TOXICITY AND ANTIMICROBIAL ACTIVITY OF 6-CHLORO-2,4-DIAMINO- PYRIMIDINE

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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 6-Chloro-2,4-diamino pyrimidine was screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cerius, Streptococcus spp, Klebsella- spp* and *salmonella spp* and fungicidal activity against *Aspergillus multi, Aspergillus niger* and *Candida albicans*. A compound exhibited low antibacterial and antifungal activity with the reference standard Streptomycin, Vancomycin 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 518.6 mg / kg of body weight.

INTRODUCTION

Heterocyclic chemistry comprises at least half of all organic chemistry research worldwide. In particular, heterocyclic structures form the basis of many pharmaceutical, agrochemical and veterinary products [1].

There are numerous biologically active molecules with six-membered rings, containing two hetero atoms. Pyrimidine is a heterocyclic aromatic organic compound similar to benzene and pyridine, containing two nitrogen atoms at positions 1 and 3 of the six-member ring. It is isomeric with two other forms of diazine, Fig 1.

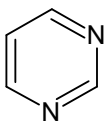
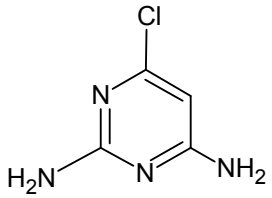


Fig 1 Pyrimidine

Nitrogen containing heterocyclic ring such as Pyrimidine is a promising structural moiety for drug designing. Pyrimidine derivatives form a component in a number of useful drugs and are associated with many biological and therapeutically activities [2]. Pyrimidine and their derivatives are considered to be important for drugs and agricultural chemicals. As pyrimidine is a basic nucleus in DNA & RNA, it has been found to be associated with diverse biological activities. Condensed pyrimidine derivatives have been reported as anti-microbial , analgesic, anti-viral, anti-inflammatory , anti-HIV, anti-tubercular , anti-tumour , anti-neoplastic, anti-malarial, diuretic, cardiovascular agents [3]. Pyrimidine compounds are also used as hypnotic drugs for the nervous system [4], calcium-sensing receptor antagonists [5] and also for antagonists of the human A2A adenosine receptor [6]. Like pyrimidine, coumarin also exhibits diverse biological properties [7, 8].

It was envisaged that these two active pharmacophores, if linked together, would generate novel molecular templates which are likely to exhibit interesting biological properties in animal models. The above-cited applications prompted us to biological study of one of pyrimidine derivative Fig 2.



6-chloropyrimidine-2,4-diamine

Fig 2: Studied compound supplied from Sigma Aldrich for Chemicals Company

MATERIALS AND METHODS

a) Antimicrobial activity

The *invitro* biological screening of the 6-Chloro-2,4-diamino pyrimidine was investigated against various bacterial species like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cerius, Streptococcus ssp., Klebsella ssp.* and *salmonella ssp.* and fungicidal activity against *Aspergillus multi, Aspergillus niger* and *Candida albicans* by well diffusion method using the disc-agar diffusion technique [9]. Muller Hinton agar was used as culture media for antibacterial activity. The antifungal activities were tested against fungus: *Aspergillus multi, Aspergillus niger* and *Candida albicans* by diffusion method using. Recommended concentration 100, 200 and 300 µg/ml of the test samples in Dimethyl sulphoxide (DMSO) solvent was introduced in the respective method. Antibiotic drug Streptomycin and Vancomycin 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* and *Candida*

albicans 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 24h at the case of bacteria and 72 h for fungi at 28°C. The inhibition zone diameters were measured in millimeters.

b) Acute toxicity (LD50)

Animals. All experiments were performed on ten of 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 [10].

Male and female mice were injected intraperitonially with different doses of the 6-Chloro-2,4-diamino pyrimidine 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).

Table (1) Shows Dixon values

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

$\mathbf{D_{50} = Xf + Kd}$

$\mathbf{LD_{50}}$ = Median Lethal Dose

xf = Last dose used in the experiment

k = Factor of change from the table

d = Difference between doses

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 (6-Chloro-2,4- diamino pyrimidine) was supplied from Sigma Aldrich for chemicals company(Germany) have molecular formula ($C_4H_5N_4Cl$),with molecular weight of 144.56 gm/mol and melting point is 199-202^0 C could be potentially biological active compounds not elaborated in the literature.

So, the results of the antimicrobial activity are shown in Table 2. The bacteria and fungi were supplied from department of Microbiology, College of Veterinary Medicine, University of Basrah. It is observed that the activity of compound have the same diameter inhibition zone in the 100, 200 and 300 µg/ml concentration of the solutions. The studied compound show low activity against *E. coli* and *salmonella spp*, but no active in *S. aureus*, *Streptococcus spp*. and *B. cerius*. The studied compound show low activity against *Klebsella spp* in 300 µg/ml. The results of antifungal activity of the compound show low activity towards *Candida albicans* at 300 µg/ml, but not active in *Aspergillus multi* and *Aspergillus niger* compared with controls, Table 2.

Table 2 : Microbial activities of 6-Chloro-2,4-diamino pyrimidine

Diameter of inhibition zone in mm for different microbial species

Microorganism	100µg/ ml	200µg/ ml	300µg/ ml	VA10	S10	NET30
E. coli	7	7	8	9	17	---
S.aureus	Non	Non	Non	20	22	---
Streptococcus	Non	Non	Non	19	20	---
Klebsella	Non	Non	7	Non	19	---
Bacillus	Non	Non	Non	Non	15	---
Salmonella	7	7	7	12	18	---
Candida albicans	Non	Non	7	---	---	12
Aspergillus multi	Non	Non	Non	---	---	13
Aspergillus niger	Non	Non	Non	---	---	15

S10= Streptomycin, VA10= Vancomycin, NET 30= Nystatin

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 [10]. 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.

$$\mathbf{D_{50} = X}f \mathbf{+ Kd}$$

$$\mathbf{LD_{50} = 500} + 0.372 \times 50$$

$$\mathbf{LD_{50}} = 518.6 \text{ mg / kg b.w}$$

$$\therefore {}^{1}/_{10}\ \mathbf{LD_{50}} = 51.86 \text{ mg / kg}$$

(1 kg = 40 mice Depending on the weight mice 25 gram**).**

$$\therefore {}^{1}/_{10}\ \mathbf{LD_{50}} = 1.2965 \text{ mg /mice Depending on the weight mice 25 gram.}$$

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 namely 6-Chloro-2,4-diamino pyrimidine 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.

دراسة السمية والفعالية الحيوية للمركب ٦-كلورو-٢و٤-ثنائي امينو بريميدين

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الخلاصة

في السنوات الأخيرة، اهتم الكيميائيين بمشتقات البايروزولات والبريميدات وذلك بسبب فعاليتها البيولوجية وامكانية استخدامها كعلاج كيميائي على نطاق واسع . في هذا البحث تمت دراسة أحد مشتقات البريميدين وهو مشتق بيريميدين- ٦ - كلورو-٢، ٤ – ثنائي أمين حيث درست فعالية المركب كمضاد بكتيري ضد *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cerius Streptococcus* وكذلك ضد *Klebsella spp.* and *salmonella spp.* انواع من الفطريات وهي: *spergillus multi, Aspergillus niger* and *Candida albicans.*

أظهرت النتائج فعالية قليلة ضد بعض انواع البكتريا والفطريات مقارنة مع مضادات حياتية مرجعية وهي الستربتوميسين، فانكوميسين والنيستاتين على التوالي. كما اختبرت السمية الحادة لتحديد قيمة الجرعة نصف القاتلة LD_{50} باستخدام طريقة ديكسون . وكانت السمية الحادة المميتة LD_{50} هي ٥١٨.٦ ملجم / كجم من وزن الجسم.

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