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Pharmacology Study of Pyrimidine Derivative

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Abstract: In recent years, pyrazole and pyrimidine derivatives attracted organic chemists due to their widespread potential biological and chemotherapeutic activities. In this study, pyrimidine derivative namely 8-ethyl-6,7-diphenyl-7,8-dihydropteridine-2,4(1*H*,3*H*)-dione was screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereys, Streptococcus*, *Klebsella*, *salmonella* and *Psedumonas* and fungicidal activity against *Aspergillus multi*, *Aspergillus niger*, *Candida albicans, Candidatrobicalis and Candida krusi*. A compound exhibited low antibacterial and no active against fungal species with the reference standard Streptomycin, gentamycin and Nystatin respectively. The toxicity of the compound was also assayed via the determination of their LD_{50} value by using Dixon's up and down method (1980). Studied compound was found to have an LD_{50} of 268.6 mg / kg of body weight.
Key Words: Pyrimidine, Antimicrobial activity, $LD_{50,}$ in vivo, Nystatin.

Introduction

Pyrimidine is an aromatic heterocyclic organic compound similar to pyridine. One of the three diazines, (6-memberd heterocyclic with two nitrogen atoms in the ring), it has the nitrogens at position 1 and 3 in the ring. Fig.1.

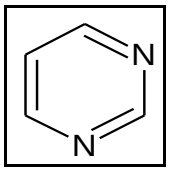
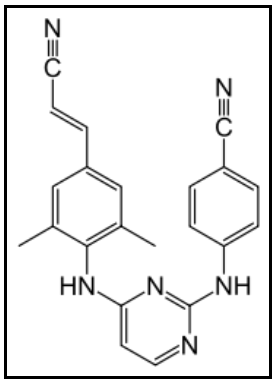
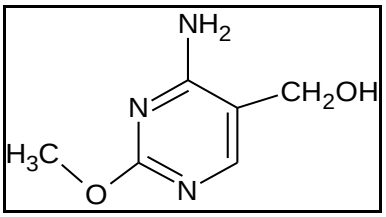


Fig. 1: Pyrimidine

Nitrogen containing heterocyclic ring such as Pyrimidine is a promising structuralmoiety for drug designing. Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological and therapeutical activities [1]. Pyrimidine derivatives have been developed as chemotherapeutic agents and have found wide clinical applications like antiviral [2, 3], antiprotozoal [4], antimicrobial, anti-insecticidal [5-8], anticancer [9-14], antihypertensive [15], antihypoglycemic and hypolipidemic [16], anti-HIV agents[17-19], in addition to their cardiovascular [20],and diuretic [21, 22] properties.

Two diarylpyrimidines (DAPY), rilpivirine (**1**)[23] and etravirine [24, 25] have been classified as non-nucleoside reverse transcriptase inhibitors, whereas bacimethrin (4-amino-5-(hydroxymethyl)-2-methoxypyrimidine)(**2**) (Fig. 2), a pyrimidine antibiotic which is active against several staphylococcal bacteria [26].





1 **2**

Fig. 2. Chemical structures of rilpivirine (1) and bacimethrin (2)

The above-cited applications prompted us to biological study of one of pyrimidine derivative, Fig 3.

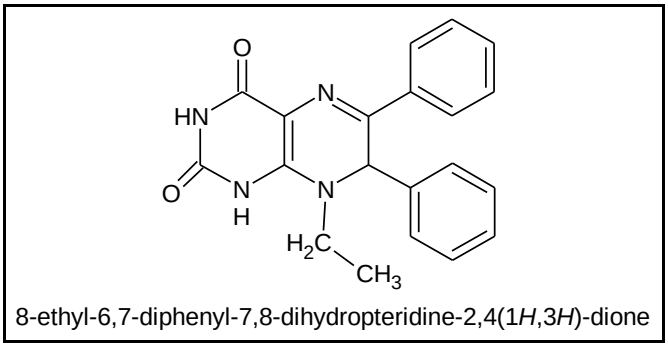


Fig. 3. Chemical structure of Pyrimidine derivative

Pharmacology Study

Materials and methods

Antimicrobial activity

The *invitro* biological screening of the 8-ethyl-6,7-diphenyl-7,8-dihydropteridine-2,4(1*H*,3*H*)-dione was investigated against various bacterial species like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Streptococcus*, *Klebsella*, *salmonella* and *Psedumonas* and fungicidal activity against *Aspergillus multi*, *Aspergillus niger*, *Candida albicans*, *Candida trobicalis and Candida krusi* by well diffusion method using the disc-agar diffusion technique [27]. Muller Hinton agar was used as culture media for antibacterial activity. The antifungal activities were tested against fungus above by diffusion method using. Recommended concentration 100, 200 and 300 µg/ml of the test samples in DMSO solvent was introduced in the respective method. Antibiotic drugs Streptomycin and Gentamycin were used as control for bacteria and Nystatin for fungi, respectively. Petri plates containing 20 ml of Mueller Hinton Agar were used for all the bacteria tested. *Aspergillus multi*, *Aspergillus niger*, *Candida albicans*, *Candida trobicalis and Candida krusi* strains was cultivated in Sabouraud's dextrose agar. Sterile Whatman no.1 filter paper disks (6mm in diameter) impregnated with the solution in DMSO of the test were placed on the Petri plates. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. The plates were incubated for 24 h in the case of bacteria and 72 h for fungi at 28 °C. The inhibition zone diameters were measured in millimeters.

Acute toxicity (LD50)

Animals: All experiments were performed on 10-14- weak old male and female Balb/c mice weighing 22-25 gm at the time of treatment by using up-and-down method, Dixon 1980 [28].

Male and female mice were injected intraperitonially with different doses of the pyrimidine derivative after conducting series of test levels. With equal spacing between doses, a series of trails were carried out using this method: increased dose following a negative response and decreased dose following a positive response.

Testing continued until chosen "nominal" sample size was reached. LD_{50} were determined after reading final result (response-dead (X) or non response alive (O) , then the following equation was applied , LD_{50} = XF + Kd.

The estimate of LD_{50} is XF + Kd, where (XF) is the final test level and (K) is the interval between dose levels. (d) is the tabulated value (Table 1).

D_{50} =X*f*+Kd
LD_{50} = Median Lethal Dose
xf = Last dose used in the experiment
k = Factor of change from the table
d = Difference between doses.

Table (1) Shows Dixon values. Dixon (1980).

	K represented serial tests started with :-				
	OOOO	**OOO**	**OO**	**O**	
OXXX	0.154-	0.154-	0.154-	0.157-	XOOO
OXXO	0.860-	0.860-	0.861-	0.878-	XOOX
OXOX	0.741	0.741	0.747	0.701	XOXO
OXOO	0.182	0.181	0.169	0.084	XOXX
OOXX	0.381	0.380	0.372	0.305	XXOO
OOXO	0.142-	0.144-	0.169	0.305-	XXOX
OOOX	1.549-	1.544	1.500	1.288	XXXO
OOOO	1.000	0.985	0.0897	0.555	XXXX
	XXXX	XXX	XX	X	
	K represented serial tests started with :-				

Results and Discussion

Generally, the pyrimidine derivatives are very interesting compounds since they have been found to have many biological and pharmacological interests. There are also a great number of biologically active nucleoside and nucleobase derivatives with antineoplastic activity. There are many reasons for searching for new agents that will cause less toxicity and which will have much greater therapeutic effects. Consequently, primidine derivative (8-ethyl-6,7-diphenyl-7,8-dihydropteridine-2,4(1*H*,3*H*)-dione) have molecular formula ($C_{20}H_{18}N_4O_2$),with molecular weight of 346.38 gm/mol could be potentially biological active compounds not elaborated in the literature.

So, the results of the antimicrobial activity are shown in Table 2. The studied compound show no activity against *E. coli* and *salmonella*, but low active in *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus*, *Klebsella* and *Psedumonas* at 300 μg/ml. The results of antifungal activity of the compound show not active towards *Aspergillus multi, Aspergillus niger, Candida albicans,Candida trobicalis andCandida krusi* compared with controls, Table 2. The bacteria and fungi were supplied from department of Microbiology, College of Veterinary Medicine, University of Basrah.

Determination of the 50% of lethal dose (LD_{50}) of the studied compound *in vivo* was detected in the mice by using the "up-and-down" procedure described by (Dixon, 1980) [9]. In the experiment we using 10 animals of white mice 10-14 weeks in age, Graded doses of injection to each one animal, a series of concentrations (350, 400, 450, 500) mg/k.g b.w) in 0.1 ml (Dimethyl sulphoxide) DMSO, were administered and chosen with equal spacing (concentrations) between doses. Mortality was recorded after 24 hrs that each one animal treated with one dose and after 24 hrs was recorded as O if the animal lives and then increased the treated dose. While X recorded for the death of animal and then decreased the dose according for the result of the animal the code which formed as being (OOXX) and according for Dixon value was get and the LD_{50} was determined according to the formula employed by Dixon (1980).

D_{50} =X*f* + Kd

LD_{50} = 250 + 0.372 x 50
LD_{50} = 268.6 mg / kg b.w
∴ $^{1}/_{10}$ **LD_{50}**= 26.86 mg / kg
(1 kg = 40 mice Depending on the weight mice 25 gram)**.**
∴ $^{1}/_{10}$ **LD_{50}**= 0.6715 mg /mice Depending on the weight mice 25 gram.

Table (2): Antimicrobial activity of 8-ethyl-6,7-diphenyl-7,8-dihydropteridine-2,4(1*H*,3*H*)-dione Diameter of inhibition zone in mm for different microbial species

Microorganism	100µg/ ml	200µg/ ml	300µg/ ml	S10	CN10	NET30
E. coli	-	-	-	15	2	-
S.aureus	-	-	7	25	20	-
Sreptococcus	-	-	6	19	20	-
Klebsella	-	-	7	25	20	-
Bacillus	-	-	6	15	18	-
Salmonella	-	-	-	15	19	-
Psedumonas	-	-	7	13	8	-
Candida albicans	-	-	-	-	-	12
Candida trobicalis	-	-	-	-	-	9
Candida krusi	-	-	-	-	-	12
Apergillus multi	-	-	-	-	-	13
Aspergillus niger	-	-	-	-	-	15

S10= streptomycin , CN10= gentamycin, NET30= nystatin

Conclusion

In conclusion the present study was, firstly, to investigate in vivo toxic effects and to find acute toxic dose (LD_{50}) of pyrimidine derivative (8-ethyl-6,7-diphenyl-7,8-dihydropteridine-2,4(1*H*,3*H*)-dione) in the first time, which have no shown strong toxicity. And secondly, to investigate in vitro antimicrobial activity, such as, antibacterial and anti fungal activity against some bacterial and fungi in hope to expansion their biological studies in future.

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