A Study of the Effect of Deprotonation Process on the Biological Activity of Seme Purine and Pyrimidine Derivatives

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Abstract: This study has been included the synthesis of four new compounds by allylation and tosylation of 2,4- dioxopurine and 5-amino-2,4,6-trioxopyrimidine.

The characterization of the products was done by elemental analysis (C,H,N %) and spectroscopic methods, 'H-NMR, UV and IR in solid state indicates that the deprotonation process has occurred at N H position of the pyrimidine and purine compounds.

The study of the biological activity of the prepared compounds represented is by the determination of their inhibition diameters and minimum inhibitory concentrations. This study shows that the biological activity of the products is not ascribed to the type of substituent group attached to the hetro-nitrogen atom instead of the proton, but it is ascribed to the deprotonation process itself.

Introduction:

The history of N- akylated and N- tosylated - heterocylic compounds, especially nitrogen bases, began in the mid of the last century. A major purpose for the synthesis of nitrogen base derivatives is the development of chemotherapeutic interest 2. Besides that these derivatives have attracted interest in connection with the investigation of carcinogenesis and an access to therapeutic agents such as antiviral pyrimidine, purine and triazole derivatives 4. Most of the known methods for the alkylation or tosylation of an anionic (deprotonated) N- H group have been used 5-9 with nitrogen base derivatives The synthesis of condensed pyrimidine and purine derivatives and their in vitro antimicrobial effects are described. Some of compounds exhibited moderate Gram positive and antifungal activity 10. Tetsuji Kametani et al. 11 reported the N- phthalidyl -5- fluorouracil derivatives have antitumor activity against leukemia Acyclovir [9-(2-hydroxyethoxymethyl)] guanine is considered as the most potent drug for treatment of herpes simplex type 1 and type 22. Several aminopyrimidine derivatives have antimalaria properties such as sulfadiazine, sulfadimthoxine and metachloridine 12.The synthesis and the biological activity of other pyrimidine and purine derivatives is the subject of 13-18 reviews The aim of this study is the achieved of deprotonation process for some pyrimidine and purine compounds and study the effect of the deprotonation on the biological activity of the products.

Experimental Section:

H-NMR spectra are achieved only for Ia, Ib and 2b compounds, Ia and Ib spectra were performed on Hitachi,

pekkin - Eimer NMR Spectrophotometer (60 MHZ) at Mosul University, 2b spectrum was recorded on a LOC ETHZ NMR GEMENI 200 MHZ at Lab Fur organic Chemistry, Zurich University, Swiss. IR spectra were taken on a pye-Unicam Sp3-300 Spectrophotometer. All the spectra were recorded as KBr discs.

Elemental analysis were taken on a Elemental Analyzer, type 1106 (Carlo Erba Stramentazion) at Mosul University. UV spectra were recorded on a PHILIPS - PU 8620 UV/Vis/NIR.

Melting Points were measured on a Gallenkamp melting points apparatus. TLC is performed on silica gel 60 F254 sheet layer (Mereck). Removal of solvents were carried out under vacuum at 30-40°C. Biological activity data were achieved at Labs. of Biological Dept. College of Science. Basrah University.

Preparation of la and 2a Compounds:

A mixture of starting material (3 mmole of 2,4 dioxopurine or 5- amino - 2,4,6 – trioxopyrimidine) and 25 ml of allyl bromide, was dissolved in 30 ml of DMF at 35 °C in the presence of 5 mmole of KOH. The mixture was stirred and reflux for about 125 minutes and thin layer chromatography (T.L.C) test reveals no further reaction The mixture was extracted from ether (60 ml)and water (30 ml). The organic extract was dried with Na2SO4, filtered and evaporated to dryness. The separated allyl product was recrystalized from acetone to obtain colorless crystals..

preparation of 1b and 2b Compounds:

A starting material (2 mmol of 2,4-dioxopurine or 5-amino-2,4,6, -trioxopyrimidine) was added to a stirred solution of p-toluene sulfonyl chloride (0.57 g, 3mmol)in pyridine (30 ml) at room temperature. After the mixture was stirred for 6 hr and the T.L.C showed no further

reaction, the mixture was poured into water (40 ml) and extracted with chloroform(60 ml). Both layers were washed with NaHCO3 solution until the medium becomes neutral. The organic extract was dried with anhydrous Na2SO4, filtered and evaporated. Then residual solid was washed with

n-hexane(50 ml x 2) and recrystalized from ethylacetate to give needly crystals of tosyl product.

Determination of the Biological Activity:

I. Inhibition Diameters: The procedure of the 19 Hahn[1] was used for studies of the products la, 1b , 2a and 2b as inhibitors of Staphylococcus aureas NCTC 6571 (Gr +) and Escherichia coli NCTS 5933(Gr-). The clear zones produced around the disk where growth of test organisms has been prevented by diffusion of the product.

II. Minimum inhibitory concentration (MIC). The MIC of products (la, 1b, 2a and 2b) against a variety of medically important micro - organisms (P.aeroginosa S.aureas, Streptococcus pneumonia and P.vulgaris) was determined according to Finegold and Baron method [20] .

Results and Discussion:

It was noted that the solubility of the products (la, 2a, 1b and 2b)in water was increased especially the tosyl products (1b and 2b) in comparison with the solubility of the starting materials in the same solvent. This factor encouraged factor to us for the study of the biological activity properties of the products.

Table 1 showed that the difference between the found values and expected calculated values for analysis results of carbon, hydrogen and nitrogen elements are situated within the range which confirmed the correctness of suggested

structures of the prepared compounds. The other physical properties of the products are summarized in Table 1. Table 2.1 and figures land 2 of 2a and 2b derivatives of the two types of the products (allyl and tosyl) show IR spectral data of the prepared compounds The spectra of allyl derivatives(la and 2a) showed six bands which ascribed to the stretching vibrations of -NH, CH, aliphatic C-H, C=O, C=N and C-C. The spectra of tosyl derivatives (1b and 2b) showed seven bands which ascribed to the stretching vibrations of -NH, aromatic C-H, aliphatic C-H, CO,C=N, C=C and S=O besides the Para substituted ring. The literature of the IR spectra of many substituted pyrimidines and purines coincide to the absorption data of the prepared compounds 21,16

The ^{1}H-NMR spectral data of three derivatives 1a, 1b and 2b are included in Table 2.2 and their representative spectra are shown in figures 3-5 The spectrum of la derivative showed two signals, the first is multiplet and attributed to the protons of allyl group within the range 3.60-3.90 ppm, the second signal is attributed to the proton of N=C-H (7.25 ppm). The spectra of tosylated derivatives (1b and 2b) consist mainly of two groups of signals, the singlet signal is attributed to the 2CH3 of tosyl group, at 1.9 ppm and 2.00 ppm (1b) and at 2.07 ppm (2b) and the multi-signal of the aromatic protons within the range 6.807.80 ppm(1b) and 7.10-7.45 ppm(2b). The disappearance of the broad peak of the proton bound to hetero-nitrogen atom in the prepared derivatives is encouraged the replacement of of allyl or tosyl group or instead of the proton which attached to the nitrogen atom of the starting materials (2,4-dioxopurine and 5- amino-2,4,6-trioxopyrimidine) .In another meaning, the deprotonation process is occurred. Also we must mention that if D_2O or

DMSO is added to the sample the broad peak will disappear. So that we noticed that the signal of the proton N-9 of 1b derivative is disappearance, because when we used D_2O or DMSO as solvent, the chemical shift of -NH group interferes with the chemical shifts of other groups 22 Also it was noted that the signal of the proton of the N=C-H group of tosyl products is disappeared, because its situation is including with the aromatic protons range.

The electronic absorption data of the investigated derivatives are gathered in Table 2.3 The spectra showed two bands attribute to π-π^* transitions. The appearance of the band II in the spectra of tosyl derivatives (1b and 2b)only is ascribed to the locally excited by π-π^* transitions within the phenyl ring of tosyl group

The biological activity properties of the products and their starting materials are summarized in Table 3. Table 3.1 shows that the inhibition zone diameters of the allyl and tosyl derivatives against standard micro-organisms are much more than of the inhibition zone diameters of the starting materials. We noted that there is no difference in the inhibition zone diameters of allyl derivatives (la and 2a) and tosyl derivatives (1b and 2b). Table 3.2 shows that the minimum inhibitory concentrations (MIC) of the prepared compounds are much less than of the famous antibiotics. For example, la, 2a, 1b and 2b exhibit of MIC of (0.6,0.7, 0.5 and 0.6) µg/ml respectively against K.pnuemonia Whereas the MICs of ampiciline cloxaciline, cephalexin and tetracycline are (3,5, 1.4, 1,8 and 30) µg/ml respectively against the same micro-organism

According to the results of elemental analysis spectroscopic methods and biological activity properties for the prepared compounds, we can fixed the following:-

the deprotonation process is occurred (replacement of allyl or tosyl group instead of the proton) and because of there is no difference in the data of biological activity properties of allyl derivatives (la and 2a) and tosyl derivatives (1b and 2b), thus we concluded that the biological activity of products is not ascribed to the type of group (allyl or tosyl) that attached to the hetro-nitrogen atom instead of the proton but it is ascribed to the deprotonation process itself. Finally we can suggested that the appropriate explanation of the biological activity of the products is included that the deprotonation process induces the ability of the biological activity of the compounds la ,2a, 1b and 2b to stop the action of enzymes which are responsible for the metabolism of bacteria and then followed by the death of bacteria This action may be ascribed to the capacity of pyrimidine and purine derivatives to make blocking of enzyme or to interference of these derivatives with chemical components of the cell of bacteria, such that these derivatives rapidly cross the plasma membrane of the cell and inter the cell to be effected as antimicrobial agents [23].

Conclusions :

1- Using of KOH as powder beside the allyl bromide (reagent) and DMF (solvent) in the preparation of the derivatives la and 2a gives product with high yield.

2- The cause of antimicrobial activity of the products is ascribed to the replacement of allyl or tosyl group instead of the proton of the hetro-nitrogen atom.

3- From the biological activity data, we can use the products as drugs after the achievement of some important studies such as industrial cost.

4- We can use the advantage of the deprotonation process as good method to prepare nucleosides from nitrogen bases by using derivatives of halosugar

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Table 1: Elemental Analysis and Physical Properties of the Products.

produ	Molecular formula	M.Wt g/mole	Elemental Analysis	C	H		Yield %	Rf	m.p °C
1a	$C_{14}H_{16}N_4O_2$	272	cal fou	61.76 61.32	5,88 5.61	20.58 20.86	62	0.74 Toluene:MeOH 8 : 2	171-174 (dec.)
1b	$C_{19}H_{16}N_4O_6S$	460	cal fou	49.56 48.96	3.47 3.14	12,17 12.55	71	0.63 EtOAC: MeOH 6 : 4	222-225
2a	$C_{10}H_{13}N_3O_3$	223	cal fou	53.81 53.33	5.82 5.54	18.83 19.23	52	0.72 Toluene : MeO 7 : 3	>300
2b	$C_{18}H_{17}N_3O_7S$	451	cal fou	47.89 47.32	3.76 3.55	9.31 9.60	67	0.67 EtOAC : MeO 7 :	219-221

R_f : retention factor g : gram m.p : melting point

Table 2: Spectral Data of the Products .

Table 2.1 : IR Spectral Data of the products recorded as KBr (cm^{-1}).

Product	NH Str.	Unsat. C-H Str.	Satu. C-H Str.	C=O Str.	C=N Str.	C=C Str.	S=O Str.	Para subs Ring
1a	----	3080 w 3060 w	2970 s 2930 s 2870 m	1650 s	1625 m	1590 m	-------	--------
1b	3518 s	3017 s	2980 m 2900 m	1710 m	1670 m	1575 m	1180 s	810-870
2a	3275 br.	2815 m	2730 m	1690 w	------	1555 w	----	-----
2b	3520 s	3110 w	2775 w	1690 s 1680 s	1650 w	1550 m 1520 w	1120 s	820-880

Note : in 1a and 2a unsat. C-H is =C-H, but in 1b and 2b is aromatic C-H . Str.= stretching s= strong w= weak m= medium br.= broad

Table 2.2 : ^1H- NMR Spectral Data of the products .

Product	Chemical Shifts (ppm)
1a	Allyl (3.60- 3.90 m) N= C – H (7.25 s)
1b	2 CH_3 (2.00 s) Aromatic protons (6.80- 7.80 m)
2b	2 CH_3 (2.07 s) NH_2 (6.00 s) Aromatic protons (7.10 – 7.45 m)

Table 2.3 : Ultra- Violet Spectral Data of the products .

	10^{-5} M H_2O				10^{-5} M MeOH			
	Band I		Band II		Band I		Band II	
product	λ_{max}	ε_{max}	λ_{max}	ε_{max}	λ_{max}	ε_{max}	λ_{max}	ε_{max}
1a	291	8979	----	----	278	8998	----	----
1b	226	9895	306	10451	221	8009	288	9875
2a	280	7998	-----	----	273	6945	----	----
2b	225	4922	298	10015	219	7998	288	9876

λ_{max} ; maximum wave length in nm .
ε_{max} : molar excitation coefficient in cm^2 $mole^{-1}$.

Table 3 : Biological Activity Data of Starting Materials and the Products .

3.1 : Inhibition diameters (mm) of starting materials and Products anti-standard microorganisms .

compoun	Micro-organisms					
	S.aureas NCTC 6571 Gr (			E.coli NCTS 5933 Gr (-)		
	Conc. of compound (µg/m			Conc. of compound (µg/ml)		
	100	150	200	100	150	200
S.M 1	1.5	2.0	4.5	1.0	1.5	4.0
S.M 2	2.0	3.0	4.0	2.5	3.5	5.0
1a	19	24	27	12	14	16
1b	21	23	26	13	15.5	19
2a	18	21.5	24.5	10.5	12	18
2b	19.5	22	23.5	11	13.5	19.5

S.M : Starting Material

3.2: Minimum inhibitory concentration (MIC) of products .

Micro-organis	MIC (µg/ml)			
	1a	1b	2a	2b
P.aeroginosa NCT 6750	0.6	0.70	0.50	0.60
S.aureas NCTC 6571	0.04	0.05	0.06	0.04
P. aeroginosa (R)	0.8	0.90	0.70	0.50
Streptococcus pneumonia NCTC 6303	0.09	0.10	0.20	0.30
P. vulgaris (R)	0.02	0.01	0.09	0.07

NC : National Collective Type Culture. TC R: Clinical Isolated .

دراسة تأثير انتزاع البروتون على الفعالية البايولوجيه لبعض مشتقات البيورين والبريميدين

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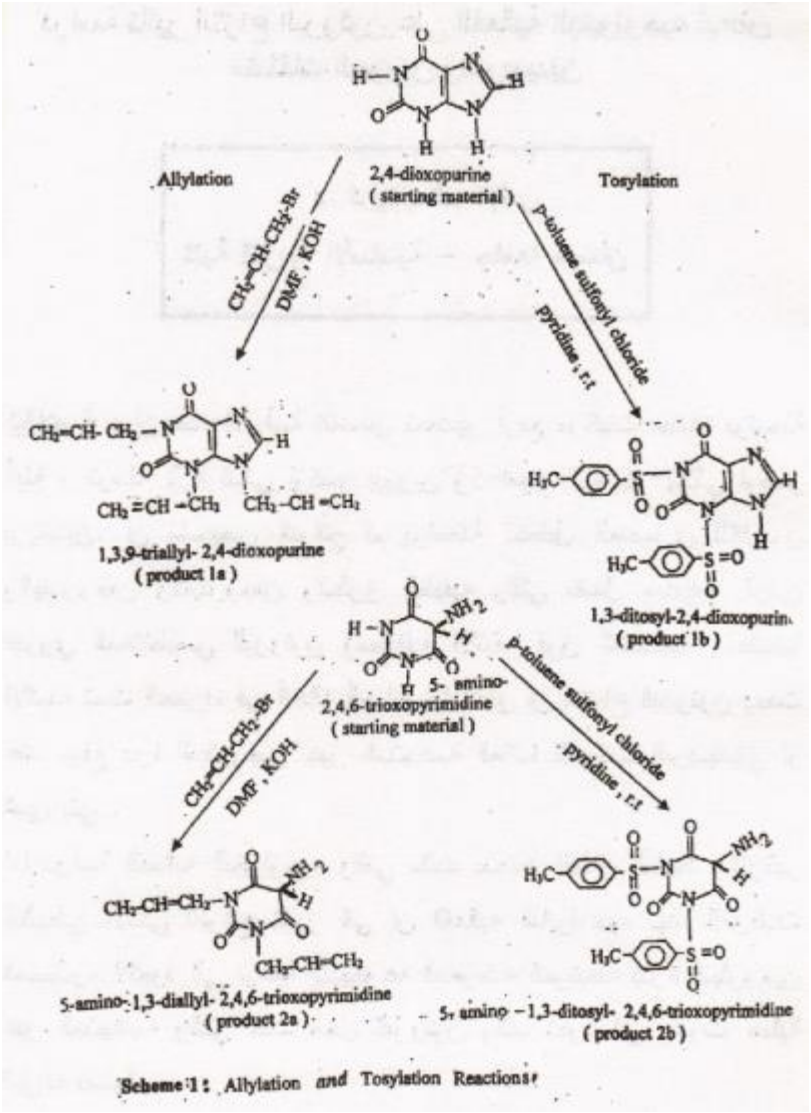
الخلاصة : إن هذه الدراسة تتضمن تحضير أربع مركبات جديدة بواسطة أليلة و توسلة ٤،٢ ثنائي اوكسو بيورين و ٥- امينو -٦،٤،٢ ثلاثي اوكسو بريميدين أن تشخيص النواتج تم بواسطة التحليل العنصري للكاربون والهيدروجين والنيتروجين والطرق الطيفيه والتي تشمل مطيافية الرنين النووي المغناطيسي للبروتون ومطيافية الاشعه فوق البنفسجيه ومطيافية الاشعه تحت الحمراء في ألحاله الصلبه تدل على ان انتزاع البروتون يحدث عند موقع ذرة النيتروجين غير المتجانسة العائدة للمركب البريميديني او البيوريني .

اما دراسة الفعاليه البايولوجيه والتي مثلت بتحديد اقطار التثبيط والتركيز التثبيطي الأدنى للنواتج تشير الى ان الفعاليه البايولوجيه لهذه المركبات المحضره لا تعود إلى نوعية المجموعه المعوضه المرتبطه بذرة النيتروجين غير المتجانسة والتي حلت محل البروتون وانما تعود الى حدوث عملية البرتنه نفسها .



Scheme 1: Allylation and Tosylation Reactions

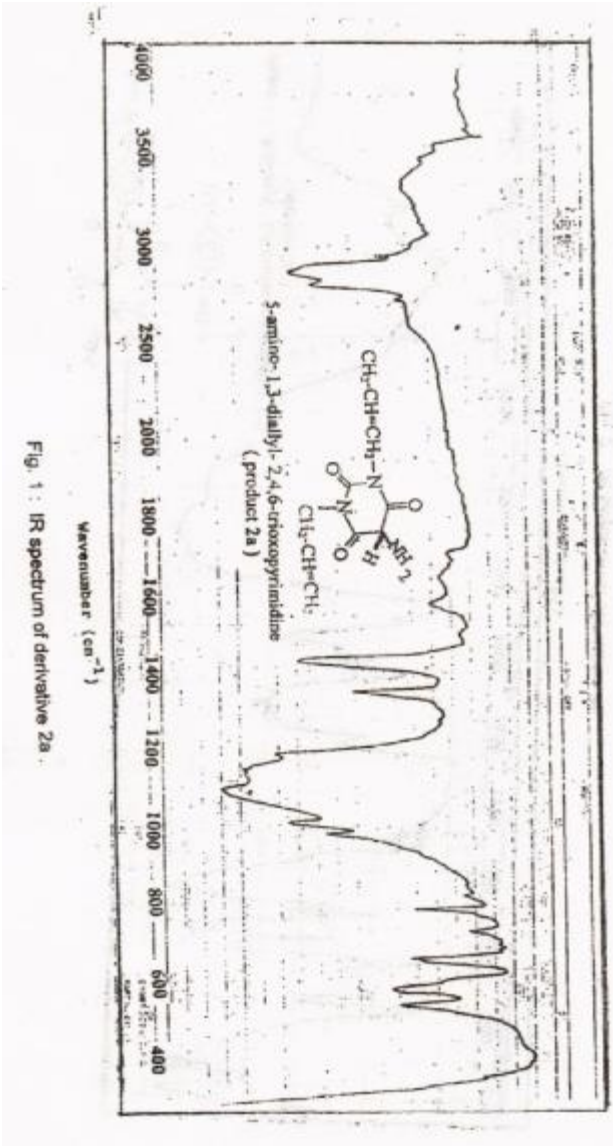


Fig. 1 : IR spectrum of derivative 2a

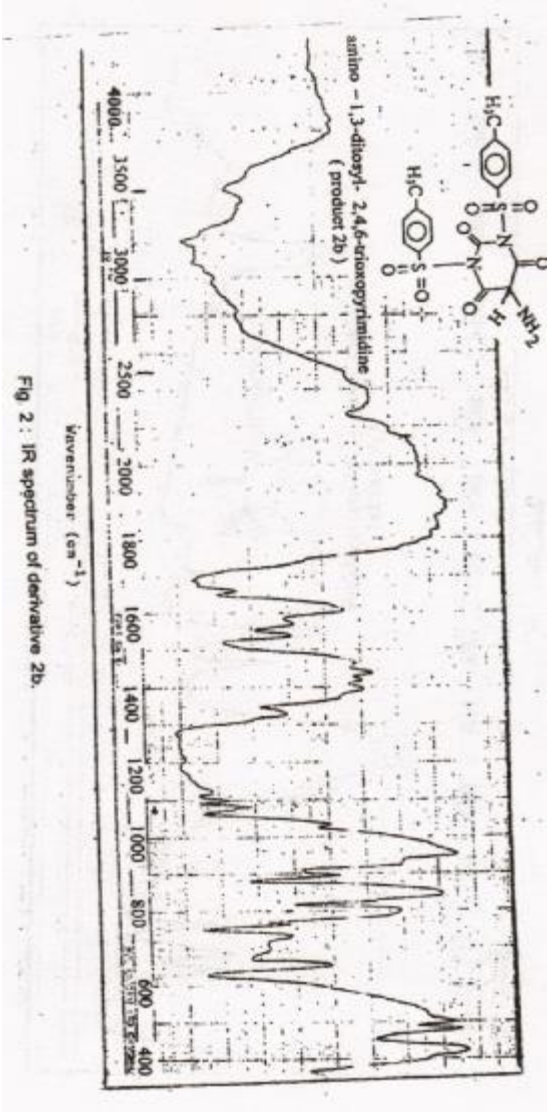


Fig 2 : IR spectrum of derivative 2b.

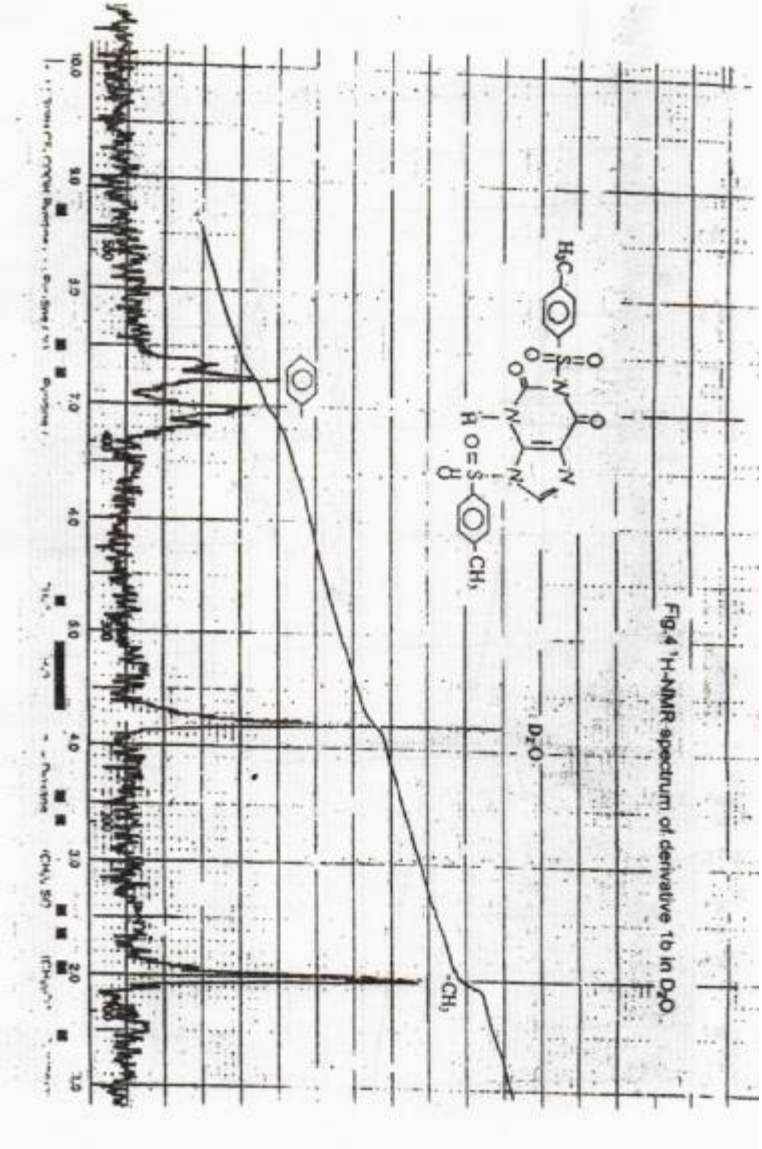


Fig.4 ^{1}H-NMR spectrum of derivative 1b in D_2O

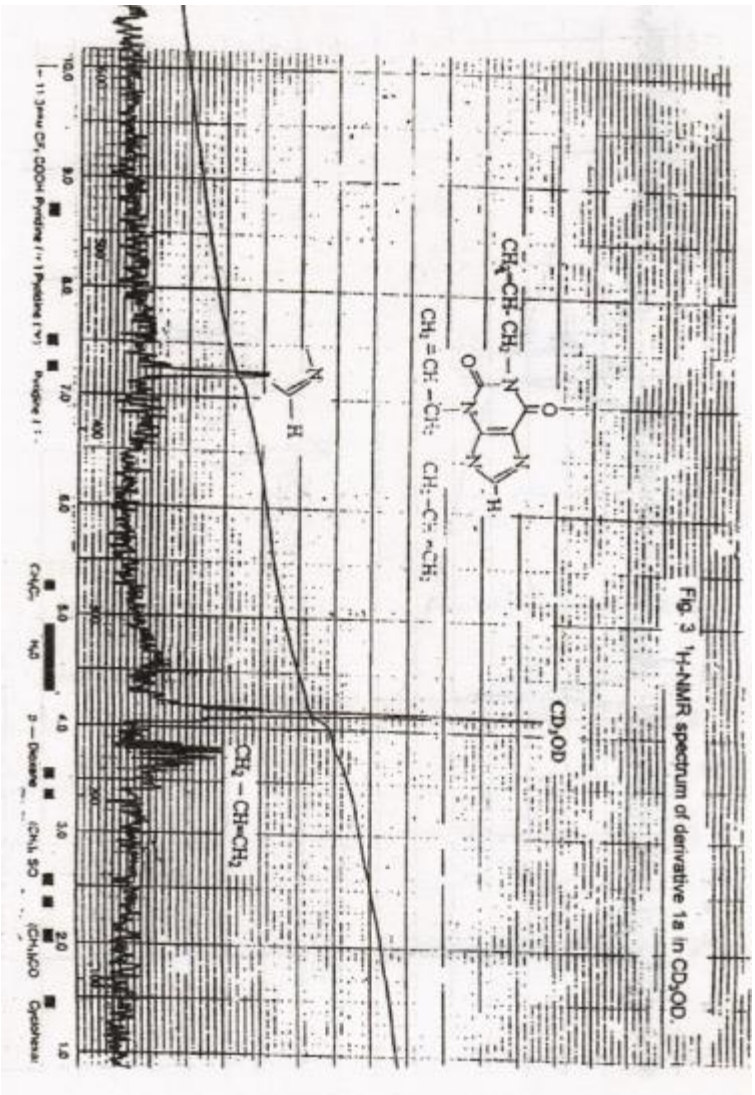


Fig. 3 ^{1}H-NMR spectrum of derivative 1a in CD_3OD.