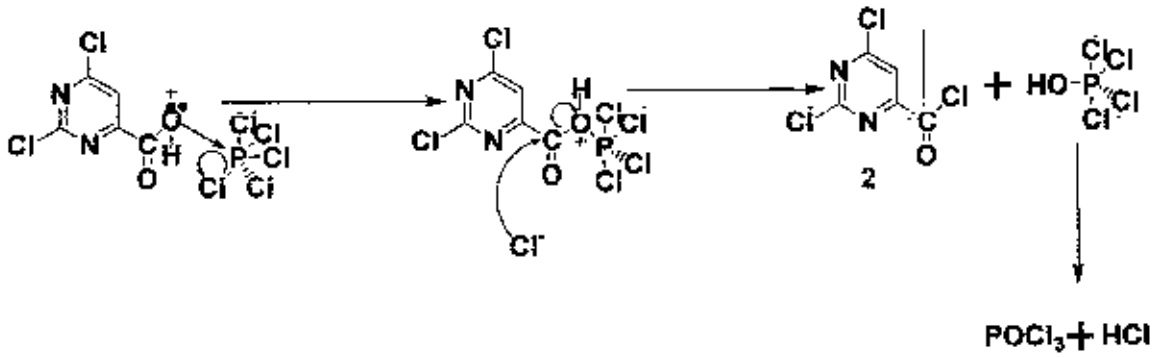
Step-1: Formation of 2, 4-dichloropyrimidine-6-carboxylic acid.



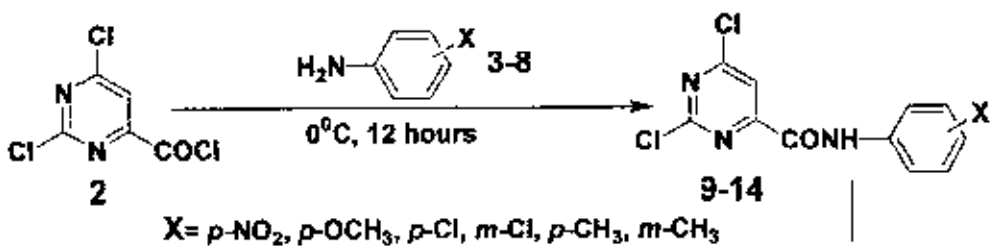
Step-2: Electron lone pair on carboxylic acid group made a coordination bond with vacant d-orbital of Phosphorous pentachloride.



Step-3: Chloride anion was eliminated from coordinated system and attacked the carbonyl carbon as a nucleophile to produce the desired product Acyl Chloride **2**.



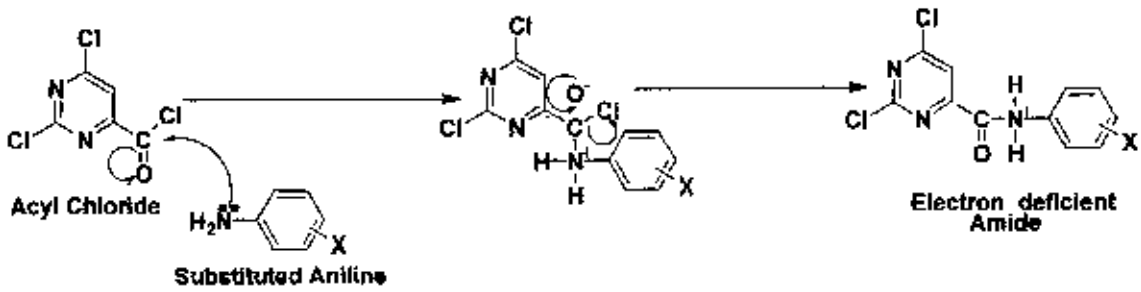
2.2. Preparation of 2, 4 - Dichloro - 6- substituted phenylamido pyrimidine-9-14: The compound 2, 4- Dichloropyrimidine-6-carbonyl chloride **2** underwent a smooth reaction with a number of substituted amine derivatives in which the acid chloride moiety was found to react predominantly to produce desired product 2, 4 - Dichloro - 6- substituted phenylamido pyrimidine **9-14,** as shown in the **Scheme-2.** The yields of the products were very good (80-89 %).



Scheme-2

Mechanism-2: The possible mechanism to form Amido compound from Acyl Chloride is given below-

Step-1: Electron lone pair on amine group of substituted Amine attacked relatively electro-positive carbonyl carbon to form electron deficient Amide compound



Step-2: A proton removed from electron deficient Amide compound to give desired product N-substituted Amide compound

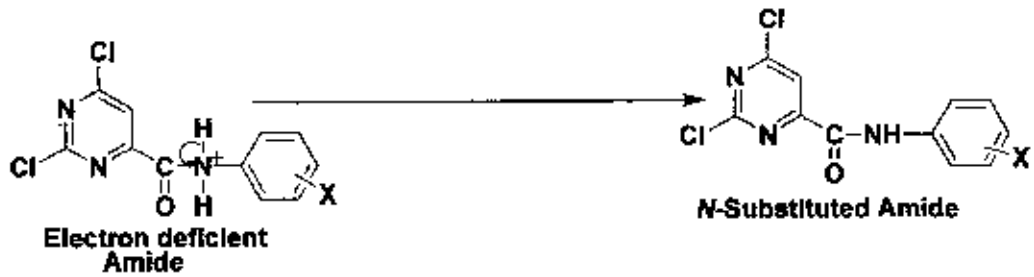
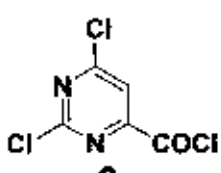
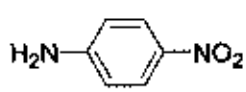
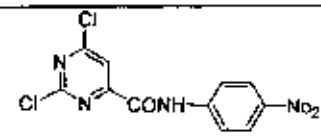
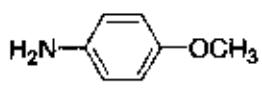
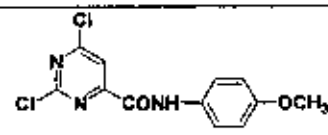
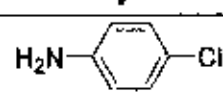
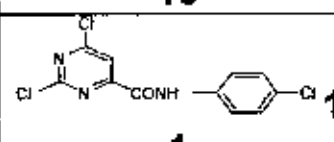
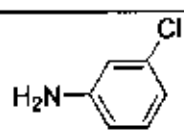
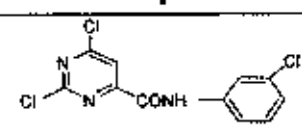
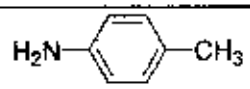
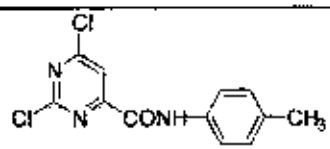
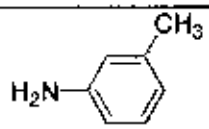
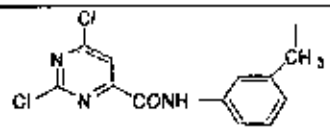


Table 1: Synthesis of 2, 4 - Dichloro - 6- substituted phenylamido pyrimidine derivatives 9-14:

Entry	Acyl Chloride	Primary Amine	Products	Yield %
I	 **2**	 **3**	 **9**	78
II		 **4**	 **10**	82
III		 **5**	 **1**	81
IV		 **6**	 **12**	83

V		 7	 13	83
VI		 8	 14	83

2.2a. Characterization of 2, 4 - Dichloro - 6-nitrophenylamido pyrimidine-9: A yellow solid was obtained with 78.05% yield, M.P. 157-159^{0}C, which was moisture sensitive. The structure of the compound was established by various spectral data. In UV (EtOH) spectrum, the λ_{max} value was found 326.00 nm.

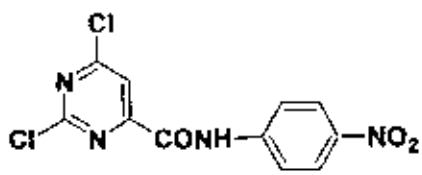
The IR (KBr) spectrum (page-123) of this compound exhibited absorption bands at ν_{max} 3363.6 and 1691.5 cm^{-1} for stretching of –NH- and -C=O groups respectively. ν_{max} 1544.9 cm^{-1} denoted presence of alkene group in aromatic ring, 1506.3, 1340.4 and 860.2 cm^{-1} revealed stretching of $-NO_2$, -C=N and -C-Cl groups respectively in the compound.

The ^{1}HNMR Spectrum (page-121) assigned chemical shift for the determination of molecular structure of the compound **9**, it was observed that two protons doublet at 7.96 (d, 2H, J= 7.68 Hz, Ar-H) revealed presence of two aromatic hydrogens, δ 8.18 (s, 1H, Ur-H) indicated one pyrimidine hydrogen at C-5 position, δ 8.29 (d, 2H, J= 7.64 Hz, Ar-H) for two benzene hydrogens, chemical shift 9.77 (s, -NH-) for one hydrogen in amide group.

The structure of the compound **9** was further confirmed by its ^{13}CNMR spectral data (page-122). Chemical shift at 118.19 indicated presence of

one Ur-CH carbon, δ 119.01, 119.89, 124.43, 126.57 were in the favor of four carbons in aromatic ring (Ar-CH), chemical shift 130.06, 131.21 were for the two terminal carbons of benzene ring (Ar-C) of the compound, δ 157.90, 159.19, 160.02 obtained for three tertiary carbons of uracil (Ur-C), δ 165.41 denoted the presence of carbonyl carbon (C=O) in amide group.

On the basis of UV, IR, ^{1}HNMR and ^{13}CNMR spectral data and other physical characteristics, the structure of **9** compound was assigned as follows-



9

2.2b. Characterization of 2, 4 - Dichloro - 6-methoxyphenylamido pyrimidine-10: A yellowish solid was obtained, yield 82.52%, M.P. 147-149^{0}C, this compound was highly moisture sensitive. The structure of the compound was established by UV, IR, ^{1}HNMR and ^{13}CNMR spectral data. Ethanol based λ_{max} was 352.00 nm for the compound.

In IR (KBr) (page-127)ν_{max} 3354.0 and 1689.5 cm^{-1} stretching bands represented –NH- and C=O groups in the compound **10**. ν_{max} 1564.2, 1529.4, 1298.0 and 829.3 indicated stretching bands of –C=C, –C=N, -C-O and –C-Cl groups in the compound respectively.

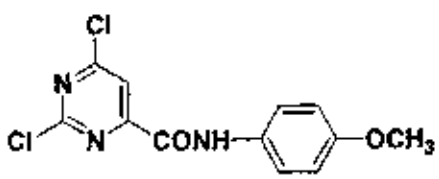
The ^{1}HNMR Spectra (page-125) showed presence of **nine (9)** hydrogens in the compound. Chemical shift position at 3.83 (s, 3H, Ar-OCH_3) was for three hydrogens in methoxy group, δ 6.94 (d, 2H, J= 9.02 Hz, Ar-H) indicated two aromatic hydrogens, δ 7.65 (d, 2H, J=9.00, Ar-H) for two

benzene hydrogens, chemical shift 8.17 (s, 1H, C_5-H) for the presence of one pyrimidine hydrogen at C-5 position in uracil structure, 9.42 (s, -NH-) denoted one hydrogen in amide group.

The ^{13}CNMR spectral (page-126) data of compound **10** enriched structural idea. Chemical shift 55.54 indicated the presence of one carbon in methoxy group (Ar-OCH_3), δ 118.22 due to the presence of Ur-CH carbon, δ 121.06, 129.75 in the favor of four carbons in aromatic ring (Ar-CH), chemical shift 133.09, 135.19 for the two terminal carbons of benzene ring (Ar-C) of the compound, chemical shift (δ) 157.60, 159.81, 160.46 obtained for the three tertiary carbons of uracil (Ur-C), δ 165.13 indicated the presence of carbonyl carbon (C=O) of amide group. Total **twelve (12)** carbon atoms were obtained in the compound by ^{13}CNMR spectra.

Elementary analysis for C, H & N elements confirmed the structure of the compound **10.** Experimental data of the three elements obtained 48.34, 3.04 & 14.09 percent respectively. Those were almost similar to the calculative data 47.40, 3.22 & 13.65 percent respectively.

The spectral data of UV, IR, ^{1}HNMR and ^{13}CNMR, and elementary data, are compatible with the structure of this compound shown below-



10

2.2c. Characterization of 2, 4 - Dichloro - 6-chlorophenylamido pyrimidine-11: A reddish crystal was obtained with 81.77% yield, M.P. 166-167°C, which was moisture sensitive. The structure of the compound was established by various spectral data. In UV λ_{max} was found 306.00 nm for the compound.

The IR (KBr) spectrum (page-131) of this compound exhibited absorption bands at ν_{max} 3359.8,1685.7 1566.1, 1527.5, 1247.9 and 837.0 cm^{-1} for the stretching of –NH-, -C=O C=C, –C=N, -C-O and –C-Cl groups in the compound respectively.

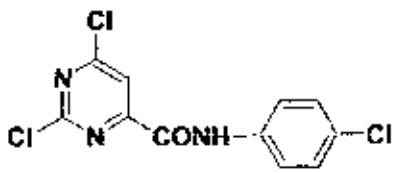
The ^{1}HNMR Spectral data (page-129) explained the presence of **six (6)** hydrogens in the compound. Chemical shift position at 7.36 (d, 2H, J= 8.2 Hz, Ar-H) showed two aromatic hydrogens, δ 7.68 (d, 2H, J= 8.14 Hz, Ar-H) for two aromatic hydrogens, δ 8.14 (s, 1H, C_5-H) revealed presence of one pyrimidine hydrogen at C-5 position, δ 9.49 (s, -NH-) for hydrogen in amide group.

The ^{13}CNMR spectral data (page-130) showed presence of **eleven (11)** carbon atoms in the compound's structure. Chemical shift 118.16 was due to the presence of Ur-CH, δ 119.01, 119.89, 124.43, 126.57 in the favor of four carbons in aromatic ring (Ar-CH), δ 130.06; 131.21 denoted two terminal carbons in benzene ring (Ar-C) of the compound, chemical shift (δ) 157.90, 159.19, 160.02 obtained for three tertiary carbons of uracil (Ur-C), δ 165.41 indicated the presence of carbonyl carbon (C=O) in amide group.

Elementary analysis boosted estimation to predict the compound's structure clearly. Calculated values of C, H & N atoms in the compound were 43.67, 2.00 & 13.89 percent respectively. These values were almost

similar to the experimental values 43.81, 2.15 & 13.85 respectively.

On the basis of UV, IR, ^{1}HNMR, ^{13}CNMR spectral and elementary data, the structure of this compound was predicted as-



11

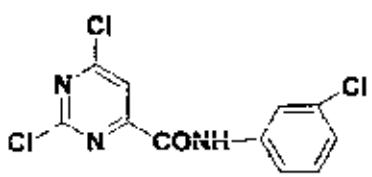
2.2d. Characterization of 2, 4 - Dichloro – 6-*m*-chlorophenylamido pyrimidine-12: A reddish solid with 83.25% yield was obtained, M.P. 147-149^{0}C, this compound was moisture sensitive. The compound's structure was established by different spectral data. λ_{max} of UV (EtOH) spectrum was 324.00 nm for the compound.

The IR (KBr) spectrum (page-135) of this compound showed absorption bands at ν_{max} 3340.5 and 1695.3 cm^{-1} for stretching of –NH- and -C=O groups. Stretching bands ν_{max} 1593.1, 1525.6, 1251.7 & 680.8 cm^{-1} indicated of –C=C, –C=N, -C-O and –C-Cl groups in the compound respectively.

In the ^{1}HNMR Spectra (page-133) of compound **12** indicated δ 7.20 (d, 1H, J= 7.22 Hz, Ar-H) for one hydrogen of aromatic ring, chemical shift δ 7.33 (q, 1H, J= 8.09 Hz, Ar-H) was for one aromatic hydrogen, doublet at δ 7.58 (d, 1H, J=8.16 Hz, Ar-H) for one hydrogen in aromatic ring, singlet at δ 7.86 (s, Ar-H) indicated one hydrogen in benzene ring singlet at δ 8.16 (s, 1H, C_5-H) for the presence of one pyrimidine hydrogen at C-5 position, δ 9.50 (s, -NH-) for hydrogen in amide group.

The structure of the compound was further confirmed by its ^{13}CNMR data (page-134). Chemical shift position at 118.16 indicated presence of one Ur-CH carbon, δ 118.36, 120.28, 125.71, 130.29 were in the favor of four carbons in aromatic ring (Ar-CH), chemical shift 135.06, 137.53 for the two terminal carbons in benzene ring (Ar-C) of the compound, chemical shift δ 157.96, 159.89, 160.02 obtained for the three tertiary carbons of uracil (Ur-C), δ 165.41 indicated the presence of carbonyl carbon (C=O) in amide group.

The data of UV, IR, ^{1}HNMR and ^{13}CNMR spectra were found to be consistent with compound follows as-



12

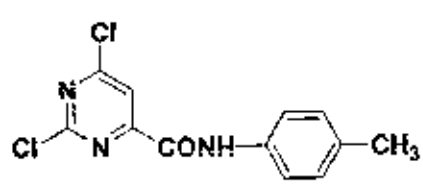
2.2e. Characterization of 2, 4 - Dichloro - 6-*p*-methylphenylamido pyrimidine-13: A lustrous silver color crystals was obtained with 83.33% yield, M.P. 159-161^{0}C, which was moisture sensitive. The structure of the compound was predicted by various spectral data. In UV (EtOH) spectrum, the λ_{max} value was found at 306.00 nm.

The IR spectrum (page-138) showed the following absorption bands at ν_{max} 3369.4, 1687.6, 1566.1, 1525.6, 1317.3, 1245.9 & 825.5 cm^{-1} indicated stretching of –NH-, -C=O, –C=C, –C=N, C-C, -C-O and –C-Cl groups in the compound respectively.

The ^{1}HNMR Spectrum of the compound revealed, three protons singlet at 2.34 (s, 3H, Ar-CH_3) of Ar-CH_3 group, the chemical shift position at 7.18 (d, 2H, J= 8.28 Hz, Ar-H) indicated two aromatic hydrogen, doublet at δ 7.59 (d, 2H, J= 8.32 Hz, Ar-H) for two aromatic hydrogen, singlet at δ 8.14 (s, 1H, C_5-H) was for the presence of one pyrimidine hydrogen at C-5 position, singlet at δ 9.43 (s, -NH-) for hydrogen in amide group.

The ^{13}CNMR spectral data of the compound further confirmed about its structure. It was observed that the chemical shift at 20.96 (Ar-CH_3) presented one carbon of Ar-CH_3, at the chemical shift position of δ 118.22 due to the presence of Ur-CH carbon, δ 120.06, 129.75 were in the favor of four carbons in aromatic ring (Ar-CH), δ 133.89, 135.39 for the two terminal carbons in benzene ring (Ar-C) of the compound, chemical shift (δ) 157.60, 159.81, 160.46 obtained for three tertiary carbons of uracil (Ur-C), δ 165.13 indicated the presence of carbonyl carbon (C=O) of amide group.

Finally, the structure of the compound **13** was confirmed by C, H, & N elementary analysis



13

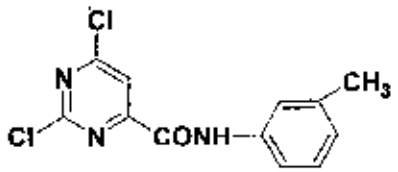
2.2f. Characterization of 2, 4 - Dichloro - 6-*m*-methylphenylamido pyrimidine-14: A reddish solid was obtained with yield of 83.25%, M.P. 147-149^{0}C, the compound was moisture sensitive. The structure of the compound was assigned by different spectral data. UV (EtOH) spectrum showed λ_{max} 356.00 nm for the compound.

The IR spectrum (page-143) of **14** compound presented absorption bands at ν_{max} 3355.9, 3068.7 and 1687.6 cm^{-1} for stretching of –NH-, Ar-C-H and C=O groups respectively. 1531.4, 1309.6, 1247.9 & 794.6 cm^{-1} indicated stretching bands of –C=N, C-C, -C-O and –C-Cl groups in the compound respectively.

The ^{1}HNMR spectra (page-141) of this product showed, three proton singlet at δ 2.41 (s, 3H), δ 7.06 (d, 1H, J= 7.43 Hz, Ar-H) was for one hydrogen in aromatic ring, at δ 7.31 (q, 1H, J= 7.61 Hz, Ar-H) denoted one aromatic hydrogen, doublet at δ 7.58 (d, 1H, J=9.42, Ar-H) indicated two aromatic hydrogens, δ 8.18 (s, 1H, C_5-H) assured one pyrimidine hydrogen at C-5 position, singlet at δ 9.46 (s, -NH-) for hydrogen in amide group. Total **six (6)** hydrogen atoms indicated in the compound.

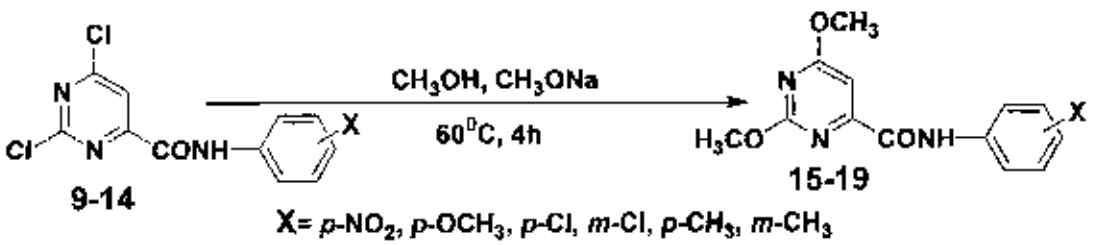
The structure of the compound was further confirmed by its ^{13}CNMR spectral data (page-142). Chemical shift at δ 21.49 indicated the presence of one carbon atom in methyl group (Ar-CH_3), at the chemical shift position of δ 117.28 due to the presence of Ur-CH carbon, δ 118.29, 120.74, 126.46, 129.12 in the favor of four carbons in aromatic ring (Ar-CH), δ 136.34, 139.32 for the two terminal carbons of benzene ring (Ar-C) of the compound, chemical shift (δ) 157.75, 159.91, 160.47 obtained for the three tertiary carbons of uracil (Ur-C), δ 165.25 indicated the presence of carbonyl carbon (C=O) of amide group. Total **twelve (12)** carbons were shown in the compound by ^{13}CNMR spectra.

The above data of UV, IR, ^{1}HNMR and ^{13}CNMR spectra were found to be consistent with the structure of this compound shown below-



14

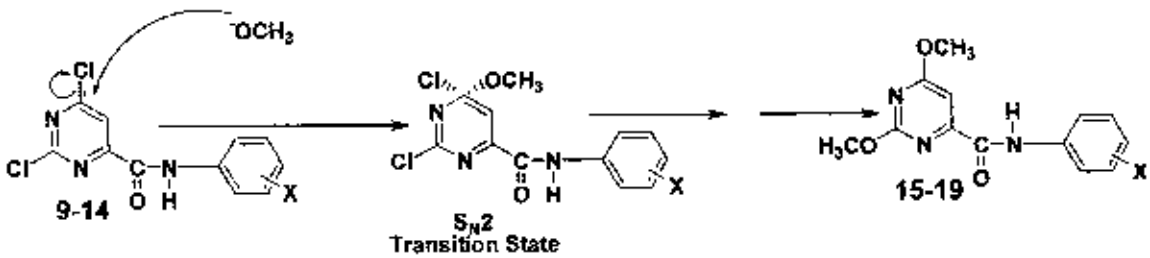
2.3. Synthesis of 2, 4 - Dimethoxy - 6- substituted phenylamido pyrimidine-15-19: 2, 4 - Dichloro-6-substituted phenylamido pyrimidine **9-14** were converted to the corresponding dimethoxy pyrimidines **15-19** on treatment with sodium methoxide in methanol as shown in the **scheme-3**. Here sodium methoxide was produced from dry methanol. The percentages of products in the reaction series were very good (85-87%).



X= *p*-NO_2, *p*-OCH_3, *p*-Cl, *m*-Cl, *p*-CH_3, *m*-CH_3

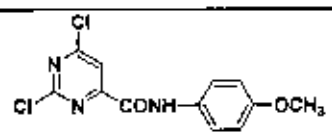
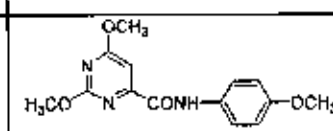
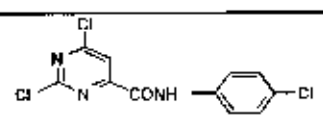
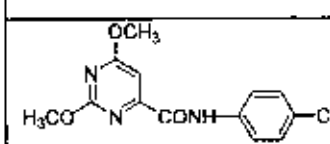
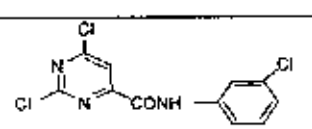
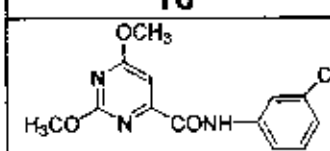
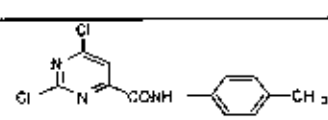
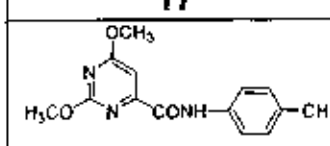
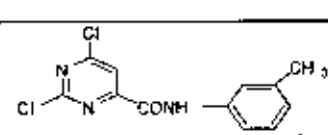
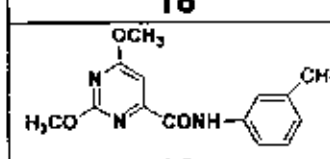
Scheme-3

Mechanism-2: The possible mechanism of the reaction given below. The reaction proceeded according to bimolecular displacement (S_N2) reaction's mechanism. This has been interpreted as involving the participation of both 2, 4 - Dichloro-6- substituted phenylamido pyrimidine and methoxide ion in the rate limiting step of the reaction. It has suggested a transition state in which the attacking methoxide ion becomes partially bonded to the reacting carbon atom before the incipient chloride ion has become wholly detached from it.



The negative charge is spread in the transition state in the course of being transferred from methoxide to chloride.

Table 2: Synthesis of 2, 4 - Dimethoxy - 6- substituted phenylamido pyrimidine derivatives 15-19:

Entry	Reactants	Base	Products	Yield %
I	 **10**	CH_3O^-	 **15**	85
II	 **11**		 **16**	86
III	 **12**		 **17**	86
IV	 **13**		 **18**	83
V	 **14**		 **19**	85

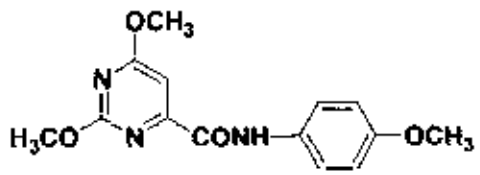
2.3a. Characterization of 2, 4 - Dimethoxy - 6-*p*-methoxyphenylamido pyrimidine-15: An off white solid was obtained with yield of 85.01%, M.P. 112-113^{0}C, which was very moisture sensitive. By UV, IR, ^{1}HNMR and ^{13}CNMR spectral data the structure of the compound was established. In UV spectrum, the λ_{max} value was found in the 286.00 nm.
3332.8, 3003.0, 2947.0 & 1685.7 cm^{-1} in IR spectrum of this compound indicated stretching bands of –NH-, Ar-C-H, -C-H and C=O groups respectively. Stretching bands 1573.8, 1535.2, 1301.9, 1263.3 & 769.5 cm^{-1} indicated –C=C, –C=N, C-C, -C-O and –C-Cl groups in the compound respectively.

The ^{1}HNMR Spectra (page-145) of the compound indicated the chemical shift position at δ 3.79 (s, 3H, Ar-OCH_3) for three hydrogens in methoxy group, two singlet spectrum at δ 4.03 and 4.08 indicated hydrogens in two methoxy groups, doublet at δ 6.89 (d, 2H, J= 6.9 Hz, Ar-H) was for two aromatic hydrogens, doublet at δ 7.62 (d, 1H, J=8.09, Ar-H) for two benzene hydrogens, singlet at δ 7.25 (s, 1H, C_5-H) for the presence of one pyrimidine hydrogen at C-5 position, at δ 9.54 (s, -NH-) represented one hydrogen in amide group. There were total **fourteen (14)** hydrogen atoms in the compound.

The structure of compound **15** was further confirmed by its ^{13}CNMR data (page-146). Chemical shift 54.50, 55.15 & 55.54 indicated the presence of three carbon atoms in three methoxy groups (-OCH_3), the chemical shift position of δ 100.395 due to the presence of Ur-CH carbon atom, δ 114.34, 121.51 in the favor of four carbons in aromatic ring (Ar-CH), δ 130.38, 133.09 for the two terminal carbon of benzene ring (Ar-C) of the compound, chemical shift (δ) 156.80, 159.22, 159.97 obtained for the three tertiary carbon of uracil (Ur-C), δ 165.13 indicated the presence of

carbonyl carbon (C=O) in amide group. ^{13}CNMR presented total **fourteen (14)** carbon atoms in the compound.

On the basis of the UV, IR, ^{1}HNMR and ^{13}CNMR spectral data, the structure of this compound was assigned as-



15

2.3b. Characterization of 2, 4 - Dimethoxy- 6-*p*-chlorophenylamido pyrimidine-16: A white color compound was obtained with 86.09% yield, M.P. 118-120^{0}C, this was moisture sensitive compound. The structure of the compound was established by spectral data. In UV spectrum, the value was found in the λ_{max} 282.00 nm.

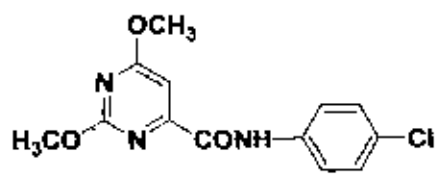
The IR (KBr) spectrum (page-151) of this compound exhibited absorption bands at 3355.9, 3107.1 and 1691.5 cm^{-1} for stretching of –NH-, Ar-C-H and C=O groups respectively. Stretching bands 1569.9, 1519.8, 1286.4, 1197.7 & 788.8 cm^{-i} indicated –C=C, –C=N, -C-O, C-C, and –C-Cl groups in the compound respectively.

The chemical shift in ^{1}HNMR Spectrum (page-149) of the compound (**16**) at 4.02 (s, 3H, Ur-OCH_3) and 4.06 (s, 3H, Ur-OCH_3) indicated presence six hydrogens in two methoxy groups, singlet δ 7.22 (s, 1H, C_5-H) for the presence of one pyrimidine hydrogen at C-5 position, doublet δ 7.31 (d, 2H, J= 8.78 Hz, Ar-H) showed two aromatic hydrogens, doublet δ 7.65 (d, 2H, J= 8.14 Hz, Ar-H) revealed two benzene hydrogens, δ 9.63 (s, -NH-) for hydrogen in amide group. ^{1}HNMR showed total **twelve (12)** hydrogen atoms in the compound.

The structural concept of the compound was further cleared by its ^{13}CNMR data (page-150). Chemical shift at 54.56 and 55.17 revealed presence of two carbons in two Ur-OCH_3 groups , δ 100.50 due to the presence of Ur-CH carbon, δ 118.83, 119.89, 124.43, 126.57 in the favor of four carbons in aromatic ring (Ar-CH), δ 131.06, 131.22 for the two terminal carbon atoms in benzene ring (Ar-C) of the compound, chemical shift (δ) 145.29, 150.81, 151.81 obtained for three tertiary carbons of uracil (Ur-C), δ 164.03 indicated the presence of carbonyl carbon (C=O) of amide group. Total carbon presences in the compound were **thirteen (13)**.

Elementary analysis for C, H & N atoms confirmed the structure of the compound **16**. Experimental data of the three elements obtained C 52.91, H 4.11 & N 14.33 percent. Those were almost similar to the calculated data C 53.16, H 4.12 & N 14.31 percent respectively.

On the basis of complete analysis of Elementary and UV, IR, ^{1}HNMR, ^{13}CNMR spectra data, the structure of this compound was referred as-



16

2.3c. Characterization of 2, 4 - Dimethoxy – 6-*m*-methylphenylamido pyrimidine-17: A pinkish solid was obtained (yield 86.51%), M.P. 120-121^{0}C, which was moisture sensitive. The structure of the compound was established by different spectral data. In UV spectrum, the λ_{max} value was found in the 276.00 nm for the compound.

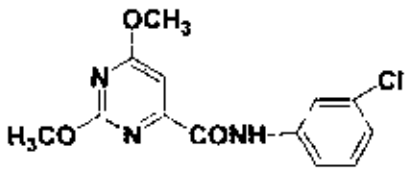
ν_{max} 3355.9, 3105.2, 1706.9, 1569.9, 1508.2, 1263.3 & 769.5 cm^{-1}

stretching bands in IR spectrum (page-155) of the compound indicated presence of–NH-, Ar-C-H, C=O, –C=C, –C=N, C-C, -C-O and –C-Cl groups respectively.

In the ^{1}HNMR Spectra (page-153) of the compound chemical shift at 4.05 and 4.09 indicated hydrogens in two methoxy groups, δ at 7.14 (d, 1H, J= 7.80 Hz, Ar-H) presented one hydrogen in aromatic ring, δ 7.25 (q, 1H, J=8.03 Hz, Ar-H) was for one hydrogen in benzene, δ 7.55 (d, 1H, J=8.01 Hz, Ar-H) indicated one hydrogen in benzene ring, chemical shift 7.85 (s, 1H, C_5-H) indicated presence of one pyrimidine hydrogen at C-5 position, 9.66 (s, -NH-) for the hydrogen in amide group. Total **twelve (12)** hydrogen atoms were indicated in the compound by ^{1}HNMR spectrum.

Structural idea of the compound was further cleared by ^{13}CNMR spectral data (page-154). Chemical shift at 54.56 and 55.17 revealed presence of two carbons in two Ur-OCH_3 groups, the chemical shift 100.54 due to the presence of Ur-CH, δ 117.85, 119.96, 124. 83, 130.08 in the favor of four carbons in aromatic ring (Ar-CH), δ 134.79, 138.23 for the two terminal carbon of benzene ring (Ar-C) of the compound, chemical shift (δ) 158.49, 160.33, 164.81 obtained for the three tertiary carbons of uracil (Ur-C), δ 173.42 indicated the presence of carbonyl carbon (C=O) in amide group. Total carbon presences in the compound were **thirteen (13).**

The data of UV, IR, ^{1}HNMR and ^{13}CNMR spectra were to be consistent with the structure of this compound shown below-



17

2.3d. Characterization of 2, 4 - Dimethoxy - 6-*p*-methylphenylamido pyrimidine-18: An off white solid was obtained with yield 83.19%, M.P. 114-115^{0}C, which was very moisture sensitive. The structure of the compound was established by different spectral data. The λ_{max} value for the compound was found at 284.00 nm.

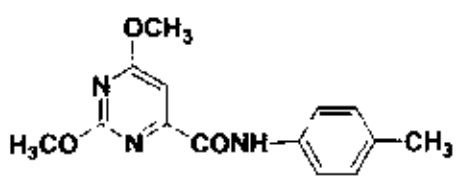
The IR spectrum (page-159) of this compound showed absorption bands at ν_{max} 3321.2 and 1676.0 cm^{-1} for stretching of –NH- and C=O groups respectively. ν_{max} 1571.9, 1525.6 & 1049 cm^{-1} stretching bands indicated –C=C, –C=N and -C-O groups in the compound respectively.

In the ^{1}HNMR Spectra (page-157) of the compound indicated the chemical shift position of 2.34 (s, 3H, Ar-CH_3) for three hydrogens in methyl group, two singlet spectrum at 4.04 and 4.09 indicated six hydrogens in two methoxy groups in uracil structure, doublet at δ 7.14 (d, 2H, J= 8.20 Hz, Ar-H) for two aromatic hydrogens, singlet at δ 7.27 (s, 1H, C_5-H) revealed the presence of one pyrimidine hydrogen at C-5 position, δ 7.59 (d, 2H, J=8.36 Hz, Ar-H) for two benzene hydrogens, singlet at δ 9.59 (s, -NH-) for hydrogen in amide group. Total hydrogen atoms indicated in the compound **fourteen (14).**

In the ^{13}CNMR spectrum data (page-158) the chemical shift 20.92 (Ar-CH_3) indicated one carbon in methyl group, Chemical shift at 54.48 and 55.13 revealed presence of two carbons in two Ur-OCH_3 groups, δ

100.42 due to the presence of Ur-CH carbon atom, δ 119.89, 129.62 in the favor of four carbons in aromatic ring (Ar-CH), δ 134.52, 134.59 for the two terminal carbons in benzene ring (Ar-C) of the compound, chemical shift (δ) 159.14, 160.07, 164.79 obtained for three tertiary carbons of uracil (Ur-C), δ 173.43 indicated the presence of carbonyl carbon (C=O) in amide group. Total **thirteen (13)** carbon atoms presented in the compound by ^{13}CNMR.

The data of UV, IR, ^{1}HNMR and ^{13}CNMR spectra were to be consistent with the structure of this compound shown below-



18

2.3e. Characterization of 2, 4 - Dimethoxy - 6-*m*-methylphenylamido pyrimidine-19: An off white solid was obtained with yield 85.20%, M.P. 117-119^{0}C, this product was very moisture sensitive. The structure of the compound was established by different spectral data. In UV spectrum, the λ_{max} value was found in the 302.00 nm.

The IR spectrum (page-163) of this compound showed absorption bands at ν_{max} 3354.0 and 1689.5 cm^{-1} for stretching of –NH- and C=O groups. ν_{max} 1571.9, 1525.6 & 1049 cm^{-1} stretching bands indicated –C=C, –C=N and -C-O groups in the compound respectively.

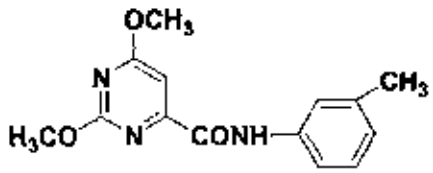
In the ^{1}HNMR Spectra (page-161) of the compound indicated the chemical shift position of 2.38 (s, 3H, Ar-CH_3) for three hydrogens in methyl group, two singlet spectrum at 4.05 and 4.10 indicated hydrogens

in two methoxy groups in uracil structure, chemical shift position of 6.52 (m, 1H, Ar-H) for one hydrogen in aromatic ring, δ 7.00 (m, 1H, Ar-H) for one hydrogen in benzene, δ 7.27 (m, 1H, Ar-H) indicated one hydrogen in benzene ring, doublet at δ 7.52 (d, 1H, J=7.99 Hz, Ar-H) for one hydrogen, δ 7.59 (s, 1H, C_5-H) for the presence of one pyrimidine hydrogen at C-5 position, singlet at δ 9.66 (s, -NH-) for hydrogen in amide group. Total **twelve (12)** hydrogen atoms indicated in the compound ^{1}HNMR Spectra.

The structure of the compound (**19**) was further confirmed by its ^{13}CNMR data (page-162). δc 21.47 (Ar-CH_3) indicated one carbon in methyl group, Chemical shift at 54.48 and 55.12 revealed presence of two carbons in two Ur-OCH_3 groups, δ 100.45 due to the presence of Ur-CH carbon, δ 116.99, 120.51, 125.64, 128.93, 137.02, 139.06 (Ar-CH) in the favor of six carbons in aromatic ring (Ar-CH), δ 134.52, 134.59 for the two terminal carbons in benzene ring (Ar-C) of the compound, chemical shift (δ) 159.06, 160.15, 164.79 obtained for three tertiary carbon of uracil (Ur-C), δ 173.42 indicated the presence of carbonyl carbon (C=O) of amide group. Total carbon presences in the compound are **thirteen (13).**

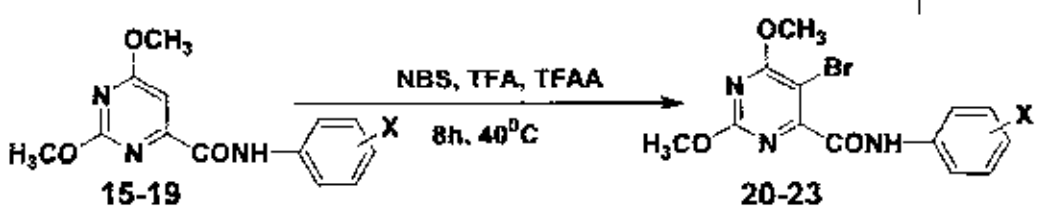
Structural concept of the compound **19** fully confirmed by Elementary analysis data of C, H & N atoms. Percentages of those elements experimentally obtained 60.93, 5.62 and 14.85 respectively. Exactly were same to the calculated data.

On the basis of the UV, IR, ^{1}HNMR and ^{13}CNMR spectra data, the structure of this compound was referred as-



19

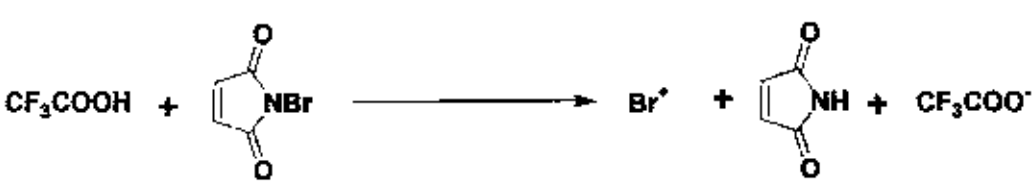
2.4. Synthesis of 2, 4 - Dimethoxy – 5-bromo-6- substituted phenylamido pyrimidine 20-23: In the view of the extensive use of halo derivatives of pyrimidine for the synthesis of the corresponding bromo-substituted derivatives, it was attempted to synthesize 5-bromo pyrimidines by using two different methods. The Bromination reaction was attempted by two methods but only NBS-TFA-TFAA method gave the desired products. The 2, 4 - Dimethoxy –5-bromo-6- substituted phenylamido pyrimidine was warmed with *N*-Bromo succenimide dissolved in trifluoro acetic acid and trifluoro acetic anhydride mixture under Nitrogen atmosphere. The yields of the products were 70-80 %.



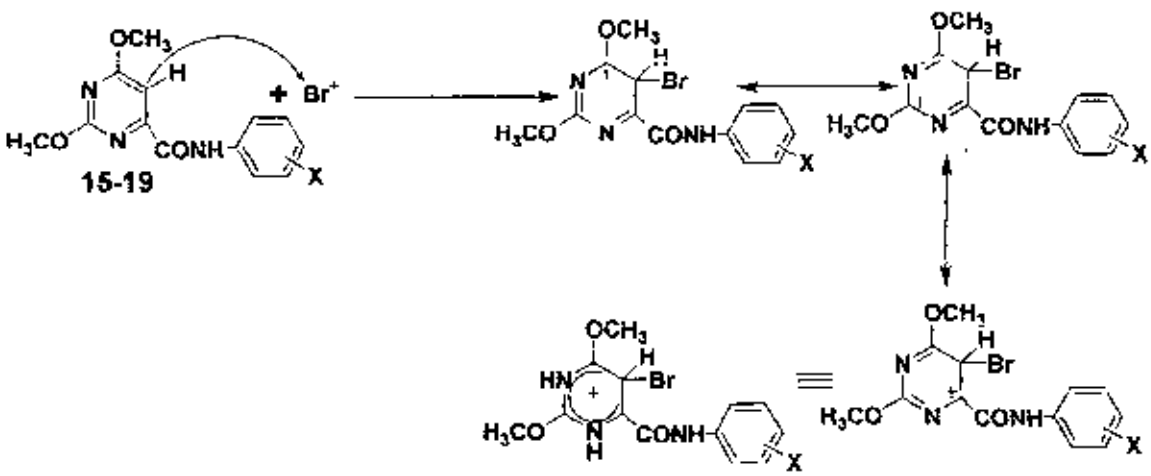
Scheme-4

Mechanism-3: the following steps are involved:

Step-1: formation of the electrophile (bromonium cation).



Step-2: the electrophile attacks the pyrimidine ring at the 5-position to give a carbonium ion.



Step-3: removal of proton from pyrimidine ring.

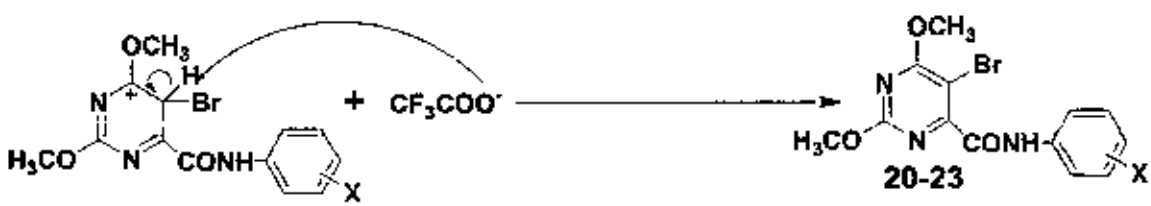
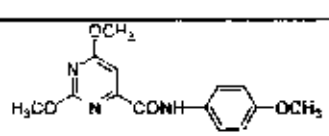
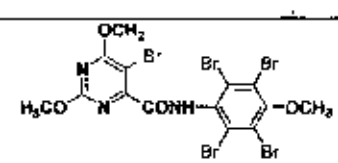
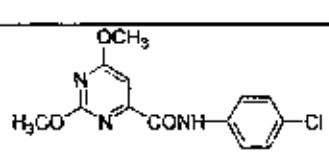
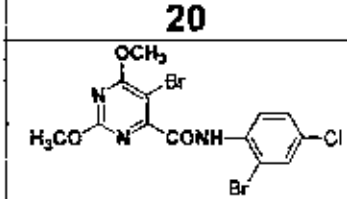
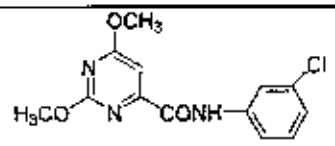
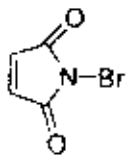
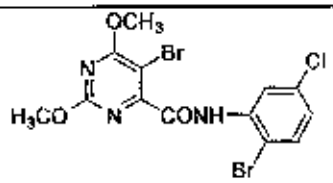
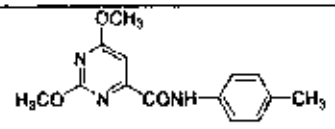
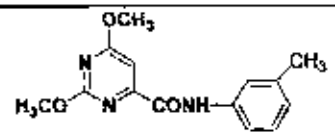
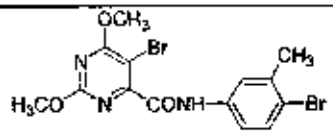


Table 3: Synthesis of 2, 4 – Dimethoxy-5-bromo - 6- substituted phenylamido pyrimidine derivatives 20-23:

Entry	Reactants	Reagent	Products	Yield %
I	 **15**		 **20**	67
II	 **16**		 **21**	72

III	 **17**		 **22**	77
IV	 **18**		Inseparable	-
V	 **19**		 **23**	85

2.4a. Characterization of 2, 4 - Dimethoxy – 5-bromo-6-(4′-methoxy-2′, 3′, 5′, 6′-tetrabromo) phenylamido pyrimidine-20: A white solid was obtained with 75.31% yield, M.P. 141-142^{0}C, which was very moisture sensitive. The structure of the compound was established by UV, IR, ^{1}HNMR and ^{13}CNMR spectral data. In UV spectrum, the λ_{max} value was found in the 246.00 nm.

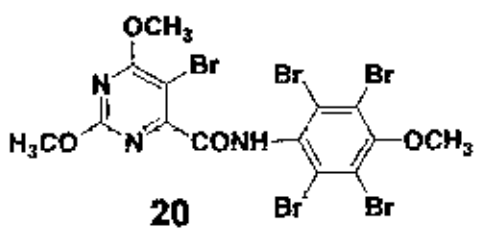
In IR spectrum (page-166) ν_{max} 3238.3, 2947.0, 1687.6, 1560.3, 1514.0, 1334.6, 1245.9 & 513.0 bands showed presence of –NH-, -C-H, C=O, –C=C, –C=N, C-C, -C-O and –C-Br groups in the compound respectively.

In the ^{1}HNMR Spectra (page-165) of the compound indicated the chemical shift position of 3.79 (s, 3H, Ar-OCH_3) for three hydrogen in methoxy group, two singlet spectrum at 4.07 and 4.12 indicated two methoxy groups in uracil structure, δ 9.43 (s, -NH-) for hydrogen in amide group. Total **ten (10)** hydrogens indicated in the compound.

The structure of compound **20** was fully confirmed by its **^{13}CNMR** data (page-167) chemical shift at 55.65 (Ur-OCH_3), 56.05 (Ur-OCH_3) showed

two carbons in methoxy groups, δ 60.65 indicated the presence of carbon in methoxy group (Ar-OCH_3), at the chemical shift position of 98.67, 121.68, 126.47, 133.09, 153.09 (Ar-C) in the favor of six carbons in aromatic ring, chemical shift (δ) 155.57, 160.03, 162.81 obtained for the three tertiary carbon atoms in uracil (Ur-C), δ 169.58 indicated the presence of carbonyl carbon (C=O) of amide group. Total carbon presences in the compound were **fourteen (14)**.

On the basis of UV, IR, ^{1}HNMR and ^{13}CNMR spectral data, the structure of this compound was estimated as-



20

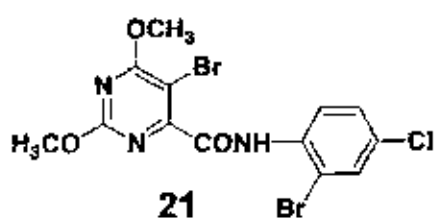
2.4b. Characterization of 2, 4 - Dimethoxy – 5-bromo-6-(2′-bromo-4′-chloro) phenylamido pyrimidine-21: A white color compound was obtained (yield 72.09%), M.P. 146--148^{0}C, which was moisture sensitive. The structure of the compound was established by spectral data. In UV spectrum, the value was found in the λ_{max} 320.00 nm which indicated the presence of amide compound.

The IR spectrum (page-172) of this compound exhibited absorption bands at ν_{max} 3303.8 and 1705.0 cm^{-1} for stretching of –NH- and -C=O groups. Stretching bands 1560.3, 1514.0, 1199.6 & 513.0 cm^{-1} indicated presence of –C=C, –C=N, -C-O and –C-Cl groups in the compound respectively.
The ^{1}HNMR Spectra (page-170) of the compound in 4.09 (s, 6H, Ar-OCH_3) indicated presence six hydrogens in two methoxy groups of uracil

structure, doublet at δ 7.36 (d, 1H, J=8.91 Hz, Ar-H) for two aromatic hydrogens, singlet at δ 7.59 (s, 1H, Ar-H) for one hydrogen in benzene ring, doublet at δ 8.60 (d, 1H, J=8.90 Hz, Ar-H) for two hydrogens in aromatic ring, singlet at δ 10.50 (s, -NH-) for hydrogen in amide group. Presences of total hydrogens in the compound were **ten (10).**

In ^{13}CNMR spectral data (page-171)chemical shift at 55.83 and 56.19 revealed presence of two carbons in two Ur-OCH_3 groups, δ 98.94 due to the presence of one Ur-C carbon, δ 113.64, 121.64, 128.66, 129.88, 131.93, 134.21 in the favor of six carbons in aromatic ring (Ar-C), chemical shift (δ) 159.91, 162.52, 169.70 obtained for three tertiary carbons of uracil (Ur-C), δ 173.06 indicated the presence of carbonyl carbon (C=O) of amide group. Total **thirteen (13)** carbons were presented by ^{13}CNMR spectra in the compound.

On the basis of complete analysis of the UV, IR, ^{1}HNMR and ^{13}CNMR spectral data, the structure of this compound was accorded as-



21

2.4d. Characterization of 2, 4 - Dimethoxy – 5-bromo-6-(2-bromo-5-chloro) phenylamido pyrimidine-22: A white solid was obtained with yield 77.50%, M.P. 143-145^{0}C, this compound was moisture sensitive. The structure of the compound was established by different spectral data. In UV spectrum, the λ_{max} value was found in the 300.00 nm for the compound.

The IR spectrum (page-173)of this compound showed absorption bands at ν_{max} 3303.8 and 1705.0 cm^{-1} for stretching of –NH- and -C=O groups. 1560.3, 1514.0 & 1296.1 cm^{-1} Stretching bands indicated –C=C, –C=N and -C-O groups in the compound respectively.

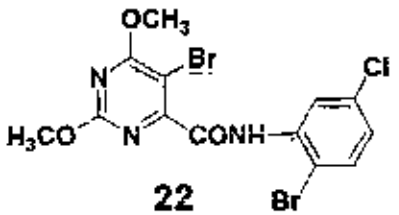
The ^{1}HNMR Spectra (page-174) of the compound (**22**) singlet at 4.13 and 4.14 indicated two methoxy groups, in the chemical shift position of δ 8.25 (d, 1H, J= 8.91 Hz, Ar-H) for one hydrogen in aromatic ring, doublet δ 8.75 (d, 1H, J= 9.01 Hz, Ar-H) indicated one hydrogen in benzene ring, singlet at δ 10.31 (s, -NH-) for hydrogen in amide group. Total hydrogens indicated in the compound **ten (10).**

The structure of the compound was further cleared by its ^{13}CNMR data (page-175). Chemical shift at 54.56 and 55.17 revealed presence of two carbons in Ur-OCH_3 groups, the chemical shift 100.54 due to the presence of Ur-CH carbon, δ 117.85, 119.96, 124. 83, 130.08 in the favor of four carbons in aromatic ring (Ar-CH), δ 134.79, 138.23 for the two terminal carbons of benzene ring (Ar-C) of the compound, chemical shift (δ) 158.49, 160.33, 164.81 obtained for the three tertiary carbons of uracil (Ur-C), δ 173.42 indicated the presence of carbonyl carbon (C=O) in amide group. Total **thirteen (13)** carbon atoms revealed by the ^{13}CNMR spectra.

Elementary analysis boosted estimation to draw the compound's structure. Calculated percentages of C, H & N in the compound were 34.58, 2.23 & 9.03 respectively. Those percentages were almost similar to the experimental data 32.43, 2.41 & 8.93 respectively.

From the UV, IR, ^{1}HNMR ^{13}CNMR spectral data and Elementary

analysis, the structure of this compound was confirmed as-



22

2.4e. Characterization of 2, 4 - Dimethoxy -5-bromo- 6-*p*-methylphenylamido pyrimidine: In the reaction there were produced four compounds. Those compounds were quite adjacent on TLC plate even high non-polar solvent system. It was difficult to separate any pure product from the four compounds.

2.4f. Characterization of 2, 4 - Dimethoxy – 5-bromo-6-(4-bromo-3-methyl) phenylamido pyrimidine-23: A white solid was obtained with yield 85.29%, M.P.139-141^{0}C, which was very moisture sensitive. The structure of the compound was established by UV, IR, ^{1}HNMR and ^{13}CNMR spectral data. In UV spectrum, the λ_{max} value was found in the 284.00 nm.

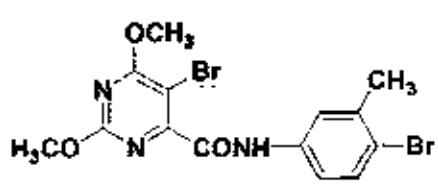
The IR spectrum (page-180) of this compound showed absorption bands at ν_{max} 3282.6 and 1705.0 cm^{-1} for stretching of –NH- and -C=O groups. Stretching bands 1616.2, 1564.2 & 1265.2 cm^{-1} indicated –C=N, –C=C and -C-O groups in the compound respectively.

In the ^{1}HNMR Spectra of **23** compound indicated the chemical shift position of 2.39 (s, 3H, Ar-CH_3) for three hydrogens in methyl group, two singlet spectrum at δ 4.04 and 4.08 indicated six hydrogens in two methoxy groups of uracil structure, chemical shift position at 7.23 (s, 1H, Ar-H) for one hydrogen in aromatic ring, δ 7.74 (s, 1H, Ar-H) for one

hydrogen in benzene, singlet at δ 8.55 (s, 1H, Ar-H) indicated one hydrogen in benzene ring, 9.66 (s, -NH-) for hydrogen in amide group. There were total **thirteen (13)** hydrogen atoms.

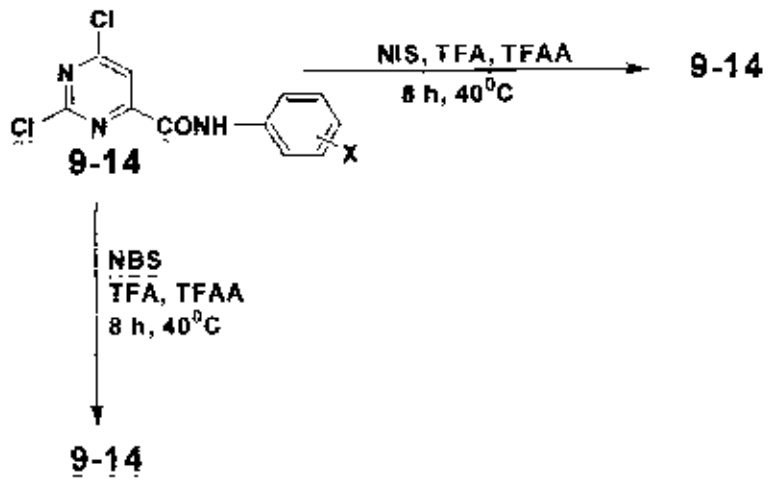
^{13}CNMR spectral data (page-179) was found for the compound as chemical shift 21.47 (Ar-CH_3) indicated one carbon in methyl group, two peaks at 54.48 and 55.12 revealed presence of two carbon in two Ur-OCH_3 groups, δ 100.45 due to the presence of one Ur-CH carbon, δ 116.99, 120.51, 125.64, 128.93, 137.02, 139.06 (Ar-CH) in the favor of six carbons in aromatic ring (Ar-CH), δ 134.52, 134.59 for the two terminal carbons in benzene ring (Ar-C) of the compound, chemical shift (δ) 159.06, 160.15, 164.79 obtained for three tertiary carbons of uracil (Ur-C), δ 173.42 indicated the presence of carbonyl carbon (C=O) in amide group. Total carbon presences in the compound were thirteen **(13).**

The Elementary analysis data for C, H & N atoms confirmed the structure of the compound **(23).** Experimental data of the three elements obtained C 38.68, H 3.08 & N 9.47 percent. These were almost similar to the calculated data C 39.01, H 3.03 & N 9.74 percent respectively.
The data of UV, IR, ^{1}HNMR and ^{13}CNMR spectra and elementary analysis, the structure of this compound was accorded as-



23

2.5. Procedure for the Preparation of 2, 4-Dichloro-5-halo-6-substituted phenylamido pyrimidine from 2, 4-Dichloro-6-substituted phenylamido pyrimidine- The Bromination and Iodination reaction of 2, 4-Dichloro-6-substitutedphenylamido pyrimidine was also carried out by using NBS-TFA-TFAA and NIS-TFA-TFAA under refluxing condition but desired compound 5-Bromo/Iodo pyrimidine was not obtained.

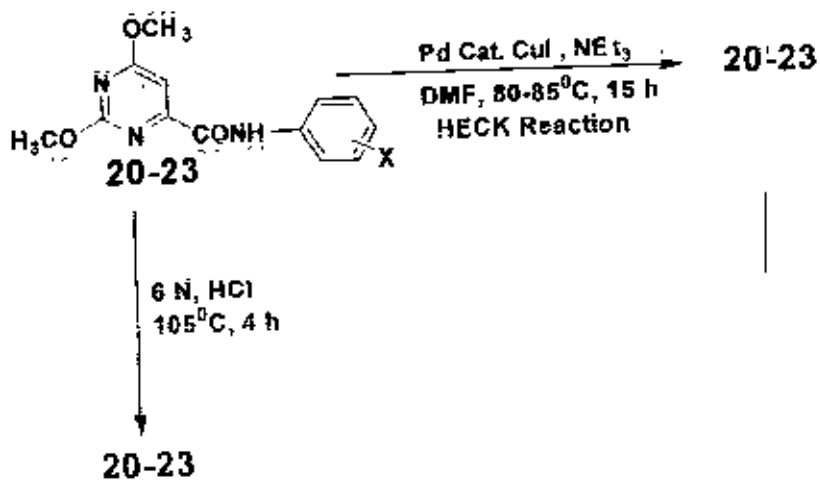


Scheme-5

2.6. Procedure for Demethylation of 5-Bromopyrimidine & Heck reaction of 2, 4 -Dimethoxy-5-bromo-6- substitutedphenylamido pyrimidine –

2.6a. Heck reaction of 2, 4 -Dimethoxy-5-bromo-6- substituted phenylamido pyrimidine: In continuation of our study on synthesis of various heterocyclic compounds through palladium-catalyzed reactions using terminal alkynes. We became interested in the palladium catalyzed carboannulation for the synthesis of 5-alkynyl pyrimidines. But bromine is not worked as a leaving group in 2, 4 -Dimethoxy-5-bromo-6-substitutedphenylamido pyrimidine. After performing Heck Reaction remained intake of original compounds.

2.6b. Demethylation of 5-Bromopyrimidine: 2, 4- Dimethyl-6-substituted phenylamido pyrimidine (1 mmol) in hydrochloric acid (6 N, 10 ml) was heated at 110°C for four hours. The reaction mixture was cooled and solid was separated out by filtration. Then it was washed with a little cold water, dried and washed again with n-hexane. IR, UV, ^{1}HNMR, ^{13}CNMR spectra of this compound was indistinguishable from those of the same compound. No desired product could be isolated.



Scheme-6

Table 4: Distinction among some spectral data of 2, 4 - Dichloro - 6- substituted phenylamido pyrimidine derivatives 9-11:

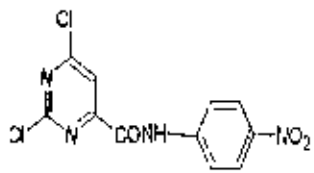
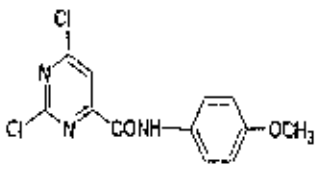
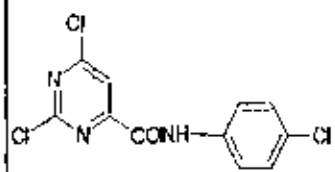
Compound	UV(nm) λ_{max}	IR ν_{max} cm^{-1}	^{1}HNMR δ_H	^{13}CNMR δ_C	MP ^{0}C	Yield %
 **9**	326.00	3363.6,1691.5, 1544.9,1533.3, 1506.3, 1407.9	7.96 (d, 2H, J= 7.68 Hz, Ar-H), 8.18 (s, 1H), 8.29 (d, 2H, J= 7.64 Hz, Ar-H), 9.77 (s, -NH-).	118.19 (Ur-CH) (Ar-C), 157.90, 159.19, 160.02 (Ur-C), 165.41 (C=O)..	157-159	78.05
 **10**	352.00	3354.0,1689.5, 1564.2,1529.4, 1508.2, 1415.7	3.83 (s, 3H, Ar- OCH_3), 6.94 (d, 2H, J= 9.02 Hz, Ar-H), 7.65 (d, 2H, J=9.00, Ar-H), 8.17 (s, 1H, C_5-H), 9.42 (s, -NH-).	54.50, 55.15 & 55.54 (-OCH_3). 100.395 (Ur-CH), 156.80, 159.22, 159.97 (Ur-C), δ 165.13 (C=O).	147-149	82.52
 **11**	306.00	3359.8, 1685.7, 1566.1, 1527.5, 1488.9, 1400.2, 1247.9, 837.0, & 758.0	7.36 (d, 2H, J= 8.2 Hz, Ar-H), 7.68 (d, 2H, J= 8.14 Hz, Ar-H), 8.14 (s, 1H, C_5-H), 9.49 (s, -NH-).	118.16 (Ur-CH), 157.90, 159.19, 160.02 (Ur-C), 165.41 (C=O).	166-167	81.77

Table 5: Distinction among some spectral data of 2, 4 - Dichloro - 6- substituted phenylamido pyrimidine derivatives 12-14:

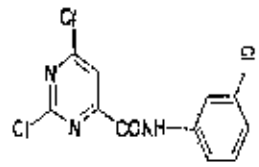
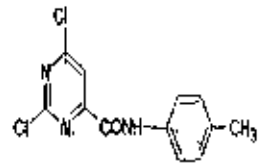
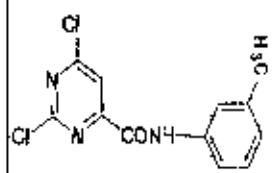
Compound	UV(nm) λ_{max}	IR ν_{max} cm^{-1}	^{1}HNMR δ_H	^{13}CNMR δ_C	MP ^{0}C	Yield %
 **12**	324.00	3340.5,1695.3, 1593.1,1525.6, 1251.7 & 680.8	7.20 (d, 1H, J= 7.22 Hz, Ar-H), 7.33 (t, 1H, J= 8.08 Hz, Ar-H), 7.58 (d, 1H, J= 8.16 Hz, Ar-H), 7.86 (s, Ar-H) 8.16 (s, 1H, C_5-H),	118.16 (Ur-CH), 118.36, 120.28,157.96, 159.89, 160.02 (Ur-C), 165.41 (C=O).	160-162	83.25
 **13**	320.00	3369.4.1687.6, 1566.1,1525.6, 1317.3,1294.1, 1245.9 & 825.5	2.34 (s, 3H, Ar-CH_3), 7.18 (d, 2H, J= 8.28 Hz, Ar-H), 7.59 (d, 2H, J= 8.32 Hz, Ar-H), 8.14 (s, 1H, C_5-H), 9.43 (s, -NH-).	20.96 (Ar-CH_3), 135.39 (Ar-C), 157.60, 159.81, 160.46 (Ur-C), 165.13 (C=O).	159-161	83.33
 **14**	356.00	3355.9, 3068.7, 1687.6, 1531.4, 1488.9, 1309.6, 1296.1, 1247.9 & 794.6	2.41 (s, 3H), 7.06 (d, 1H, J= 7.43 Hz, Ar-H), 7.31 (q, 1H, J= 7.61 Hz, Ar-H), 7.58 (d, 2H, J=9.42, Ar-H) 8.18 (s, 1H, C_5-H), 9.46 (s, -NH-).	21.49 (Ar-CH_3), 117.28 (Ur-C), 118.29, 120.74, 126.46, 129.12 (Ar-CH), 159.91, 160.47 (Ur-C), 165.25 (C=O).	147-149	83.07

Table 7: Distinction among some spectral data of 2, 4 - Dimethoxy - 6- substituted phenylamido pyrimidine derivatives 15-17:

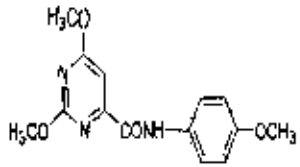
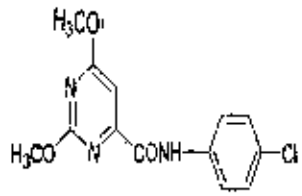
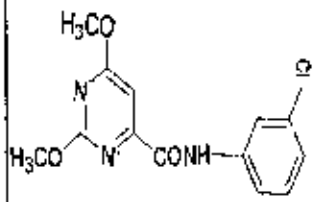
Compound	UV(nm) λ_{max}	IR ν_{max} cm^{-1}	^{1}HNMR δ_H	^{13}CNMR δ_C	MP ^{0}C	Yield %
 **15**	286.00	3332.8,3003.0, 2947.0,1685.7, 1589.2,1573.8, 1535.2,1514.0, 1483.2,1465.8, 1384.8,1355.9,	3.79 (s, 3H, Ar-OCH_3), 4.03 (s, 3H, Ur-OCH_3), 6.89 (d, 1H, J= 6.9 Hz, Ar-H), 7.25 (s, 1H, C_5-H), 7.62 (d, 1H, J=8.09, Ar-H) 9.54 (s,	54.50 (Ar-CH_3), 55.15, 55.54, 156.80, 159.22, 159.97 (Ur-C), 165.13 (C=O).	112-113	85.01
 **16**	282.00	3355.9,3107.1, 1691.5,1606.6, 1583.4,1569.9, 1519.8,1492.8, 1415.7,1398.3,	4.02 (s, 3H, Ur-OCH_3), 4.06 (s, 3H, Ur-OCH_3), 7.22 (s, 1H, C_5-H), Ar-H), 7.65 (d, 2H, J=8.8 Hz, Ar-H), 9.63 (s, -NH-).	54.56 (Ur-OCH_3), 55.17, 100.50 (Ur-CH), 151.81 (Ur-C), 164.03 (C=O).	118-120	86.09
 **17**	276.00	3355.9, 3105.2; 1706.9, 1610.5; 1598.9, 1569.9; 1508.2, 1481.2, 1475.4, 1419.5, 1396.4, 1367.4,	4.05 (s, 3H, Ar-OCH_3), 4.09 (s, 3H, Ar-OCH_3), 7.14 (d, 1H, J= 7.80 Hz, Ar-H), 7.55 (d, 1H, J=8.01 Hz, Ar-H), 7.85 (s, 1H, C_5-H), 9.66 (s, -NH-).	54.53 (Ur-OCH_3), 134.79, 138.23 (Ar-C), 158.49, 160.33, 164.81 (Ur-C), 173.42 (-C=O).	120-121	86.51

Table 8: Distinction among some spectral data of 2, 4 - Dimethoxy - 6- substituted phenylamido pyrimidine derivatives 18-19 & 2, 4 – Dimethoxy-5-bromo - 6- substituted phenylamido pyrimidine derivatives 20:

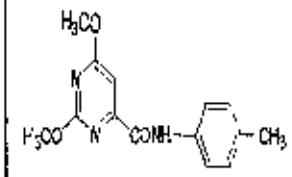
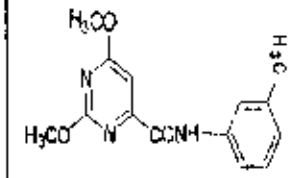
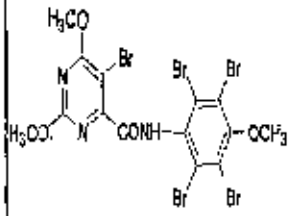
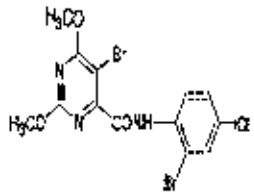
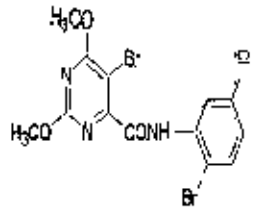
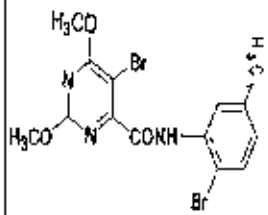
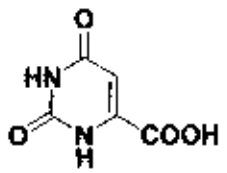
Compound	UV(nm) λ_{max}	IR ν_{max} cm^{-1}	^{1}HNMR δ_H	^{13}CNMR δ_C	MP ^{0}C	Yield %
 **18**	284.00	3321.2,1676.0, 1606.6,1585.4, 1571.9,1525.6, 1481.2,1458.1, 1404.1,1390.6, 1361.7 & 1049	2.34 (s, 3H, Ar-CH_3), 4.04 (s, 3H, Ar-OCH_3), 4.09 (s, 3H, Ar-OCH_3) 7.27 (s, 1H, C_5-H), 7.59 (d, 2H, J=8.36 Hz, Ar-H), 9.59 (s, -NH-).	20.92 (Ar-CH_3), 54.48 (Ur-OCH_3), 159.14, 160.07, 164.79 (Ur-C), 173.43	114-115	83.19
 **19**	302.00	3319.1,1673.9, 1609.6,1587.0, 1571.9,1527.6, 1495.2,1452.1, 1404.1,1391.6, 1360.7 & 1049	2.38 (s, 3H, Ar-CH_3), 4.05 (s, 3H, Ar-OCH_3), 4.10 (s, 3H, Ar-OCH_3), 7.52 (d, 1H, J=7.99 Hz, Ar-H), 7.59 (s, 1H, C_5-H), 9.66 (s, -NH-).	21.47 (Ar-CH_3),55.12 (Ur-OCH_3), 159.06,160.15, 164.79 (Ur-C), 173.42 (-C=O).	117-119	85.20
 **20**	246.00	3238.3, 2947.0, 1687.6, 1560.3, 1514.0, 1452.3, 1384.8, 1199.6, 1107.1, 1031.8, 1014.5, 1002.9, 929.6 & 513.0	3.79 (s, 3H, Ar-OCH_3), 4.07 (s, 3H, Ur-OCH_3), 4.12 (s, 3H, Ur-OCH_3) 9.43 (s, -NH-).	55.65 (Ur-OCH_3), 56.05 (Ur-OCH_3), 155.57, 160.03, 162.81 (Ur-C), 169.58 (-C=O).	141-142	67.31

Table 8: Distinction among some spectral data of 2, 4 – Dimethoxy-5-bromo - 6- substituted phenylamido pyrimidine derivatives 21-23:

Compound	UV(nm) λ_{max}	IR ν_{max} cm^{-1}	^{1}HNMR δ_H	^{13}CNMR δ_C	MP ^{0}C	Yield %
 **21**	320.00	3303.8,1705.0, 1560.3,1514.0, 1487.0,1460.0, 1388.7,1353.9, 1199.6 & 1029.9	4.09 (s, 6H, Ar-OCH_3), 7.36 (d, 1H, J=8.91 Hz, Ar-H), 7.59 (s, 1H, Ar-H), 8.60 (d, 2H, J=8.90 Hz, Ar-H), 10.50 (s, -NH-).	55.83, 56.19 (Ur-OCH_3), 98.94 (Ur-C), 159.91, 162.52, 169.70 (Ur-C), 173.06	146-148	72.09
 **22**	300.00	3303.8,1705.0, 1589.2,1560.3, 1514.0,1487.0, 1487.0,1460.0, 1353.9, 1172.6 & 1029.9	4.13 (s, 3H, Ar-OCH_3), 4.14 (s, 3H, Ar-OCH_3), 8.25 (d, 1H, J= 8.91 Hz, Ar-H) 8.75 (d, 1H, J= 9.01 Hz, Ar-H), 10.31 (s, -NH-).	55.01, 55.79 (Ur-OCH_3), 98.04 (Ur-CBr), 159.90, 169.09 (Ur-C), 173.07 (C=O).	143-145	77.50
 **23**	284.00	3282.6, 1705.0, 1616.2, 1564.2, 1517.9, 1488.9, 1469.7, 1442.7, 1396.4, 1369.4, 1265.2, 1203.5, 1114.8 & 1020.3	2.39 (s, 3H, Ar-CH_3), 4.04 (s, 3H, Ar-OCH_3), 4.08 (s, 3H, Ar-OCH_3), 7.23 (s, 1H, Ar-H), 7.74 (s, 1H, Ar-H) 8.55 (s, 1H, Ar-H), 10.42 (s, -NH-).	55.55 (Ur-OCH_3), 56.01 (Ur-OCH_3), 61.61 (Ar-CH_3), 98.07 (Ur-C-Br), 162.80 (Ur-C), 169.56 (-C=O).	139-141	85.29

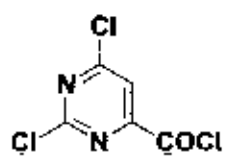
3.1. Preparation of 2, 4 - Dichloropyrimidine-6-carbonyl chloride-2:

A mixture of 2, 4 - Dioxo - 1, 3, 5 - trihydro - pyrimidine - 6- carboxylic acid (Orotic acid) 5.0 g (0.032 mol) and phosphorus oxychloride ($POCl_3$, 40 ml) was refluxed for 24 hours at 105-108^{0}C, then phosphorus pentachloride (15 g, 0.072 mol) was added into the reaction mixture.



1

The mixture was then refluxed for 24 hours. $POCl_3$ was removed under reduced pressure. The residue was distilled with the help of short path distillation set under reduced pressure and ~4.0 g 2, 4 – Dichloropyrimidine- 6- carbonyl chloride **2** was obtained as dense colorless liquid.



2

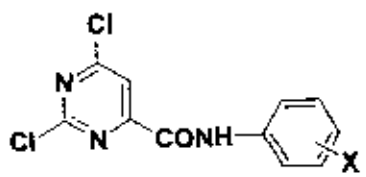
3.2. General procedure for synthesis of 2, 4 - Dichloro - 6- substituted phenylamido pyrimidine 9-14:

The substituted amine compounds were dissolved in benzene and added to the cold solution of 2, 4 – Dichloropyrimidine -6- carbonyl chloride (5.0g) drop wise. The mixture was then allowed to warm up to room temperature and stirred at room temperature (25^{0}C) for 2 hours. The mixture was kept at 0-5^{0}C for over night. Then the mixture was washed with distilled water followed by saturated aqueous solution of Sodium hydrogen carbonate ($NaHCO_3$). Then the mixture was extracted in

Chapter 3

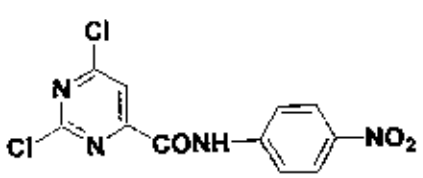
Experimental

Chloroform (3×50 ml). The organic portion was washed with distilled water (2×50 ml). Then the organic layer was dried over anhydrous Sodium Sulfate (Na_2SO_4). The solvent was removed under reduced pressure to obtain crude product. The product was purified by column chromatography then the isolated product was crystallized from methanol.



9-14

3.2a. Synthesis of 2, 4 - Dichloro - 6-*p*-nitrophenylamido pyrimidine-9: 2, 4 - Dichloro - 6-*p*-nitrophenylamido pyrimidine (6.10 g, 18.54 mmol) was produced from the reaction of 2, 4 – Dichloropyrimidine- 6-carbonyl chloride (5.0 g, 23.76 mmol) and *p*-Nitroaniline (3.45 g, 25 mmol). Data obtained from the synthesized compound **9** are given below-



9

MF: $C_{11}H_6N_4O_4Cl_2$ **MW:** 329.1

Physical Analysis

Shape and Color: Amorphous and Reddish

Melting Point: 157-159^{0}C

Odor: Bad smell

Percentage of Yield: 78.05

Analytical Analysis

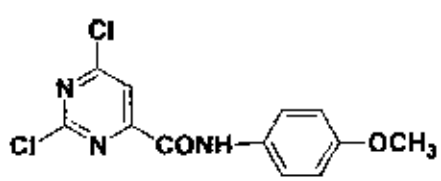
UV (EtOH): λ_{max} 326.00 nm.

IR (KBr): ν_{max} 3363.6, 1691.5, 1544.9, 1533.3, 1506.3, 1407.9, 1340.4, 1309.6, 1292.2, 1253.6, 1110.9 & 860.2 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 7.96 (d, 2H, J= 7.68 Hz, Ar-H), 8.18 (s, 1H), 8.29 (d, 2H, J= 7.64 Hz, Ar-H), 9.77 (s, -NH-).

^{13}CNMR (100MHz, $CDCl_3$): δ_C 118.19 (Ur=CH) 119.01, 119.89, 124.43, 126.57 (Ar-CH), 130.06, 131.21 (Ar-C), 157.90, 159.19, 160.02 (Ur-C), 165.41 (C=O).

3.2b. Preparation of 2, 4 - Dichloro - 6-*p*-methoxyphenylamido pyrimidine- 10: 2, 4 - Dichloro - 6-*p*-methoxyphenylamido pyrimidine (6.94 g, 19.61 mmol) was produced from 2, 4 – Dichloropyrimidine- 6-carbonyl chloride (5.0 g, 23.76 mmol) and *p*-Anisidine (3.00 g, 25 mmol). The reaction's conditions were followed as mentioned in general procedure (3.2). Physical and analytical data of the compound (**10**) are given below-



10

MF: $C_{12}H_9N_3O_2Cl_2$ **MW:** 354.14

Physical Analysis

Shape and Color: Amorphous and Yellowish

Melting Point: 147-149^{0}C

Odor: Rotten fish

Percentage of Yield: 82.52

Analytical Analysis

UV (EtOH): λ_{max} 352.00 nm.

IR (KBr): ν_{max} 3354.0, 1689.5, 1564.2, 1529.4, 1508.2, 1415.7, 1298.0, 1253.6, 1250.0, 1033.8, 829.3 & 788.8 cm^{-1}

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 3.83 (s, 3H, Ar-OCH_3), 6.94 (d, 2H, J= 9.02 Hz, Ar-H), 7.65 (d, 2H, J=9.00, Ar-H), 8.17 (s, 1H, C_5-H), 9.42 (s, -NH-).

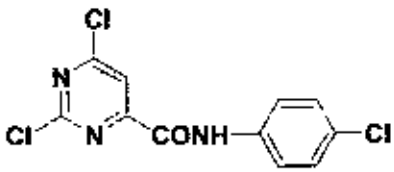
^{13}CNMR (100MHz, $CDCl_3$): δ_C 54.50, 55.15 & 55.54 (-OCH_3), 100.395 (Ur-CH) δ 114.34, 121.51 (Ar-CH), δ 130.38, 133.09 (Ar-C), 156.80, 159.22, 159.97 (Ur-C), δ 165.13 (C=O).

CHN Analysis

Elementary Data	C %	H %	N %
Calculated	48.34	3.04	14.09
Experimental	47.40	3.22	13.65

3.2c. Synthesis of 2, 4 - Dichloro - 6-*p*-chlorophenylamido pyrimidine-11: 2, 4 - Dichloro - 6-*p*-chlorophenylamido pyrimidine (6.15 g, 19.43 mmol) was prepared from the reaction of 2, 4 – Dichloropyrimidine- 6-carbonyl chloride (5.0 g, 23.76 mmol) and *p*-Chloroaniline (3.64 g, 28.51

mmol) using the same reaction conditions as described in general procedure **3.2**. Physical properties and all analytical data obtained from the compound **11** are given below-



11

MF: $C_{11}H_6N_3OCl_3$ **MW:** 316.56

Physical Analysis

Shape and Color: Red and fine Crystal

Melting Point: 166-167^{0}C

Odor: Rotten fish

Percentage of Yield: 81.77

Analytical Analysis

UV (EtOH): λ_{max} 306.00 nm.

IR (KBr): ν_{max} 3359.8, 1685.7, 1566.1, 1527.5, 1488.9, 1400.2, 1247.9, 837.0, & 758.0 cm^{-1}.

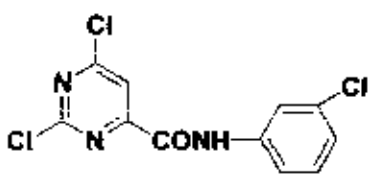
^{1}HNMR (400 MHz, $CDCl_3$): δ_H 7.36 (d, 2H, J= 8.2 Hz, Ar-H), 7.68 (d, 2H, J= 8.14 Hz, Ar-H), 8.14 (s, 1H, C_5-H), 9.49 (s, -NH-).

^{13}CNMR (100MHz, $CDCl_3$): δ_C 118.16 (Ur-CH), 119.01, 119.89, 124.43, 126.57 (Ar-CH), 130.06, 131.21 (Ar-C), 157.90, 159.19, 160.02 (Ur-C), 165.41 (C=O).

CHN Analysis

Elementary data	C %	H %	N %
Calculated	43.67	2.00	13.89
Experimental	43.81	2.15	13.85

3.2d. Preparation of 2, 4 - Dichloro - 6-*m*-chlorophenylamido pyrimidine- 12: The mixture of 2, 4 – Dichloropyrimidine- 6- carbonyl chloride (5.0 g, 23.76 mmol) and *m*-Chloroaniline (3.57 g, 28.05 mmol) saturated in benzene was afforded the desired product 2, 4 - Dichloro - 6-*m*-chlorophenylamido pyrimidine (6.26 g, 19.78 mmol). Necessary information to erect the structure of the compound are given below-



12

MF: $C_{11}H_6N_3OCl_3$ **MW:** 316.56

Physical Analysis

Shape and Color: Amorphous and Reddish

Melting Point: 160-162^{0}C

Odor: Bad smell

Percentage of Yield: 83.25

Analytical Analysis

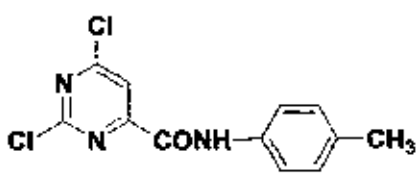
UV (EtOH): λ_{max} 324.00 nm.

IR (KBr): ν_{max} 3340.5, 1695.3, 1593.1, 1525.6, 1251.7 & 680.8 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 7.20 (d, 1H, J= 7.22 Hz, Ar-H), 7.33 (t, 1H, J= 8.08 Hz, Ar-H), 7.58 (d, 1H, J= 8.16 Hz, Ar-H), 7.86 (s, Ar-H) 8.16 (s, 1H, C_5-H), 9.50 (s, -NH-).

^{13}CNMR (100MHz, $CDCl_3$): δ_C 118.16 (Ur-CH), 118.36, 120.28, 125.71, 130.29 (Ar-CH), 135.06, 137.53 (Ar-C), 157.96, 159.89, 160.02 (Ur-C), 165.41 (C=O).

3.2e. Synthesis of 2, 4 - Dichloro - 6-*p*-methylphenylamido pyrimidine-13: A mixture of acid chloride (2, 4 – Dichloropyrimidine-6- carbonyl chloride, 5.0 g, 23.76 mmol) and *p*-Methylaniline (3.03 g, 28.25 mmol) saturated in benzene was taken in a two-neck round bottom flask and stirred at room temperature for 2 hours. The reaction system was stored at 0^0C for 12 hours. A lustrous silver color compound was obtained. All analytical data and physical properties of the compound **13** are given below-



13

MF: $C_{12}H_9N_3OCl_2$ **MW:** 282.12

Physical Analysis

Shape and Color: Crystal and Silver

Melting Point: 159-161^{0}C

Odor: Odorless

Percentage of Yield: 83.33

Analytical Analysis

UV (EtOH): λ_{max} 320.00 nm.

IR (KBr): ν_{max} 3369.4, 1687.6, 1566.1, 1525.6, 1317.3, 1294.1, 1245.9 & 825.5 cm^{-1}.

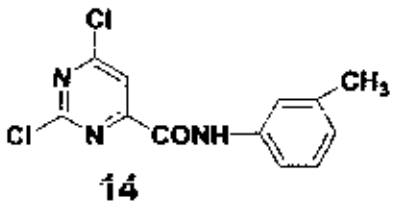
^{1}HNMR (400 MHz, $CDCl_3$): δ_H 2.34 (s, 3H, Ar-CH_3), 7.18 (d, 2H, J= 8.28 Hz, Ar-H), 7.59 (d, 2H, J= 8.32 Hz, Ar-H), 8.14 (s, 1H, C_5-H), 9.43 (s, -NH-).

^{13}CNMR (100MHz, $CDCl_3$): δ_C 20.96 (Ar-CH_3), 118.22 (Ur-CH), 120.06, 129.75 (Ar-CH), 133.89, 135.39 (Ar-C), 157.60, 159.81, 160.46 (Ur-C), 165.13 (C=O).

CHN Analysis

Elementary data	C %	H %	N %
Calculated	51.09	3.22	14.89
Experimental	49.07	3.28	14.08

3.2f. Preparation of 2, 4 - Dichloro - 6-*m*-methylphenylamido pyrimidine- 14: A mixture of 2, 4 – Dichloropyrimidine- 6- carbonyl chloride (5 g, 23.76 mmol) and *m*-Methylaniline (3.02 g, 28.15 mmol) in benzene yielded the expected product 2, 4 - Dichloro - 6-*p*-nitrophenylamido pyrimidine (5.57 g, 19.74 mmol). Analytical data and physical properties of the compound are given below-



14

MF: $C_{12}H_9N_3OCl_2$ **MW:** 282.12

Physical Analysis

Shape and Color: Crystal and Reddish

Melting Point: 147-149^{0}C

Odor: Odorless

Percentage of Yield: 83.07

Analytical Analysis

UV (EtOH): λ_{max} 356.00 nm.

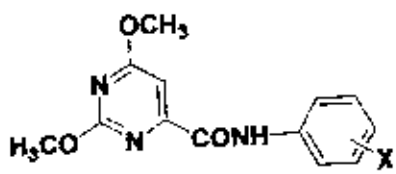
IR (KBr): ν_{max} 3355.9, 3068.7, 1687.6, 1531.4, 1488.9, 1309.6, 1296.1, 1247.9 & 794.6 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 2.41 (s, 3H), 7.06 (d, 1H, J= 7.43 Hz, Ar-H), 7.31 (q, 1H, J= 7.61 Hz, Ar-H), 7.58 (d, 2H, J=9.42, Ar-H) 8.18 (s, 1H, C_5-H), 9.46 (s, -NH-).

^{13}CNMR (100MHz, $CDCl_3$): δ_C 21.49 (Ar-CH_3), 117.28 (Ur-C), 118.29, 120.74, 126.46, 129.12 (Ar-CH), 136.34, 139.32 (Ar-C), 157.75, 159.91, 160.47 (Ur-C), 165.25 (C=O).

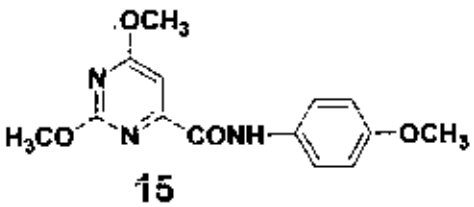
3.3. General procedure for the Synthesis of 2, 4 - Dimethoxy - 6-substituted phenylamido pyrimidine 15-19:

2, 4 - Dimethoxy - 6- substituted benzoamido pyrimidine (1 mmol) was added to the cold solution of sodium methoxide solution prepared by dissolving sodium (3 mmol) in methanol (30 ml). The mixture was refluxed at 60^0C for 4 hours under Nitrogen atmosphere. After removal of solvent and neutralization with dilute hydrochloric acid solution the mixture was extracted with chloroform (3×50 ml). The chloroform layer was washed with water (2×30 ml) dried over anhydrous Na_2SO_4 and solvent was removed under reduced pressure. Then the residue was crystallized from methanol.



15-19

3.3a. Synthesis of 2, 4 - Dimethoxy - 6-*p*-methoxyphenylamido pyrimidine- 15: Reaction of 2, 4 - Dichloro - 6-*p*-methoxyphenylamido pyrimidine (2.5 g, 8.64 mmol) and sodium methoxide prepared by dissolving sodium (0.59 g, 25.92 mmol in dry methanol (50 ml) at 60^0C for 4 hours produced desired 2, 4 - Dimethoxy - 6-*p*-methoxyphenylamido pyrimidine (7.34 mmol, 2.12 g). All data obtained from the compound **15** are given below-



15

MF: $C_{14}H_{15}N_3O_4$ **MW:** 289.29

Physical Analysis

Shape and Color: Amorphous and Off -White

Melting Point: 112-113^{0}C

Odor: Pleasant smell

Percentage of Yield: 85.01

Analytical Analysis

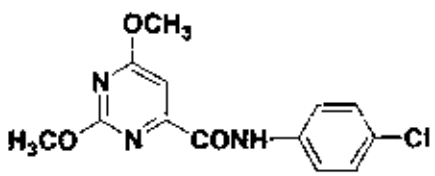
UV (EtOH): λ_{max} 286.00 nm.

IR (KBr): ν_{max} 3332.8, 3003.0, 2947.0, 1685.7, 1589.2, 1573.8, 1535.2, 1514.0, 1483.2, 1465.8, 1384.8, 1355.9, 1315.4, 1301.9 1263.3, 1232.4, 1218.9, 1201.6, 1186.1, 1186.1, 1126.4, 1076.2 1043.4, 987.5, 933.5 & 769.5 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 3.79 (s, 3H, Ar-OCH_3), 4.03 (s, 3H, Ur-OCH_3), 4.08 (s, 3H, Ur-OCH_3) 6.89 (d, 1H, J= 6.9 Hz, Ar-H), 7.25 (s, 1H, C_5-H), 7.62 (d, 1H, J=8.09, Ar-H) 9.54 (s, -NH-).

^{13}CNMR (100MHz, $CDCl_3$): δ_C 54.50 (Ar-CH_3), 55.15, 55.54 (Ur-OCH_3), 100.39 (Ur-CH), 130.38, 133.09 (Ar-CH), 156.80, 159.22, 159.97 (Ur-C), 165.13 (C=O).

3.3b. Preparation of 2, 4 - Dimethoxy - 6-*p*-chlorophenylamido pyrimidine- 16: 2, 4 - Dimethoxy - 6-*p*-chlorophenylamido pyrimidine (6.18 mmol, 1.82 g) was synthesized from the reaction of 2, 4 - Dichloro - 6-*p*-chlorophenylamido pyrimidine (2.11 g, 7.18 mmol) and sodium methoxide in dry methanol (50 ml) using the conditions mentioned in general procedure **3.3**. Physical properties and analytical obtained from the desired compound **16** are given below-



16

MF: $C_{13}H_{12}N_3O_3Cl$ **MW:** 293.71

Physical Analysis

Shape and Color: Amorphous and white

Melting Point: 118-120^{0}C

Odor: Pleasant smell

Percentage of Yield: 86.09

Analytical Analysis

UV (EtOH): λ_{max} 282.00 nm.

IR (KBr): ν_{max} 3355.9, 3107.1, 1691.5, 1606.6, 1583.4, 1569.9, 1519.8, 1492.8, 1479.3, 1460.0, 1415.7, 1398.3, 1382.9, 1348.1, 1303.8, 1286.4, 1303.8, 1259.4, 1238.2, 1197.7, 1176.5, 1093.6, 1093.6, 1024.1, 1010.1, 983.6, 929.6 & 829.3 cm^{-1}.

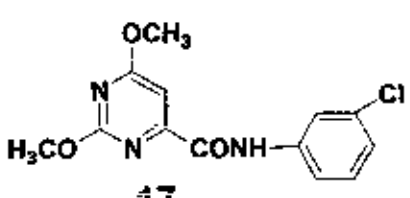
^{1}HNMR (400 MHz. $CDCl_3$): δ_H 4.02 (s, 3H. Ur-OCH_3), 4.06 (s, 3H, Ur-OCH_3), 7.22 (s, 1H, C_5-H), 7.31 (d, 2H, J=8.78 Hz, Ar-H), 7.65 (d, 2H, J=8.8 Hz, Ar-H), 9.63 (s, -NH-).

^{13}CNMR (100MHz, $CDCl_3$): δ_C 54.56 (Ur-OCH_3), 55.17 (Ur-OCH_3), 100.50 (Ur-CH), 118.83, 119.89, 124.43, 126.57 (Ar-CH), 131.06, 131.22 (Ar-C), 145.29, 150.81, 151.81 (Ur-C), 164.03 (C=O).

CHN Analysis

Elementary data	C %	H %	N %
Calculated	53.16	4.12	14.31
Experimental	52.91	4.11	14.33

3.3c. Synthesis of 2, 4 - Dimethoxy - 6-*m*-chlorophenylamido pyrimidine-17: 2, 4 - Dimethoxy - 6-*m*-chlorophenylamido pyrimidine (6.79 mmol, 1.99 g) was prepared from the reaction of 2, 4 - Dichloro - 6-*m*-chlorophenylamido pyrimidine (2.31 g, 7.86 mmol) and sodium methoxide (CH_3ONa). Various data obtained from the compound are given below-



17

MF: $C_{13}H_{12}N_3O_3Cl$ **MW: 293.71**

Physical Analysis

Shape and Color: Amorphous and Pinkish

Melting Point: 120-121^{0}C

Odor: Pleasant smell

Percentage of Yield: 86.51

Analytical Analysis

UV (EtOH): λ_{max} 276.00 nm.

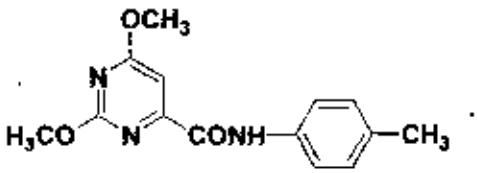
IR (KBr): ν_{max} 3355.9, 3105.2, 1706.9, 1610.5, 1598.9, 1569.9, 1508.2, 1481.2, 1475.4, 1419.5, 1396.4, 1367.4, 1290.3, 1263.3, 1201.6, 1188.1 & 1112.9 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 4.05 (s, 3H, Ar-OCH_3), 4.09 (s, 3H, Ar-OCH_3), 7.14 (d, 1H, J= 7.80 Hz, Ar-H) 7.25 (q, 1H, J=8.03 Hz, Ar-H), 7.55 (d, 1H, J=8.01 Hz, Ar-H), 7.85 (s, 1H, C_5-H), 9.66 (s, -NH-).

^{13}CNMR (100 MHz, $CDCl_3$): δ_C 54.53 (Ur-OCH_3), 55.17 (Ur-OCH_3), 100.54 (Ur-CH), 117.85, 119.96, 124. 83, 130.08 (Ar-CH), 134.79, 138.23 (Ar-C), 158.49, 160.33, 164.81 (Ur-C), 173.42 (-C=O).

3.3d. Preparation of 2, 4 - Dimethoxy - 6-*p*-methylphenylamido pyrimidine-18: The mixture of 2, 4 - Dichloro - 6-*p*-methylphenylamido pyrimidine (2.40 g, 8.78 mmol) and sodium methoxide was afforded the desired product 2, 4 - Dimethoxy - 6-*p*-methylphenylamido pyrimidine

(7.3 mmol, 2.0 g). Physical and analytical data are given below-



18

MF: $C_{14}H_{15}N_3O_3$ **MW:** 273.29

Physical Analysis

Shape and Color: Amorphous and Off-white

Melting Point: 114-115^{0}C

Odor: Pleasant smell

Percentage of Yield: 83.19

Analytical Analysis

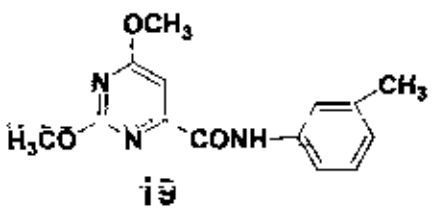
UV (EtOH): λ_{max} 284.00 nm.

IR (KBr): ν_{max} 3321.2, 1676.0, 1606.6, 1585.4, 1571.9, 1525.6, 1481.2, 1458.1, 1404.1, 1390.6, 1361.7 & 1049 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 2.34 (s, 3H, Ar-CH_3), 4.04 (s, 3H, Ar-OCH_3), 4.09 (s, 3H, Ar-OCH_3), 7.14 (d, 2H, J= 8.20 Hz, Ar-H) 7.27 (s, 1H, C_5-H), 7.59 (d, 2H, J=8.36 Hz, Ar-H), 9.59 (s, -NH-).

^{13}CNMR (100 MHz, $CDCl_3$): δ_C 20.92 (Ar-CH_3), 54.48 (Ur-OCH_3), 55.13 (Ur -OCH_3), 100.42 (Ur-CH), 119.89, 129.62 (Ar-CH), 134.52, 134.59 (Ar-C) 159.14, 160.07, 164.79 (Ur-C), 173.43 (-C=O).

3.3e. Synthesis of 2, 4 - Dimethoxy - 6-*m*-methylphenylamido pyrimidine-19: 2, 4 - Dimethoxy - 6-*m*-methylphenylamido pyrimidine (6.08 mmol, 1.66 g) was synthesized from the reaction of 2, 4 - Dichloro - 6-*p*-nitrophenylamido pyrimidine (1.95 g, 7.14 mmol) and sodium methoxide in dry methanol (50 ml). Data obtained from the compound **19** are given below-



19

MF: $C_{14}H_{15}N_3O_3$ **MW:** 273.29

Physical Analysis

Shape and Color: Amorphous and Off-white

Melting Point: 117-119^{0}C

Odor: Pleasant smell

Percentage of Yield: 85.20

Analytical Analysis

UV (EtOH): λ_{max} 302.00 nm.

IR (KBr): ν_{max} 3319.1, 1673.9, 1609.6, 1587.0, 1571.9, 1527.6, 1495.2, 1452.1, 1404.1, 1391.6, 1360.7 & 1049 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 2.38 (s, 3H, Ar-CH_3), 4.05 (s, 3H, Ar-OCH_3), 4.10 (s, 3H, Ar-OCH_3), 6.52 (m, 1H, Ar-H), 7.00 (m, 1H, Ar-H) 7.27 (m,

1H, Ar-H) 7.52 (d, 1H, J=7.99 Hz, Ar-H), 7.59 (s, 1H, C_5-H), 9.66 (s, -NH-).

^{13}CNMR (100 MHz, $CDCl_3$): δ_C 21.47 (Ar-CH_3), 54.48 (Ur-OCH_3), 55.12 (Ur-OCH_3), 100.45 (Ur-CH), 116.99, 120.51, 125.64, 128.93, 137.02, 139.06 (Ar-CH), 159.06, 160.15, 164.79 (Ur-C), 173.42 (-C=O).

CHN Analysis

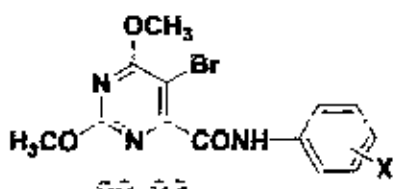
Elementary data	C %	H %	N %
Calculated	61.53	5.53	15.38
Experimental	60.93	5.62	14.85

3.4. General procedure for the preparation of 2, 4-Dimethoxy-5-bromo-6-substituted phenylamido pyrimidine-20-23:

3.4a. Bromine (Br_2) in Pyridine: A mixture of 2, 4-Dimethoxy-6-substituted phenylamido pyrimidine (500 mg), Bromine (0.56 ml) and Pyridine (10 ml) was stirred for 4 hours under inert atmosphere. The solvent was then removed at high temperature under reduced pressure. The residue was triturated with water and filtered, crystallized from methanol. The desired product could not be isolated.

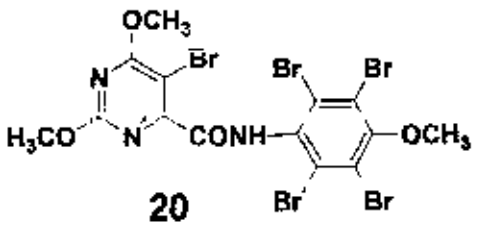
3.4b. *N*-Bromo succinimide (NBS) in Trifluroacetic acid and trifluroacetic anhydrate mixture: A mixture of 2, 4-Dimethoxy-6-substituted phenylamido pyrimidine (1.6954 mmol), Trifluroacetic acid (50 ml) and trifluroacetic anhydrate (5 ml) was refluxed for 20 minutes.

N-Bromo succinimide (1.2 equiv.) was added and the reaction mixture was further refluxed for 10 hours at 40^{0}C. The progress of this reaction was observed with TLC. Then the reaction mixture was allowed to cool at room temperature and the solvent was removed under reduced pressure. Then the mixture was washed with distilled water followed by saturated aqueous solution of Sodium hydrogen carbonate ($NaHCO_3$). Then the mixture was extracted in Chloroform (3×50 ml). The organic portion was washed with distilled water (2×50 ml). Then the organic layer was dried over anhydrous Sodium Sulfate (Na_2SO_4). The solvent was removed under reduced pressure to obtain crude product. The product in this series was separated by column chromatography. The separated product then crystallized from methanol. General structure of the compounds in this series is given below-



20-23

3.4b.1. Synthesis of 2, 4 - Dimethoxy – 5-bromo-6-(4′-methoxy-2′, 3′, 5′, 6′-tetrabromo) phenylamido pyrimidine-20: Reaction of 2, 4 - Dimethoxy -6-*p*-methoxyphenylamido pyrimidine (0.5 g, 0.34 mmol) and *N*-Bromosuccinimide (0.51 mmol) in presence of TFA and TFAA produced 2, 4 - Dimethoxy – 5-bromo-6-(4′-methoxy-2′, 3′, 5′, 6′-tetrabromo) phenylamido pyrimidine (0.23 mmol, 0.16 g) **20**. Physical and analytical data of the compound **20** are given below-



20

MF: $C_{14}H_{10}N_3O_4Br_5$ **MW:** 683.77

Physical Analysis

Shape and Color: Amorphous and White

Melting Point: 141-142^{0}C

Odor: Odorless

Percentage of Yield: 67.31

Analytical Analysis

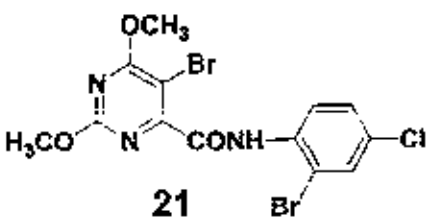
UV (EtOH): λ_{max} 246.00 nm.

IR (KBr): ν_{max} 3238.3, 2947.0, 1687.6, 1560.3, 1514.0, 1452.3, 1384.8, 1353.9, 1334.6, 1299.9, 1286.4, 1245.9, 1199.6, 1107.1, 1031.8, 1014.5, 1002.9, 929.6 & 513.0 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 3.79 (s, 3H, Ar-OCH_3), 4.07 (s, 3H, Ur-OCH_3), 4.12 (s, 3H, Ur-OCH_3) 9.43 (s, -NH-).

^{13}CNMR (100 MHz, $CDCl_3$): δ_C 55.65 (Ur-OCH_3), 56.05 (Ur-OCH_3), 60.65 (Ar-OCH_3), 98.67 (Ur-CH), 121.68, 126.47, 133.09, 153.09 (Ar-C), 155.57, 160.03, 162.81 (Ur-C), 169.58 (-C=O).

3.4b.2. Preparation of 2, 4 - Dimethoxy – 5-bromo-6-(2′-bromo-4′-chloro) phenylamido pyrimidine-21: 2, 4 - Dimethoxy – 5-bromo-6-(2′-bromo-4′-chloro) phenylamido pyrimidine (1.10 mmol, 0.49 g) was synthesized from the reaction of 2, 4 - Dimethoxy - 6-*p*-chlorophenylamido pyrimidine (0.69 g, 1.53 mmol) and NBS (0.32 g, 1.83 mmol). Other procedures were followed as general procedure 3.4b. Analytical data and physical properties of the compound **21** are given below-



21

MF: $C_{13}H_{10}N_3O_3Br_2Cl$ **MW:** 451.50

Physical Analysis

Shape and Color: Amorphous and White

Melting Point: 146-148^{0}C

Odor: Odorless

Percentage of Yield: 72.09

Analytical Analysis

UV (EtOH): λ_{max} 320.00 nm.

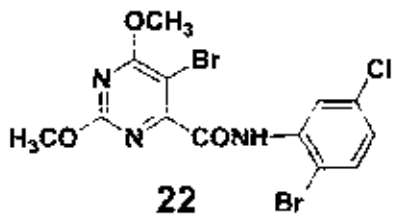
IR (KBr): ν_{max} 3303.8, 1705.0, 1560.3, 1514.0, 1487.0, 1460.0, 1388.7, 1353.9, 1199.6 & 1029.9 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 4.09 (s, 6H, Ar-OCH_3), 7.36 (d, 1H, J=8.91 Hz, Ar-H), 7.59 (s, 1H, Ar-H), 8.60

(d, 2H, J=8.90 Hz, Ar-H), 10.50 (s, -NH-).

^{13}CNMR (100 MHz, $CDCl_3$): δ_C 55.83, 56.19 (Ur-OCH_3), 98.94 (Ur-C), 113.64, 121.64, 128.66, 129.88, 131.93, 134.21 (Ar-C), 159.91, 162.52, 169.70 (Ur-C), 173.06 (C=O).

3.4b.3. Synthesis of 2, 4 - Dimethoxy – 5-bromo-6-(2-bromo-5-chloro) phenylamido pyrimidine-22: 2, 4 - Dimethoxy – 5-bromo-6-(2-bromo-5-chloro) phenylamido pyrimidine (1.36 mmol, 0.61 g) was prepared from the reaction of 2, 4 - Dimethoxy - 6-*m*-chlorophenylamido pyrimidine (0.79 g, 1.75 mmol) and NBS (0.37 g, 2.1 mmol) in presence of TFA and TFAA. Physical properties and analytical data obtained from the synthesized compound **22** are given below-



22

MF: $C_{13}H_{10}N_3O_3Br_2Cl$ **MW:** 451.50

Physical Analysis

Shape and Color: Amorphous and White

Melting Point: 143-145^{0}C

Odor: Odorless

Percentage of Yield: 77.50

Analytical Analysis

UV (EtOH): λ_{max} 300.00 nm.

IR (KBr): ν_{max} 3303.8, 1705.0, 1589.2, 1560.3, 1514.0,

1487.0, 1487.0, 1460.0, 1446.5, 1388.7, 1353.9, 1296.1, 1199.6, 1172.6 & 1029.9 cm^{-1}.

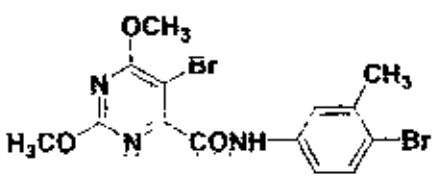
^{1}HNMR (400 MHz, $CDCl_3$): δ_H 4.13 (s, 3H, Ar-OCH_3), 4.14 (s, 3H, Ar-OCH_3), 8.25 (d, 1H, J= 8.91 Hz, Ar-H) 8.75 (d, 1H, J= 9.01 Hz, Ar-H), 10.31 (s, -NH-).

^{13}CNMR (400 MHz, $CDCl_3$): δ_C 55.01, 55.79 (Ur-OCH_3), 98.04 (Ur-CBr), 115.60, 121.14, 127.66, 129.01, 130.73, 134.21 (Ar-C), 159.90, 163.51, 169.09 (Ur-C), 173.07 (C=O).

CHN Analysis

Elementary Data	C %	H %	N %
Calculated	34.58	2.23	9.03
Experimental	34.43	2.41	8.93

3.4b.4. Synthesis of 2, 4 - Dimethoxy – 5-bromo-6-(4-bromo-3-methyl) phenylamido pyrimidine-23: 2, 4 - Dimethoxy – 5-bromo-6-(4-bromo-3-methyl) phenylamido pyrimidine (1.65 mmol, 0.7 g) was synthesized from the reaction of 2, 4 - Dimethoxy-6-*m*-methylphenylamido pyrimidine (0.83g, 1.93 mmol) and NBS (0.41g, 2.32mmol) in presence of TFA and TFAA under the same conditions as general procedure **3.4**. Physical properties and analytical data of the compound **23** are given below.



23

MF: $C_{14}H_{13}N_3O_3Br_2$ **MW:** 431.08

Physical Analysis

Shape and Color: Amorphous and White

Melting Point: 139-141^{0}C

Odor: Odorless

Percentage of Yield: 85.29

Analytical Analysis

UV (EtOH): λ_{max} 284.00 nm.

IR (KBr) : ν_{max} 3282.6, 1705.0, 1616.2, 1564.2, 1517.9, 1488.9, 1469.7, 1442.7, 1396.4, 1369.4, 1265.2, 1203.5, 1114.8 & 1020.3 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$): δ_H 2.39 (s, 3H, Ar-CH_3), 4.04 (s, 3H, Ar-OCH_3), 4.08 (s, 3H, Ar-OCH_3), 7.23 (s, 1H, Ar-H), 7.74 (s, 1H, Ar-H) 8.55 (s, 1H, Ar-H), 10.42 (s, -NH-).

^{13}CNMR (100 MHz, $CDCl_3$): δ_C 55.55 (Ur-OCH_3), 56.01 (Ur-OCH_3), 61.61 (Ar-CH_3), 98.07 (Ur-C-Br), 123.68, 129.47, 133.06, 151.09 (Ar-C), 155.57, 160.03, 162.80 (Ur-C), 169.56 (-C=O).

CHN Analysis

Elementary data	C %	H %	N %
Calculated	39.01	3.03	9.74
Experimental	38.68	3.08	9.47

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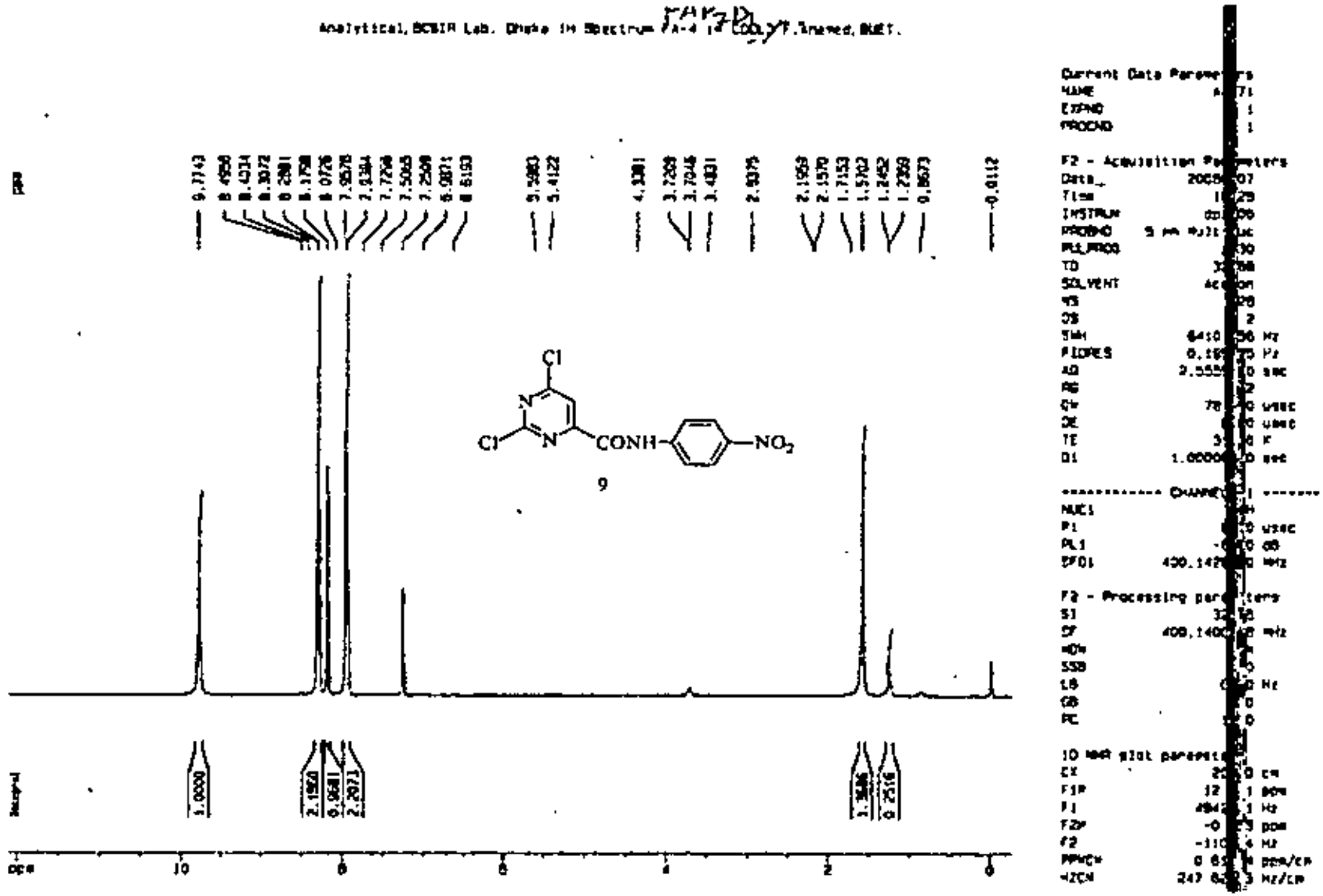
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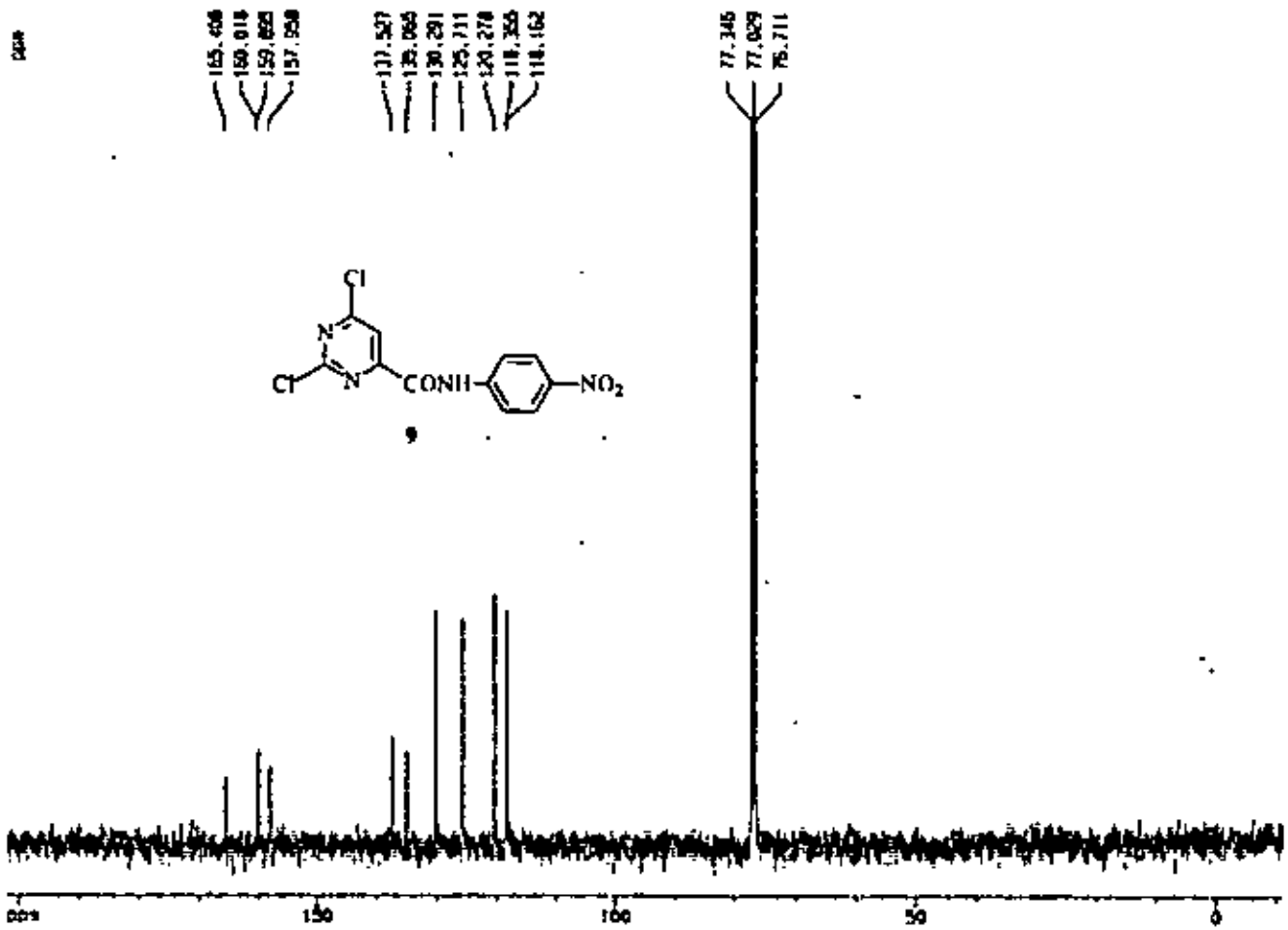

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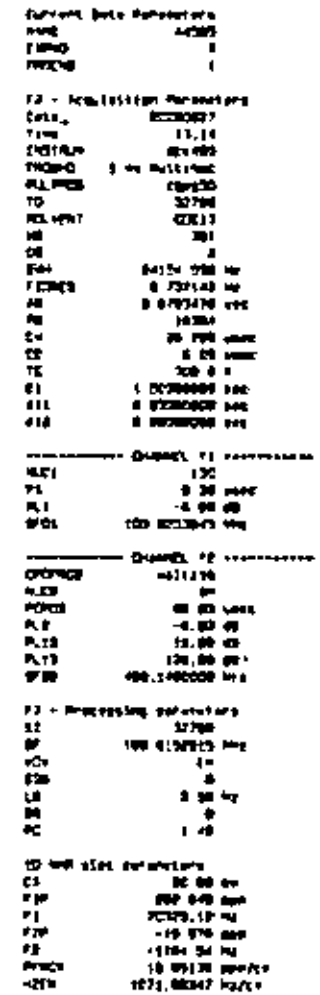

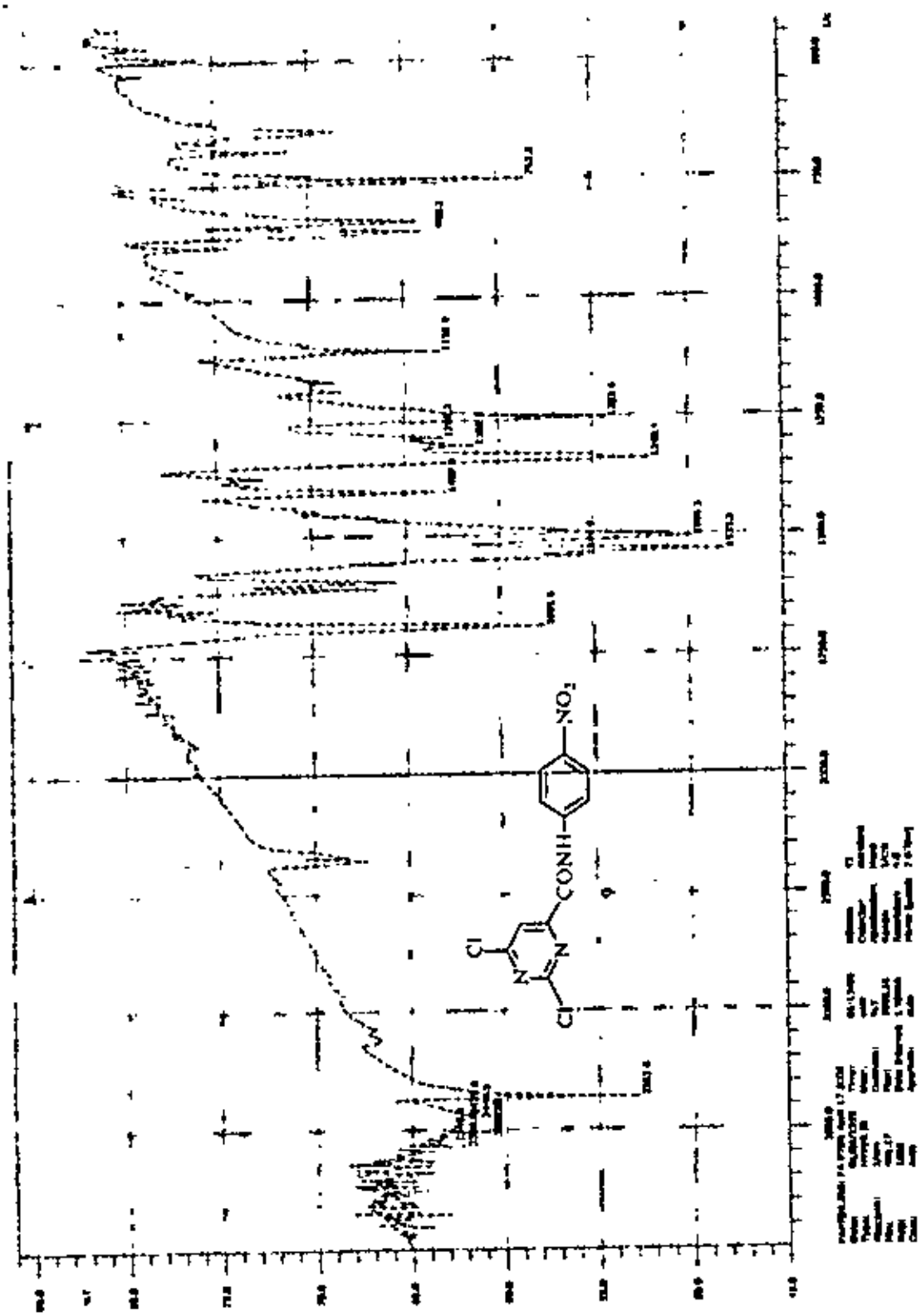
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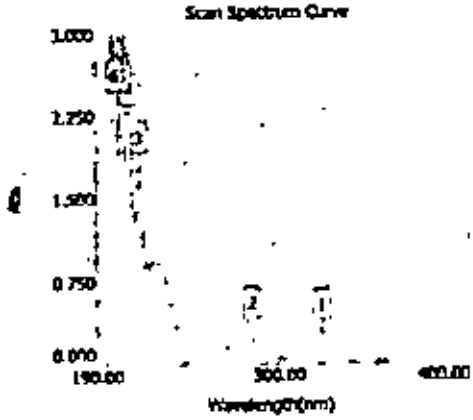

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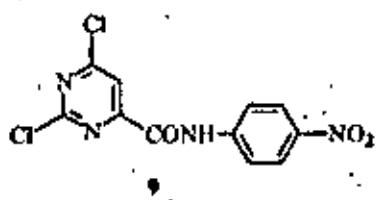
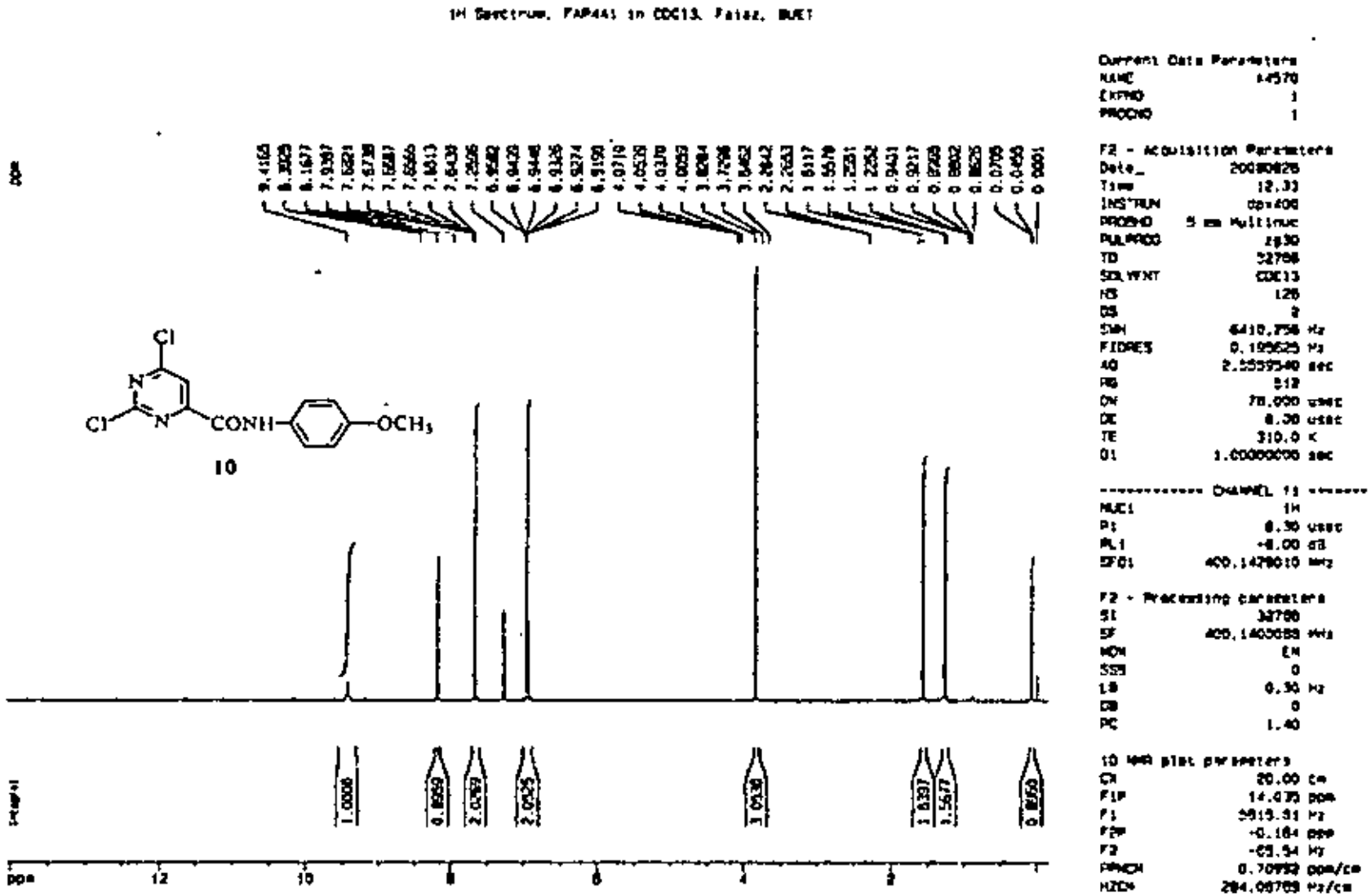
5	Peak	206.00	9.999	
6	Peak	202.00	9.999	



9



Current Data Parameters
NAME 441570
EXPNO 1
PROCNO 1

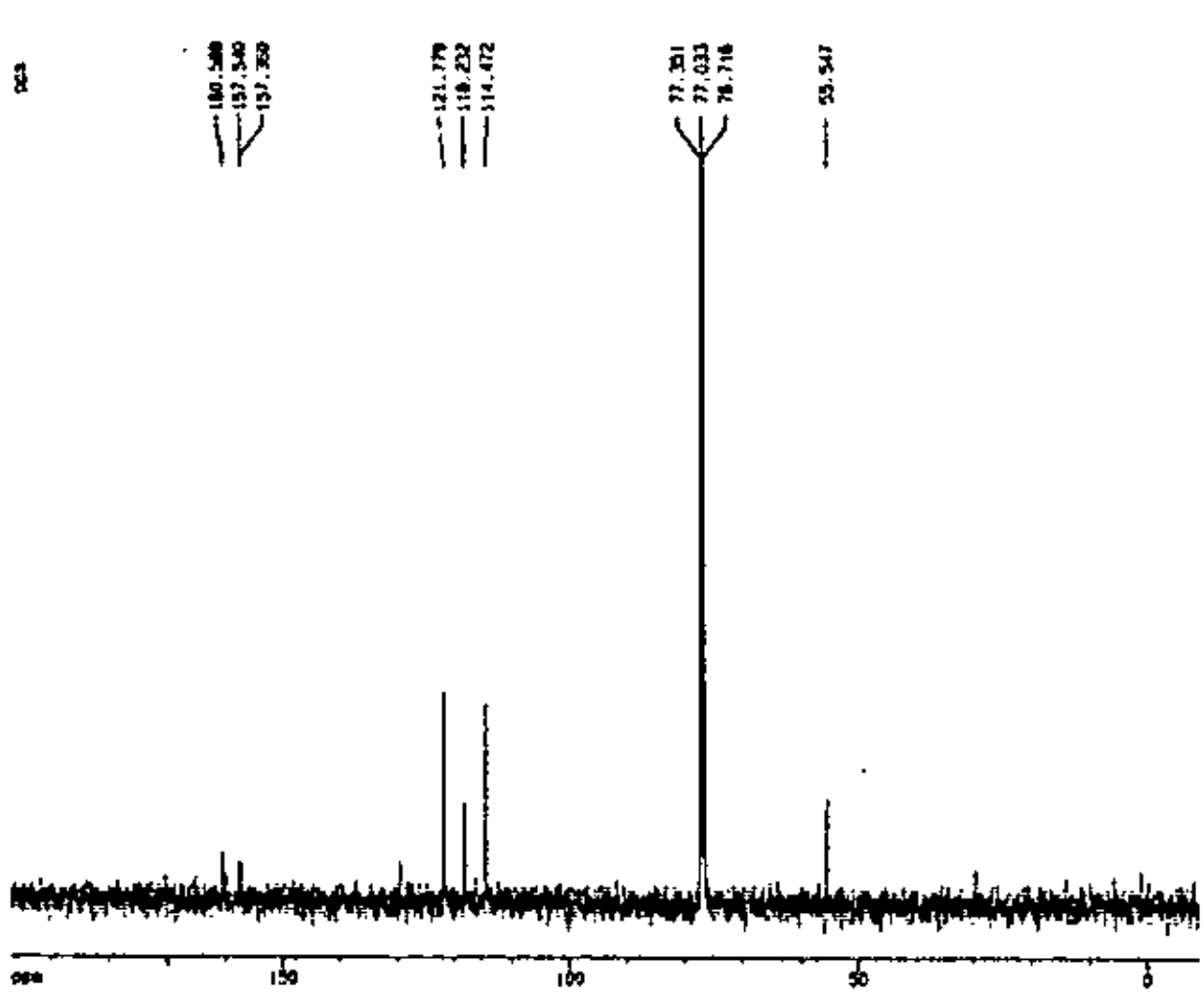
F2 - Acquisition Parameters
Date_ 20030825
Time 12.33
INSTRUM dpx400
PROBHD 5 mm Multinuc
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 128
DS 2
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 512
DW 78.000 usec
DE 6.50 usec
TE 310.0 K
D1 1.00000000 sec

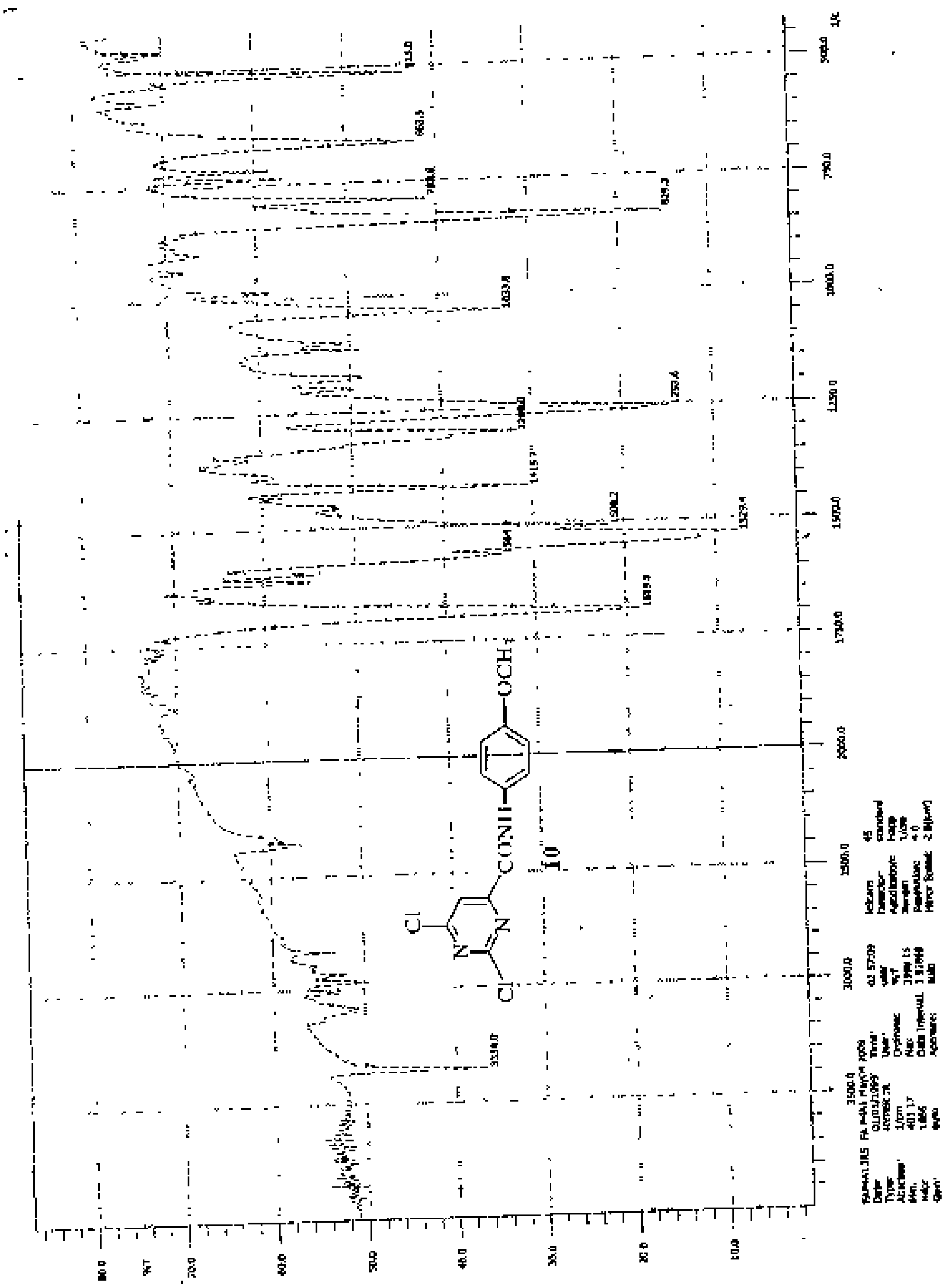
------------ CHANNEL f1 ========
NUC1 1H
P1 8.30 usec
PL1 +0.00 dB
SFO1 400.1478010 MHz

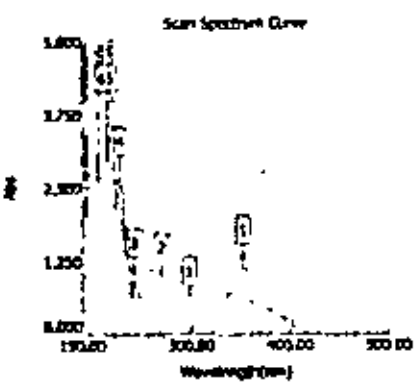
F2 - Processing parameters
SI 32768
SF 400.1400000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 14.470 ppm
F1 5915.31 Hz
F2P -0.184 ppm
F2 -69.54 Hz
PPMCM 0.70992 ppm/cm
HZCM 284.06769 Hz/cm

Analytical, BCSIR Lab. Dhaka 13C Spectrum FAP4A1 in CDCl3, Faiz, BUET.



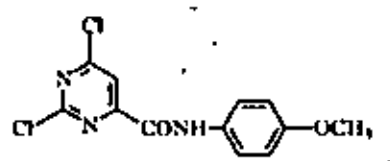




1	Peak	352.00	1.154
2	Peak	278.00	0.875
3	Peak	242.00	0.939
4	Peak	225.00	1.671
5	Peak	213.00	0.999
6	Peak	210.00	0.997
7	Valley	206.00	0.463

- Instrument Performance
  Model : SPECTROPHOTOMETER
  Spectral Bandwidth : 2.00 nm
- Scan Spectrum Performance
  Scan Range : 190.00 to 500.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled3.spd
  Create Date/Time : Thursday, April 15, 2999 11:52:51 AM
  Data Type : Original
  Method File:
- Analyze Note
  Analyst : Administrator
  Sample Name :
  Comment :

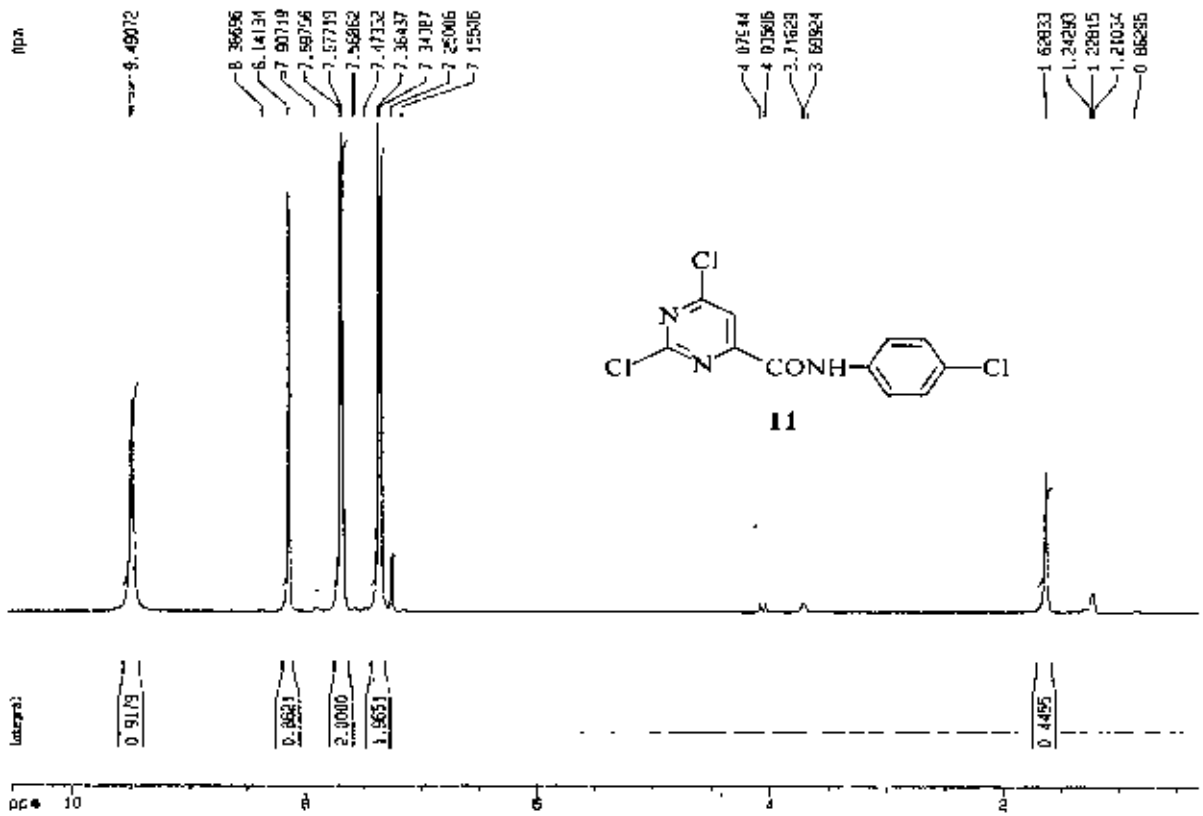
No.	P/V	Wavelength(nm)	Abs	Comment



10

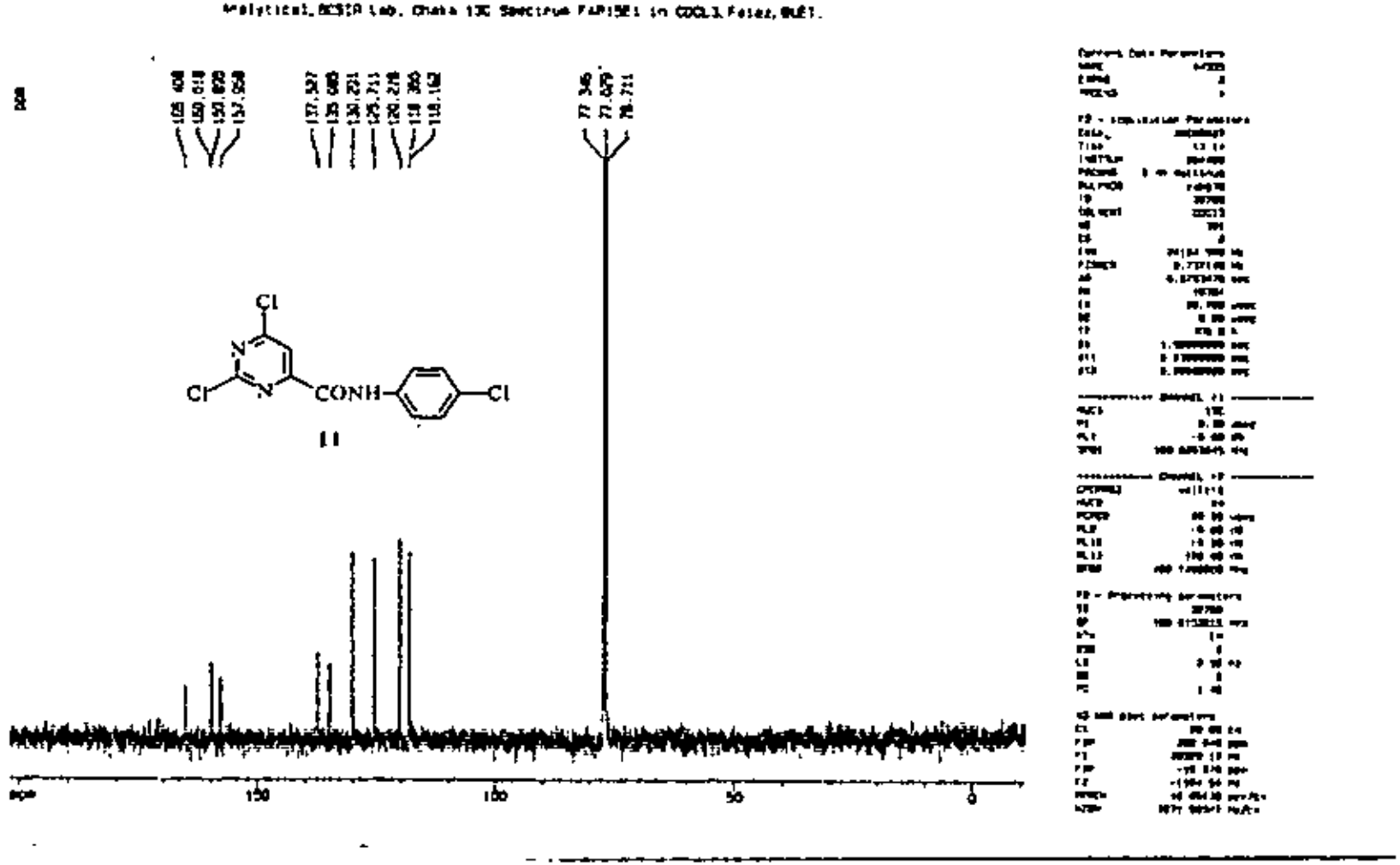
FAP6C1

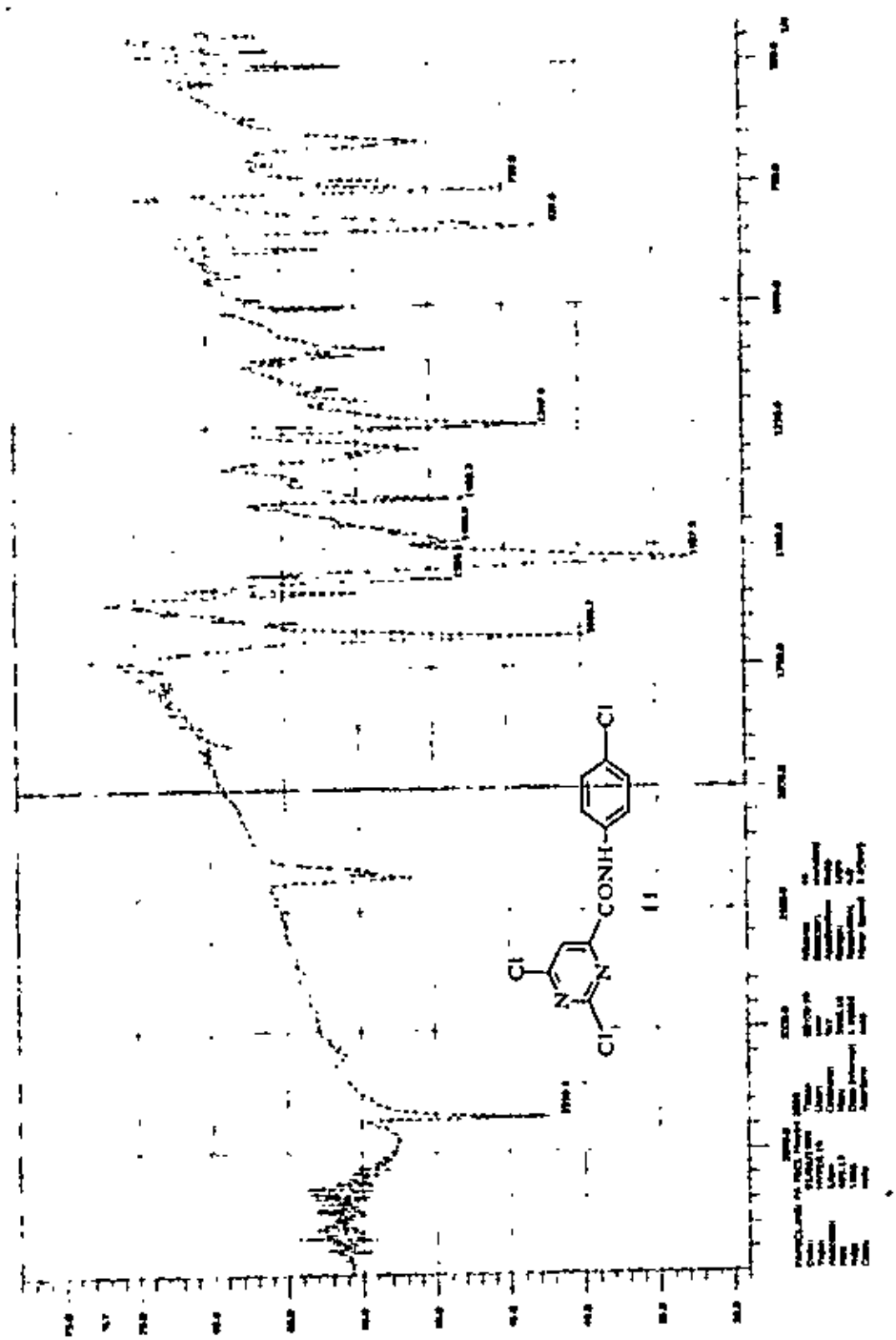
Analytical, BCSIR lab. Dhaka 1H Spectrum FA-9 in CDCL3, F.Ahamed, BUET

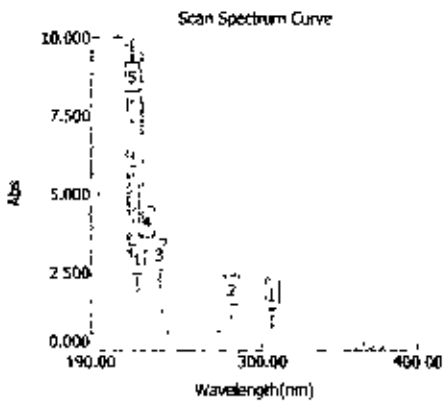


11

```
Current Data Parameters
NAME                  A4270
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20080607
Time                  16 16
INSTRUM             dpx400
PROBHD     5 mm Multinuc
PULPROG               zg30
TD                   32768
SOLVENT               CDCl3
NS                      128
DS                        2
SWH               6410.256 Hz
FIDRES            0.195625 Hz
AQ                2.5559640 sec
RG                      362
DW                 78.000 usec
DE                  6.00 usec
TE                  310.0 K
D1                1.0000000 sec

------------ CHANNEL f1 ------------
NUC1                    1H
P1                   8.30 usec
PL1                 -6.00 dB
SFO1          400.1420010 MHz

F2 - Processing parameters
SI                   32768
SF              400.1400176 MHz
WDW                     EM
SSB                      0
LB                   0.30 Hz
GB                       0
PC                   1.40

1D NMR plot parameters
CX                  20.00 cm
F1P                 10.535 ppm
F1                4215.49 Hz
F2P                 0.320 ppm
F2                128.19 Hz
PPMCM             0.51073 ppm/cm
HZCM            204.35023 Hz/cm
```

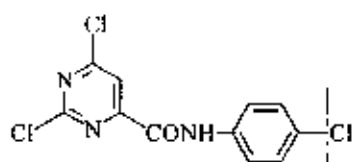

Cl
N
N
Cl
CONH
Cl
11
150
100
50
0


Cl
HNOC
CONH
Cl
N
N
Cl



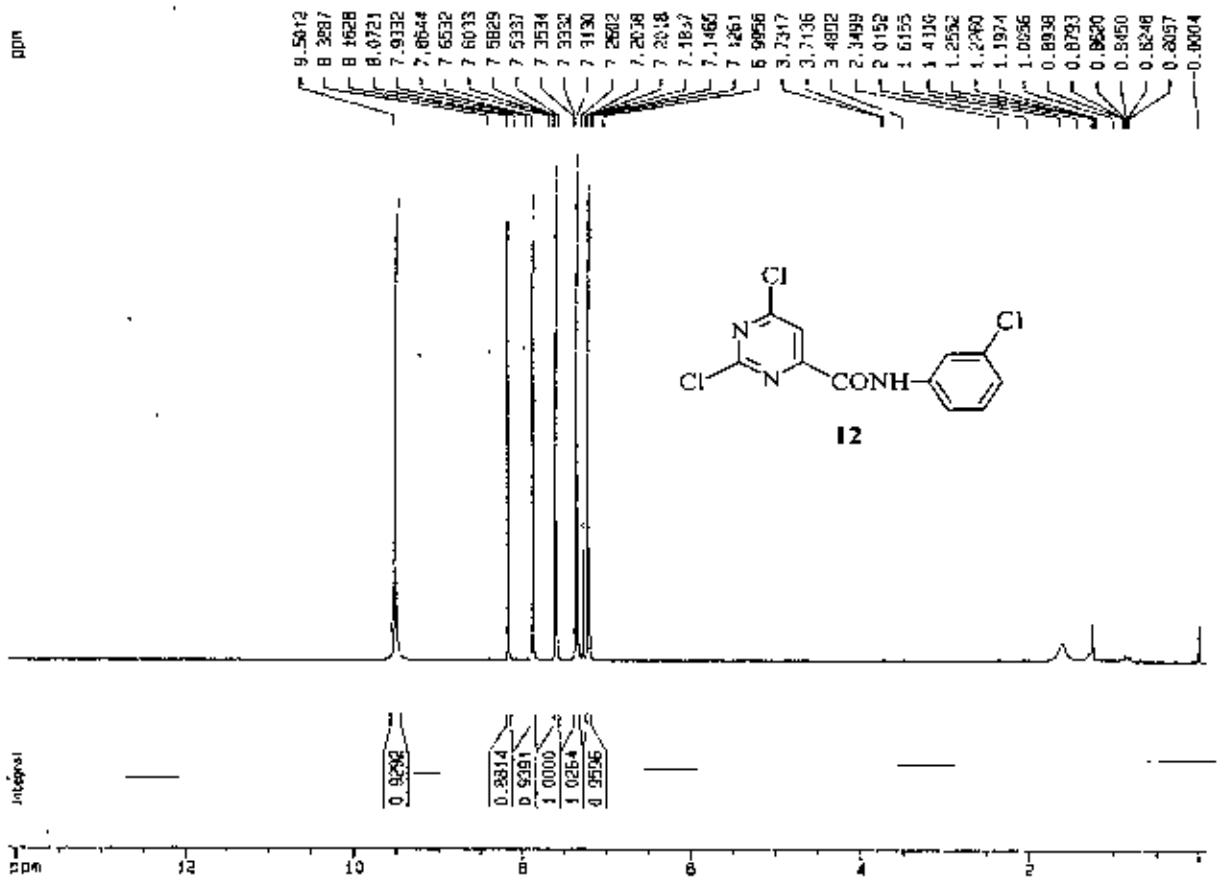
1	Peak	306.00	0.527
2	Peak	280.00	0.676
3	Peak	234.00	1.804
4	Peak	226.00	2.851
5	Peak	216.00	9.990
1	Valley	220.00	1.652

- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 400.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled6.cpd
  Create Date/Time : Wednesday, April 08, 2009 9:16:00 PM
  Data Type : Original
  Method File:
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment :
- **No. P/V Wavelength(nm) Abs Comment**



I I

Analytical BCSIR, 1H Spectrum, FAPiSE1 in CDCl3, Falaz BJET



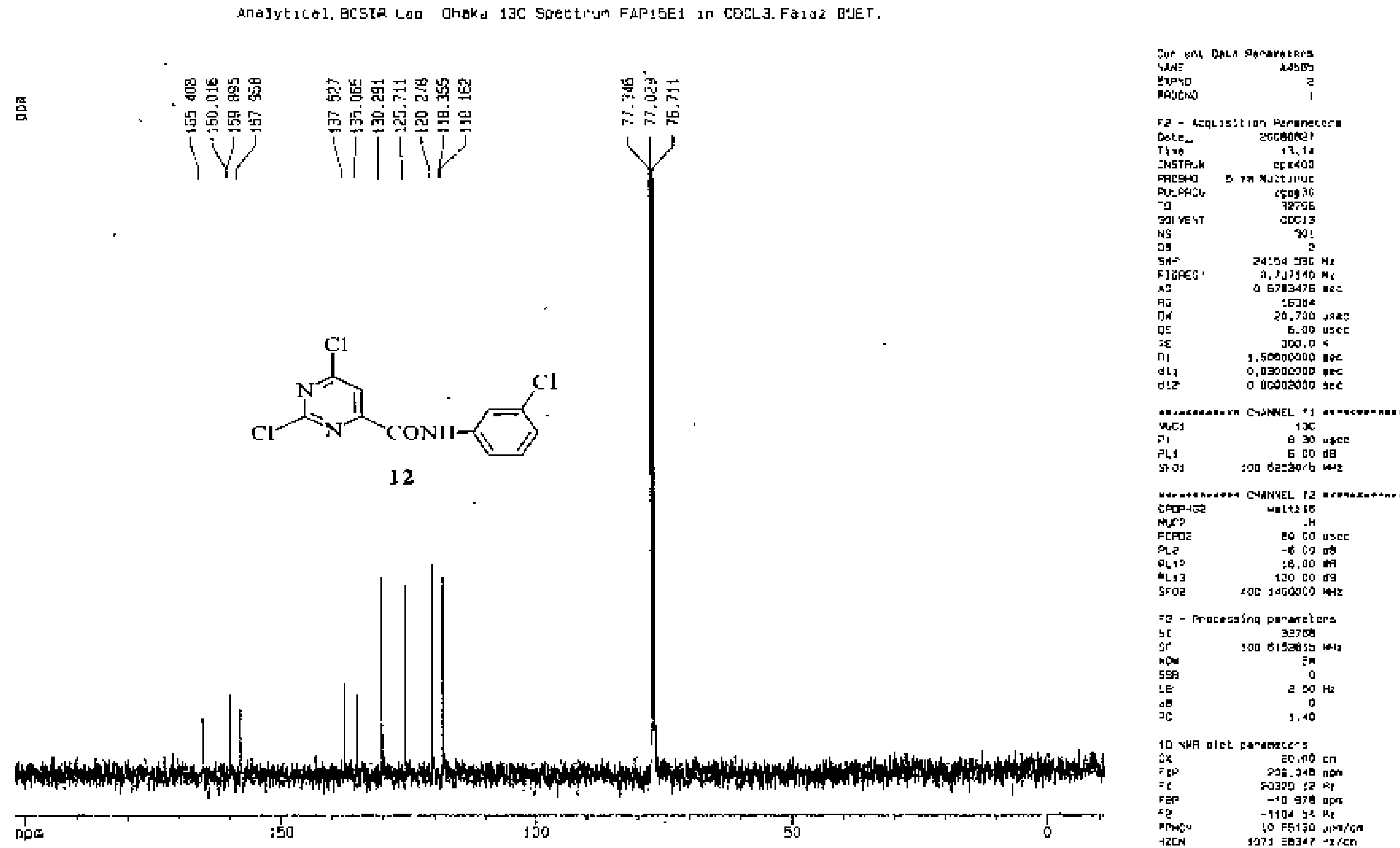
Current Data Parameters
NAME A4585
EXPNO 5
PROCNO 5

F2 - Acquisition Parameters
Date_ 20080826
Time 10 56
INSTRUM dpx400
PROBHD 5 mm Multinuc
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 88
DS 2
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 406.4
DW 78.000 usec
DE 6.00 usec
TE 310.0 K
D1 1.00000000 sec

---------- CHANNEL F1 ----------
NUC1 1H
P1 8.30 usec
PL1 -0.00 dB
SF01 400.1428010 MHz

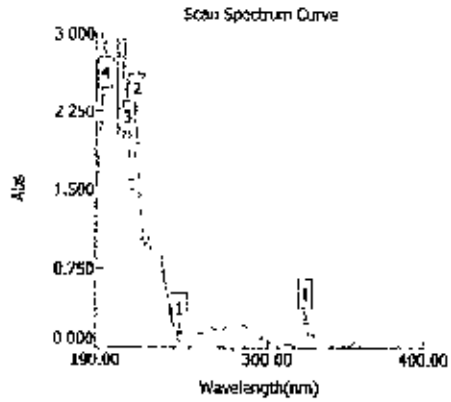
F2 - Processing parameters
SI 32768
SF 400.1400084 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 14.070 ppm
F1 5629.83 Hz
F2P -0.095 ppm
F2 -37.90 Hz
PPMCM 0.70822 ppm/cm
HZCM 283.38651 Hz/cm


Analytical, BCSIR Lab Dhaka 13C Spectrum FAP15E1 in CDCL3 Faiaz BUET.
165.408
160.016
159.895
157.558
137.527
135.065
130.291
125.711
120.278
118.355
118.162
77.346
77.029
76.711
Cl
N
Cl
N
CONH
Cl
ppm
150
100
50
0
Current Data Parameters
NAME A4505
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20080827
Time 13.14
INSTRUM spect
PROBHD 5 mm Multinuc
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 391
DS 2
SWH 24154.590 Hz
FIDRES 0.737140 Hz
AQ 0.6783476 sec
RG 16384
DW 20.700 usec
DE 6.00 usec
TE 300.0 K
D1 1.50000000 sec
d11 0.03000000 sec
d12 0.00002000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 8.30 usec
PL1 6.00 dB
SFO1 100.6238076 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -6.00 dB
PL12 16.00 dB
PL13 120.00 dB
SFO2 400.1460000 MHz
F2 - Processing parameters
SI 32768
SF 100.6128615 MHz
WDW EM
SSB 0
LB 2.50 Hz
GB 0
PC 1.40
1D NMR plot parameters
CX 20.00 cm
F1P 206.348 ppm
F1 20320.12 Hz
F2P -10.978 ppm
F2 -1104.54 Hz
PPMCM 10.86130 ppm/cm
HZCM 1071.58347 Hz/cm

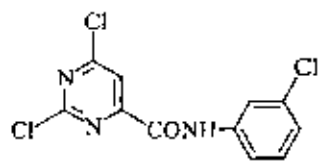
12

Cl
CONH
Cl
N
N
Cl
12



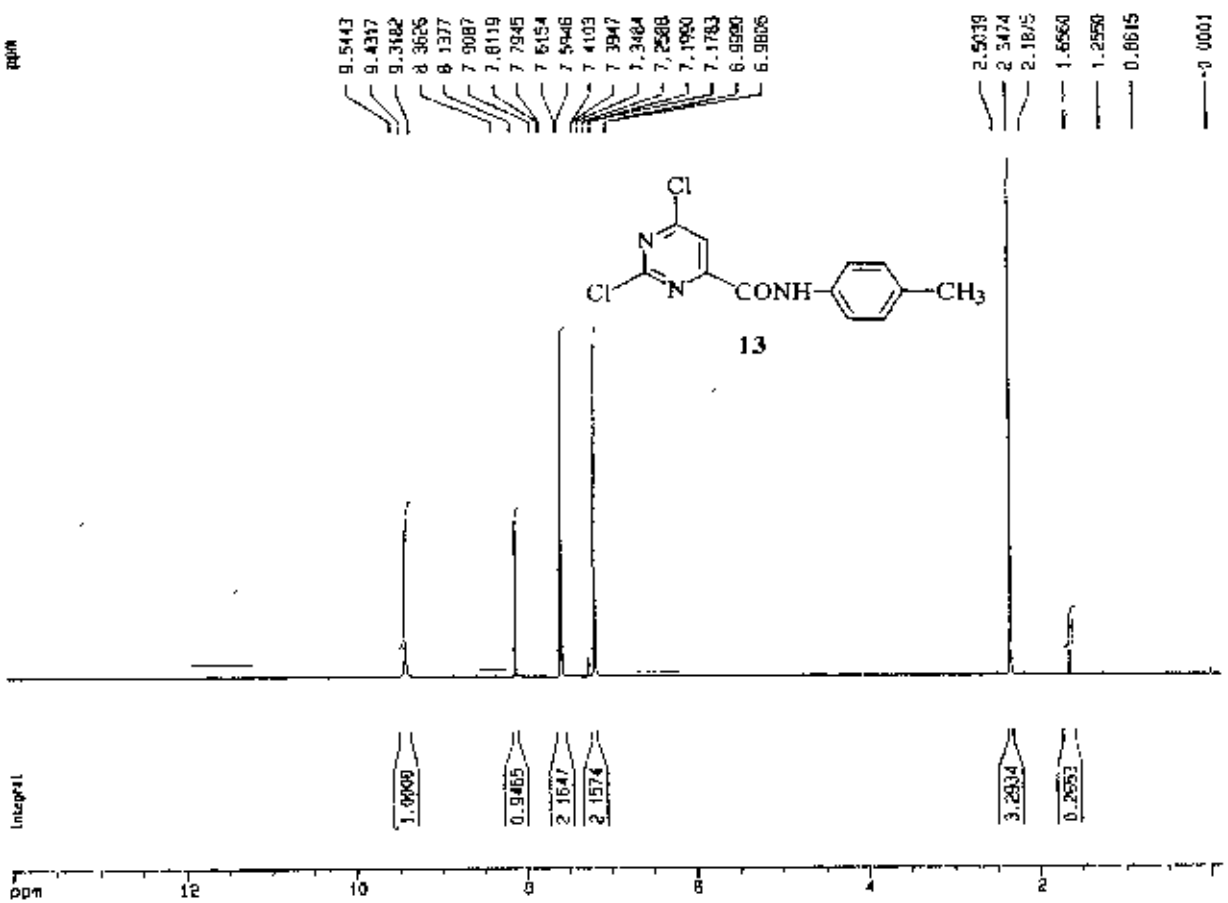
- **Instrument Performance**
  Model . SPECTROPHOTOMETERS
  Spectral Bandwidth . 2.00 nm
- **Scan Spectrum Performance**
  Scan Range . 190.00 to 400.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File Untitled9.spd
  Create Date/Time . Wednesday, February 04, 2009 9:30:05 PM
  Data Type : Original
  Method File
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment

No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	324.00	0.136	
2	Peak	216.00	2.100	
3	Peak	210.00	2.576	
4	Peak	196.00	9.999	
1	Valley	244.00	0.000	



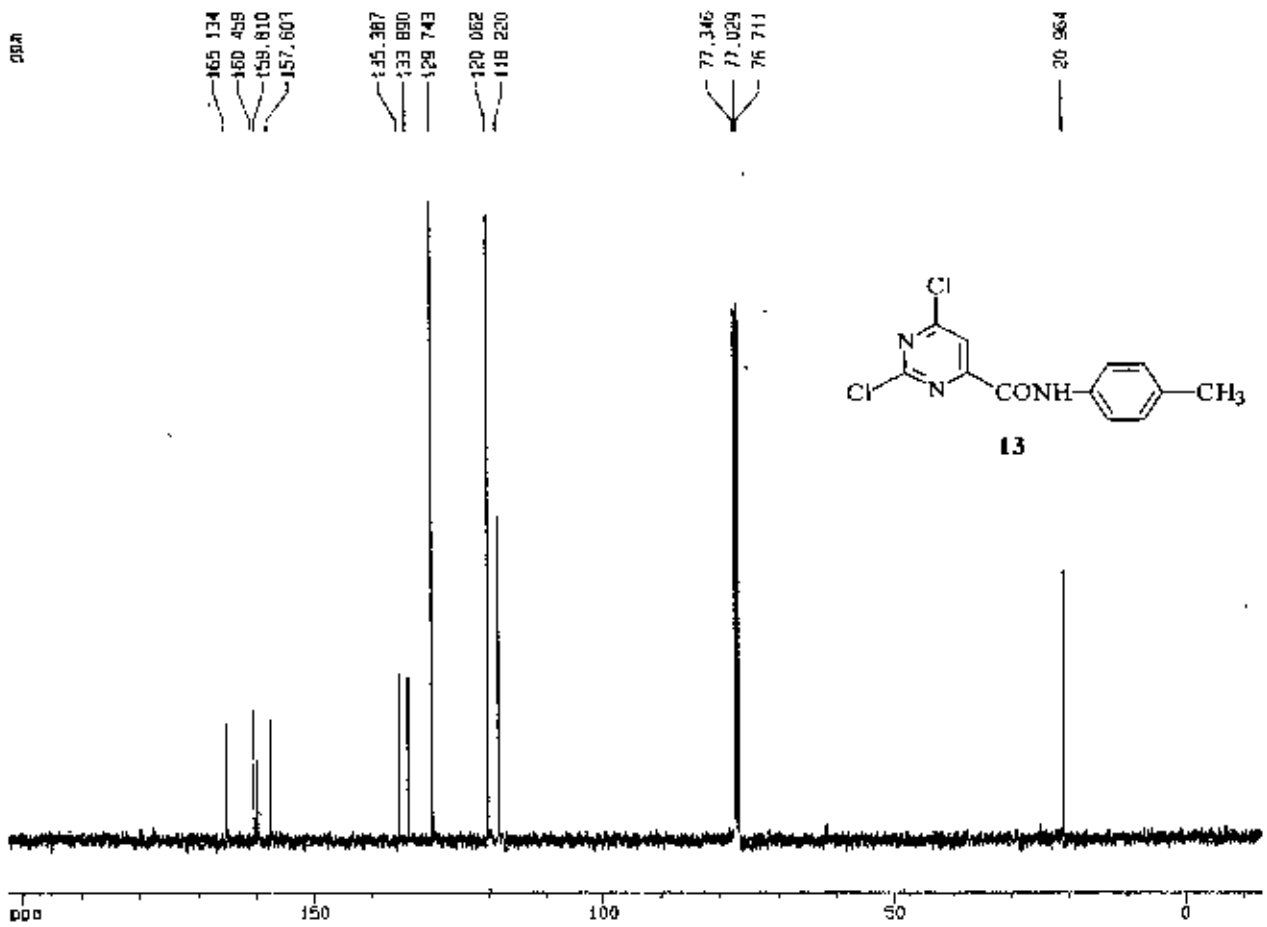
12

Analytical, BCSIR 1H Spectrum, PAP16F1 in CDCl3, Falaz, BUET




```
Current Data Parameters
NAME                  A4567
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20080826
Time                  11.17
INSTRUM               cpx400
PROBHD     5 mm Multinuc
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                      112
DS                        2
SWH                6410.256 Hz
FIDRES             0.195625 Hz
AQ                 2.5559540 sec
RG                      181
DW                   78.000 usec
DE                    6.00 usec
TE                   310.0 K
D1                 1.00000000 sec

========== CHANNEL f1 ==========
NUC1                      1H
P1                     6.30 usec
PL1                   -6.00 dB
SFO1            400.1428010 MHz

F2 - Processing parameters
SI                        32768
SF                 400.1400092 MHz
WDW                          EM
SSB                           0
LB                     0.30 Hz
GB                            0
PC                         1.40

1D NMR plot parameters
CX                       20.00 cm
F1P                      14.051 ppm
F1                     5622.23 Hz
F2P                     -0.134 ppm
F2                      -52.31 Hz
PPMCM                 0.70907 ppm/cm
HZCM                283.72705 Hz/cm
```

Analytical, BCSIR Lab,Dhaka 13C Spectrum FAP16P1 in CDCL3,Talat, BUET.



Current Data Parameters
NAME A4567
EXPNO 2
PROCNO 1

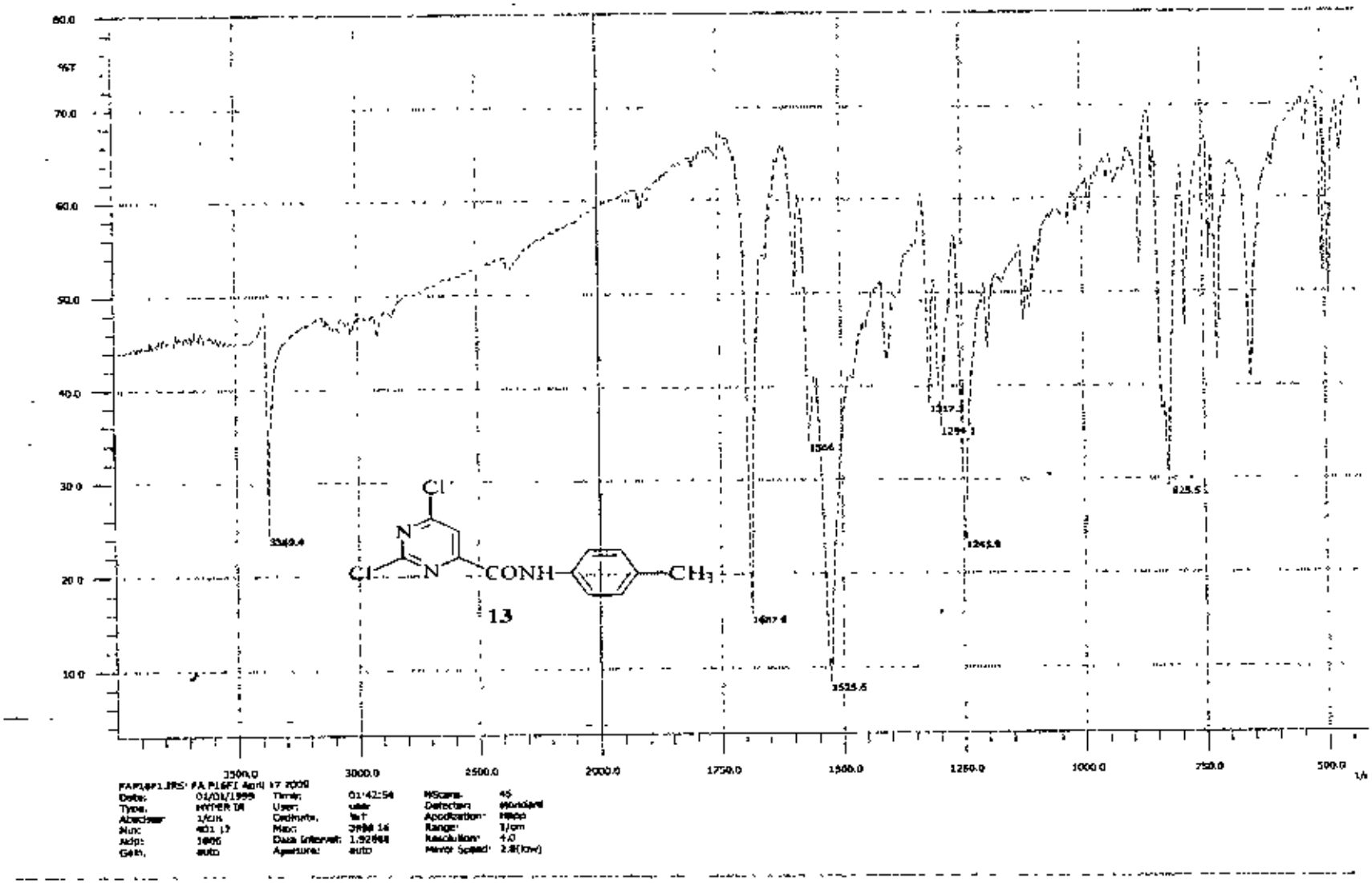
F2 - Acquisition Parameters
Date_ 20080827
Time 13.51
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 968
DS 2
SWH 29761.904 Hz
FIDRES 0.727140 Hz
AQ 0.8834176 sec
RG 8384
DW 20.700 usec
DE 6.00 usec
TE 300.0 K
D1 1.50000000 sec
D11 0.03000000 sec
D12 0.00002000 sec

CHANNEL f1
NUC1 13C
P1 8.10 usec
PL1 +6.00 dB
SFO1 100.6253045 MHz

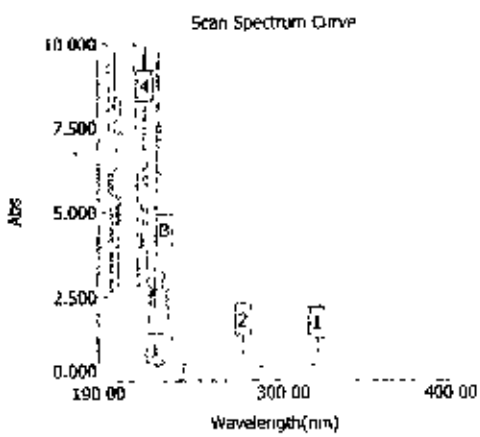
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 +6.00 dB
PL12 16.00 dB
PL13 120.00 dB
SFO2 400.1400000 MHz

F2 - Processing parameters
SI 32768
SF 100.6152845 MHz
WDW EM
SSB 0
LB 2.50 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 202.274 ppm
F1 20351.85 Hz
F2P -13.046 ppm
F2 -1312.84 Hz
PPMCM 10.76810 ppm/cm
HZCM 1083.23450 Hz/cm

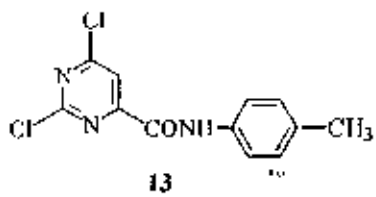

3349.4
1697.4
1535.6
1566
1317.3
1294.3
1245.8
825.5
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
Cl
N
Cl
N
CONH
CH3

13



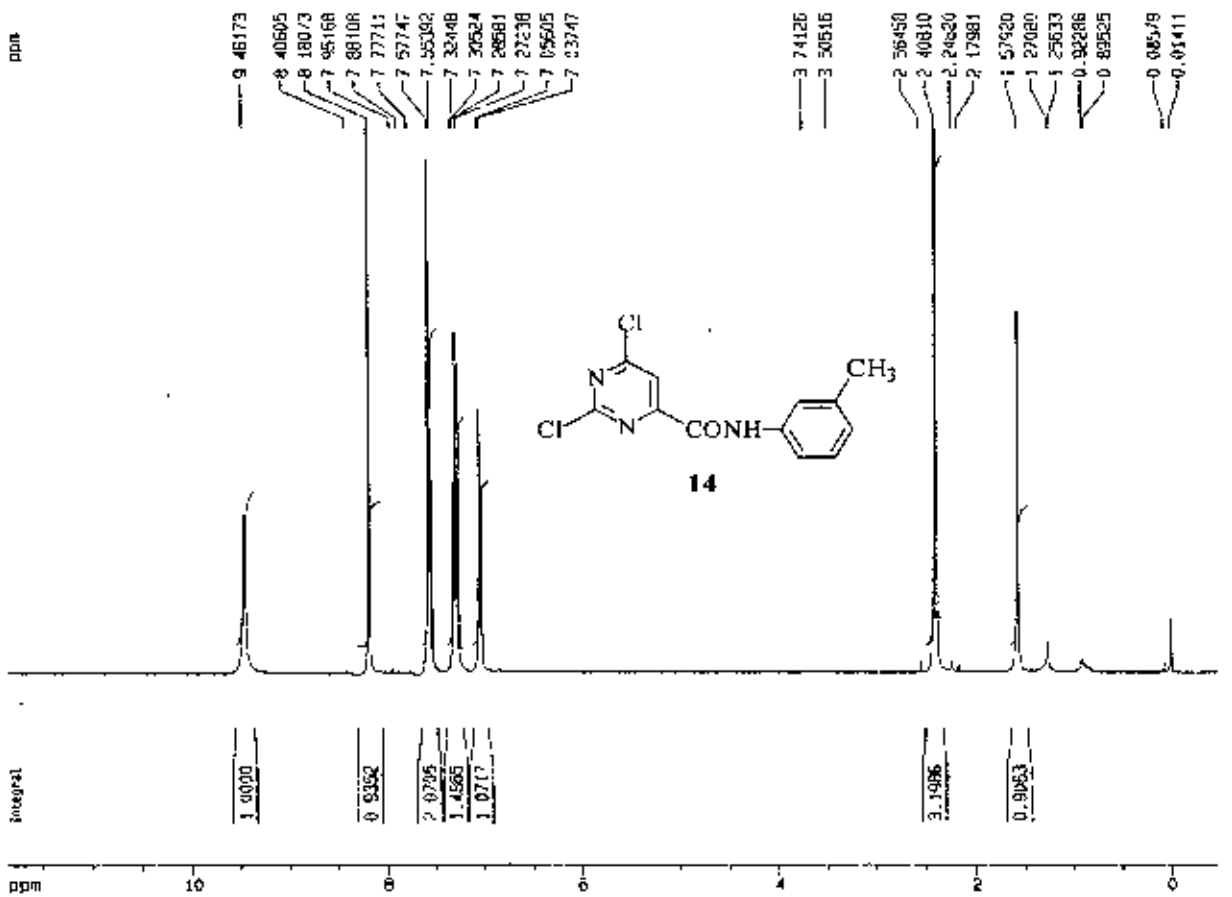
1	Peak	320.00	0.474
2	Peak	276.00	0.561
3	Peak	230.00	3.204
4	Peak	218.00	9.999
1	Valley	224.00	2.181

- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 400.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled5.spd
  Create Date/Time : Wednesday, April 08, 2009 9:12:54 PM
  Data Type : Original
  Method File:
- **Analyse Note**
  Analyster : Administrator
  Sample Name :
  Comment :
- **No. P/V Wavelength(nm) Abs Comment**



13

Analytical BCSIR Lab. Dhaka 1H Spectrum in CDCl3 Perez .BUET.



Current Data Parameters
NAME A4776
EXPNO 1
PROCNO 1

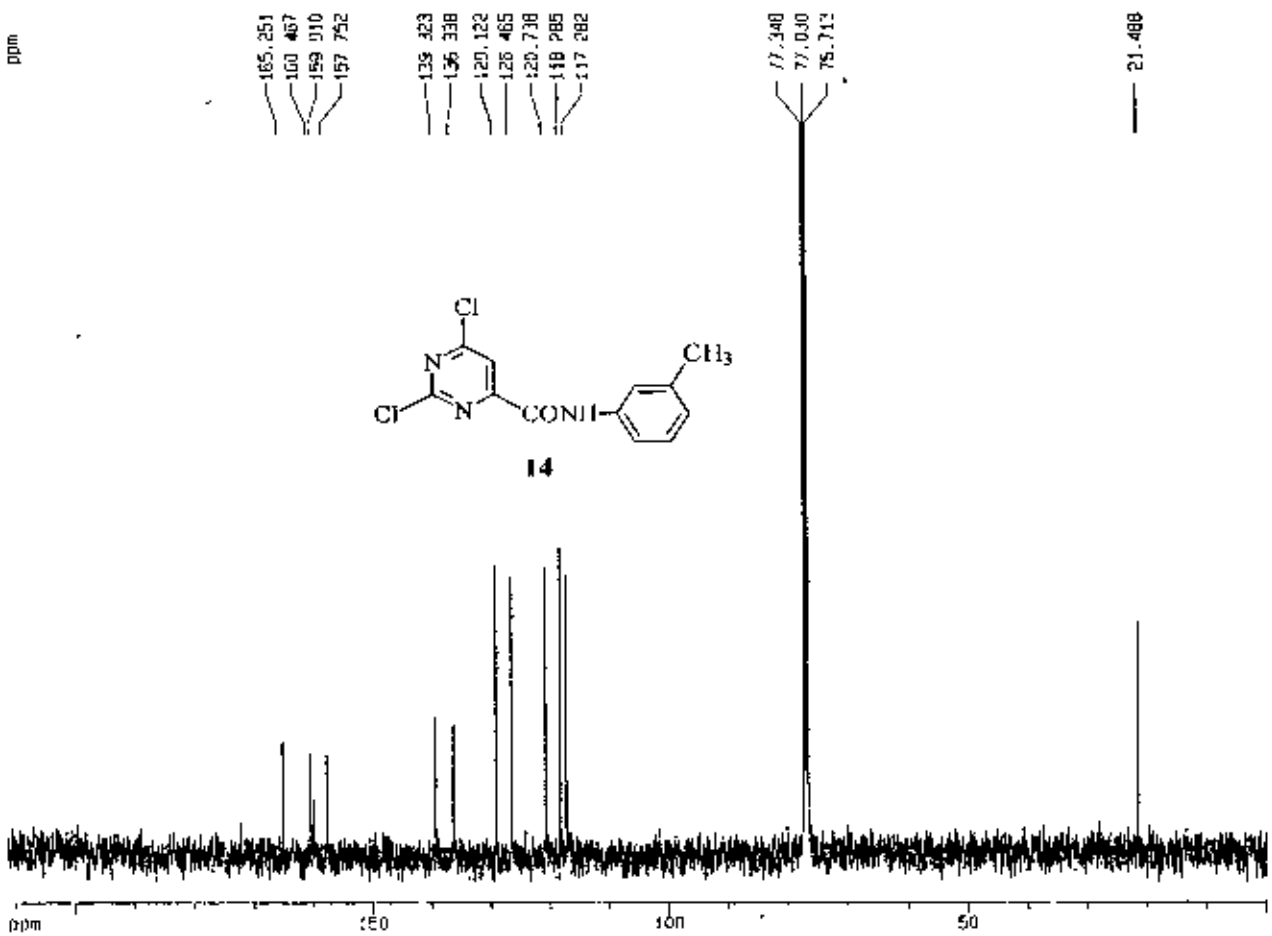
F2 - Acquisition Parameters
Date_ 20081104
Time 9 52
INSTRUM dpx400
PROBHD 5 mm Multinuc
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 128
DS 2
SWH 6410.256 Hz
FIDRES 0.195628 Hz
AQ 2.5550540 sec
RG 362
DW 78.000 usec
DE 5.00 usec
TE 310.0 K
D1 1.00000000 sec

CHANNEL f1
NUC1 1H
P1 8.30 usec
PL1 -0.00 dB
SFO1 400.1428010 MHz

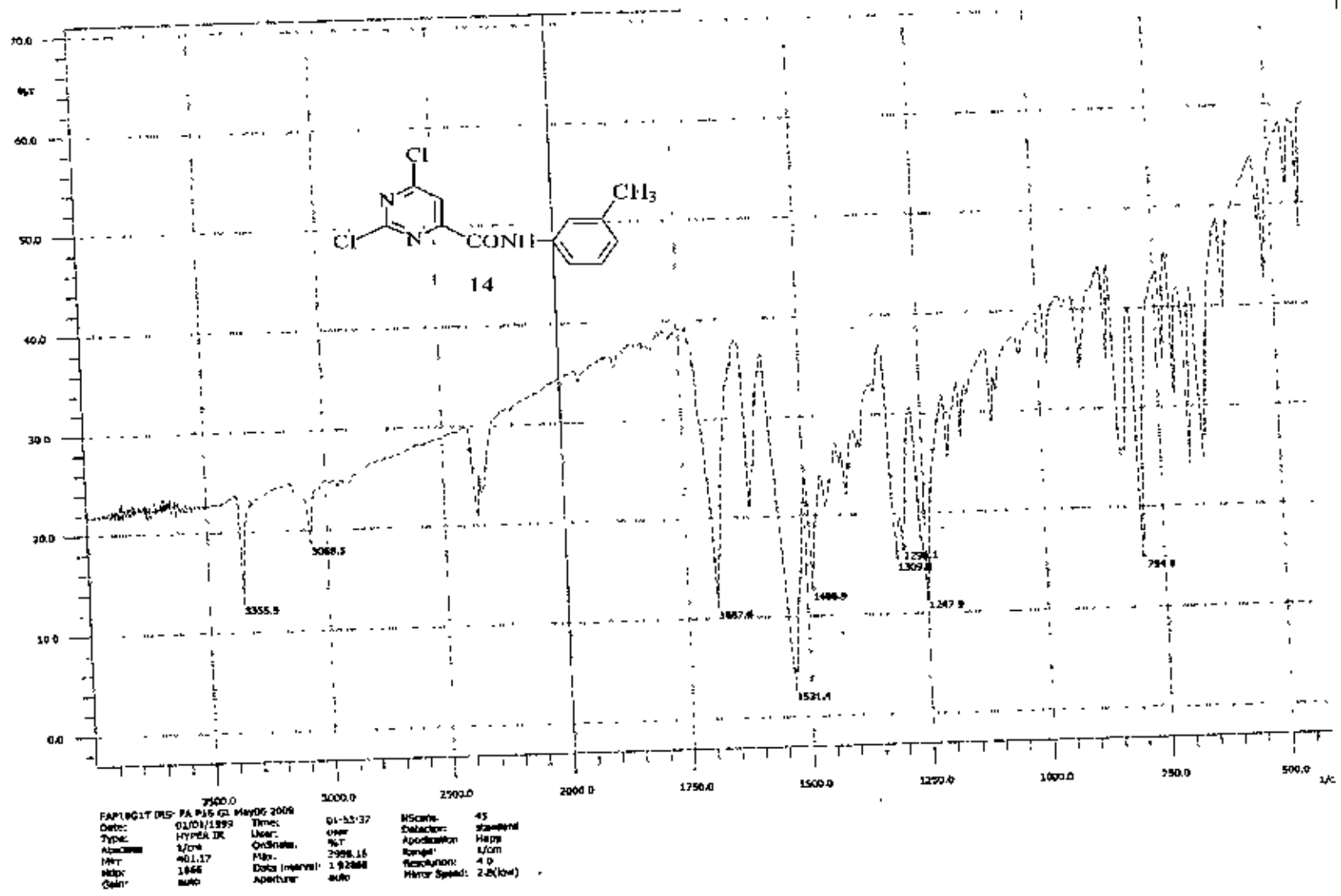
F2 - Processing parameters
SI 32768
SF 400.1400036 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

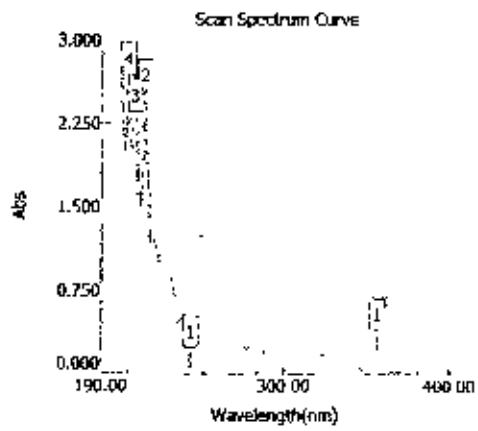
1D NMR plot parameters
CX 20.00 cm
F1P 11.851 ppm
F1 4742.21 Hz
F2P -0.440 ppm
F2 -176.18 Hz
PPMCM 0.81458 ppm/cm
HZCM 245.91961 Hz/cm

13C Spectrum, FAP'864 in CDCl3, Faiaz, BUET




```
Current Data Parameters
NAME                  aa276
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_              20081105
Time                   11 09
INSTRUM                spx400
PROBHD     5 mm Multinuc
PULPROG              zgpg30
TD                    32768
SOLVENT               CDCl3
NS                      593
DS                        2
SWH                24154.530 Hz
FIDRES              0.737140 Hz
AQ                  0.6783476 sec
RG                      16384
DW                     20.700 usec
DE                      6.00 usec
TE                     303.0 K
D1                 1.50000000 sec
D11                0.03000000 sec
D12                0.00002000 sec

======== CHANNEL f1 ========
NUC1                      13C
P1                      8.50 usec
PL1                     -6.00 dB
SFO1               100.6253045 MHz

======== CHANNEL f2 ========
CPDPRG2                 waltz16
NUC2                       1H
PCPD2                   80.00 usec
PL2                     -1.00 dB
PL12                    16.00 dB
PL13                    20.00 dB
SFO2               400.1324000 MHz

F2 - Processing parameters
SI                        32768
SF                 100.6150815 MHz
WDW                          EM
SSB                           0
LB                         0.50 Hz
GB                            0
PC                         1.40

1D NMR plot parameters
CX                        20.00 cm
F1P                     231.232 ppm
F1                    23253.21 Hz
F2P                     -0.000 ppm
F2                        0.75 Hz
PPMCM                 11.58201 ppm/cm
HZCM                  1165.69934 Hz/cm
```

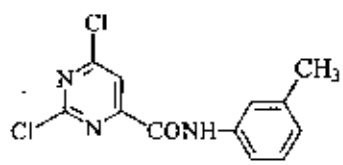

Cl
N
Cl
N
CONH
CH3
14
%T
3355.5
3068.5
1887.6
1531.4
1309.8
1247.9
784.9
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0



No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	356.00	0.162	
2	Peak	216.00	2.305	
3	Peak	210.00	2.874	
4	Peak	206.00	2.453	
1	Valley	244.00	0.000	

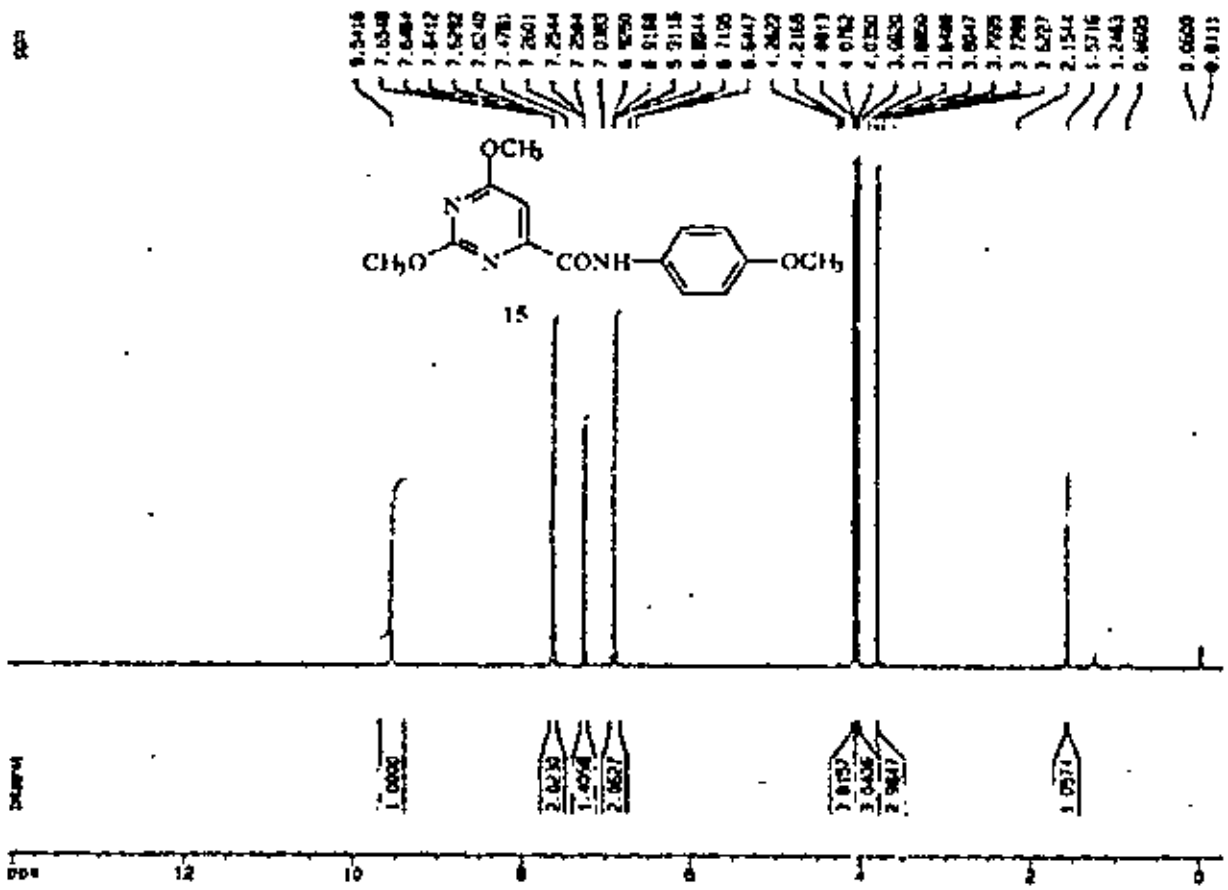
- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 400.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled10.spd
  Create Date/Time : Wednesday, February 04, 2009 9:31:55 PM

  Data Type : Original
  Method File:
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment :



14

1H Spectrum, FAP042 in CDCl3, Filter, DUET



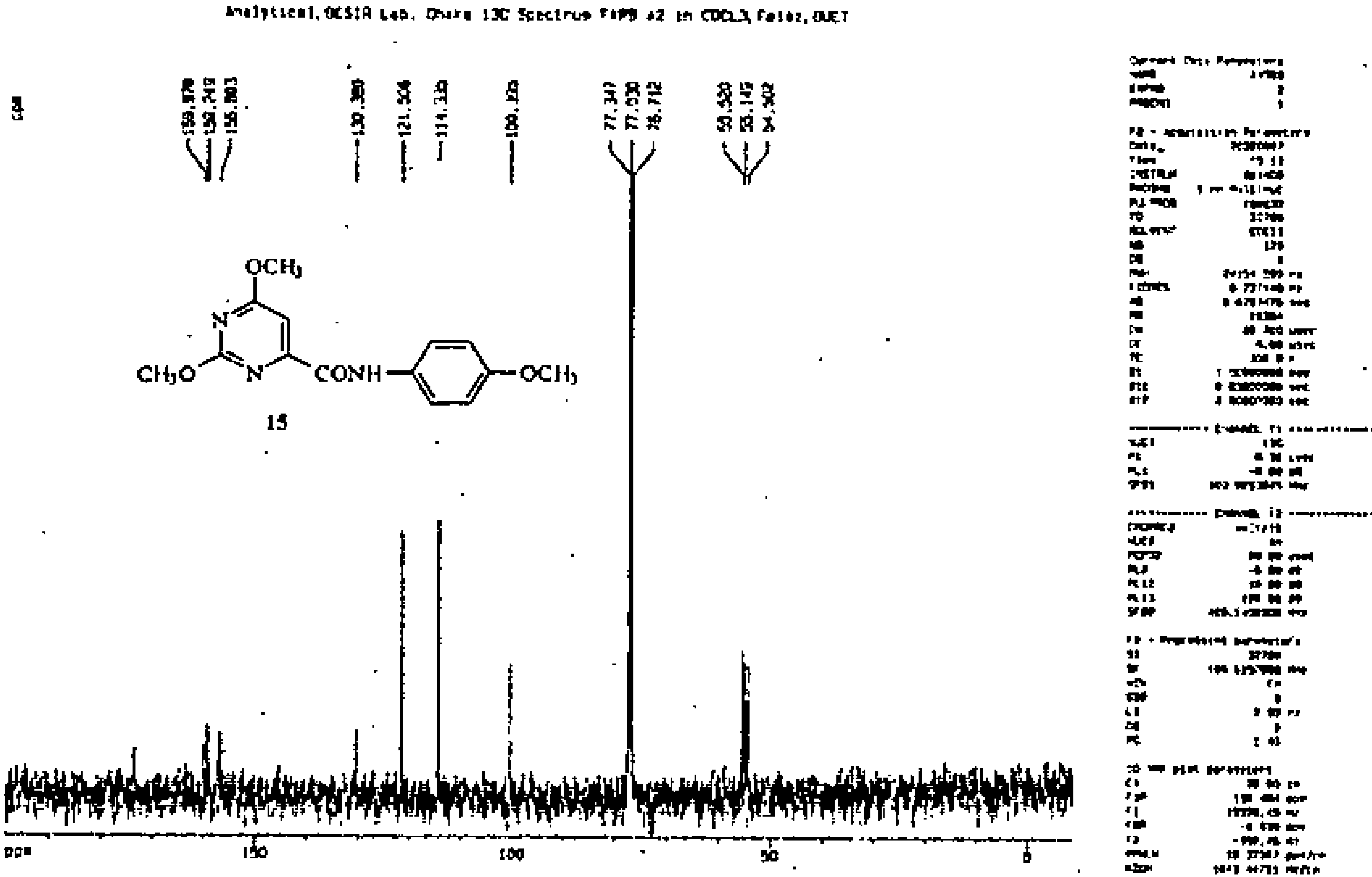
Current Data Parameters
NAME 44565
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20060619
Time 11.38
INSTRUM dpx400
PROBHD 5 mm Multinuc
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 128
DS 2
SWH 8410.256 Hz
FIDRES 0.195825 Hz
AQ 2.5560540 sec
RG 322.5
DW 78.000 usec
DE 6.00 usec
TE 310.0 K
D1 1.00000000 sec

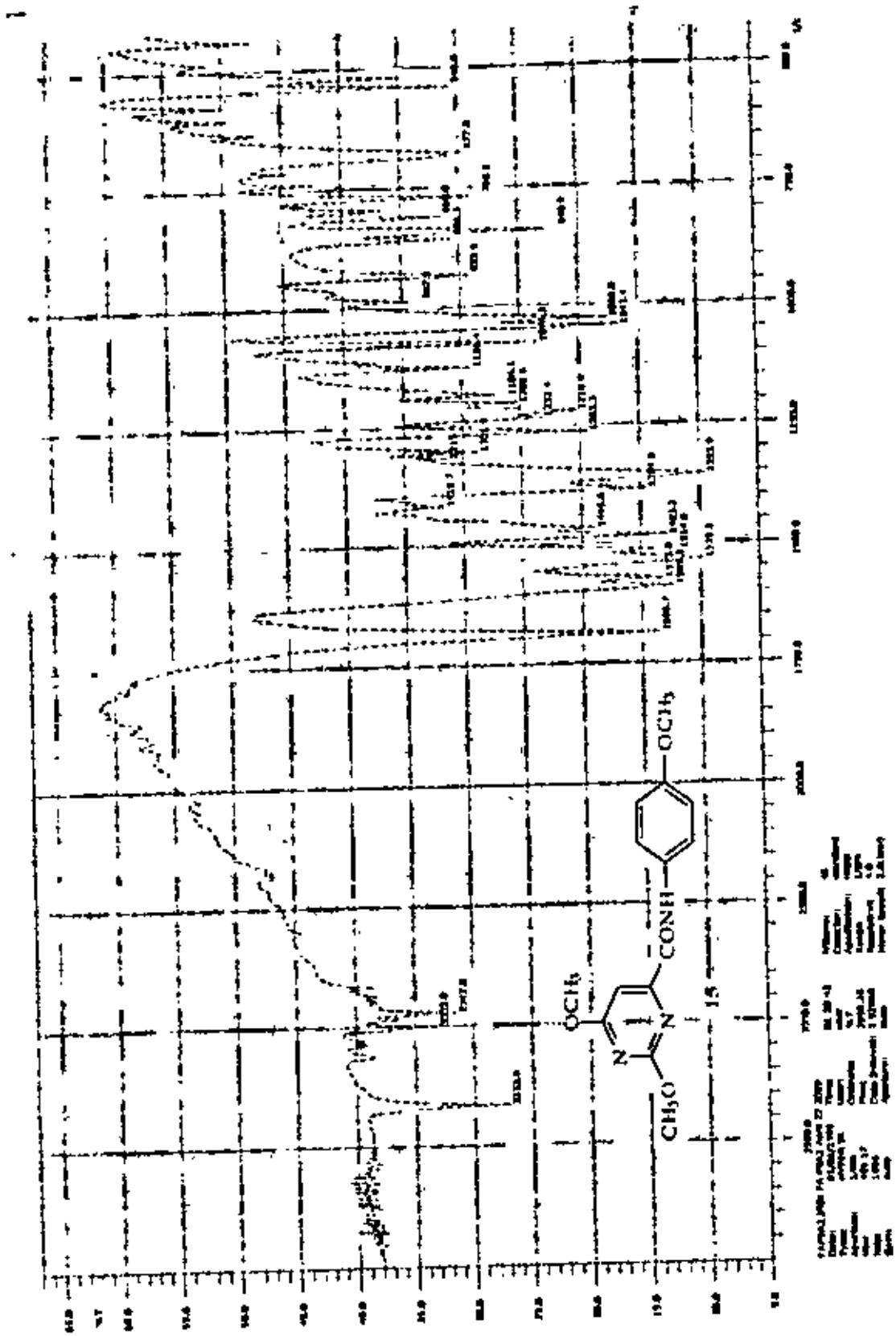
------------ CHANNEL f1 ------------
NUC1 1H
P1 8.30 usec
PL1 -6.00 dB
SFO1 400.1478010 MHz

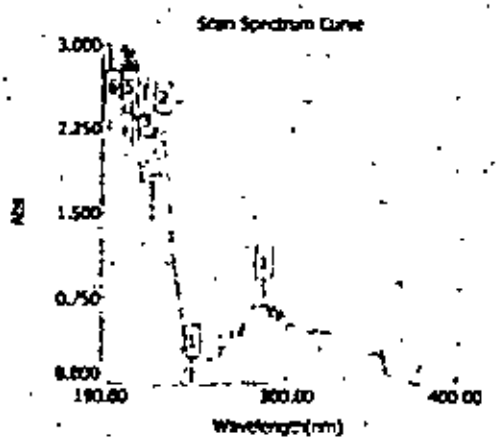
F2 - Processing parameters
SI 32768
SF 400.1400129 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 14.058 ppm
F1 5625.41 Hz
F2P -0.276 ppm
F2 -110.44 Hz
PPMCM 0.71073 ppm/cm
HZCM 288.78254 Hz/cm


Analytical, BCSIR Lab, Dhaka 13C Spectrum FAPB #2 in CDCL3, Faiez, BUET
159.878
158.249
155.803
130.380
121.504
114.330
100.195
77.347
77.030
76.712
55.520
55.145
54.502
OCH3
N
N
CH3O
CONH
OCH3
ppm
150
100
50
0

15

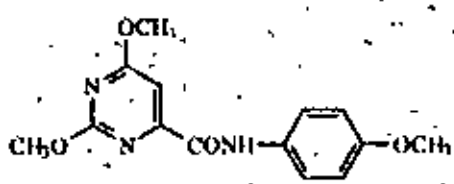

OCH3
CONH
OCH3
N
N
CH3O



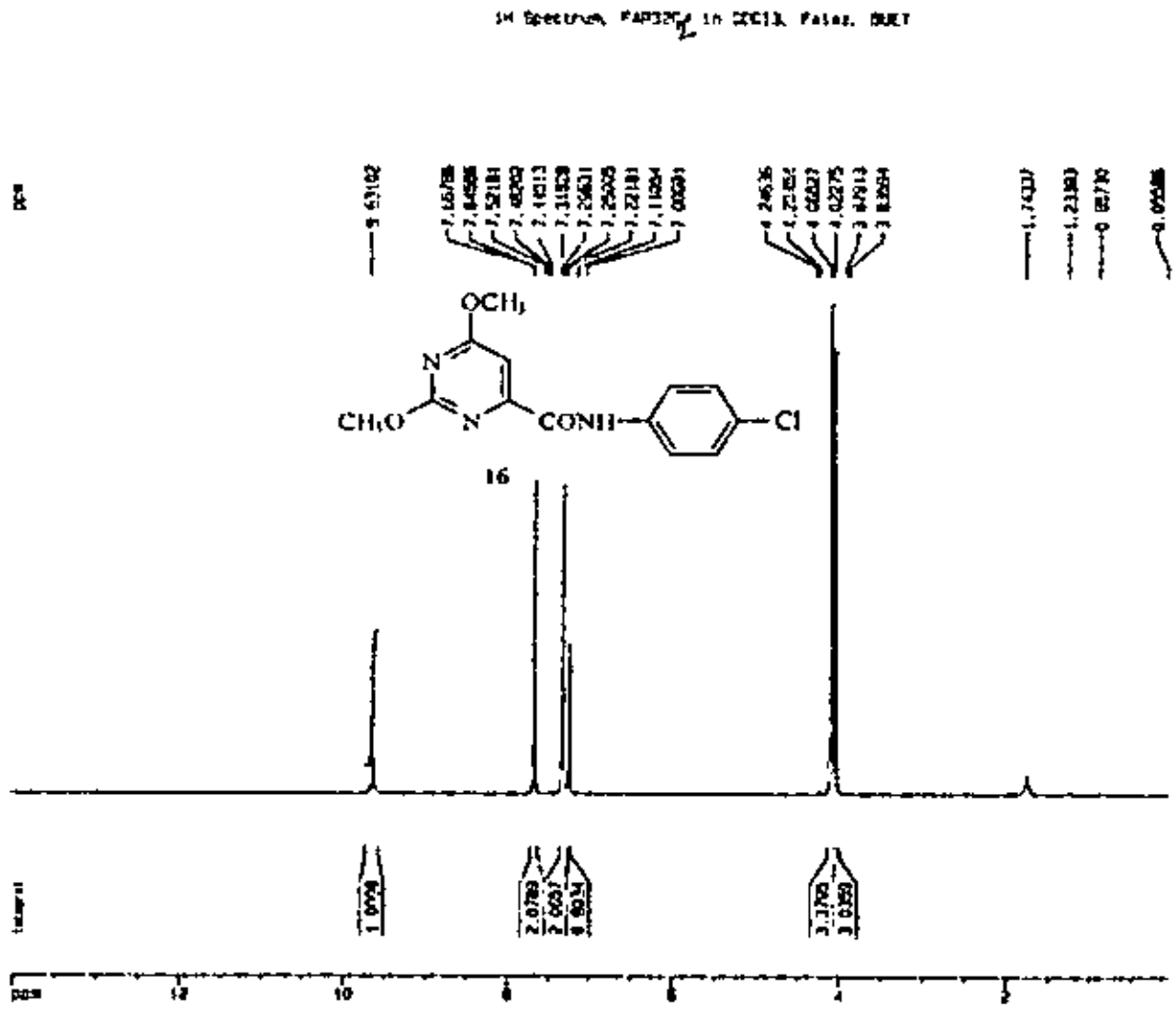
No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	286.00	0.689	
2	Peak	226.00	2.157	
3	Peak	216.00	2.703	
4	Peak	210.00	2.878	
5	Peak	206.00	9.999	
6	Peak	196.00	9.999	
1	Valley	244.00	0.000	

- Instrument Performance
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- Scan Spectrum Performance
  Scan Range : 190.00 to 400.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled11.spd
  Create Date/Time : Wednesday, February 04, 2009 9:37:39 PM

  Data Type : Original
  Method File:
- Analyse Note
  Analyser : Administrator
  Sample Name :
  Comment :



15



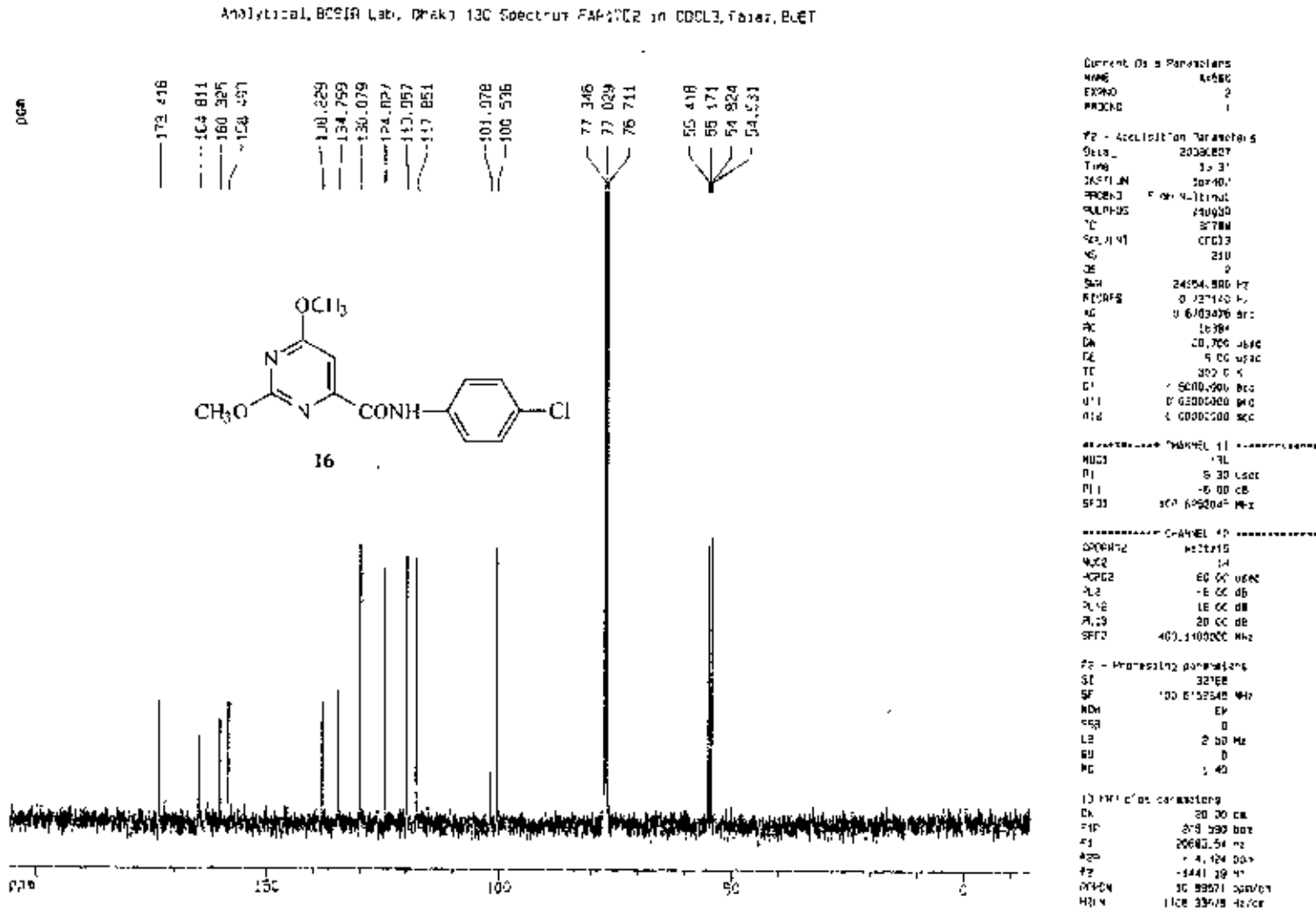
Current Data Parameters
NAME A4935
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20080525
Time 11.50
INSTRUM spr400
PROBHD 5 mm Multinuc
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 105
DS 8
SWH 8410.926 Hz
FIDRES 0.125625 Hz
AQ 3.8994540 sec
RG 181
DW 78.000 usec
DE 6.00 usec
TE 310.0 K
D1 1.00000000 sec

------------ CHANNEL f1 ------------
NUC1 1H
P1 8.30 usec
PL1 -6.00 dB
SFO1 400.1428910 MHz

F2 - Processing parameters
SI 32768
SF 400.1400128 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 14.000 ppm
F1 5605.17 Hz
F2P 0.040 ppm
F2 16.18 Hz
PPMCM 0.68500 ppm/cm
HZCM 278.25918 Hz/cm



Current Data Parameters
NAME
EXPNO
PROCNO

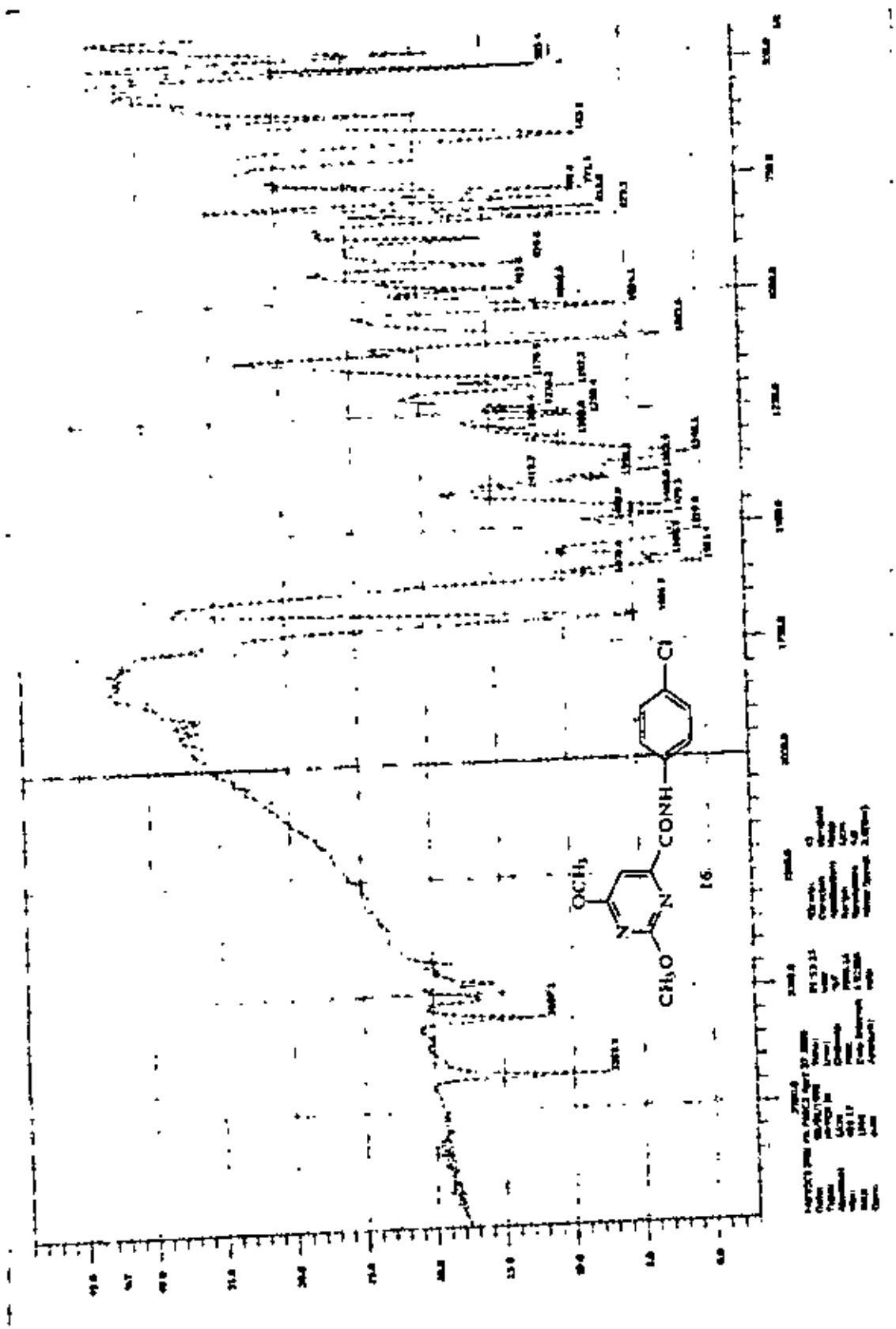
F2 - Acquisition Parameters
Date_
Time
INSTRUM
PROBHD
PULPROG
TD
SOLVENT
NS
DS
SWH
FIDRES
AQ
RG
DW
DE
TE
D1
D11
D16

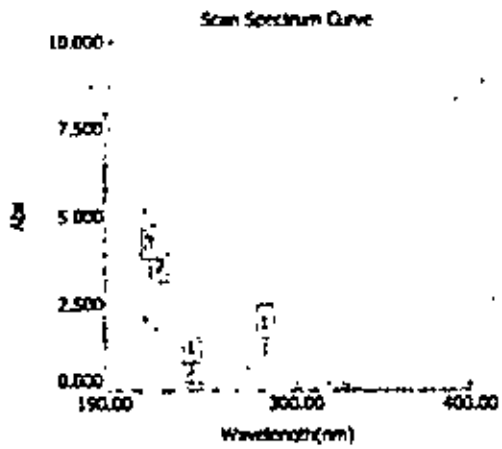
CHANNEL f1
NUC1
P1
PL1
SFO1

CHANNEL f2
CPDPRG2
NUC2
PCPD2
PL2
PL12
PL13
SFO2

F2 - Processing parameters
SI
SF
WDW
SSB
LB
GB
PC

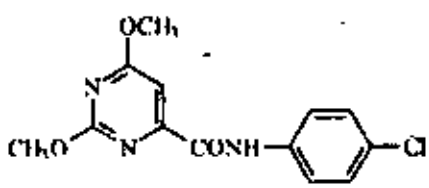
1D NMR plot parameters
CX
F1P
F1
F2P
PPMCM
HZCM


Cl
CONH
OCH3
N
N
CH3O



1	Peak	282.00	0.706
2	Peak	272.00	2.344
3	Peak	216.00	3.001
4	Valley	240.00	0.000

- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 400.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled7.spd
  Create Data/Time : Wednesday, April 08, 2009 9:18:07 PM
  Data Type : Original
  Method File :
- **Analyse Note**
  Analyst : Administrator
  Sample Name :
  Comment :
- No. P/V Wavelength(nm) Abs Comment



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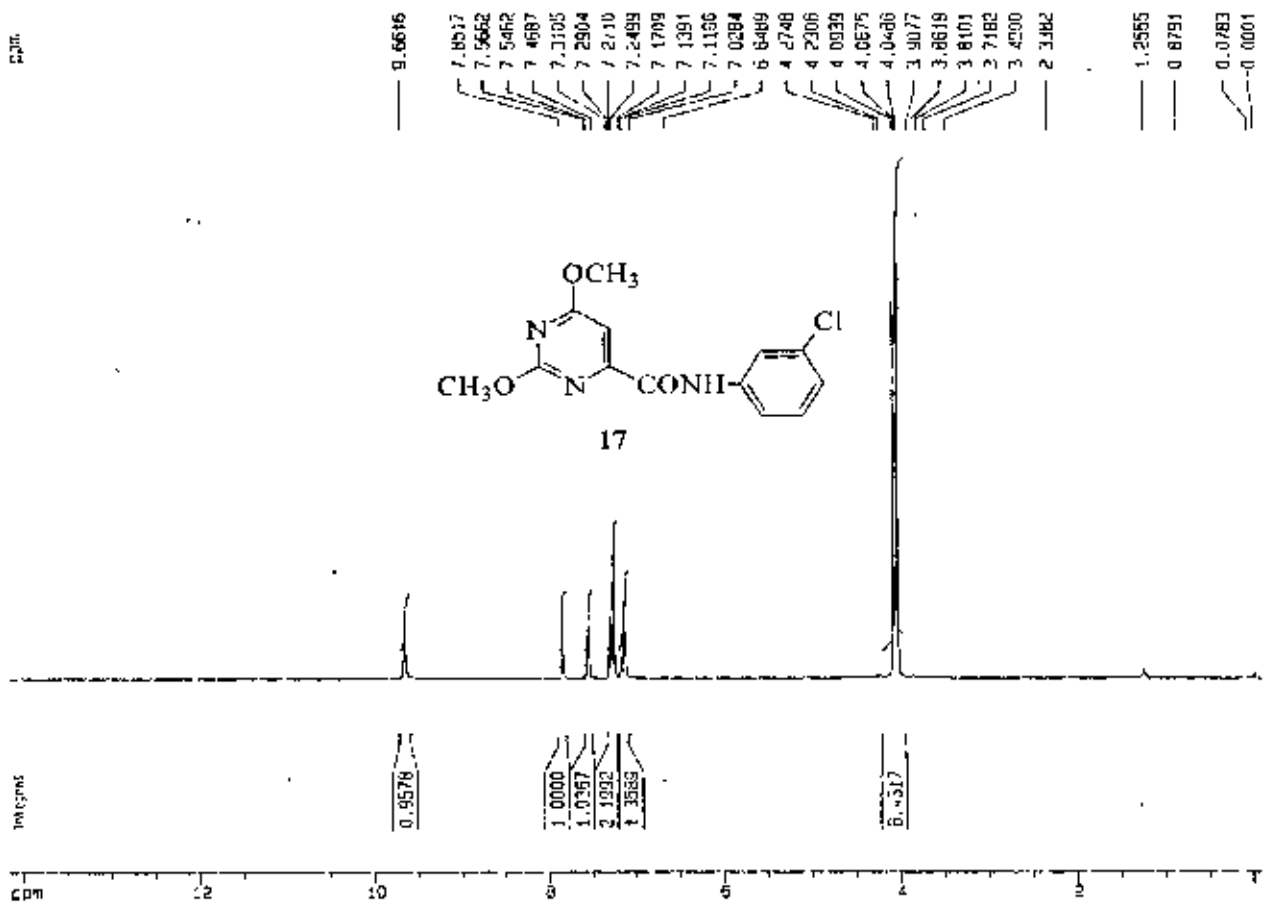
Analytical RCSIR, 1H Spectrum, FAM-17EJ in CDCl3, Falan, BJET



17

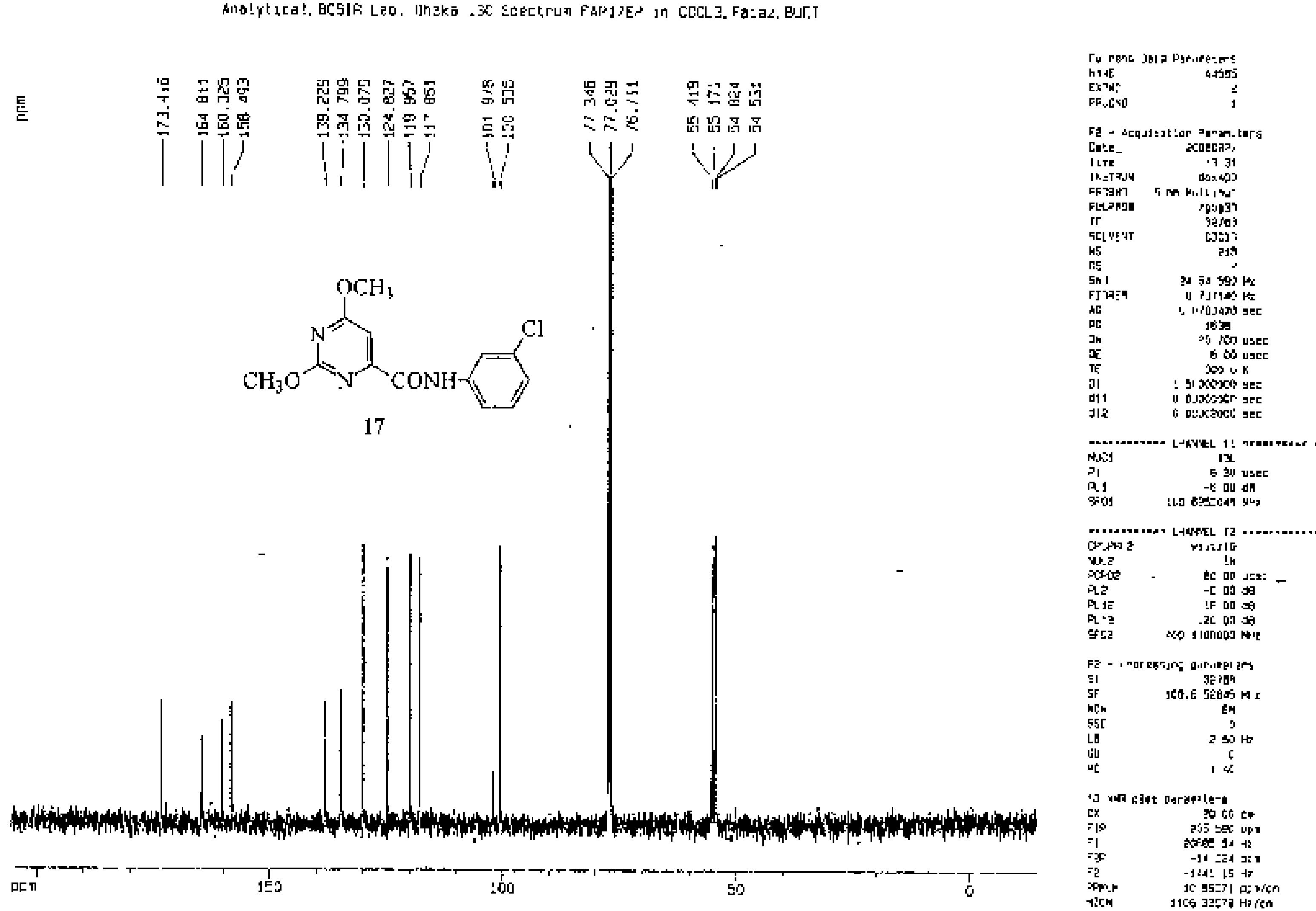
Current Data Parameters
NAME A4586
EXPNO 1
PROCNO 1

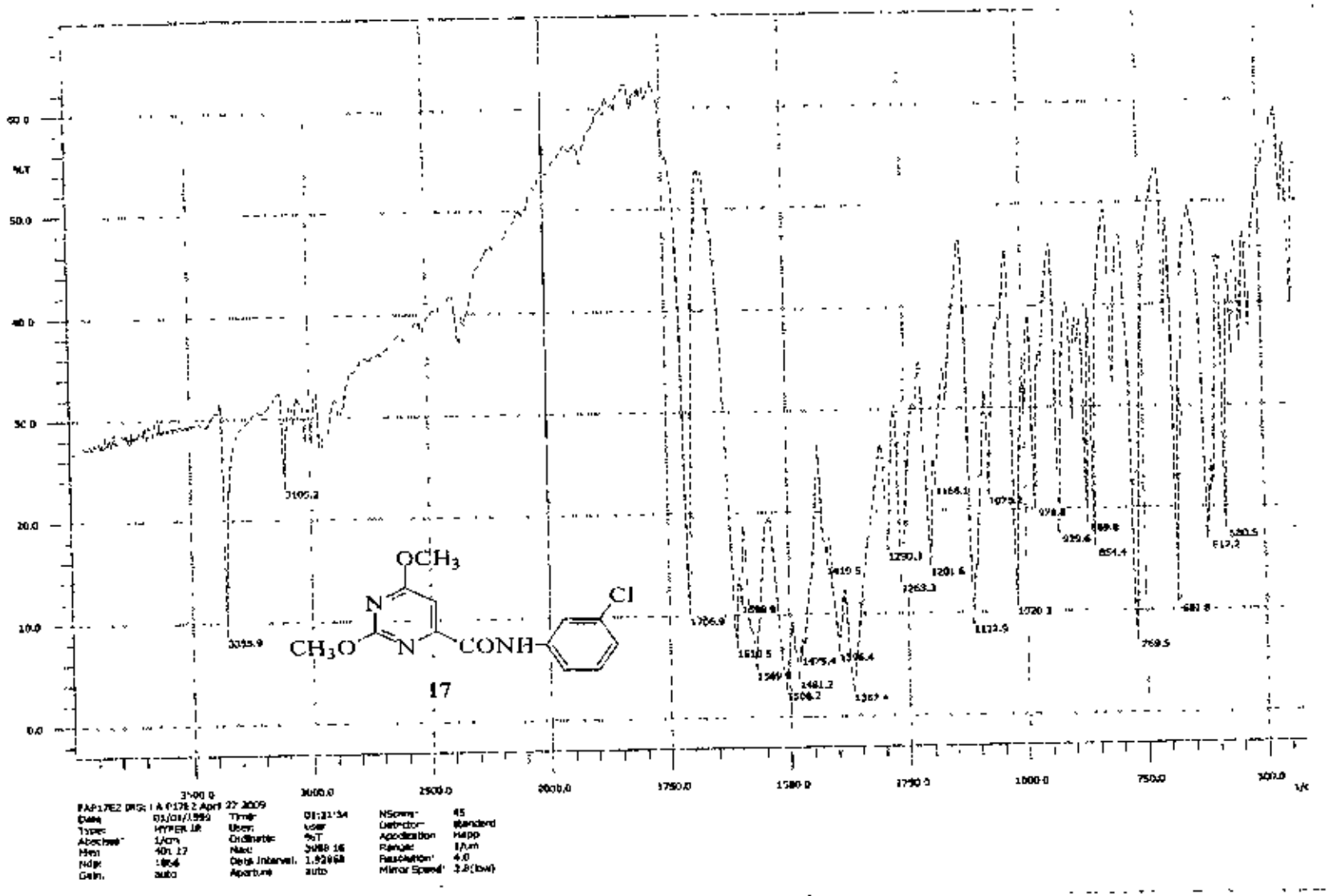
F2 - Acquisition Parameters
Date_ 20080826
Time 11 02
INSTRUM dpx400
PROBHD 5 mm Multinuc
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 128
DS 2
SWH 6710.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 181
DW 78.000 usec
DE 6.00 usec
TE 310.0 K
D1 1.00000000 sec

CHANNEL f1
NUC1 1H
P1 8.30 usec
PL1 -6.00 dB
SFO1 400.1428010 MHz

F2 - Processing parameters
SI 32768
SF 400.1400033 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 14.180 ppm
F1 5682.16 Hz
F2P -0.079 ppm
F2 -39.63 Hz
PPMCM 0.71247 ppm/cm
HZCM 285.08951 Hz/cm

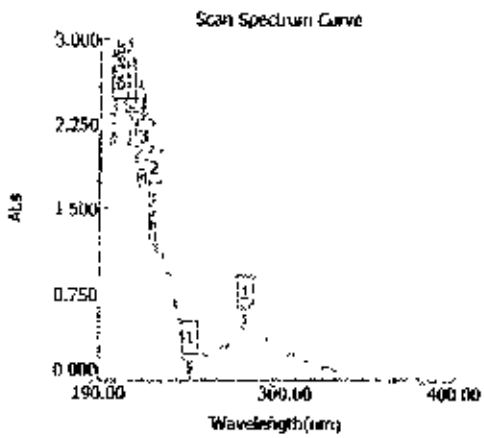

Analytical, BCSIR Lab, Dhaka 13C Spectrum FAP17EP in CDCL3, Fataz, BUET
ppm
173.416
164.811
160.325
158.493
139.225
134.799
130.075
124.827
119.957
117.851
101.976
100.536
77.346
77.029
76.711
55.419
55.171
54.824
54.534
OCH3
N
CH3O
N
CONH
Cl
17
ppm
150
100
50
0



17

FAP17E2 IRS: A 0178.2 April 27 2009

Date:	01/01/1999	Time:	01:21:34	NScans:	45
Type:	HYPER IR	User:	user	Detector:	standard
Abscissa:	1/cm	Ordinate:	%T	Application:	Happ
Min:	401.17	Max:	3988.16	Range:	1/cm
Npx:	1864	Data Interval:	1.92868	Resolution:	4.0
Gain:	auto	Aperture:	auto	Mirror Speed:	3.8(low)



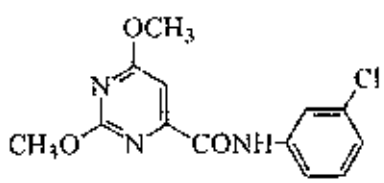
No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	276.00	0.396	
2	Peak	227.00	1.492	
3	Peak	216.00	2.525	
4	Peak	210.00	2.874	
5	Peak	206.00	9.999	
6	Peak	202.00	9.999	
1	Valley	244.00	0.000	

Instrument Performance
Model : SPECTROPHOTOMETERS
Spectral Bandwidth : 2.00 nm

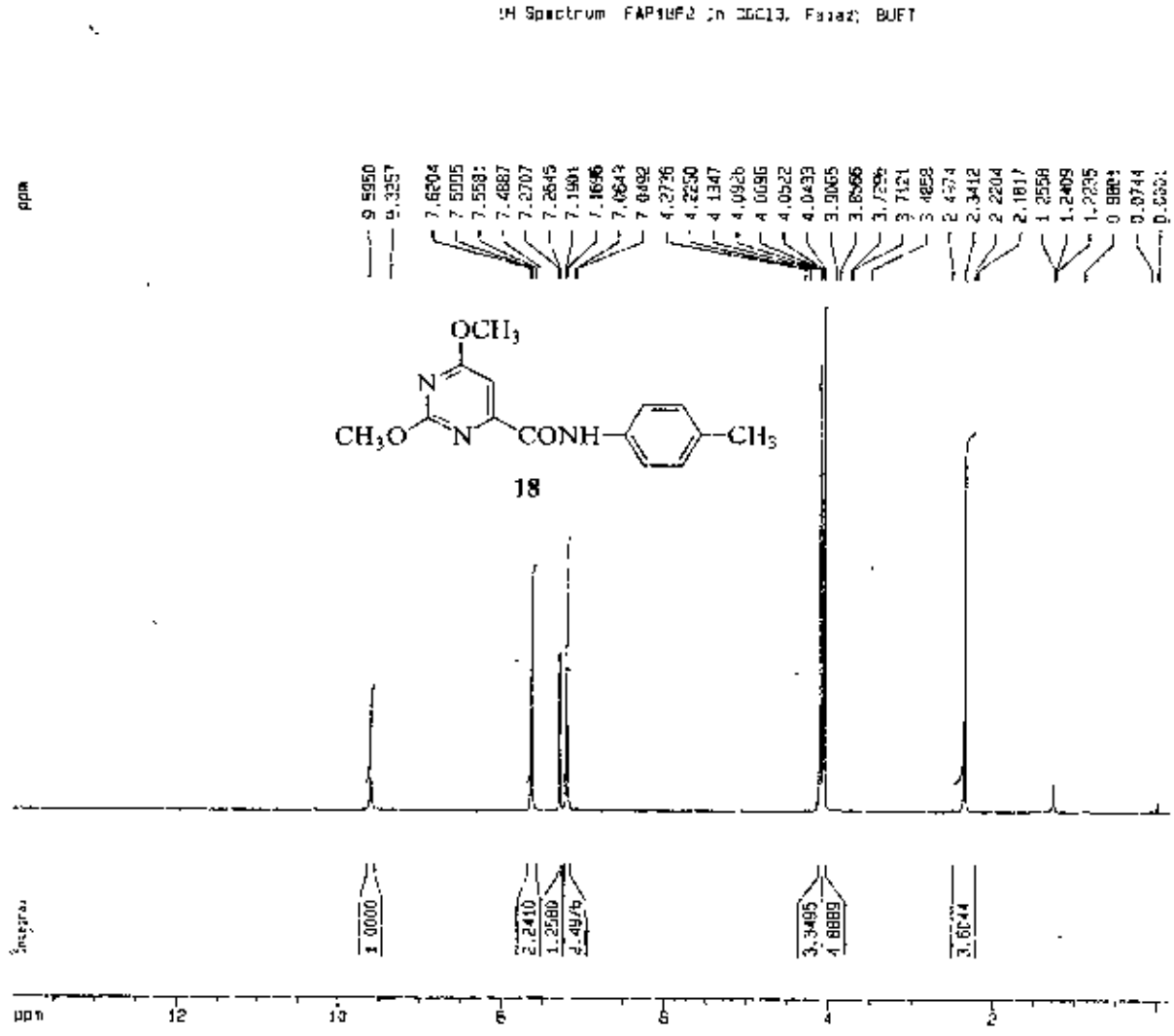
Scan Spectrum Performance
Scan Range : 190.00 to 400.00 nm
Measure Mode : Abs
Interval : 2.00 nm
Speed : Fast
Data File : Untitled6.spd
Create Date/Time : Wednesday, February 04, 2009 9:23:36 PM

Data Type : Original
Method File

Analyse Note
Analyser : Administrator
Sample Name :
Comment :



17



18

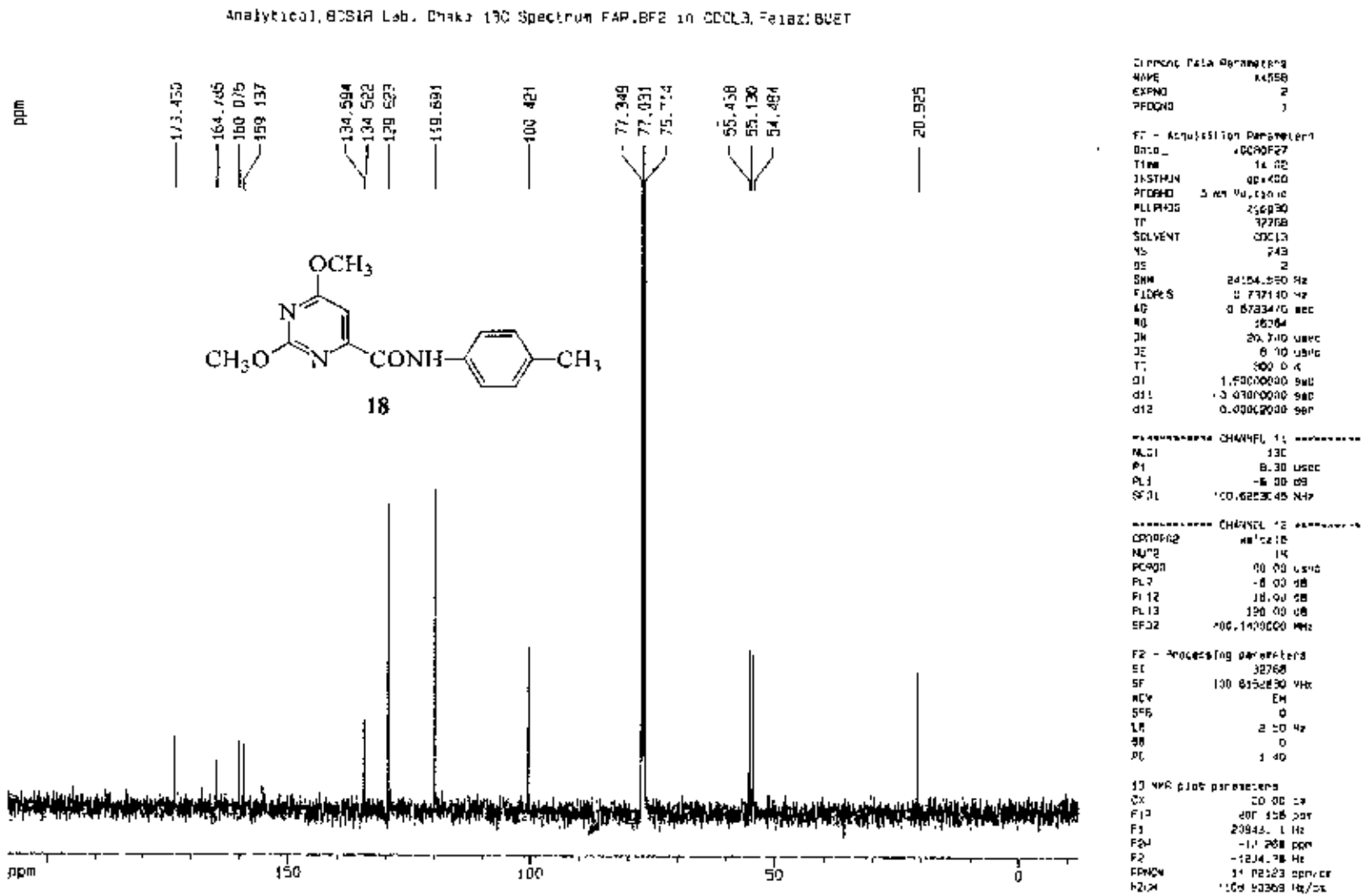
Current Data Parameters
NAME A4568
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20080926
Time 11.25
INSTRUM dpx400
PROBHD 5 mm Multinuc
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 30
DS 2
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 181
DW 78.000 usec
DE 6.00 usec
TE 310.0 K
D1 1.00000000 sec

CHANNEL f1
NUC1 1H
P1 8.30 usec
PL1 -6.00 dB
SFO1 400.1423010 MHz

F2 - Processing parameters
SI 32768
SF 400.1400065 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 13.989 ppm
F1 5597.72 Hz
F2P -0.158 ppm
F2 -63.20 Hz
PPMCM 0.70737 ppm/cm
HZCM 283.04567 Hz/cm



Current Data Parameters
NAME kk558
EXPNO 2
PROCNO 1

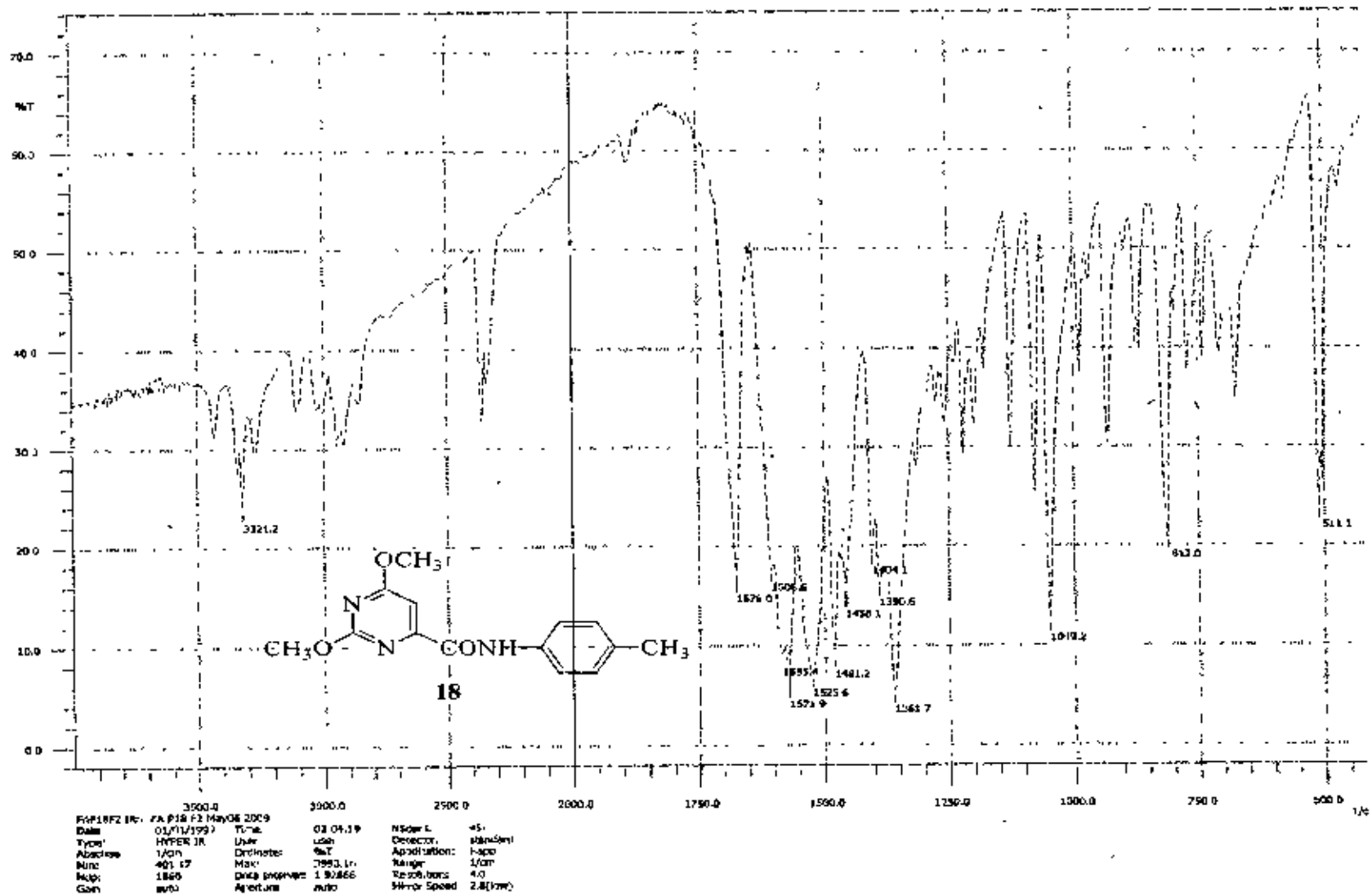
F2 - Acquisition Parameters
Date_ 20040527
Time 14.02
INSTRUM qnp400
PROBHD 5 mm Multinuc
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 243
DS 2
SWH 24154.550 Hz
FIDRES 0.737110 Hz
AQ 0.6783470 sec
RG 16384
DW 20.700 usec
DE 6.00 usec
TE 300.0 K
D1 1.50000000 sec
d11 0.03000000 sec
d12 0.00002000 sec

CHANNEL f1
NUC1 13C
P1 8.30 usec
PL1 -6.00 dB
SFO1 100.6253645 MHz

CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -6.00 dB
PL12 18.90 dB
PL13 120.00 dB
SFO2 400.1400000 MHz

F2 - Processing parameters
SI 32768
SF 100.6152830 MHz
WDW EM
SSB 0
LB 2.50 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 207.156 ppm
F1 20845.11 Hz
F2P -1.268 ppm
F2 -127.54 Hz
PPMCM 11.78523 ppm/cm
HZCM 1186.90368 Hz/cm



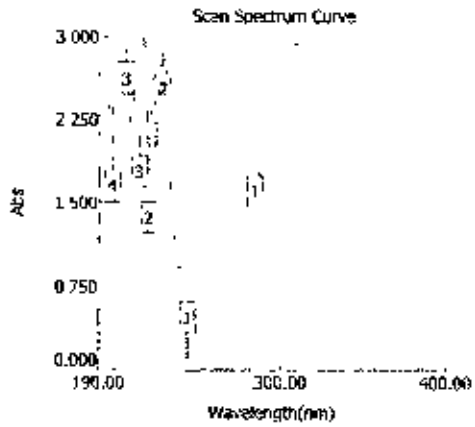
OCH_3

CH_3O

CONH

CH_3

18



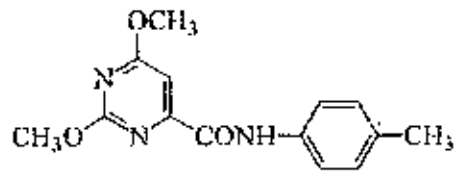
● **Instrument Performance**
Model : SPECTROPHOTOMETERS
Spectral Bandwidth : 2.00 nm

● **Scan Spectrum Performance**
Scan Range : 190.00 to 400.00 nm
Measure Mode : Abs
Interval : 2.00 nm
Speed : Fast
Data File : Untitled8.spd
Create Date/Time : Wednesday, February 04, 2009 9:28:09 PM

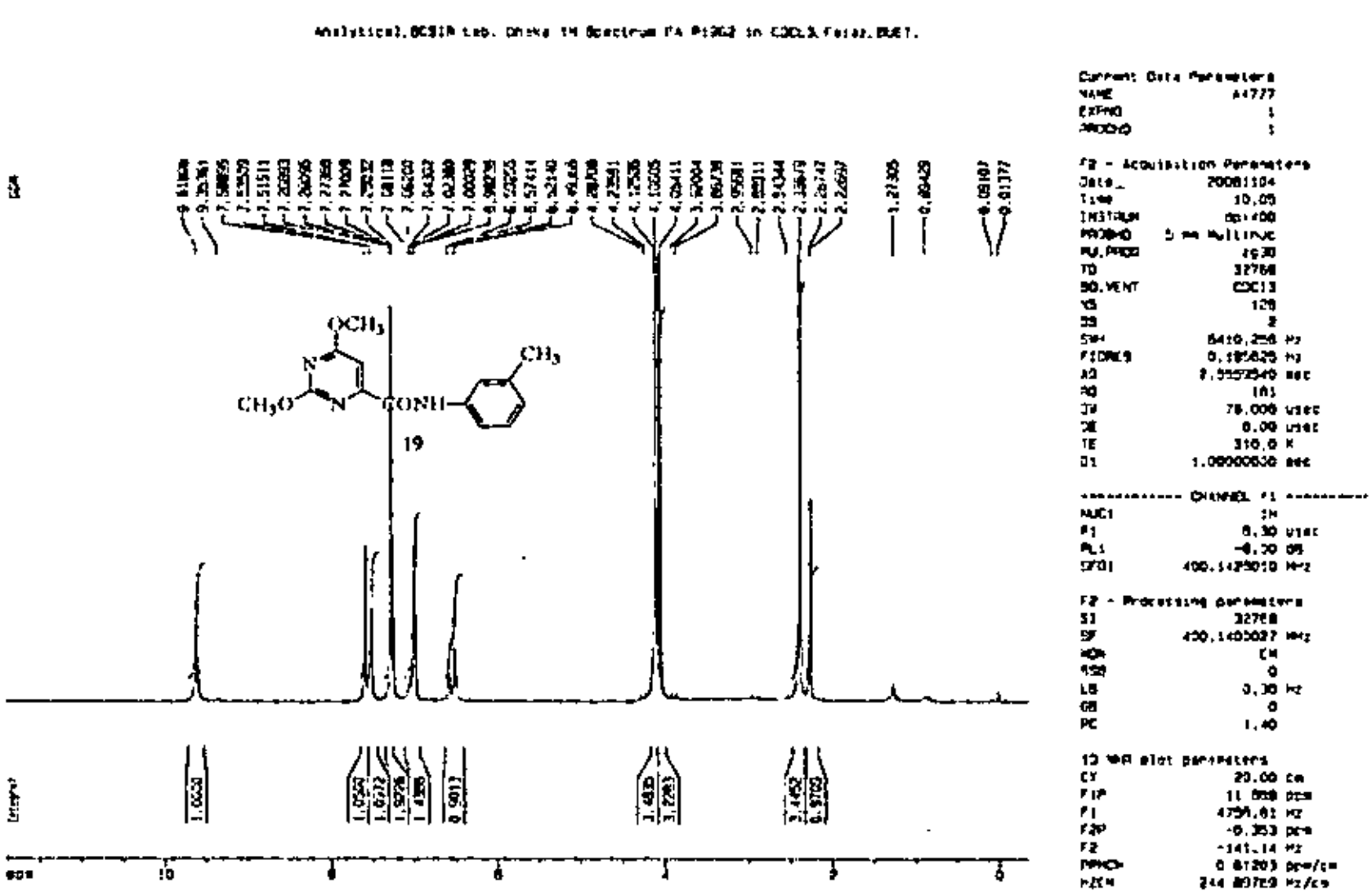
Data Type : Original
Method File :

● **Analyse Note**
Analyser : Administrator
Sample Name :
Comment :

● No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	284.00	1.232	
2	Peak	228.00	2.901	
3	Peak	206.00	9.999	
1	Valley	244.00	0.092	
2	Valley	220.00	1.739	
3	Valley	214.00	2.155	
4	Valley	198.00	2.023	



18



Current Data Parameters

NAME	A1777
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20081104
Time	10.05
INSTRUM	dpx400
PROBHD	5 mm Multinuc
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	5410.256 Hz
FIDRES	0.165120 Hz
AQ	3.0277540 sec
RG	181
DW	78.000 usec
DE	6.00 usec
TE	310.0 K
D1	1.00000000 sec

---------- CHANNEL f1 ----------

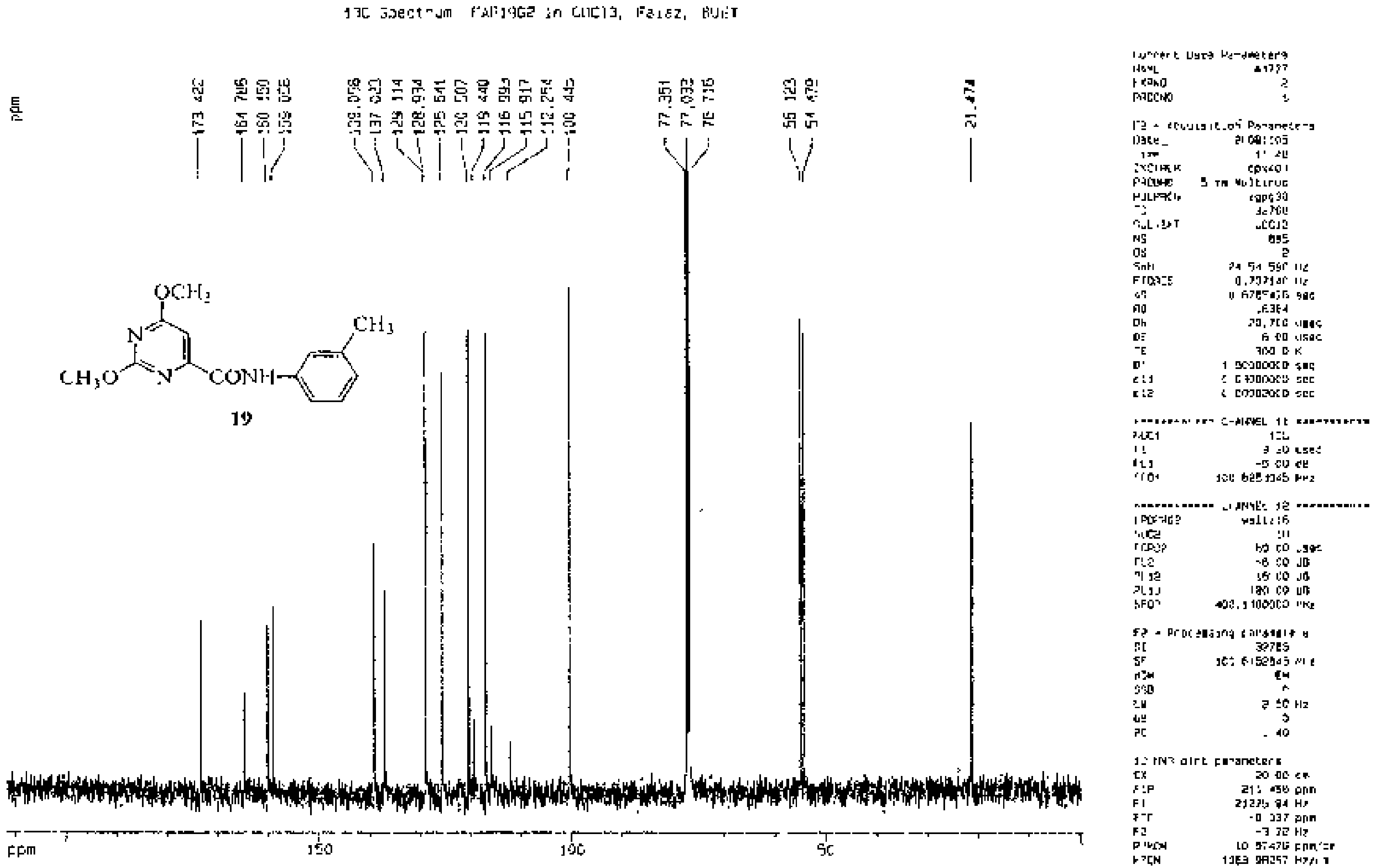
NUC1	1H
P1	8.30 usec
PL1	-0.30 dB
SFO1	400.1425010 MHz

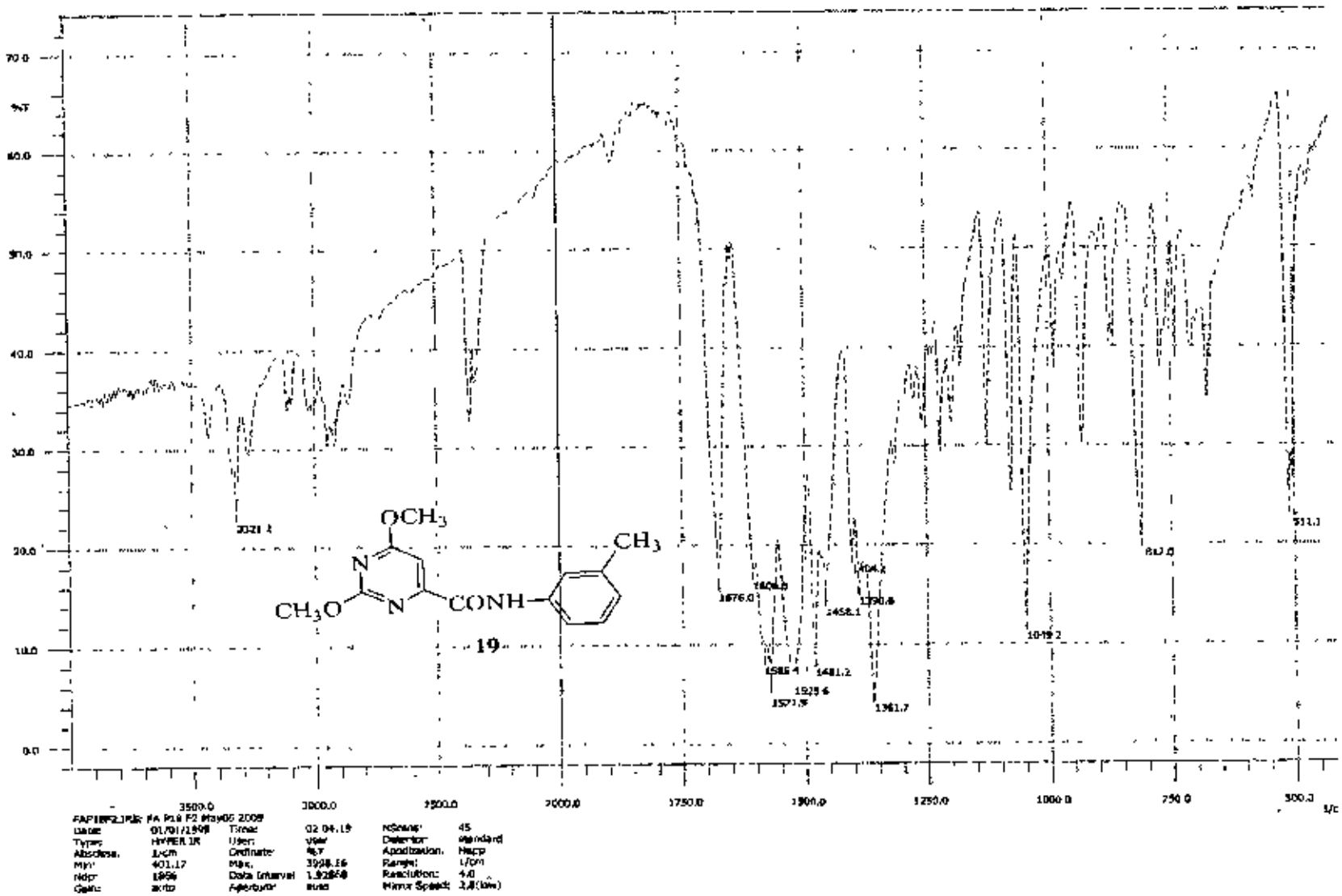
F2 - Processing parameters

SI	32768
SF	400.1400027 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.40

1D NMR plot parameters

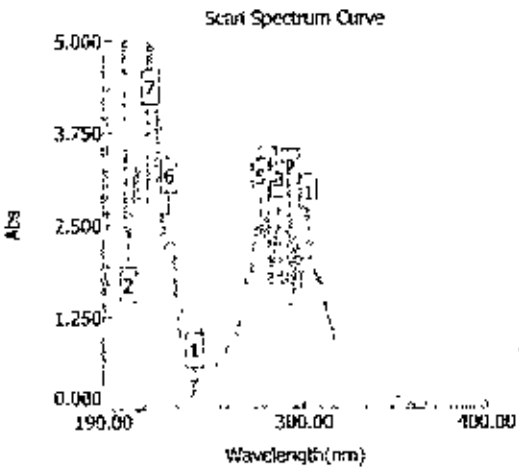
CX	20.00 cm
F1P	11.000 ppm
F1	4759.61 Hz
F2P	-0.353 ppm
F2	-141.14 Hz
PPMCM	0.61203 ppm/cm
HZCM	244.89769 Hz/cm


13C Spectrum FAP19G2 in CDCl3, Faiez, BUET
ppm
173.422
164.785
160.150
159.056
139.098
137.023
129.114
128.934
125.541
120.507
119.440
116.993
115.917
112.254
100.445
77.351
77.032
76.715
55.123
54.479
21.474
OCH3
CH3
CH3O
N
CONH
19
150
100
50
ppm


%T
3021.3
1676.0
1458.1
1361.7
1019.2
811.0
531.1
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/c

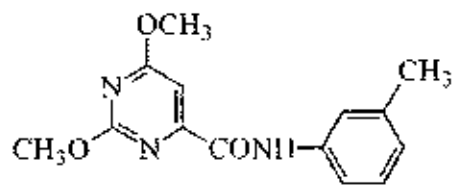
OCH3
CH3
N
CH3O
N
CONH

19

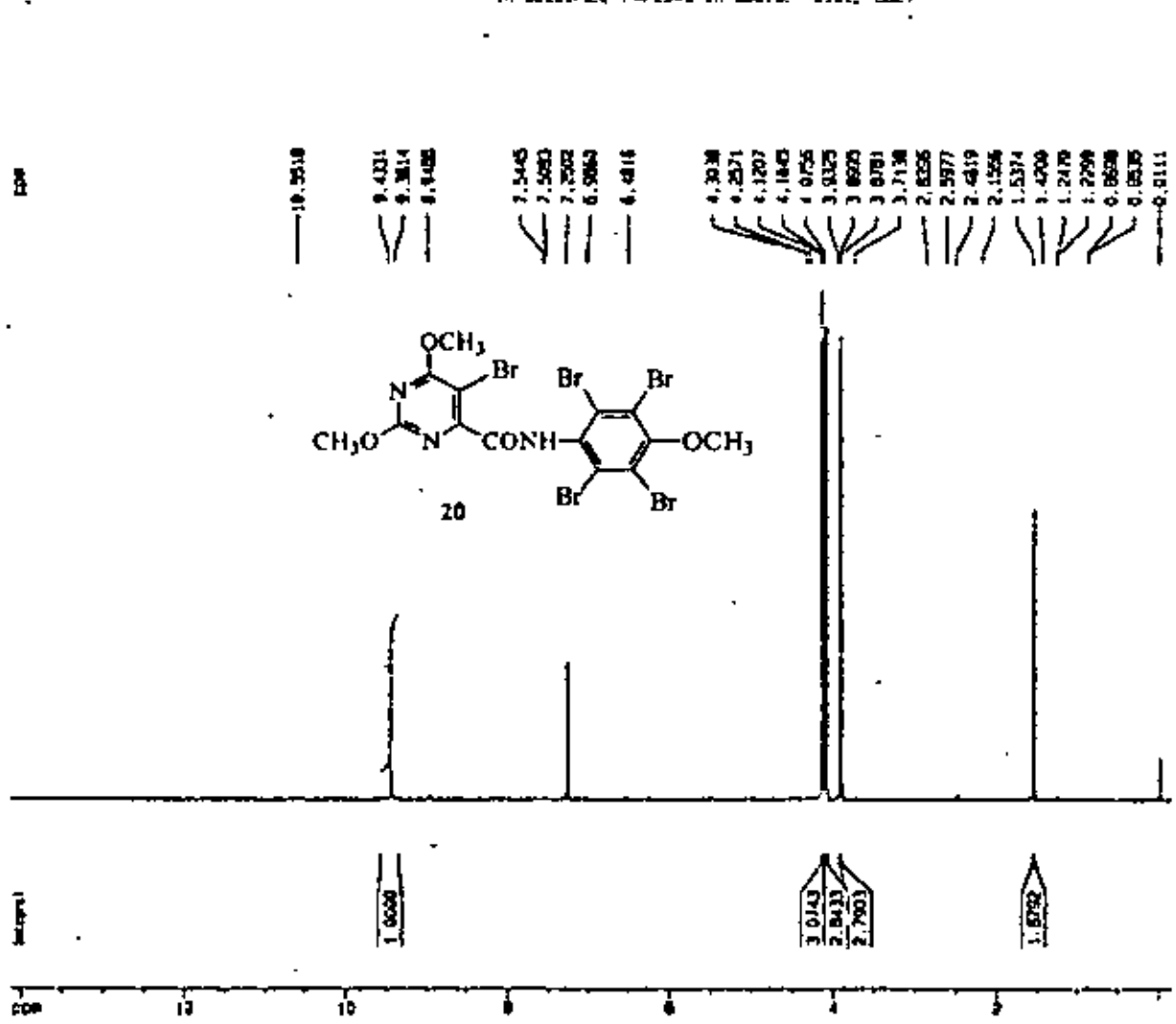


1	Peak	302.00	2.315
2	Peak	292.00	2.667
3	Peak	286.00	2.497
4	Peak	280.00	2.695
5	Peak	276.00	2.523
6	Peak	226.00	2.546
7	Peak	216.00	0.999
1	Valley	240.00	0.170
2	Valley	204.00	2.312

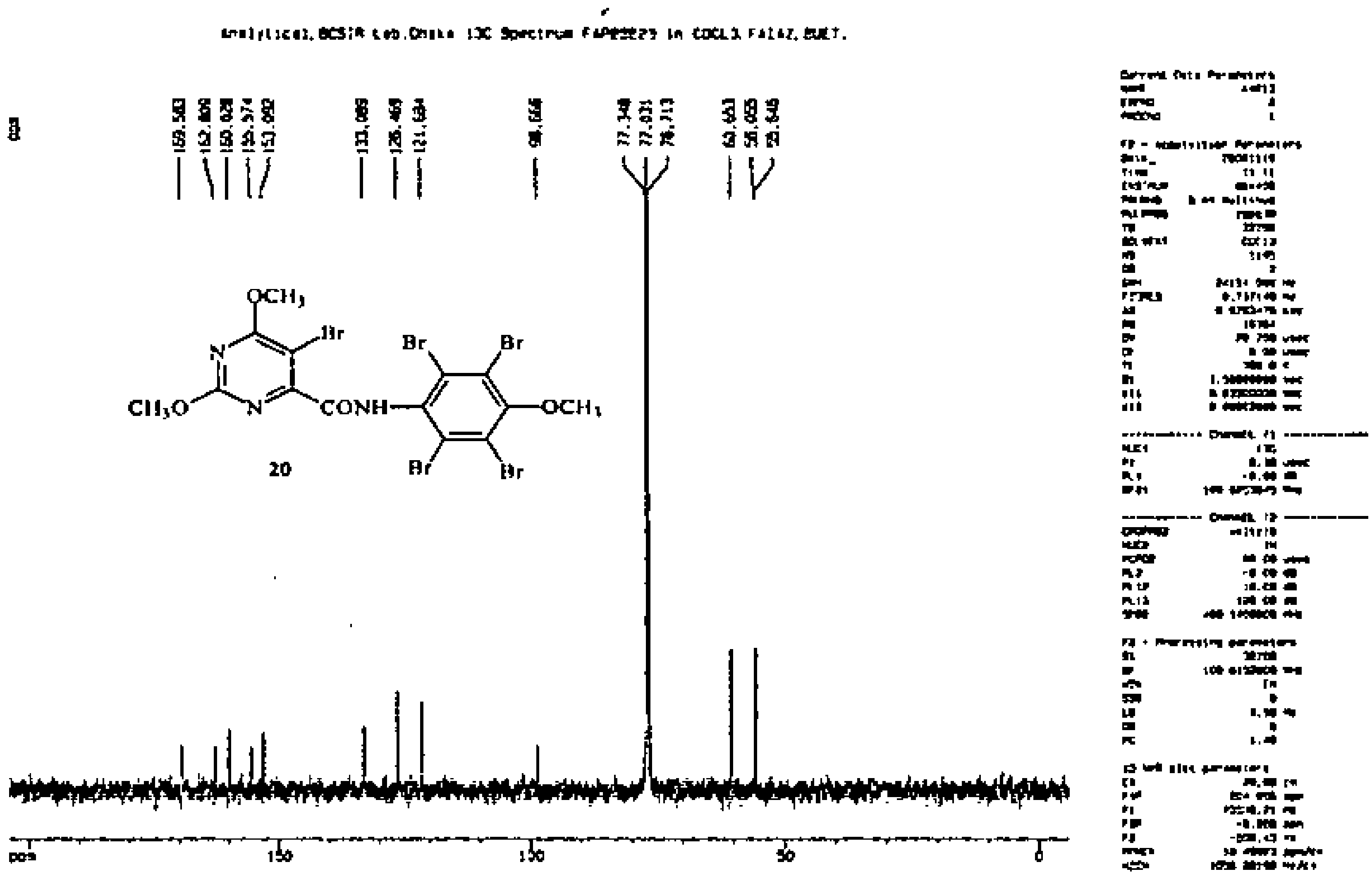
- **Instrument Performance**
  Model : SPECTROPHOTOMETERS
  Spectral Bandwidth : 2.00 nm
- **Scan Spectrum Performance**
  Scan Range : 190.00 to 400.00 nm
  Measure Mode : Abs
  Interval : 2.00 nm
  Speed : Fast
  Data File : Untitled2.spd
  Create Date/Time : Thursday, April 16, 2009 11:49:24 AM
  Data Type : Original
  Method File:
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment :
- **No. P/V Wavelength(nm) Abs Comment**

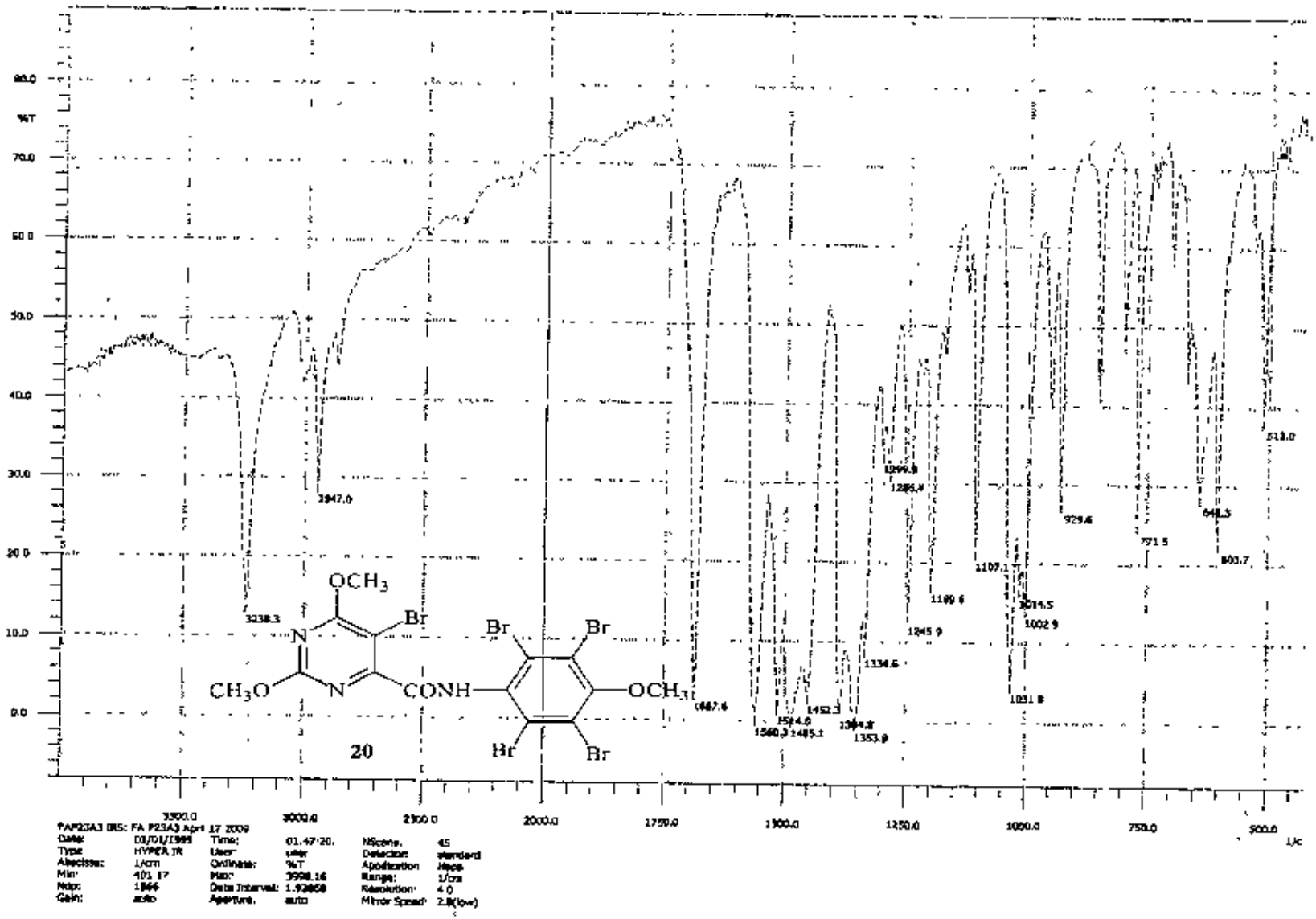


19



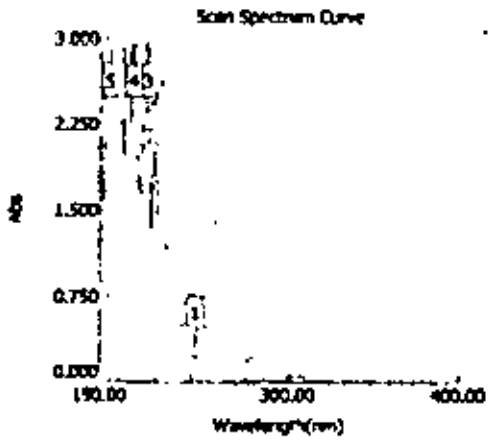

```
Current Data Parameters
NAME                 44813
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20081113
Time                 11.18
INSTRUM              dpx400
PROBHD     5 mm Multinuc
PULPROG              zg30
TD                  32768
SOLVENT              CDCl3
NS                     128
DS                       2
SWH              8410.256 Hz
FIDRES           0.195625 Hz
AQ               2.5559540 sec
RG                     362
DW                78.000 usec
DE                 6.00 usec
TE                310.0 K
D1             1.00000000 sec

------------ CHANNEL f1 ------------
NUC1                   1H
P1                  8.30 usec
PL1                -8.00 dB
SFO1         400.1428010 MHz

F2 - Processing parameters
SI                   32768
SF            400.1400128 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.40

1D NMR plot parameters
CX                   20.00 cm
F1P                  14.110 ppm
F1                 5645.85 Hz
F2P                 -0.157 ppm
F2                  -62.76 Hz
PPMCM              0.71333 ppm/cm
HZCM             285.43015 Hz/cm
```


13C Spectrum
in CDCL3
169.563
162.806
160.028
155.574
153.092
133.089
126.469
121.684
98.666
77.348
77.031
76.713
60.653
56.055
55.646
OCH3
Br
N
CH3O
N
CONH
Br
Br
OCH3
Br
Br
20
ppm
150
100
50
0



OCH_3, Br, N, CH_3O, N, CONH, Br, Br, OCH_3, Br, Br

20



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3	Peak	218.00	3.061	
4	Peak	210.00	9.999	
5	Peak	196.00	9.999	

- **Instrument Performance**
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  Sample Name :
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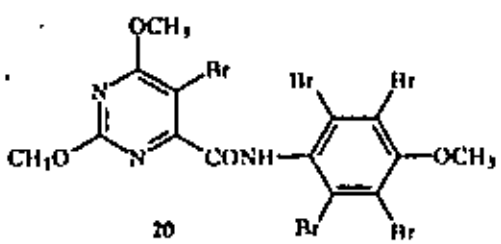
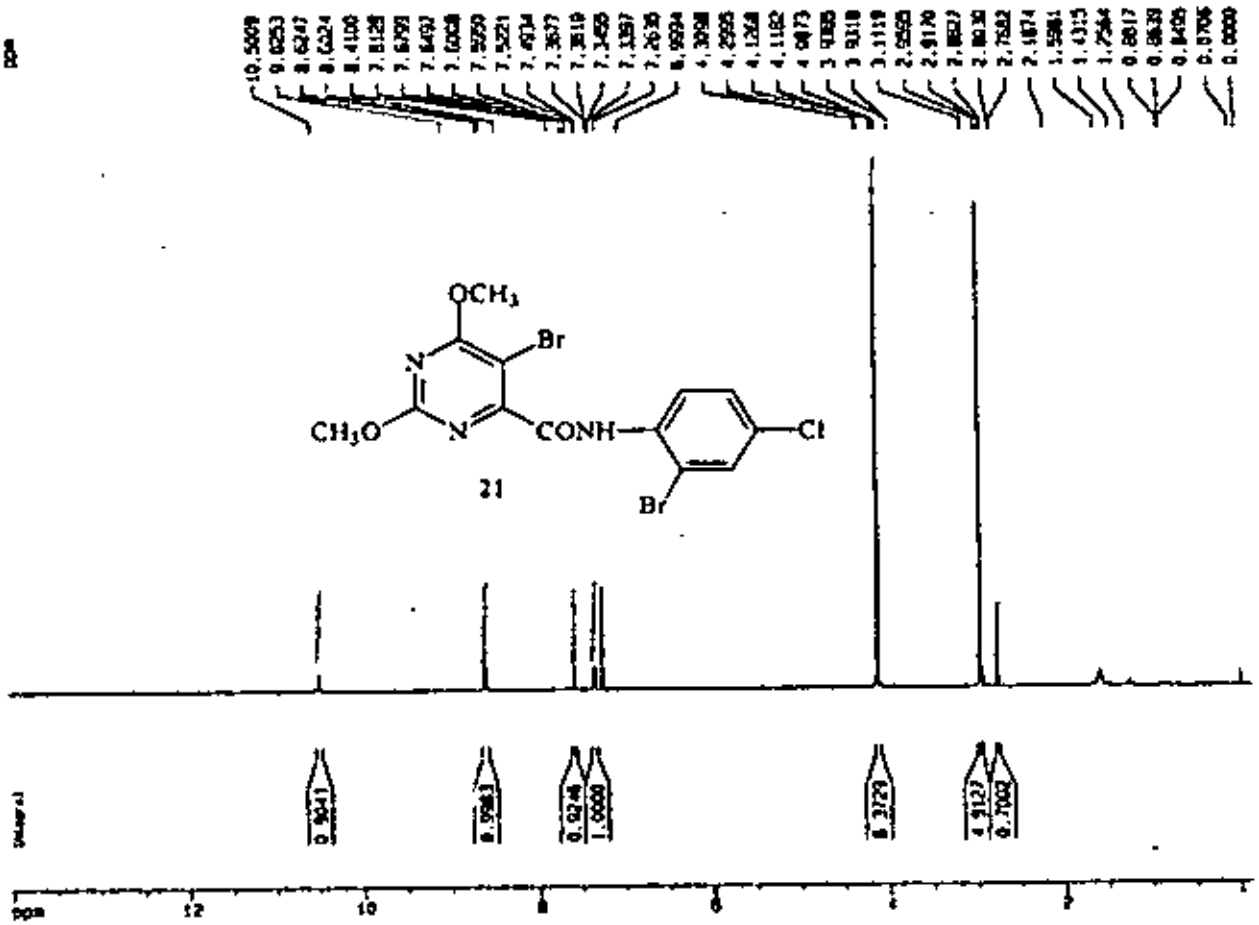


20

Analytical, RCSIR, 1H Spectrum, FAP22C3 in CDCl3, Faiaz, BUET

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PROCNO                    1

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TD                    32768
SOLVENT                Aceton
NS                        47
DS                         2
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FIDRES            0.195625 Hz
AQ                2.5559540 sec
RG                    456.1
DW                  78.000 usec
DE                   6.00 usec
TE                  310.0 K
D1                1.00000000 sec

------------ CHANNEL f1 ----------
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P1                   8.30 usec
PL1                 -6.00 dB
SFO1           400.1428010 MHz

F2 - Processing parameters
SI                    32768
SF                400.1400074 MHz
WDW                       EM
SSB                        0
LB                      0.30 Hz
GB                         0
PC                      1.40

1D NMR plot parameters
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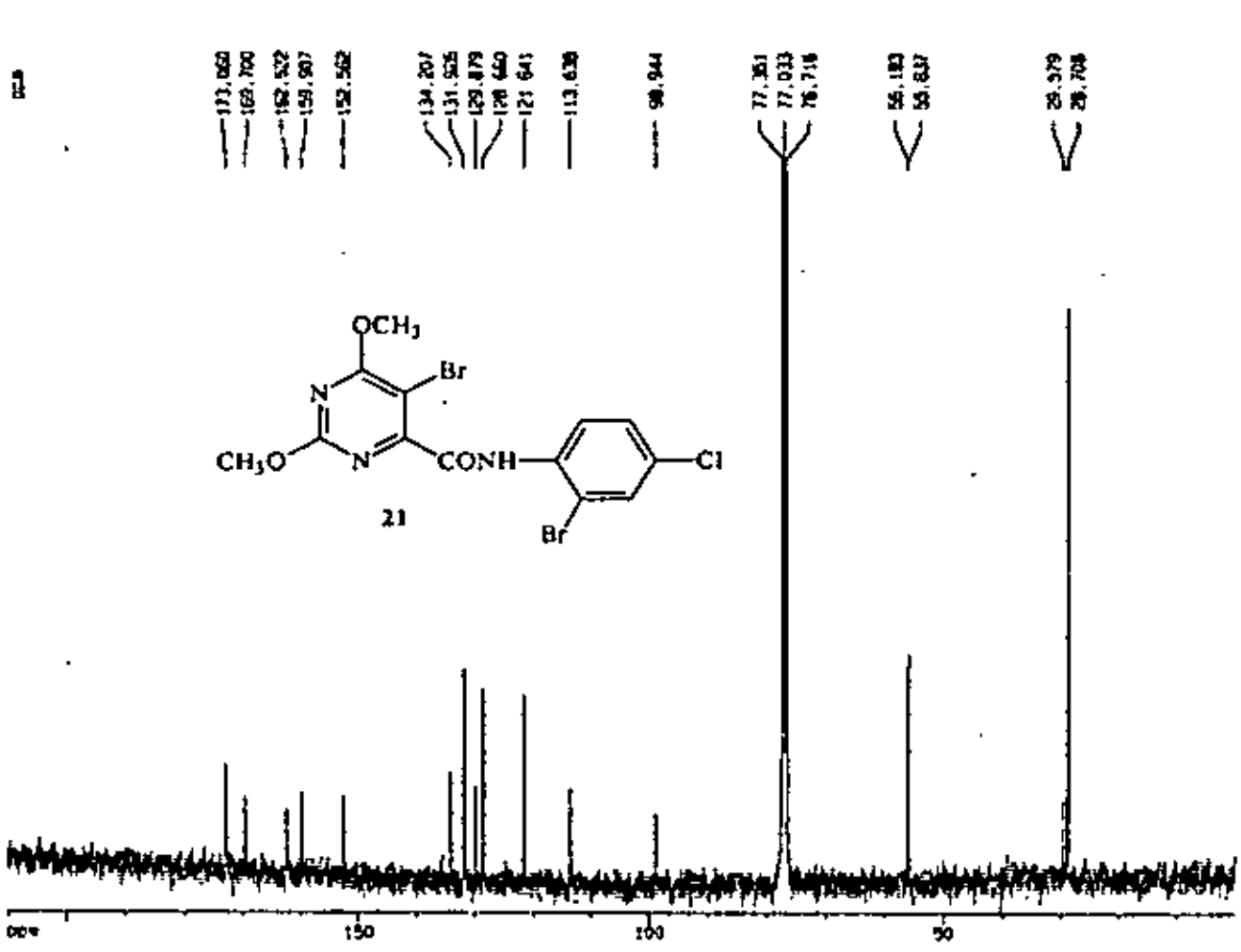
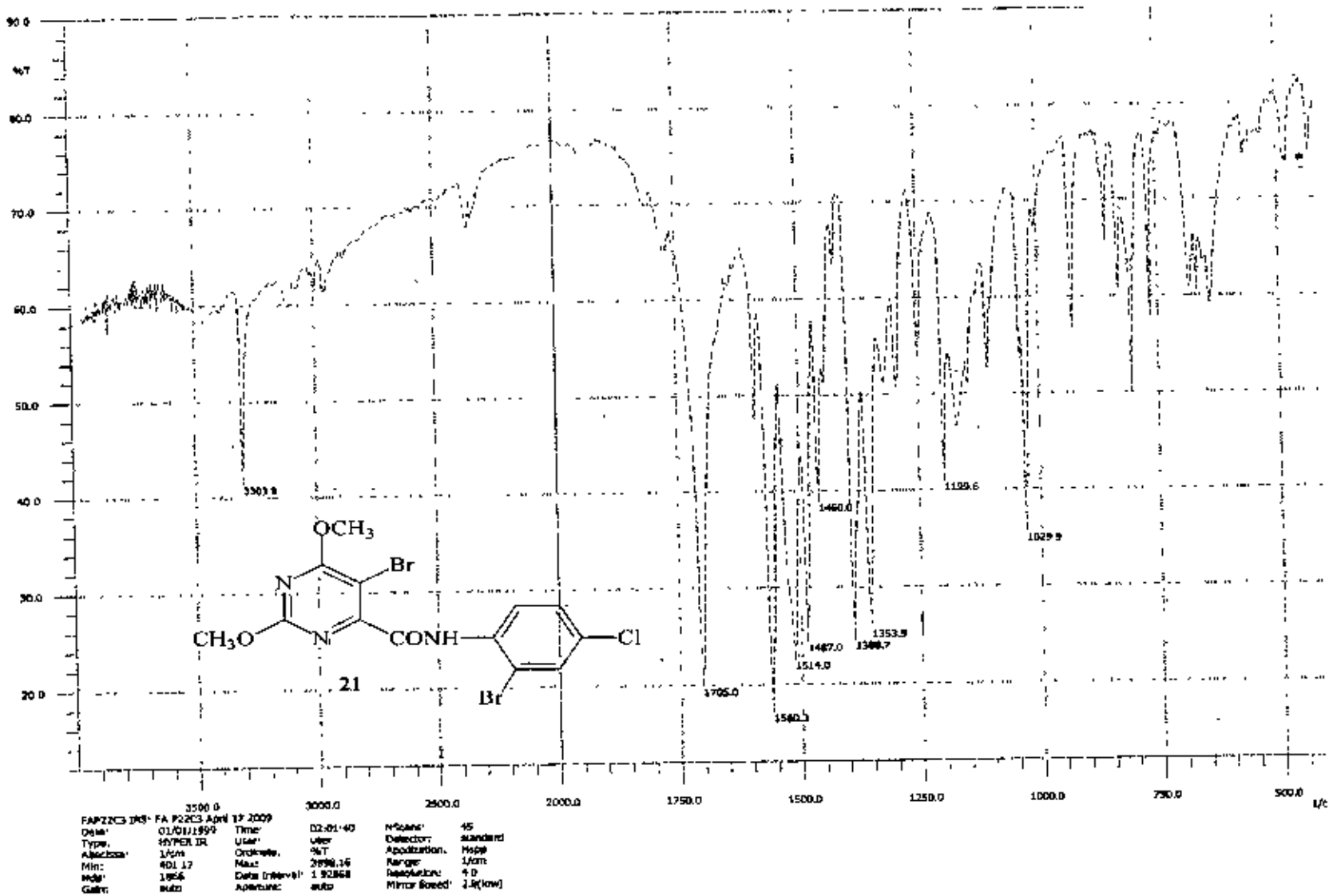

OCH3
Br
N
N
CH3O
CONH
Cl
Br
21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1580.3
1514.0
1487.0
1460.0
1388.7
1353.9
1199.6
1029.9
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/c
FAPZ2C3 IR9 FA P22C3 April 17 2009
Date: 01/01/1999
Time: 02:01:40
nScans: 45
Type: HYPER IR
User: user
Detector: standard
Abscissa: 1/cm
Ordinate: %T
Apodization: Happ
Min: 401.17
Max: 3998.16
Range: 1/cm
Pnts: 1856
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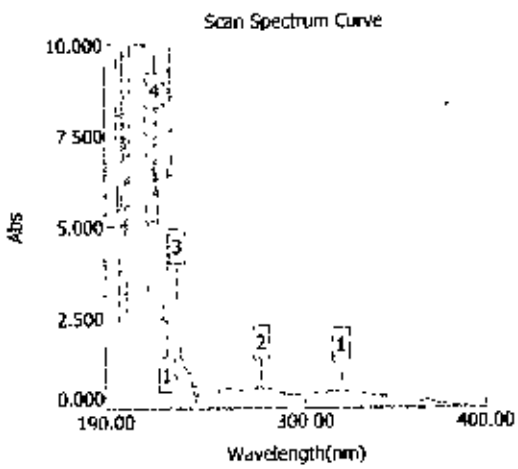
Resolution: 4.0
Gain: auto
Aperture: auto
Mirror Speed: 1.9[low]

OCH3
Br
N
CH3O
N
CONH
Cl
Br

21



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3	Peak	230.00	3.204
4	Peak	218.00	9.999
1	Valley	224.00	2.181

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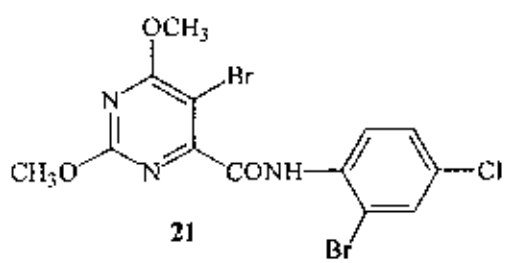
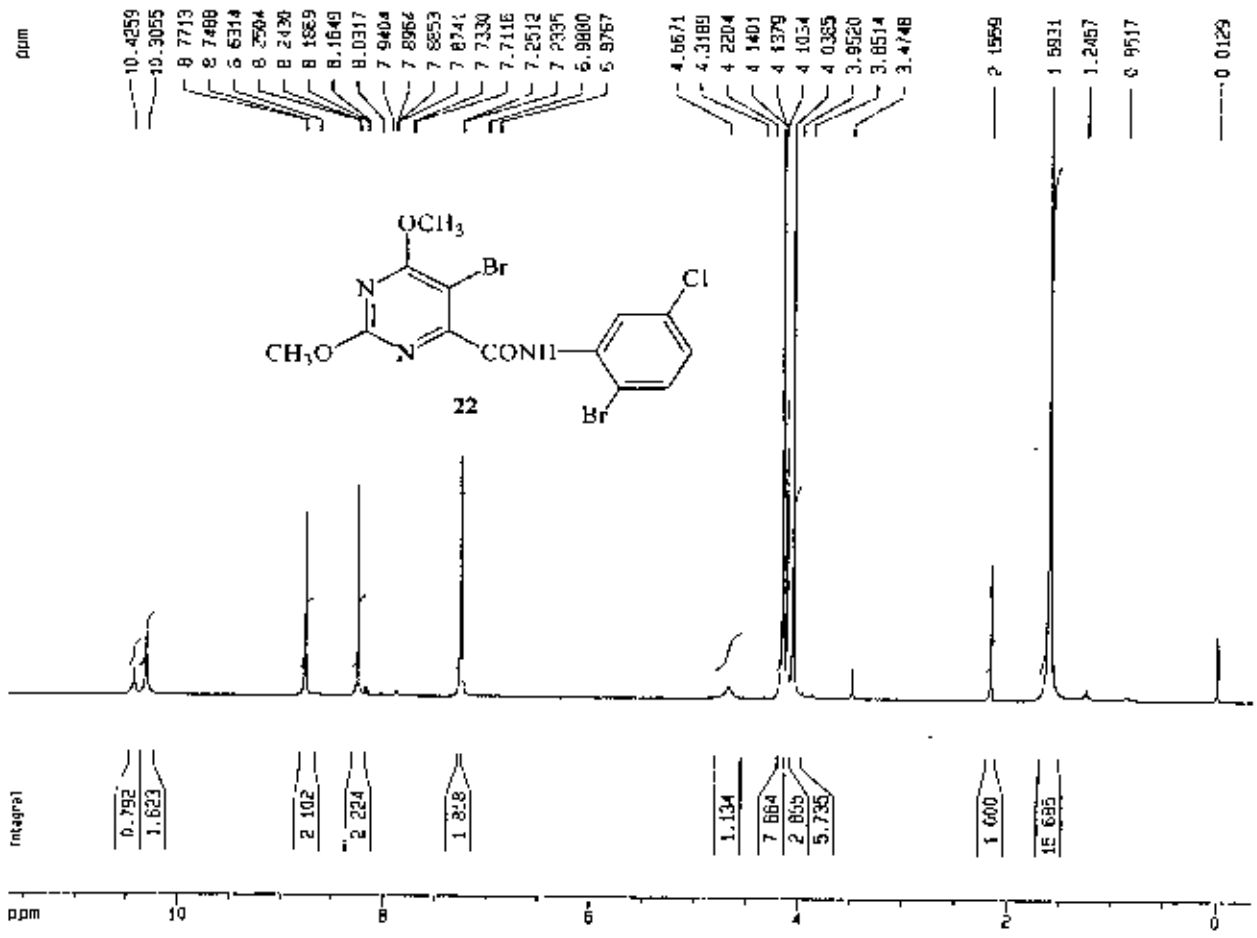
- **No. P/V Wavelength(nm) Abs Comment**



21

Analytical BCSIR Lab Dhaka 1H Spectrum FAP21E3 in CDCL3, Faiaz, BUET



22

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PROCNO                    1

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TD                    32768
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DS                        2
SWH               6410 256 Hz
FIDRES          0 195625 Hz
AQ              2 5559540 sec
RG                      512
DW                78 000 usec
DE                 6 00 usec
TE                310 0 K
D1              1 00000000 sec

========== CHANNEL f1 ==========
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P1                      8.30 usec
PL1                    -6.00 dB
SFO1          400.1428010 MHz

F2 - Processing parameters
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SF          400 1400115 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1 40

1D NMR plot parameters
CX                   20 00 cm
F1P                11.645 ppm
F1                 4659 45 Hz
F2P                -0 341 ppm
F2                 -136 32 Hz
PPMCM           0 59926 ppm/cm
HZCM          239.78856 Hz/cm
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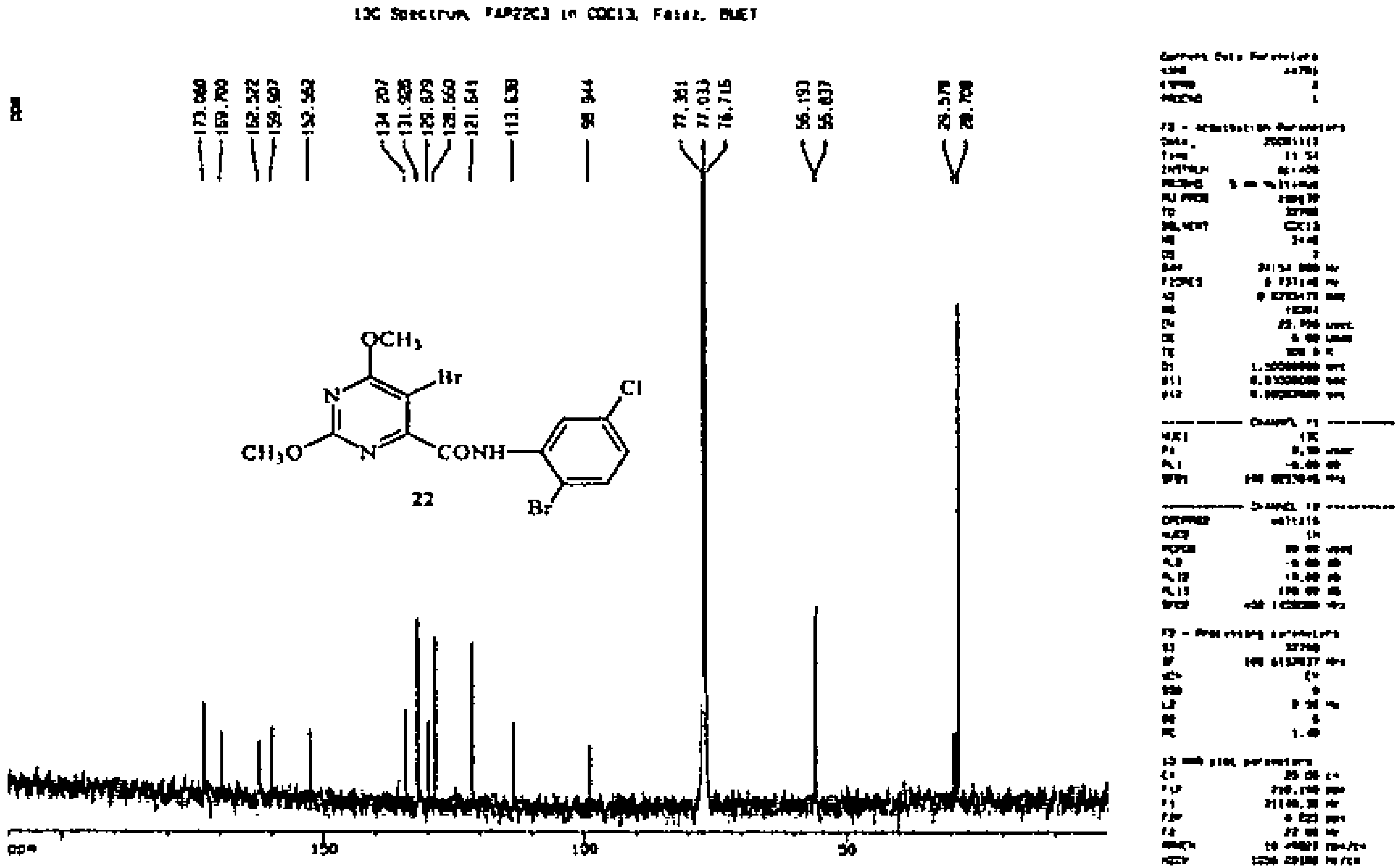
13C Spectrum FAP22C3 in CDCl3, Fatal, BUET
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131.926
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128.560
121.641
113.638
98.944
77.351
77.033
76.715
56.193
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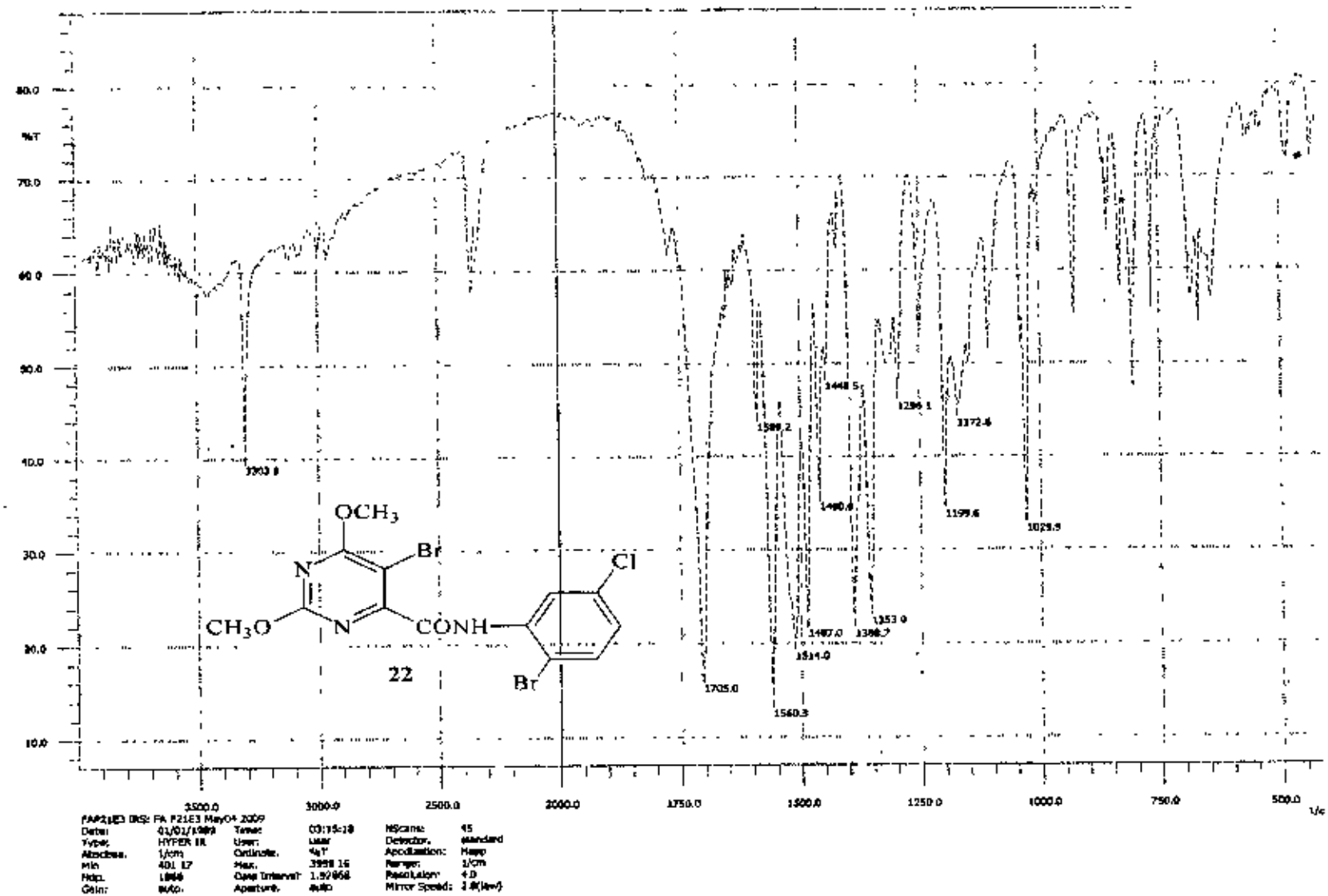
CH3O
N
CONH
Cl
Br
22
ppm
150
100
50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40.0
30.0
20.0
10.0
3500.0
3000.0
2500.0
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1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/c
3393.9
1705.0
1589.2
1560.3
1531.0
1497.0
1448.5
1388.7
1353.9
1296.1
1199.6
1372.4
1029.9
1490.0
OCH3
Br
N
CH3O
N
CONH
Cl
Br
22
FAR21E3 DRS: FA F21E3 May04 2009
Date: 01/01/1989
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Ndp. 1868
Gain: auto
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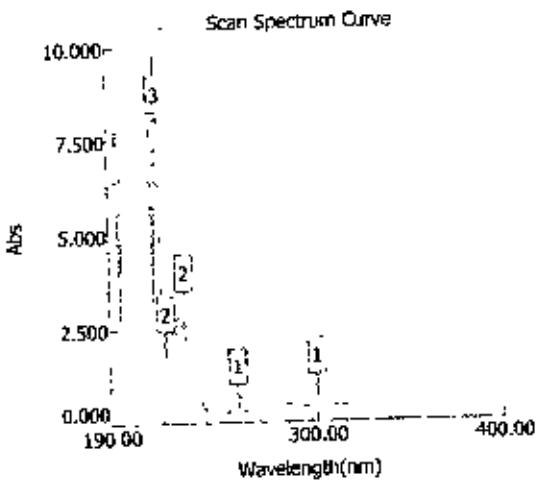

1	Peak	300.00	0.493
2	Peak	230.00	2.728
3	Peak	216.00	9.939
1	Valley	258.00	0.246
2	Valley	220.00	1.649

- **Instrument Performance**
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- **Analyse Note**
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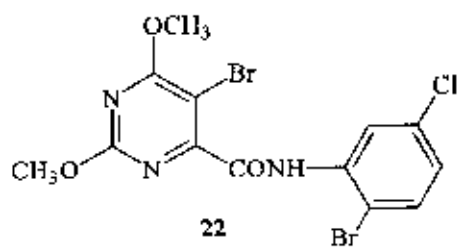
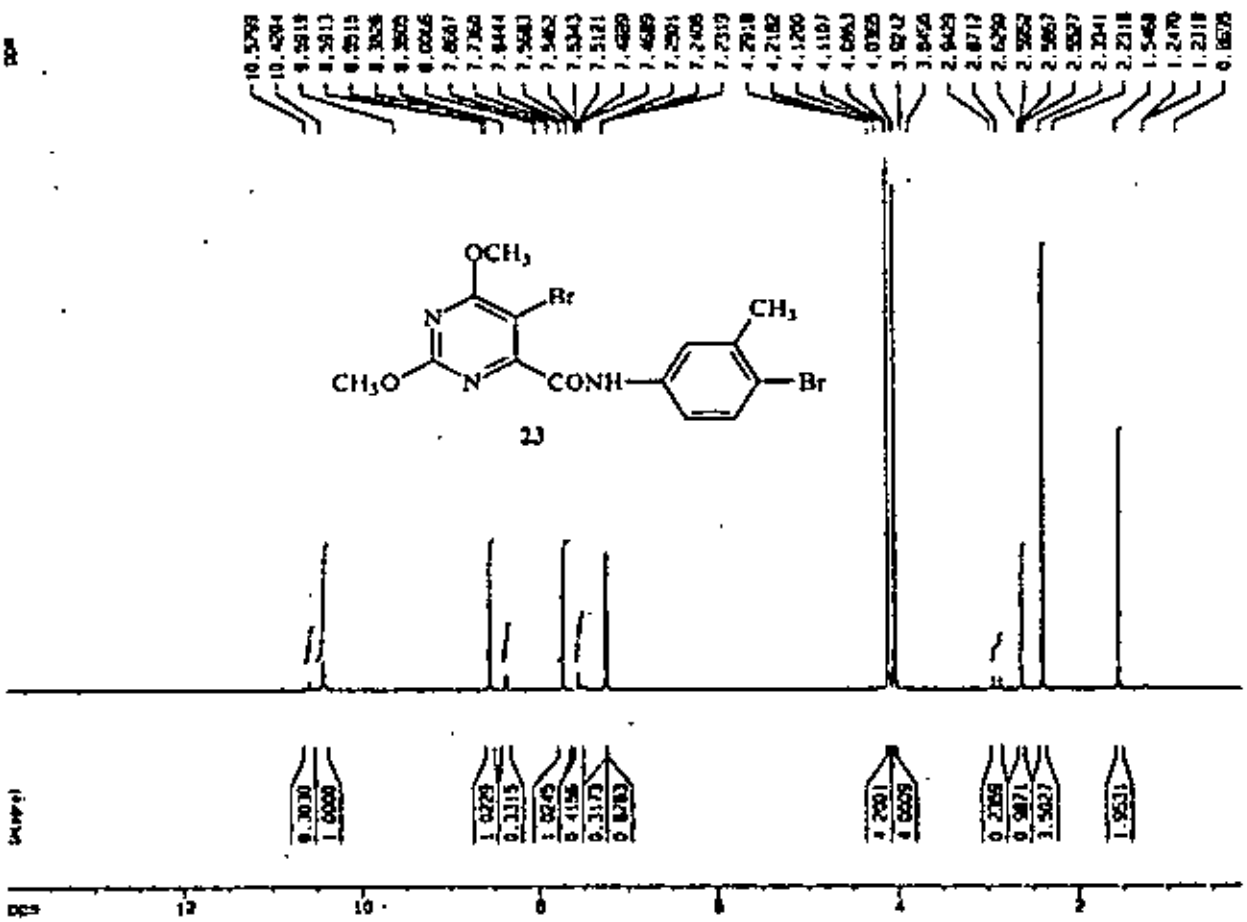
  Comment :
- **No. P/V Wavelength(nm) Abs Comment**



22

1H Spectrum FAP2503 in CDCl3. Fatez. DUE7

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PROCNO                  1

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TD                 32768
SOLVENT             CDCl3
NS                     31
DS                      2
SWH             8410.256 Hz
FIDRES          0.128525 Hz
AQ              2.5559540 sec
RG                 406.4
DW                 70.000 usec
DE                  6.00 usec
TE                310.0 K
D1              1.00000000 sec

------------ CHANNEL f1 ------------
NUC1                  1H
P1                  8.30 usec
PL1                -6.00 dB
SFO1          400.1428010 MHz

F2 - Processing parameters
SI                 32768
SF            400.1400128 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.40

1D NMR plot parameters
CX                 20.00 cm
F1P                17.996 ppm
F1                 5398.45 Hz
F2P                 0.184 ppm
F2                  73.47 Hz
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HZCM            376.15045 Hz/cm
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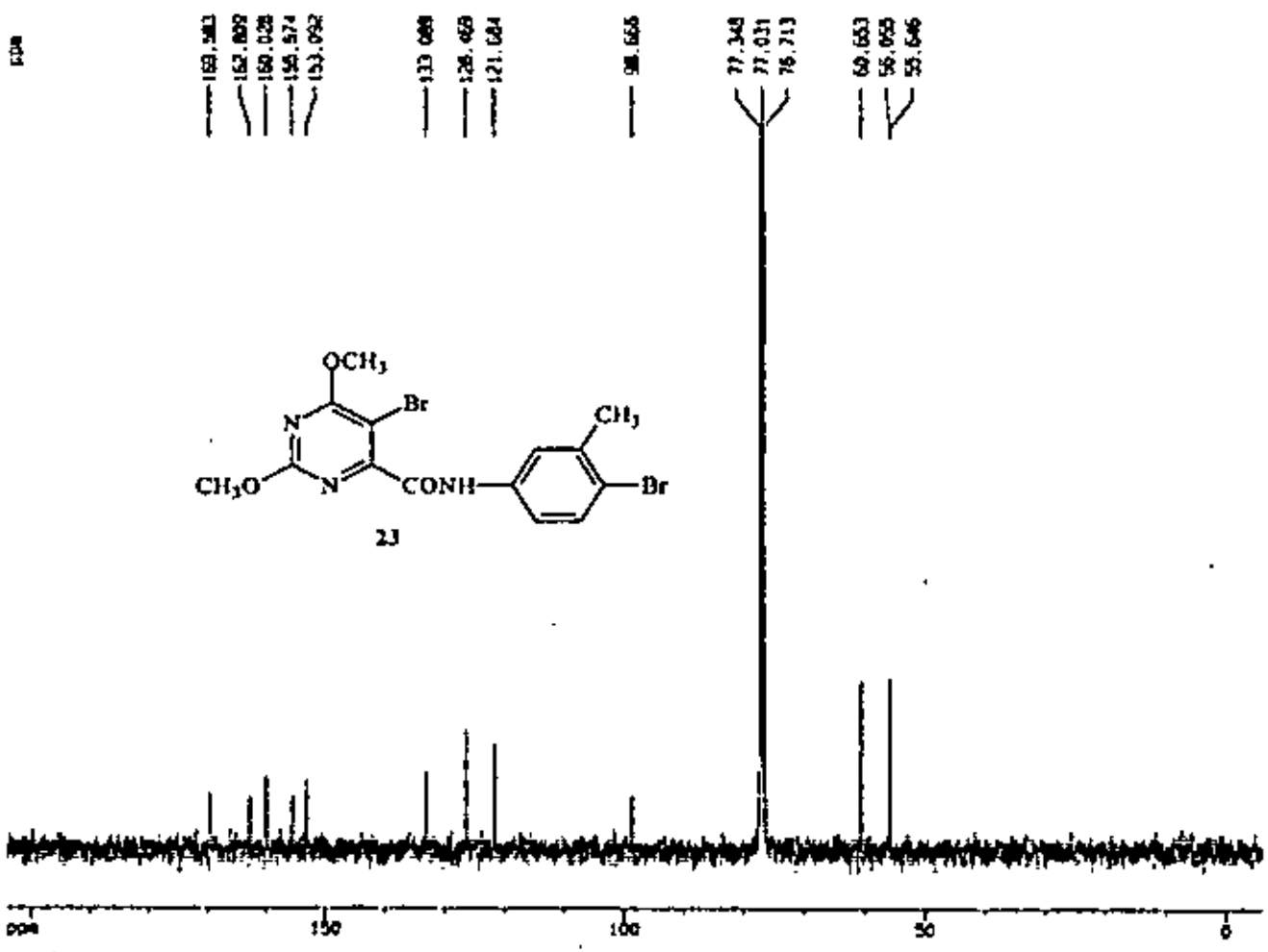
Analytical, BCSIR Lab, Dhaka 13C Spectrum FAP23023 in CDCL3, FAIAZ, BUET.



Current Data Parameters
NAME
EXPNO
PROCNO

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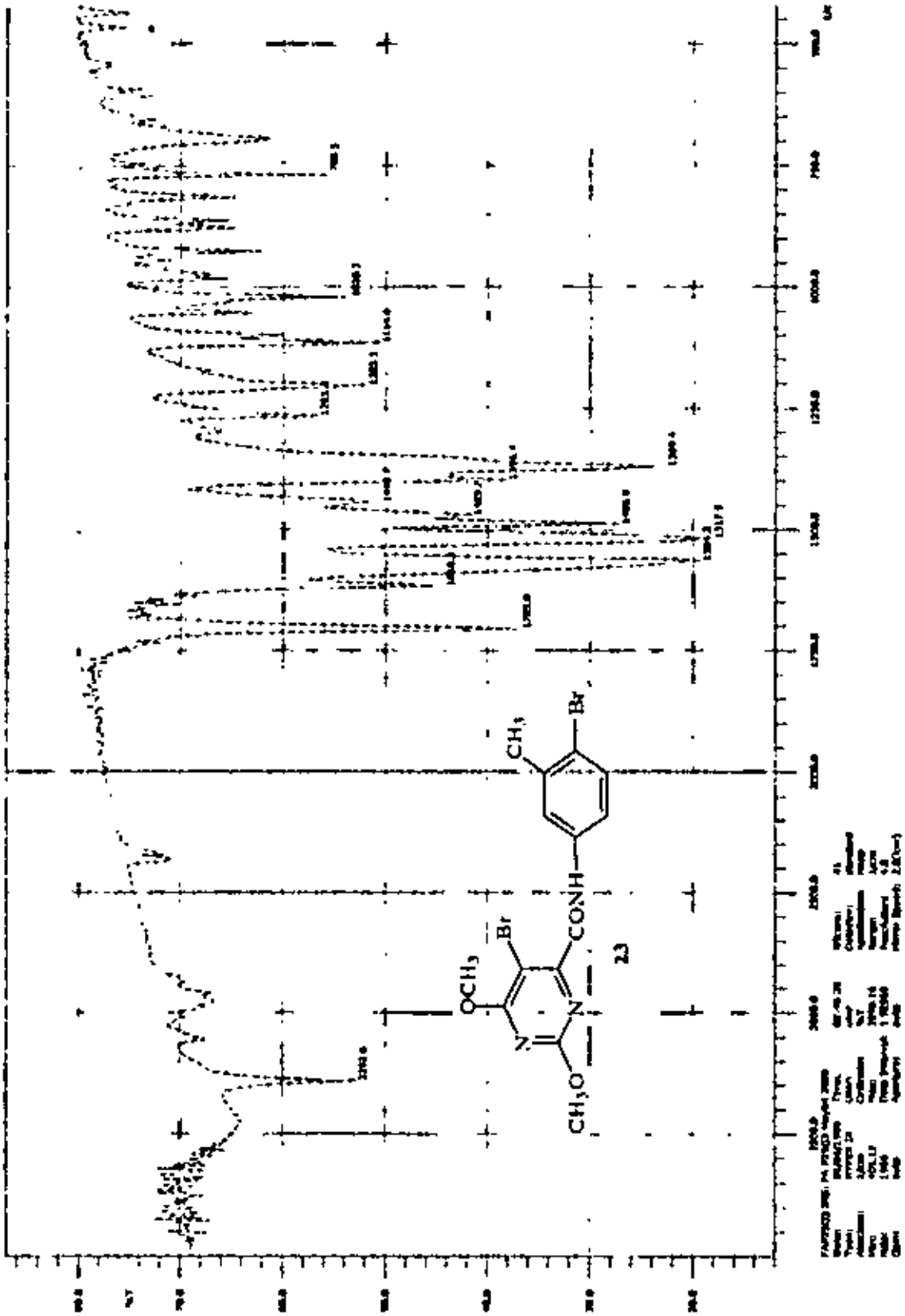
Date_
Time
INSTRUM
PROBHD
PULPROG
TD
SOLVENT
NS
DS
SWH
FIDRES
AQ
RG
DW
DE
TE
D1
D11
D12

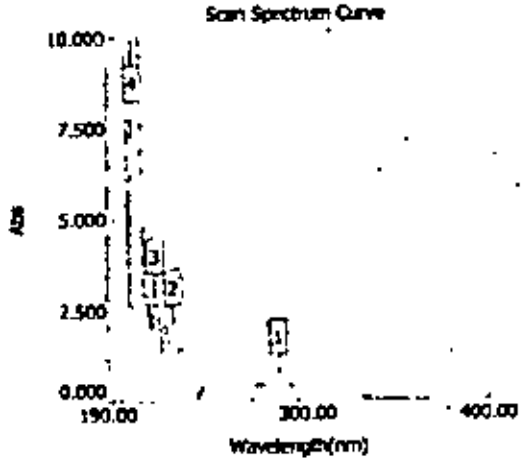
CHANNEL f1
NUC1
P1
PL1
SF01

CHANNEL f2
CPDPRG2
NUC2
PCPD2
PL2
PL12
PL13
SFO2

F2 - Processing parameters
SI
SF
WDW
SSB
LB
GB
PC

1D NMR plot parameters
CX
F1P
F1
F2P
F2
PPMCM
HZCM


Br
CH3
CONH
Br
OCH3
N
N
CH3O
2.3



1	Peak	284.00	0.490
2	Peak	226.00	1.851
3	Peak	216.00	2.700
4	Peak	204.00	9.999

- **Instrument Performance**
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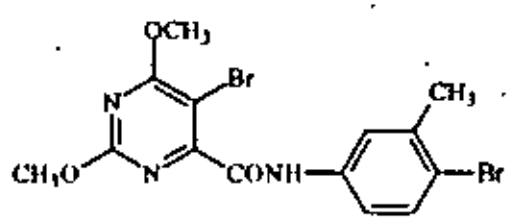
- **Analyse Note**
  Analyser : Administrator
  Sample Name :
  Comment :
- No. P/V Wavelength(nm) Abs Comment



23

Chapter 4

Antimicrobial Screening

INTRODUCTION

Bacteria and fungi are responsible for many infectious diseases. The increasing clinical implications of drug resistant fungal and bacterial pathogens have lent additional urgency to antimicrobial drug research. The deterioration of human population due to enhance of prevalence of infections diseases is becoming a global problem[1]. It was found from the literature that nitrogen and sulfur containing compounds showed marked microbial activities[2-6]. When heterocyclic part of the compounds, such as; imidazole, nitroimidazole etc. become attached to carbohydrates[7], their efficiency to inhibit bacteria of fungus sharply increased. It was also found that a large number of biologically active compounds possesses aromatic and heteroaromatic nucleus. If an active nucleus is linked to another nucleus, the resulting molecule may possess greater potential for biological activity[8]. In *vitro* antimicrobial activities of fused pyrimidines were successfully evaluated in our laboratory[9].

M. shaheb[10] a post graduate student carried out in *vitro* antimicrobial activates of fused pyrimidine derivatives. M. S. Rahman[11] showed that antimicrobial activities of alkaloids plant leaves. The alkaloids were screened against several pathogenic bacteria.

S. M. Shahed[12, 13] a former research student of organic laboratory carries out antifungal activities of a series of acylated D- Mannose derivatives.

M. fakruddin[14] also a research student of organic laboratory carries out antifungal activities of a series of fused pyrimidine derivatives. He used five human pathogenic bacteria *viz. Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureusand* and four pathogenic fungi, *viz. Vibrio mimicus, Vibrio parahemolyticus, Aspergillus niger and panicillum sp.* S. M. Abe Kawsar [15, 16] also a former research student of

organic laboratory carried out in *vitro* antibacterial activities of a series of acylated uridine derivatives.

Recently, our groups synthesized 2-substituted benzofurans[17], isoindolinone and isoquinolinone[18] and tested their antibacterial and antifungal activities. Plants are the natural reservoir of many antimicrobial agents. In recent times, traditional medicine as an alternative form of health care and to overcome microbial resistance has led the researchers to investigate the antimicrobial activity of medicinal plants.

4.1. Materials and methods:

The anti bacterial activities of pyrimidine derivatives were studied against twelve bacteria and the activities of the same compounds were also studied against four fungi. For the detection of antibacterial activities the disc diffusion method[19] was followed.

The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the *in vitro* fungal and bacterial growth. This ability may be estimated by any of the following three methods.

a) Disc diffusion method

b) Serial dilution method

c) Bioautographic method

But there is no standardized method for expressing the results of antimicrobial screening. Some investigators use the diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of microorganisms. However, a great number of factors viz., the extraction

methods, inoculum volume, culture medium composition[19], p^H, and incubation temperature can influence the results.

Among the above mentioned techniques the disc diffusion[19] is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method[20].

4.1a. Principle of disc diffusion method:

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (kanamycin) discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media. The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimetre.

In the present study the crude extracts, fractions as well as some pure compounds were tested for antimicrobial activity by disc diffusion method. The experiment was carried out more than once and the mean of the readings was taken.

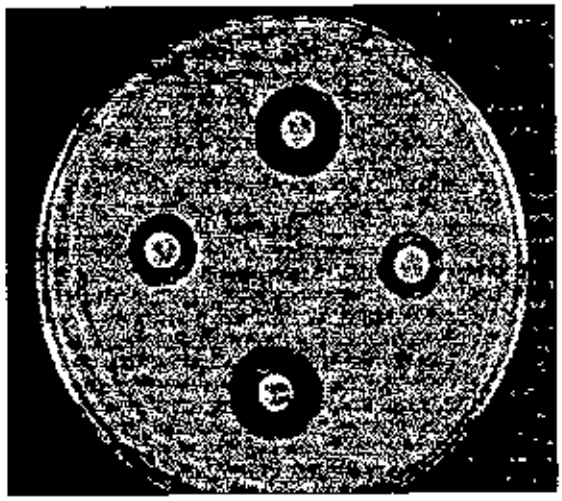


Fig.2: Disc diffusion method

4.2. Experimental:

4.2a. Apparatus and reagents:

Filter paper discs Petri dishes Inoculating loop

Sterile cotton Sterile forceps Spirit burner

Micropipette Screw cap test tubes Nose mask and Hand gloves

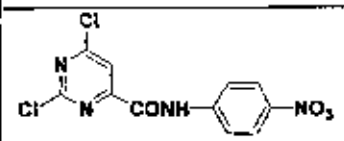
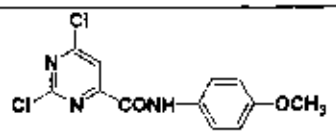
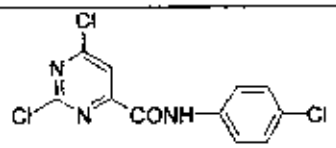
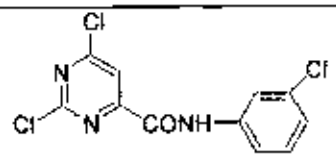
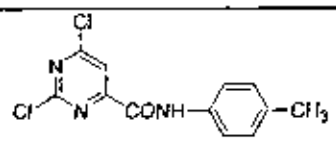
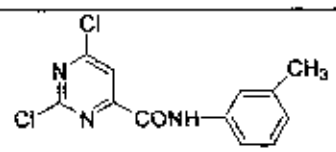
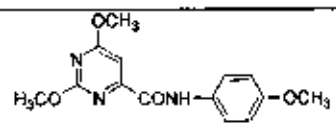
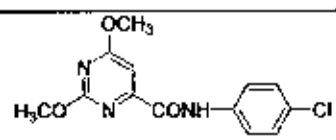
Laminar air flow hood Autoclave Incubator

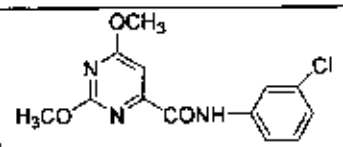
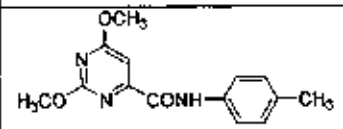
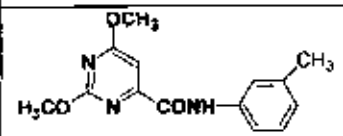
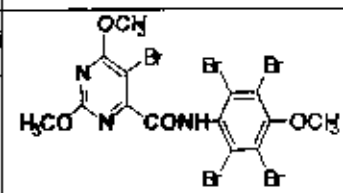
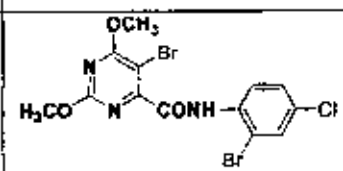
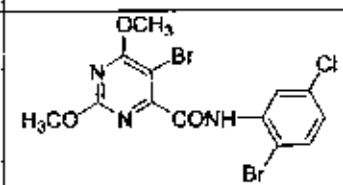
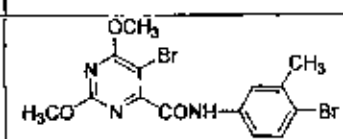
Refrigerator Nutrient agar medium Ethanol

Chloroform

4.2b. Test materials:

Table 4.1a: List of compounds used for antibacterial activities:

Comp. no.	Name of the test chemicals	Structure
9	2,4-Dichloro-6-*p*-nitrophenylamido pyrimidine	
10	2,4-Dichloro-6-*p*-methoxyphenylamido pyrimidine	
11	2,4-Dichloro-6-*p*-chlorophenylamido pyrimidine	
12	2,4-Dichloro-6-*m*-chlorophenylamido pyrimidine	
13	2,4-Dichloro-6-*p*-methylphenylamido pyrimidine	
14	2,4-Dichloro-6-*m*-methylphenylamido pyrimidine	
15	2,4-Dimethoxy-6-*p*-methoxyphenylamido pyrimidine	
16	2,4-Dimethoxy-6-*p*-chlorophenylamido pyrimidine	

17	2,4-Dimethoxy-6-*m*-chlorophenylamido pyrimidine	
18	2,4-Dimethoxy-6-*p*-methylphenylamido pyrimidine	
19	2,4-Dimethoxy-6-*m*-methylphenylamido pyrimidine	
20	2, 4 - Dimethoxy – 5-bromo-6-(4′-methoxy-2′, 3′, 5′, 6′-tetrabromo) phenylamido pyrimidine	
21	2, 4 - Dimethoxy – 5-bromo-6-(2′-bromo-4′-chloro) phenylamido pyrimidine	
22	2, 4 - Dimethoxy – 5-bromo-6-(2′-bromo-5′-chloro) phenylamido pyrimidine	
23	2, 4 - Dimethoxy – 5-bromo-6-(4′-bromo-3′-methyl) phenylamido pyrimidine	

4.2c. Test organisms:

The microbial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram positive and gram-negative organisms were taken for the test and they are listed in the Table 7.1.

Table 4.1b: List of test microorganisms:

Gram positive Bacteria	Gram negative bacteria	Fungi
Bacillus cereus	*Escherichia coli*	*Candida albicans*
Bacillus megaterium	*Pseudomonas aeruginosa*	*Aspergillus niger*
Bacillus subtilis	*Salmonella paratyphi*	*Sacharomyces cerevaceae*
Staphylococcus aureus	*Salmonella typhi*	
Sarcina lutea	*Shigella boydii*	
	Shigella dysenteriae	
	Vibrio mimicus	
	Vibrio parahemolyticus	

The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram positive and gram-negative organisms were taken for the test and they are listed in the Table 5.1.

4.2d. Composition of culture medium:

Nutrient agar medium (DIFCO) was used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

Table 4.2: Composition of nutrient agar medium:

a. Nutrient agar medium

Ingredients	Amounts
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto agar	2.0 gm
Distilled water q.s.	100 ml
pH	7.2-7.6 at 25°C

a. Nutrient broth medium :

Ingredients	Amounts
Bacto beef extract	0.3 gm
Bacto peptone	0.5 gm
Distilled water q.s.	100 ml
pH	7.2 ±0.1 at 250C

b. Muller – Hunton medium:

Ingredients	Amounts
Beef infusion	30 gm
Casamino acid	1.75 gm
Starch	0.15 gm
Bacto agar	1.70 gm
Distilled water q.s.	100 ml
pH	7.3 ±0.2 at 250 C

d. Tryptic soya broth medium (TSB):

Ingredients	Amounts
Bacto tryptone	1.7 gm
Bacto soytone	0.3 gm
Bacto dextrose	0.25 gm
Sodium chloride	0.5 gm
Di potassium hydrogen Phosphate	0.25 gm
Distilled water q.s.	100 ml
pH	7.3 ± 0.2 at 250c

Nutrient agar medium (DIFCO) is the most frequently used and also used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

4.2e. Preparation of medium:

Calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25°C) was adjusted at 7.2-7.6 using NaOH or HCl 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs pressure at 121°C for 20 minutes. The slants were used for making fresh culture of microorganisms that were in turn used for sensitivity study.

4.2f. Sterilization procedures:

To avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were strictly maintained. UV light was switched on

an hour before working in the Laminar Hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs./sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized by UV light.

4.2g. Preparation of subculture:

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37°C for their optimum growth. These fresh cultures were used for the sensitivity test.

4.2h. Preparation of the test plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The microbial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

4.2i. Preparation of discs

Measured amount of each test sample (specified in table 7.4) was dissolved in specific volume of solvent (chloroform or methanol) to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

Standard Kanamycin (30 µg/disc) discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. Blank discs were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

4.2j. Diffusion and incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test microorganisms. The plates were then kept in a refrigerator at 4°C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours.

4.2k. Determination of the zone of inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition.

After incubation, the antimicrobial activity of the test materials was determined by measuring the diameter of the zones of inhibition in millimetre with transparent scale.

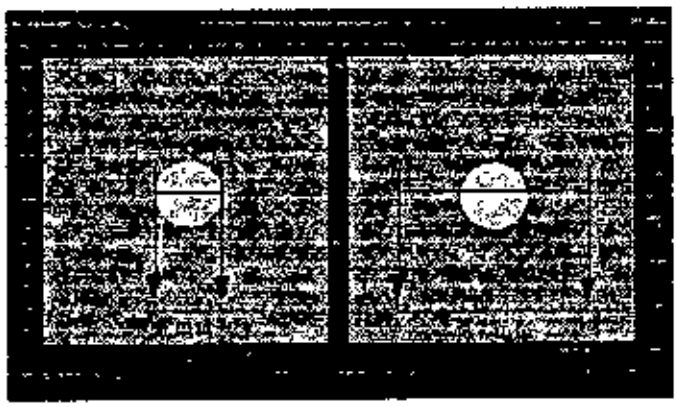


Fig 3: Determination of the zone of inhibition

4.3 RESULTS AND DISCUSSION OF THE TEST SAMPLES:

The antimicrobial activities of new pyrimidine derivatives were examined in the present study. The antibacterial activities of pyrimidine derivatives were studied against thirteen bacteria such as *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Vibrio mimicus, Vibrio parahemolyticus* and the activities of the same compounds were also studied against three fungi such as *Candida albicans, Aspergillus niger, Sacharomyces cerevaceae.*

All compounds were soluble in chloroform and showed mild to moderate inhibitory activity against microbial growth & the average zone of inhibition produced by them 7-14 mm.

The result of the diameter of inhibition zone and percentage of inhibition of microbial growth due to the effect of chemicals, are presented in table 4.3 to table 4.5.

The antibacterial activities were measured in terms of diameters of zone of inhibition in (mm). All experiments were performed thrice to minimize the experimental plus individual errors. The mean value of the diameters

of zone inhibition (M.DIZ) was taken as in disc for determining antimicrobial spectra. Sensitivity test results are in table 4.3 to 4.5 and were compared with a standard antibiotic kanamycin (30-40 µm/disc).

The gram positive and gram negative as well as pathogenic fungi used in the present investigation, three synthesized compounds (**10, 12 and 14**) were found comparatively inhabitant activity against most of the tested organisms, at a dose of 200 µm/disc shown in tables 4.3 and 4.4. There were completely no activities of the synthesized compounds **9, 11, 13, 15, 16, 17, 18** and **19**.

All 5-bromo-6-amido uracil compounds showed mild activity against all tested organisms. Compounds **20, 22** and **23** showed moderate activity against all bacterias, and fungis.

Table 4.3: Antimicrobial activity of test samples 9-13:

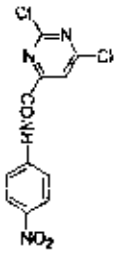
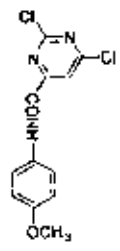
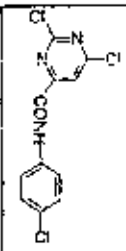
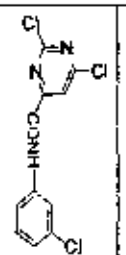
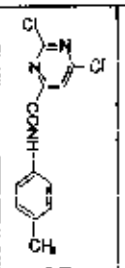
Test microorganisms	Diameter of zone of inhibition (mm)					
	 **9**	 **10**	 **11**	 **12**	 **13**	**KAN**
Gram positive bact.						
Bacillus cereus	-	7	-	7	-	33
Bacillus megaterium	-	7	-	7	-	35
Bacillus subtilis	-	7	-	7	-	35
Staphylococcus aureus	-	7	-	7	-	33
Sarcina lutea	-	7	-	7	-	33
Gram negative bact.						
Escherichia coli	-	7	-	9	-	33
Pseudomonas aeruginosa	-	8	-	8	-	35
Salmonella paratyphi	-	7	-	7	-	33
Salmonella typhi	-	7	-	7	-	32
Shigella boydii	-	7	-	7	-	32
Shigella dysenteriae	-	7	-	7	-	33
Vibrio mimicus	-	7	-	7	-	35
Vibrio parahemolyticus	-	7	-	7	-	33
Fungi						
Candida albicans	-	7	-	7	-	32
Aspergillus niger	-	7	-	7	-	32
Sacharomyces cerevacae	-	7	-	7	-	32

Table 4.4: Antimicrobial activity of test samples of 14-18:

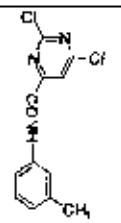
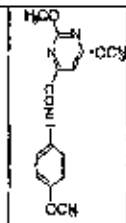
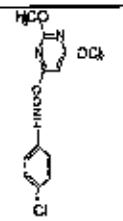
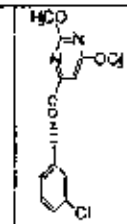
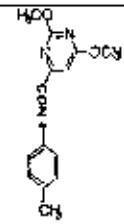
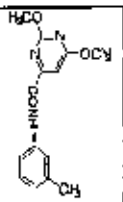
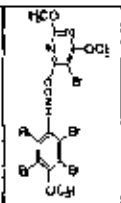
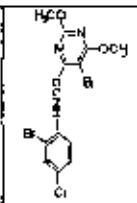
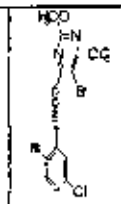
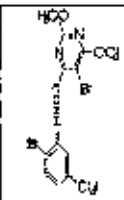
Name of microorganisms	Diameter of zone of inhibition (mm)					
Dose	200µg/ disc	200µg/ disc	200µg/ disc	200µg/ disc	200µg/ disc	Stand 30
	Number of test sample					
	 **14**	 **15**	 **16**	 **17**	 **18**	**KAN**
Gram positive bact.						
Bacillus cereus	7	-	-	-	-	33
Bacillus megaterium	8	-	-	-	-	35
Bacillus subtilis	7	-	-	-	-	35
Staphylococcus aureus	7	-	-	-	-	33
Sarcina lutea	7	-	-	-	-	32
Gram negative bact.						
Escherichia coli	-	-	-	-	-	33
Pseudomonas aeruginosa	7	-	-	-	-	35
Salmonella paratyphi	-	-	-	-	-	33
Salmonella typhi	7	-	-	-	-	32
Shigella boydii	7	-	-	-	-	32
Shigella dysenteriae	9	-	-	-	-	33
Vibrio mimicus	-	-	-	-	-	35
Vibrio parahemolyticus	-	-	-	-	-	33
Fungi						
Candida albicans	-	-	-	-	-	32
Aspergillus niger	9	-	-	-	-	32
Sacharomyces cerevacae	7	-	-	-	-	32

Table 4.5: Antimicrobial activity of test samples of 20-23:

Name of microorganisms	Diameter of zone of inhibition (mm)					
Dose	200µg/ disc	200µg/ disc	200µg/ disc	200µg/ disc	200µ/ disc	Stand 30
	Number of test samle					
	 **19**	 **20**	 **21**	 **22**	 **23**	**KAN**
Gram positive bact.						
Bacillus cereus	-	12	7	12	10	36
Bacillus megaterium	-	12	7	12	12	40
Bacillus subtilis	-	13	9	11	10	40
Staphylococcus aureus	-	12	8	12	10	36
Sarcina lutea	-	12	8	12	10	36
Gram negative bact.						
Escherichia coli	-	13	7	12	11	39
Pseudomonas aeruginosa	-	12	7	11	11	35
Salmonella paratyphi	-	12	9	12	10	35
Salmonella typhi	-	12	8	12	10	37
Shigella boydii	-	12	8	12	10	33
Shigella dysenteriae	-	13	7	12	10	34
Vibrio mimicus	-	13	7	12	10	37
Vibrio parahemolyticus	-	12	9	12	13	38
Fungi						
Candida albicans	-	13	7	12	12	33
Aspergillus niger	-	12	7	12	10	38
Sacharomyces cerevacae	-	12	9	12	10	34

Interpretation of sensitivity test results:

Gram (+) Bacteria:		**Gram (-) bacteria**	
18mm (M.DIZ)	= Sensitive	>16mm (M.DIZ)	= Sensitive
14-18 mm (M.DIZ)	= Intermediate	13-16 mm (M.DIZ)	= Intermediate
>14 mm (M.DIZ)	= resistant	>13 mm (M.DIZ)	= resistant

KAN : Standard kanamycin disc

"-" indicated no sensitivity or zone of inhibition lower than 6 mm.

4.4. Conclusion:

Fifteen synthesized pyrimidine substances have been tested for in antimicrobial activity against five gram-positive and eight gram –negative bacteria as well as three human fungal pathogens. Some of this compound demonstrated mild to moderate antimicrobial activity against most of the test organisms. From these structures we found that the substituted halide causes relatively were microbial growth inhibition.

Among tested compounds, 5-bromo-6-amido uracil derivatives (**20-23**) exhibited relatively greater inhibition of growth of the microorganisms. The highest activity of the compounds (**20, 21, 23 and 24**) could be due to their bromide substitution at C-5 position of uracil ring, which subsequently facilitated the diffusion of the chemical entities through the microbial cell wall.

On the other hand, 2, 4-dichloropyrimidine substances (**9-14**) showed relatively inhibition of growth of the microorganisms to 2, 4-dimethylpyrimidine substances (**15-19**). That's why it can be said chloride group is more active than methyl group against gram positive bacteria, gram negative bacteria and fungi.

4.5. References:

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Chapter 5

Conclusion

Conclusion

- In this thesis, we demonstrated a convenient and facile method for the synthesis of 5-bromo-6-amido uracil from orotic (uracil-6-carboxylic acid) acid.

- *N*-bromosuccinimide in trifluoroacetic acid and trifluoroacetic anhydride was found to be an excellent reagent for the bromination at C-5 position of 2, 4-dimethoxy-6-substituted pyrimidine.

- The most important features of the synthesis are that readily available starting materials are used under relatively mild reaction conditions. Also, no toxic and hazardous compounds are produced by these syntheses.

- A variety of functional groups can be introduced at the C-5 and C-6 positions of the pyrimidine ring by this procedure.

- Finally, all synthesized compounds were tested antibacterial and antifungal activities but 2, 4-Dimethoxy-6-amido-5-bromo-pyrimidines **20-23** showed the highest activities against 13 bacteria and 3 fungi.

- Therefore this methodology could be utilized to synthesize the biologically important uracil derivatives. This method will be attractive to both organic and medicinal chemists.