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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND PHARMACEUTICAL STUDY OF COBALT(II) COMPLEX OF PYRIMIDINE DERIVATIVE

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Abstract

A new cobalt(II) complex of pyrimidine derivative has been prepared from reaction of cobalt chloride and 6-chloro-2-(methylsulfanyl)pyrimidin-4-amine. The synthesized compound was characterized by elemental analysis (CHN), IR and ^{1}HNMR spectroscopy. The new compound was screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus,* and fungicidal activity against *Candida albicans* and *Aspergillus niger*, which exhibited some potent antibacterial and antifungal activity. The toxicity of the synthesized compound was determined using Balb/c mice model. Dixon's up and down method (1980) was found to have an LD_{50} of 192.8 mg/ kg of body weight.

Keywords: Pyrimidine, Antimicrobial, Cobalt complex, LD_{50}, Pharmaceutical study.

Introduction

Pyrimidine is the most important diazine and is essential for any form of life. Since its discovery in 1818 [1]. And its isolation by Gabriel and Colman in 1899[2] there has been a great interest in this heterocyclic system as a component of many biological active substances.

Several pyrimidine derivatives possess pharmacological interest such as antibacterial [3], antifungal [4] , antimalarial [5], anticonvulsant [6], and antitumor [7] activities, on the other hand, some pyrimidine derivatives have shown a remarkable activity as PDE4 inhibitors, antileukemia, bronchodilators, vasodilators, antiallergic, antihypertensive and anticancer agents [8-14]. pyrimidine derived metal ion complexes have been extensively studied in recent years owing to their great variety of biological activity [15]. The recent findings of the presence of metal–sulfur and metal–nitrogen bonds at the active sites of several oxidoreductases such as hydrogenase, xanthine-oxidase and nitrogenase [16,17] have stimulated interest in pyrimidine chemistry [15] with mixed sulfur and nitrogen donor atoms. The coordination of nitrogen atoms in pyrimidine has played an important role for connecting different metals transmitting antiferromagnetic interactions and for obtaining magnetic system of high nuclearity [18].

Jasim Sh. S. *et.al.* were prepared cobalt(II) and some metal ions complexes of pyrimidine derivative by reaction pyrimidine-2-ylimino acetic acid with metal ions [19]. In this paper, the synthesis of new cobalt(II) complex of 6-chloro-2-(methylsulfanyl)pyrimidin-4-amine and study of their biological activity are reported.

Experimental

Physical measurements

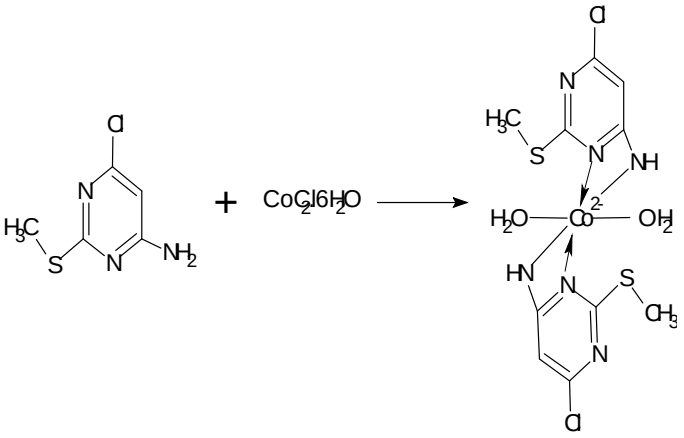
The IR spectra were recorded in the range 4000-200 cm^{-1} on a Pye-Unicam SP3-300 spectrometer using KBr discs at Department of Chemistry, College of Education for pure Sciences, University of Basrah. ^1H NMR spectra were measured on a Brucker at 600 MHz, with TMS as internal reference at Konstanz university, Germany. Microanalysis for carbon, hydrogen and nitrogen were carried out by a Perkin-Elmer 240B Elemental Analyzer (Germany). Melting points were measured by a Philip Harris melting point apparatus and uncorrected.

Synthesis

To a hot solution of 6-chloro-2-(methylsulfanyl)pyrimidin-4-amine (ligand) (0.351g. 2mmole) in (10ml) ethanol, a hot solution of cobalt(II) Chloride hexahydrate (0.238g. 1m mole) in (10 ml) of ethanol was added. The mixture was refluxed for 90 min., filtered off and recrystallized from a hot methanol (10ml), a blue precipitate was obtained.

Yield; 76% , M.P.= 192-194^{0}C. FT-IR (KBr,ν, cm^{-1}): 3370-3250 (NH) and (OH), 2890 (CH-aliphatic), 1640,1580(C=C, C=N), 810 (C-Cl). ^1H NMR (600 MHz,$CDCl_3$, δ, ppm): 7.20 (s,4H, $2NH_2$), 6.43 (s,2H, H-5), 2.31 (s,6H, $2S\text{-}CH_3$). Analytical calculated for $C_{10}H_{14}Cl_2CoN_6O_2S_2$ (444.24): C, 27.03; H, 3.17; N, 18.92. Found: C, 26.62, H, 2.96, N, 18.60.

Scheme 1: Preparation of cobalt complex 6-chloro-2-(methylsulfanyl)pyrimidin-4-amine



Pharmaceutical Study

Antimicrobial activity

The synthesized compound was screened *in vitro* for their antibacterial activity against bacteria: *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cerius,* using the disc-agar diffusion technique [20]. Muller Hinton agar was used as culture media for antibacterial activity. The antifungal activities were tested against fungus: *Candida albicans* and *Aspergillus niger* by diffusion method using. Recommended concentrations 100, 200 and 500 µg/ml of the test samples in DMSO solvent was introduced in the respective method. Petri plates containing 20 ml of Mueller Hinton Agar were used for all the bacteria tested. *Candida albicans* strain 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 was 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 using a caliper vernia.

Acute toxicity (LD_{50}) study

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 [21].

Male and female mice were injected intraperitonially with different doses of the synthesized compound 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 3).

Result and Discussion

The newly synthesized cobalt(II) complex of 6-chloro - 2 - (methylsulfanyl) pyrimidin – 4 - amine $[(Co(L)_2(H_2O)_2]$ is very stable in room temperature in the solid state. Polydentate complex was obtained upon reacting between cobalt ion and pyrimidine derivative (ligand) at 1:2 molar ratio. The new complex compound was confirmed elemental

analysis (CHN), IR and NMR spectral analysis. IR spectra for synthesized compound displayed common features in certain regions and show broad strong bands in the rang 3370-3250 cm^{-1} due to ν(N-H) and (OH) groups. The IR spectra confirm the presence of (C=C,C=N) groups with a sharp region around 1640,1580cm^{-1}, respectively. The IR spectra of compounds show a band at 810 cm^{-1} range can be attributed to (C-Cl) group. In the far-infrared region, ν (Co-Cl) band is observed in the spectra of the $[(Co(L)_2(H_2O)_2]$ at 245 cm^{-1}.

The ^1H NMR spectra of studied compound was recorded in DMSO*d6* solution and show all the expected protons with proper intensity ratio. It is worthy to note that the proton resonance of the NH_2 as a singlet at 7.20 ppm this value is in agreement with previously reported data [22].

The cyclic proton H-5 was appeared 6.34 ppm by singlet signal. The ^1H NMR spectra of prepared characteristic bands in the fingerprint and other regions. The IR spectra of all prepared compound

compound showed a singlet signal at 2.31 ppm due to methyl protons [23].

Antimicrobial activity

The antimicrobial activity of the synthesized compounds was evaluated according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997) using the agar disk diffusion method [24].

The results of the antimicrobial activity are shown in Table 2. It is observed that the activity of compounds increases with an increase in the concentration of the solutions. The antibacterial activity of cobalt complex compound show high activity than pyrimidine derivative (L) especially at concentration 500µg/ml.

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

	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 :-				

LD_{50} =Xf +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 **2**: Antimicrobial activity of the Cobalt(II) complex pyrimidine derivative (Diameter of inhibition zone in mm for different microbial species)

Comp.	**Conc.** µg/ml	***E.coli***	***B.cerus***	***S.aureus***	***C.albicans***	***A.niger***
		100 200 500	100 200 500	100 200 500	100 200 500	100 200 500
Ligand		10 10 10	10 10 12	9 9 14	10 10 11	- - -
Co(II)-Complex		9 12 14	9 12 12	9 10 13	10 10 14	- - -

The results of antifungal activity of ligand(L) and complex compounds were inactive against *Aspergillus niger* and fungicidal activity against *Candida albicans*, Table 2.

Median lethal dose (LD_{50})

The LD_{50} of cobalt (II) complex of pyrimidine derivative was detected in the mice by using the "up-and-down" procedure described by (Dixon, 1980) [21] in the experiment, we using 10 animals of white mice 10-14 weeks in age, Graded doses of complex compound in traperitonial to each one animal, a series of concentrations (50, 100, 150, 200, 250, mg/k.g b.w) in 0.1 ml DMSO were administered and chosen with equal spacing (concentrations) between doses .

Mortality was recorded after 24hrs that each one animal treated with one dose and after 24hrs 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 (XOXO) and according for Dixon value was get and the LD_{50} was determined according to the formula employed by Dixon.

$LD_{50} = Xf + Kd$
$LD_{50} = 200 + (-0.144)50$
$LD_{50} = 192.8$ mg / kg b.w
$^1/_{10}\, LD_{50} = 19.28$mg / kg
(1 kg = 40 mice depending on the weight mice 25-28 gram)**.**
$^1/_{10}\, LD_{50} = 2.09$ mg /mice depending on the weight mice 25 gram

This dose (2.09 mg / mice) solution in 0.1 ml DMSO and given intrapritonial for 1 week.

Conclusion

In conclusion a new complex of Co(II) with pyrimidine derivative was prepared by convenient methods. Spectroscopic characterization of new compounds such as, (CHN) analysis, infra red (IR) and nuclear magnetic resonance (nmr) Spectroscopy were supported the structure of synthesized compounds. The antimicrobial activity was evaluated against three bacterial strains and two fungal species which exhibited some antibacterial and antifungal activities. The metal complex compound has better antibacterial than pyrimidine (L). The toxicity of synthesized compound was reported. It's LD_{50}, calculated by Dixon. Is 192.8 mg/ kg of body weight.

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