PREPARATION OF A NEW PYRIMIDONE DERIVATIVE AND STUDY OF ITS ABILITY AS ANTIMICROBIAL AGENT AGAINST GRAM NEGATIVE AND GRAM POSITIVE BACTERIA

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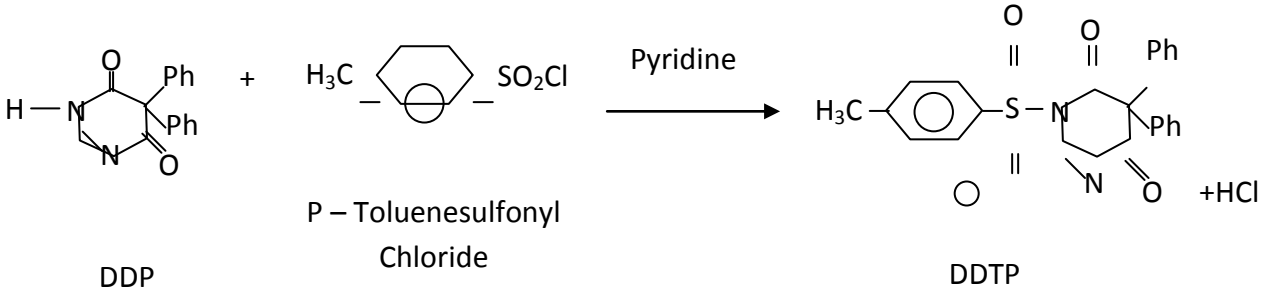
المستخلص:-

البحث يتضمن تحضير المشتق البريميدوني(٥،٥-ثنائي فنيل-٦،٤-ثنائي أوكسو-٣-توسايل بريميدين)(DDTP) من المركب (٥،٥- ثنائي فنيل-٦،٤-اوكسو بريميدين)(DDP) بإستخدام الكاشف بارا-تلوين سلفونايل كلوريد وبوجود البريدين. لوحظ إن الناتج قد عوض بمجموعة التوسايل عند الموقع N-٣ وهذا ماأشارت إليه القياسات الفيزياويه والتحليل العنصري الدقيق والتشخيصات الطيفيه المتمثله بمطيافيات الاشعه فوق البنفسجيه والاشعه تحت الحمراء والرنين النووي المغناطيسي للبروتون. كذلك تضمنت الدراسه تحديد خواص الفعاليه المضاده للمايكروب للمركب الناتج والمتمثله بتحديد أقطار التثبيط والتركيز التثبيطي الادنى والجرعه القاتله الوسطى .

ABSTRACT

In this paper a new pyrimidone derivative (5,5-diphenyl-4, 6-dioxo-3-tosylpyrimidine)(DDTP) has been prepared for 5,5-diphenyl-4, 6-dioxo-pyrimidine (DDP) by using p-toluenesulfonyl chloride and dry pyridine.



DDP

P – Toluenesulfonyl Chloride

DDTP

It was noted that the product has substituted tosyl group at position N-3, as indicated by physical measurements, C, H, N analysis, and UV,IR, and ^{1}H-NMR spectroscopy identifications. Also the work was determined the antimicrobial activity properties of the product, represented by inhibition diameters (IZ.mm), minimum inhibitory concentration(MIC), and median iethal dose (LD_{50}).

INTRODUCTION

A major purpose for the synthesis of nitrogen base derivatives is the development of new compounds of chemotherapeutic interest.[1] The term chemotherapy was introduced by Ehrlich (1909), and it now used in the sense of the treatment of disease due bacteria invasion by chemical compounds which destroy the micro-organisms without affecting the host. It has been found that a given compound is specific in its toxicity towards a particular micro-organisms[2].

The N-alkylation and N-tosylation of nitrogen base derivatives have attracted interest in connection with investigation of carcinogenesis[3] and as 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 with nitrogen base derivatives[5-11]. Some derivatives of nitrogen bases exhibit significant in vitro and vivo, antiviral, antibacterial, and antitumour activity. One of the reasons that nitrogen base derivatives have been studies as potent antimicrobial agents is that these compounds rapidly cross the plasma membrane of the cell by faciliated transport mechanism, thus gaining rapid entry into the cell. This overcomes one of the major barriers for the antiviral agent , which must reach the viral infection within the cell to be effective[1].The relationship between chemical structure and chemotherapeutic action is extremely complicated ,but some progress has been in this field[12]. 5-Fluorouracil is now a valuable drug for treatment of tumors of the breast, colon, or rectum, and to less extend, gastric, hepatic, pancreatic, uterine, ovarian, and bladder carcinomas[13]. The biological activity of nitrogen base derivatives is the subject of reviews[14-21].

The aim of this study is a preparation of new nitrogen base derivative by introduction tosyl group instead of the proton, which may attached to the nitrogen atom (proton replacement). This modification will help us to obtain derivative with high biological activity properties and development of new compound and of chemotherapeutic interest.

EXPERIMENTAL SECTION

GENERAL : Melting point was measured on a Gallenkamp Melting Point Apparatus.Removal of solvents were carried out under vacum at 30- 40 ^{0}C. Elemental analysis was performed at Chemistry Department , College of Science , Mosul University , Iraq . IR spectrum as KBr disc was taken on a Schimadzu IR- 470 Compatras , at Technical Institute of Basrah , Iraq . ^{1}H-NMR specrum of DDTP derivative is performed on a LOC ETHZ NMR GEMENI 200MHz at Lab. Fur Organic Chemistry , Zurich University ,Swiss. UV spectrum is recorded on a PHILIPS- PU 8620 UV/ Vis/ NIR . TLC was performed on silica gel 60 F254 sheet layer (Merck) .

The determination of antimicrobial activity properties was achieved at Lab. Of Biology Department , Basrah University , Iraq .

PREPARATION OF THE DDTP DERIVATIVE

Equimolar amounts of DDP (0.528 , 2 mmole) and recrystallized p-toluenesulfonyl chloride (0.380 , 2 mmole) were dissolved in dry pyridine (15 ml). The reaction mixture was stirred for 3 hr at room temperature and the TLC showed no further reaction. The mixture was extracted from chloroform (60 ml) and water (40 ml). Both layers were washed with sodium bicarbonate solution until the medium become neutral. The organic extract was dried with anhydrous sodium sulfate, filtered and

evaporated .The crude derivative was recrystallized from methanol to obtain needle crystals.

STUDY OF THE ANTIMICROBIAL ACTIVITY PROPERTIES

1- Inhibition Diameter (IZ .mm) : The procedure of Hahn [22] was used for the study of the DDTP derivative as inhibitor of the *Staphylococcus aureas* NCTC 6571 (Gr +) and *Escherischia coli* NCTC 5933 (Gr -) . The clear zone produced around disck where growth of test organism has been prevented by diffusion of the DDTP derivative .

2- Minimum Inhibitory Concentration (MIC) : The MIC of DDTP derivative against a variety of medically important micro-organisms (*S.aureas ,E. coli , P. aeroginosa , K. pneumonia , P. vulgaris , B. subtilis , B.pumulis , P.aeroginosa , S. pneumonia) was* determined according to Finegold and Baron method [23] .

3-**Medium Lethal Dose (LD 50)** : The acute toxicity of the product was measured in male mice (Albino mice of BALB / C) according to Armitage method [24].

RESULTS AND DISCUSSION

The physical properties of DDTP derivative are summarized in Table 1. This Table is appeared that the product is crystalline solid of high melting point . The structure of DDTP derivative was confirmed by its elemental analysis as shown in Table 1 , which showed that difference between the found values and expected calculated values of carbon , hydrogen and nitrogen elements are situated within the range which confirmed the correctness of suggested structure of DDTP derivative .The spectroscopic data of the investigated derivative are gathered in Table 2 . The electronic absorption of the DDTP showed, two bands attribute to $\pi \rightarrow \pi^*$ transitions . The short band at 227 nm (ε_{max} = 7780) is ascribed to the locally excited by $\pi \rightarrow \pi^*$ transitions within the phenyl ring of tosyl group , while the long band at 298 nm (ε_{max} = 6850) could be attributed to the $n \rightarrow \pi^*$ transitions within the heterocyclic ring , two phenyl rings and tosyl residue . Concerning the solvent effect , it is clear that the two bands are blue shift on going from water to methanol as a result of the $n \rightarrow \pi^*$ origin of these bands which is agreement with their assignments . The IR spectrum of the DDTP derivative (KBr disc) is shown in Table 2 and Fig 1 .The absorption data showed seven stretching vibration bands which confirmed the correctness of the proposed structure. These bands are aromatic C-H , aliphatic C-H ,C= O , C= N , C = C ,S=O and para substituted ring .The ^{1}H- NMR spectral data of the investigated (DDTP) are included in Table 2 and Fig 2.[1] H-NMR spectrum of DDTP derivative is characterized by two groups of signals . The aliphatic and the aromatic proton signals . The aliphatic signal is singlet and attributed to the methyl of tosyl group at 2.474 ppm , this signal leads us to say in confidence that the replacement of proton by the tosyl group is carried out . For the aromatic region , the situation is different since the close similarity of the aromatic environments of the aromatic protons makes their resonance crowded within a narrow range of the chemical shift . Inspite

of the formal similarity of the signals , there is a clear difference between the two principal signals of the tosyl group . The first signal is ascribed to the chemical shift value of H1 and H2 at 7.776 ppm . Whereas the second signal is ascribed to the chemical shift value of H3 and H4 at 7.503 ppm . The type of the two principal signals is doublet . The other aromatic protons are appeared within the range 9.990-8.199 ppm .

The results of antimicrobial activity properties are summarized in Table3 . The data of Table 3a confirm that the inhibition zone diameters of the DDTP derivative against standard micro-organisms are much more than of the IZ of the starting material (DDP) .So that we can concluded that the biological activity of DDTP derivative was ascribed to the replacement of the tosyl group instead of the proton in position N- 3 of DDP . The MIC of the DDTP derivative is much less than of the famous antibiotics as shown in Table 3b . Wherease the data of Table 3c are shown that the toxicity of the DDTP derivative is less compared with the famous antibiotics such as tetracycline .

In general the biological activity of the chemotherapeutic drugs is represented by using these compounds as inhibitors of bacterial synthesis in one or more of the following components [25 , 26] : folic acid , peptidoglycan , and protien or as inhibitors of bacterial nucleic acid function in one of two ways :

1- Interaction with DNA and some times RNA templates causing interference with transcription or replication .

2- Interaction with polymerase involved in transcription or replication. So that we can suggested that the appropriate explanation of the biological activity of the DDTP derivative is that the interference of the DDTP (pyrimidine derivative) directly with bacterial DNA replication may effect RNA which then inhibits the protein synthesis [25.]

Table 1 : Physical Properties of DDTP Derivative.

R_f value	Yield %	MP °C	Molecular Formula	Molecular Weight	Elemental Analysis					
					C%		H%		N%	
					Cal.	Fou.	Cal.	Fou.	Cal.	Fou.
0.87 EtOAC:MeOH 3 : 2	76	>300	$C_{23}H_{18}N_2O_4S$	418.451	66.01	66.06	4.33	4.30	6.69	7.72

R_f = Retention factor MP = Melting Point Cal. = Calculate Fou. = Found

Table 2 : Spectroscopic Data of DDTP Derivative.

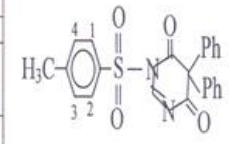
IR Spectral Data		1H-NMR Spectral Data	UV Spectral Data							
Group (str.)	Cm^{-1}	Chemical Shift (ppm) [JHz]	10^{-4} M H_2O				10^{-4} M MeOH			
			Band I		Band II		Band I		Band II	
Aromatic C-H	3064and		λ_{max}	ε_{max}	λ_{max}	ε_{max}	λ_{max}	ε_{max}	λ_{max}	ε_{max}
Alinhatic C-H	2928									
C=O	1742 and 1675		227	7780	298	6850	220	5600	290	6889
C=N	1616									
C=C	1580	CH_3 (2.472 s)								
S=O	1187	H1,H2 (7.776 d) [8.919]								
Para substituted ring	813-838	H3,H4 (7.503 d) [9.240]	M = Molarity MeOH = Methanol							
		Other aromatic protons : (8.990 – 8.199 m)	λ_{max} = Maximum wave length (nm)							
Str. = stretching		d = doublet m = multiplet	ε_{max} = Molar extinction coefficient (cm^2 $mole^{-1}$)							

Table (3): Antimicrobial Activity Results.

3a: Inhibition diameters (mm) of starting material (DDP) and I derivative (DDTP) anti standard micro-organisms.

	Micro-organism

Compound	*Staphylococcus aureas*				*Escherischia coli*			
	Concentration μg/ml				Concentration μg/ml			
	50	100	150	200	50	100	150	200
DDP	1.3	2.8	3.1	4.2	1	3.1	3.6	4.1
DDTP	13.1	17.5	19.5	23	14	16	18.5	21.5

R = Clinical Isolation

3b: Minimum inhibitory concentration (MIC) of DDTP and some famous antibiotics.

Micro-organisms	MIC μg/ml				
	DDTP	Cep	Clo	Amp	Tc
S. aureas NCTC 6571	0.04	0.3	0.1	0.7	0.9
E. coli NCTC 5933	0.02	1.5	0.9	1	1
P. aeroginosa NCTC 6750	0.8	15.5	7	10	30
K. pneumoniae (R)	0.09	1.8	1.5	3.5	30
P. vulgaris (R)	0.02	0.9	1.5	1.5	2
B. subtilis PC1 219	0.04	0.9	1.8	00.9	1.5
B. pumulis NTCT 8241	0.02	0.7	1.5	0.8	1.8
P. aeroginosa (R)	0.7	< 50	40	<50	40
S. pneumonia NTCT 6303	0.09	7	7.3	5.5	30

3c: Median lethal dose (LD_{50})

Compound	LD_{50} μg/kg
DDTP	1250
TC	850

Amp = Ampicillin

Cep = Cephalexin

Clo = Cloxacillin

Tc = Tetracycline

NCTC = National Collective Type culture.

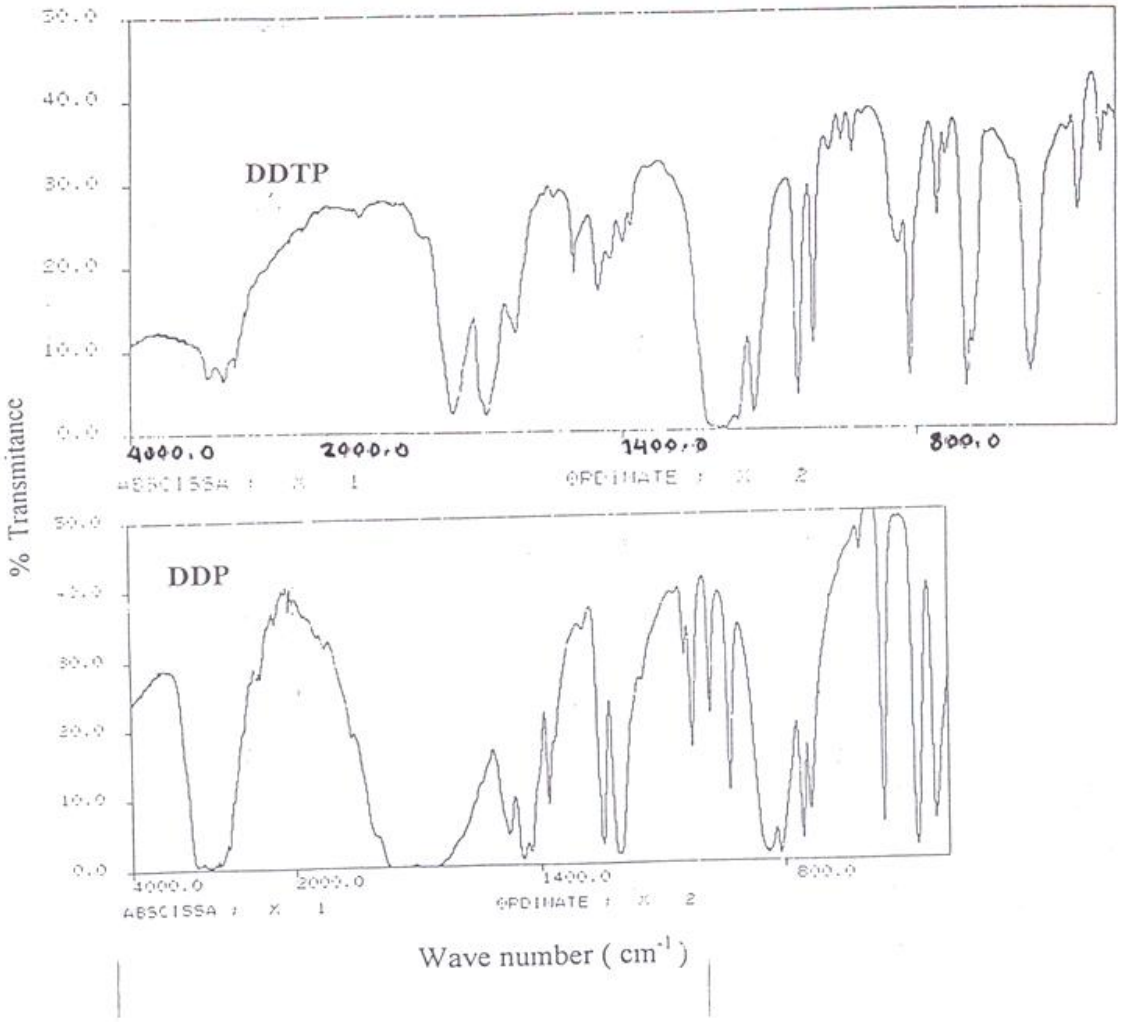


Fig 1:IR spectra of DDTP and DDP.

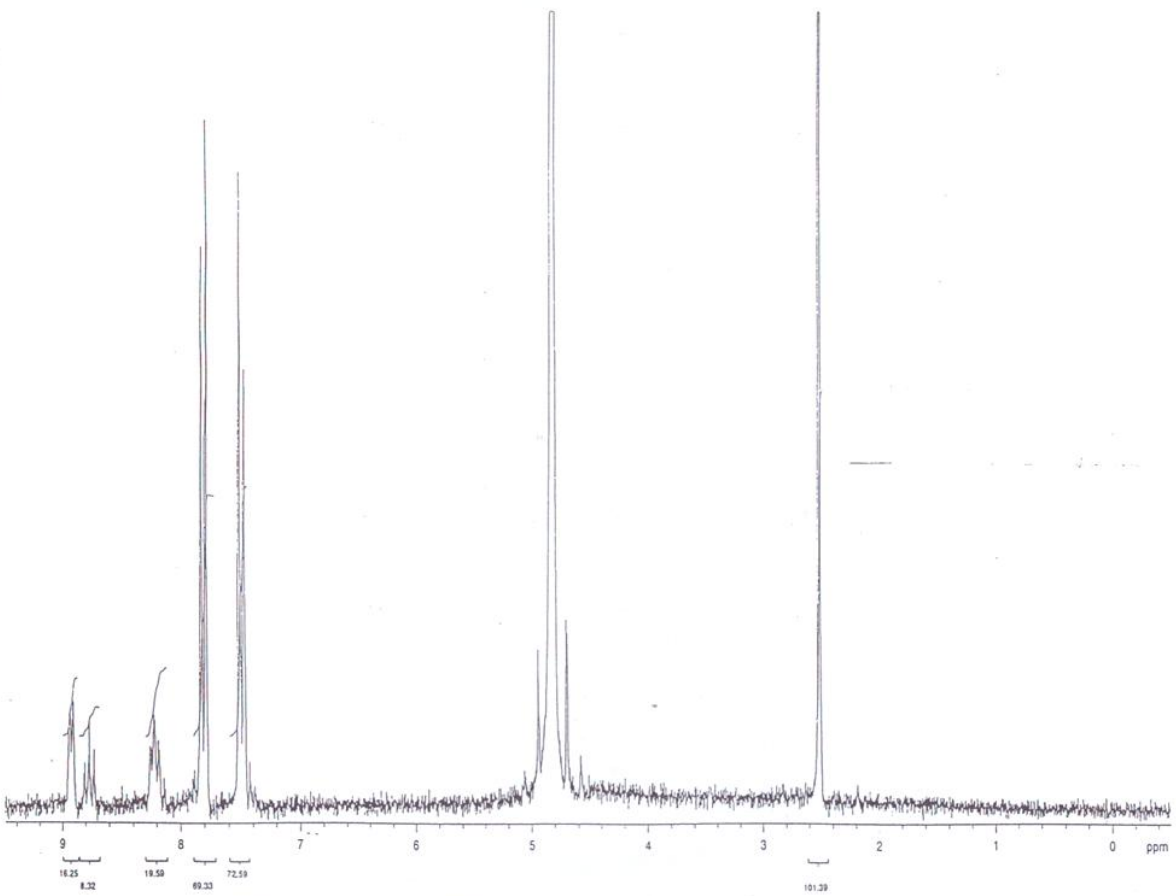


Fig 2 : ^1H – NMR spectrum of compound DDTP in D_2O .

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