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ORGANOMETALLIC COMPLEXES OF CHOLIC ACID & ITS ANTIFUNGAL ACTIVITY

TRIPATHI KISHU*, KUMAR T. SIVA[a]
Institute of Pharmaceutical Sciences & Research Center, Bhagwant University, Ajmer, Rajasthan, India.
[a]Principal , Nandha College of Pharmacy , Tamil Nadu, India.

Cholic acid & metal ions both have antifungal activity , therefore, their organometallic complexes were prepared to have synergistic effect. Cholic acid is one of the lead molecule for preparing organometallic complexes & their complexes were found to have more active antifungal activity.

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Keywords: Cholic acid, Organometallic complexes

1. Introduction

Cholic acid, a main bile acid, is a biosurfactant involved in the digestion of dietary lipids .It is commercially available at low cost. Futhermore , it has an unusual molecular structure with some special characteristics, such as the facial amphiphilicity . The carboxylic acid & three hydroxylic groups can act as synthesis handles. For these reasons cholic acid is a suitable building block for new functional molecules.

Cholic acid, a natural biodetergent has been reported to exhibited antibacterial [11-14], antiviral[5], antifungal [4], antimalarial [10] , antitubercular[10], anticancer[9], spermicidal [2, 3] , antiallergic [6,7,8] etc. Since cholic acid is a suitable building block for new molecules or in other words, it is a lead compound for the development of various compounds, therefore, it is thought worthwhile to select it for the above research work.

The antimicrobial activity of metal chelates was found to be in the order[1]:

$Cd^{II} > Ni^{II} > Mn^{II} > Cu^{II} > Zn^{II} > Co^{II} > Fe^{II}$

Cholic acid is one of the lead molecule for preparing organometallic complexes & their complexes were found to have more active antifungal activity because of synergistic effect of cholic acid as well as metal ions.

2. Experiment

Method for preparation of Organometallic Complexes of Cholic Acid:

The methanolic solution of cholic acid (AR grade) with methanolic solution of inorganic metallic salts were mixed with frequent stirring. If required , refluxed the above mixture on waterbath for an appropriate interval of time. Cooled it, filtered it under vacuum & washed it with water, alcohol & ether. Dried it completely & collected it for further analysis. First physical and then spectral characterization will be performed for its structural elucidation.

*Corresponding author: kishutripathi@rediffmail.com

Table 1:Physical Characterisation of Organometallic Complexes of Cholic Acid.

COMPOUND CODE	MELTING POINT	SOLUBILITY	COLOUR	Rf VALUE
BSN I	260 ºC	DMF,DMSO	Off white	0.723a
CN II	262 ºC	DMF,DMSO	White	0.724a
ZA III	245 ºC	DMF,DMSO	White	0.612a
MCL IV	281 ºC	DMF,DMSO	Off white	0.677a
NA V	258 ºC	DMF,DMSO	Sea green	0.671a
MA VI	265 ºC	DMF,DMSO	Grey	0.672a
CA	198 ºC	DMF,DMSO	White	0.666a

Solvent system for TLC a: Hexane: Ethylacetate : Acetic acid (7:23:3)

UV spectra was recorded by JASCO 7800 UV/VISIBLE spectrophotometer after dissolving few micrograms of the solute in DMSO. The values of λ max : BSN I 202.0 (0.138), CN II 208.8 (0.084), ZA III 212.8 (0.091) , MCL IV 223.0 (0.058) , NA V 202.4 (0.079) , MA VI 217.6 (0.057) , CA 207.2 (0.126).

IR spectras of Organometallic Complexes of Cholic Acid were taken on JASCO 100 IR spectrophotometer.

BSN I : 3000-2500 cm^{-1} absent(O-H(s) of COOH absent), 1725-1700 cm^{-1} absent (C=O(s) of COOH absent),3650-3590 cm^{-1} present(O-H (s) of free alcohol present), 3248.65,3066.73 cm^{-1} present (Bonded OH(s) of COOH present), 1243.91 cm^{-1} present (C-O(s) of alcoholic O-H present), 1187.70-1097.43 cm^{-1} present (C-O(s) of O-H deformation of alcoholic O-H present), 1097.43 cm^{-1} present(Sec., alicyclic 5 or 6-membered C-O of alcohol of free OH present), 1371.18, 1325.33 cm^{-1} present(Free OH (b) present), 1646.08, 1611.87, 1551.21 cm^{-1} present (Carboxylate anion(s) asymmetric present), 1403.37 cm^{-1} present(Carboxylate anion(s) symmetric present).

CN II: 3000-2500 cm^{-1} absent(O-H(s) of COOH absent), 1725-1700 cm^{-1} absent (C=O(s) of COOH absent),3650-3590 cm^{-1} present(O-H (s) of free alcohol present), 3248.65,3066.73 cm^{-1} present (Bonded OH(s) of COOH present), 1243.91 cm^{-1} present (C-O(s) of alcoholic O-H present), 1187.70 cm^{-1} present (C-O(s) of O-H deformation of alcoholic O-H present), 1097.43 cm^{-1} present(Sec., alicyclic 5 or 6-membered C-O of alcohol of free OH present), 1325.33 cm^{-1} present(Free OH (b) present), 1611.87, 1551.21 cm^{-1} present (Carboxylate anion(s) asymmetric present), 1403.37 cm^{-1} present(Carboxylate anion(s) symmetric present).

ZA III: 3000-2500 cm^{-1} absent(O-H(s) of COOH absent), 1725-1700 cm^{-1} absent (C=O(s) of COOH absent),3650-3590 cm^{-1} present(O-H (s) of free alcohol present), 3251. 36,3066.35 cm^{-1} present (Bonded OH(s) of COOH present), 1243.88 cm^{-1} present (C-O(s) of alcoholic O-H present), 1175.22 cm^{-1} present (C-O(s) of O-H deformation of alcoholic O-H present), 1096.68 cm^{-1} present(Sec., alicyclic 5 or 6-membered C-O of alcohol of free OH present), 1325.34 cm^{-1} present(Free OH (b) present), 1646.08 cm^{-1} present (Carboxylate anion(s) asymmetric present), 1399.07 cm^{-1} present(Carboxylate anion(s) symmetric present).

MCL IV: 3000-2500 cm^{-1} absent(O-H(s) of COOH absent), 1725-1700 cm^{-1} absent (C=O(s) of COOH absent), 3650-3590 cm^{-1} present(O-H (s) of free alcohol present), 3247.23,3180.81,3065.93 cm^{-1} present (Bonded OH(s) of COOH present), 1243.35 cm^{-1} present (C-O(s) of alcoholic O-H present), 1187.60,1012.88 cm^{-1} present (C-O(s) of O-H deformation of alcoholic O-H present), 1097.01 cm^{-1}present(Sec., alicyclic 5 or 6-membered C-O of alcohol of free OH present), 1399.07,1369.58,1324.34 cm^{-1} present(Free OH (b) present), 1646.08 cm^{-1} present (Carboxylate anion(s) asymmetric present), 1369.58 cm^{-1} present(Carboxylate anion(s) symmetric present).

NA V: 3000-2500 cm^{-1} absent(O-H(s) of COOH absent), 1725-1700 cm^{-1} absent (C=O(s) of COOH absent), 3650-3590,3414.40 cm^{-1} present(O-H (s) of free alcohol present,3248.40,3065.40 cm^{-1} present (Bonded OH(s) of COOH present), 1240.55 cm^{-1} present (C-O(s) of alcoholic O-H present), 1174.19 cm^{-1} present (C-O(s) of O-H deformation of alcoholic O-H present), 1096.90 cm^{-1} present(Sec., alicyclic 5 or 6-membered C-O of alcohol of free OH present), 1321.65 cm^{-1} present(Free OH (b) present), 1679.26,1614.18,1546.54 cm^{-1} present (Carboxylate anion(s) asymmetric present), 1402.82 cm^{-1} present(Carboxylate anion(s) symmetric present).
MA VI: 3000-2500 cm^{-1} absent(O-H(s) of COOH absent), 1725-1700 cm^{-1} absent (C=O(s) of COOH absent), 3650-3590cm^{-1} present(O-H (s) of free alcohol present, 3251.36,3065.35 cm^{-1} present (Bonded OH(s) of COOH present), 1243.88 cm^{-1} present (C-O(s) of alcoholic O-H present), 1175.22,1013.25 cm^{-1} present (C-O(s) of O-H deformation of alcoholic O-H present), 1096.68 cm^{-1} present(Sec., alicyclic 5 or 6-membered C-O of alcohol of free OH present), 1399.07, 1325.34 cm^{-1} present(Free OH (b) present), 1646.08 cm^{-1} present (Carboxylate anion(s) asymmetric present), 1399.07 cm^{-1} present(Carboxylate anion(s) symmetric present).
CA: 2930.6,2591.3 cm^{-1} present(O-H(s) of COOH present), 1641.3 cm^{-1} present (C=O(s) of COOH present), 3677.8,3653.9,3449.4 cm^{-1}present(O-H (s) of free alcohol present, 3248.40,3065.40 cm^{-1} absent (Bonded OH(s) of COOH absent), 1294.7 cm^{-1} present (C-O(s) of alcoholic O-H present), 1154.2 cm^{-1} present (C-O(s) of O-H deformation of alcoholic O-H present), 1099.00 cm^{-1} present(Sec., alicyclic 5 or 6-membered C-O of alcohol of free OH present), 1336.00 cm^{-1} present(Free OH (b) present), 1614.18,1546.54 cm^{-1} absent (Carboxylate anion(s) asymmetric absent), 1402.82 cm^{-1} absent (Carboxylate anion(s) symmetric absent).
1H NMR of following compounds was taken by JEOL FX 90 Q FOURIER TRANSFORM NMR SPECTROMETER:
For **CA** δ_H (400 MHz, $CDCl_3$): 0.67 (3H,s, 18-CH_3), 0.88(3H,s, 19-CH_3), 0.99(3H,d, 21- CH_3), 2.32-1.02 (24H ,m, aliphatic H), 3.48 (1H,m, 3- CH), 3.83 (1H,bs, 7- CH), 3.97 (1H,bs, 12- CH), 4.03 (2H,d, CH_2), 11.8 (1H,s, COOH).
In all the organometallic complexes of cholic acid, the peak at 11.8 ppm for –COOH was absent which confirms that –COOH group was involved in complexation.

Antifungal activity of organometallic compounds of cholic acid:
Material and Methods:
The antifungal activity of organometallic compounds of cholic acid was based on Poisoned Food Technique [15, 16, 17] and done against *Aspergillus flavus* and *Aspergillus niger* collected from NBRI, Lucknow. The antifungal activity of these compounds was tested by agar plate diffusion method using Ketoconazole as standard and 10, 20 , 50 , 100μg/ml of compounds.

Procedure
The compounds were prepared and tested against *Aspergillus flavus* and *Aspergillus niger*. The one ml of each compound of particular concentration was poured into a Petridishes having about 20-25 ml of molten potato dextrose agar medium. As the medium gets solidify, Petri dishes were inoculated at the centre of plate separately with the fungal isolates and kept at 37°C for 96 hours in BOD incubator. All the values (zone of inhibition, % inhibition) was recorded by known mathematical calculation.
Detection
Antifungal activity of organometallic compounds of cholic acid was measured from zone of inhibition, % inhibition.

Table 2: Antifungal Activity of organometallic compounds of cholic acid.

Compound Code	Concentration (µg/ml)	Aspergillus flavus (dia.mm)	% Inhibition	Aspergillus niger (dia.mm)	% Inhibition
BSN-I	10	0.8	73.3	1.5	25.0
	20	0.6	80.0	1.0	50.0
	50	0.4	86.7	0.6	70.0
	100	0.2	93.3	0.1	95.0
CN-II	10	1.4	50.3	1.4	30.0
	20	1.2	60.0	1.2	40.0
	50	1.0	66.6	0.5	75.0
	100	0.8	73.3	0.1	95.0
ZA-III	10	1.2	60.0	1.5	25.0
	20	0.7	76.6	1.2	40.0
	50	0.5	83.3	0.8	75.0
	100	0.0	96.7	0.5	90.0
MCL-IV	10	1.2	60.0	1.4	30.0
	20	0.7	76.6	1.0	50.0
	50	0.5	83.3	0.8	60.0
	100	0.0	96.7	0.4	80.0
NA-V	10	1.4	53.3	1.5	25.0
	20	1.0	66.6	1.0	50.0
	50	0.7	76.6	0.6	70.0
	100	0.4	86.7	1.0	95.0
MA-VI	10	1.4	53.3	1.0	50.0
	20	1.2	60.0	0.8	60.0
	50	1.0	66.6	0.5	75.0
	100	0.8	73.3	0.2	90.0
CA	10	1.2	60.0	1.0	50.0
	20	1.0	66.6	0.8	60.0
	50	0.8	73.3	0.5	75.0
	100	0.5	83.3	0.2	95.0
Control	100	3.0	-	2.0	-

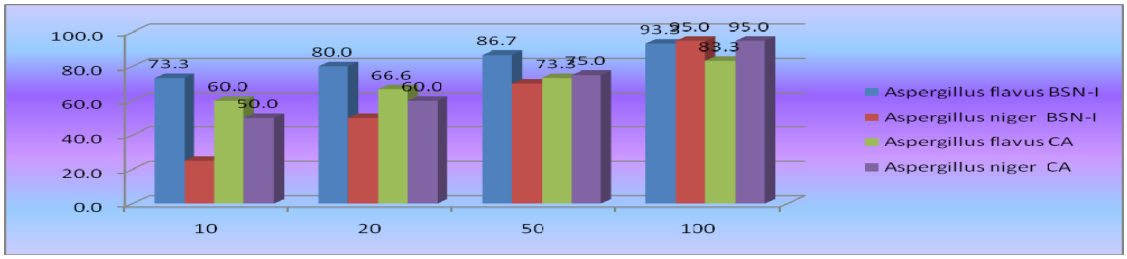

Fig.1. Antifungal Activity of BSN-I

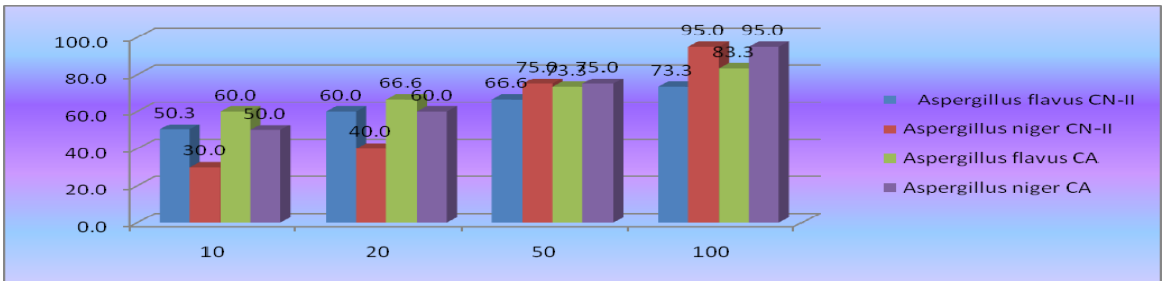

Fig.2. Antifungal Activity of CN-II

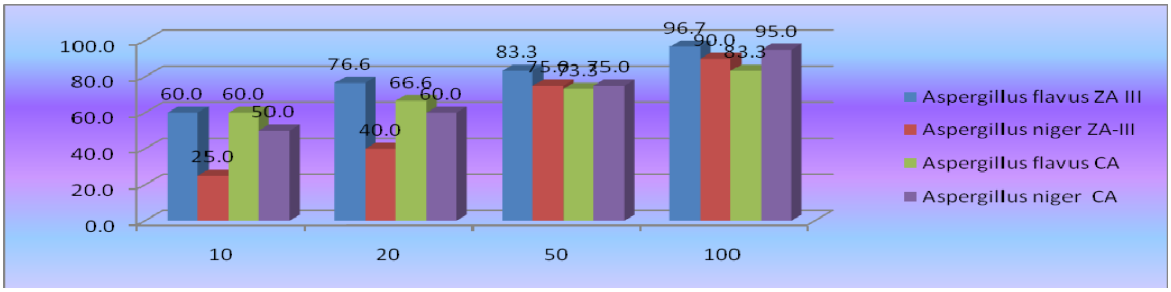

Fig.3. Antifungal Activity of ZA-III

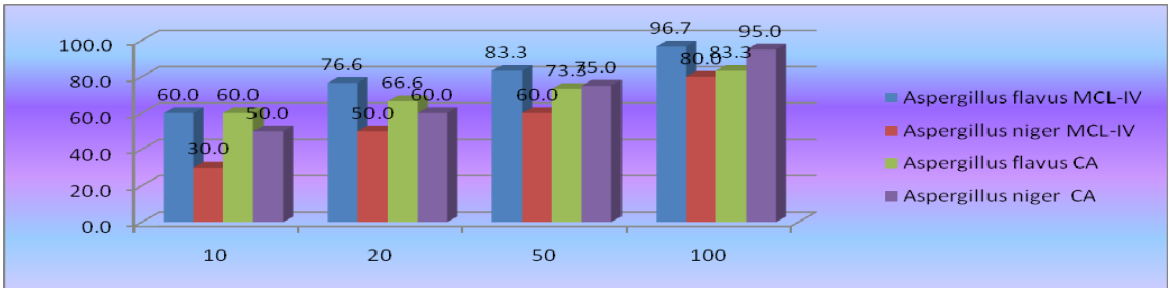

Fig.4. Antifungal Activity of MCL-IV

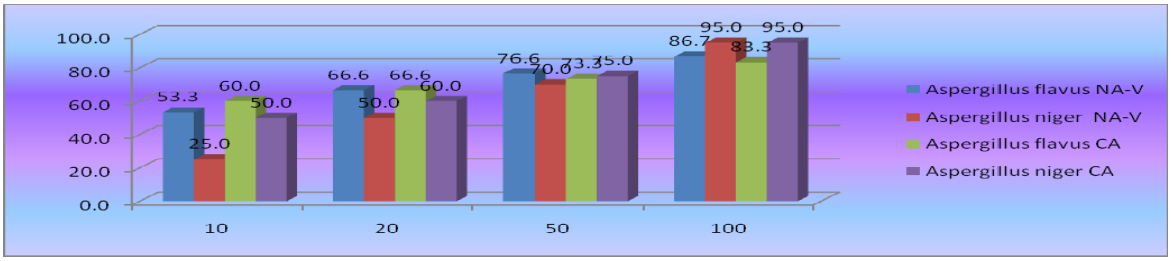

Fig.5. Antifungal Activity of NA-V

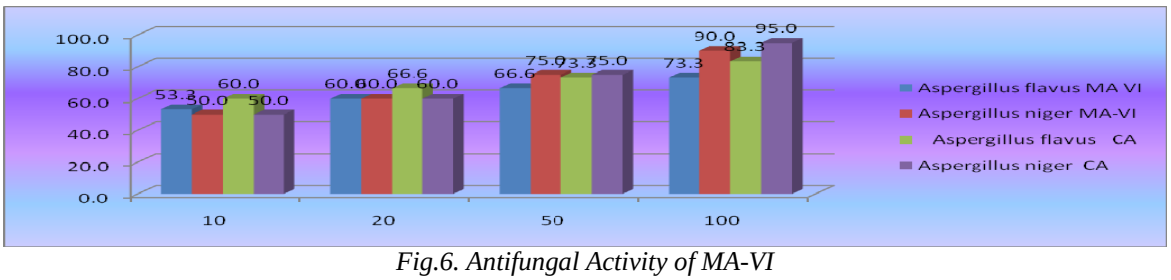

Fig.6. Antifungal Activity of MA-VI

3.Results and discussion

The melting point of CA(cholic acid) was 198 ºC whereas organometallic complexes of CA were found to be more than CA .

Colour of CA is white whereas organometallic complexes of CA were found to be coloured. R_f of CA was 0.666 using Solvent

system for TLC a: Hexane: Ethylacetate : Acetic acid (7:23:3) whereas organometallic complexes of CA were found to be

different. λ max of CA was 207.2 for C=O of COOH whereas organometallic complexes of CA were found to be shifted to higher

or lower due to complexation. In IR Spectra of CA 2930.6,2591.3 cm^{-1} present(O-H(s) of COOH present), 1641.3 cm^{-1} present

(C=O(s) of COOH present) were found to be absent in organometallic complexes of CA , 1614.18,1546.54 cm^{-1} absent

(Carboxylate anion(s) asymmetric absent), 1402.82 cm^{-1} absent (Carboxylate anion(s) symmetric absent), 3248.40,3065.40 cm^{-1}

absent (Bonded OH(s) of COOH absent) were found to be present in organometallic complexes of CA suggesting that COOH

group was involved in complexation. In NMR Spectra all the organometallic complexes of cholic acid, the peak at 11.8 ppm for

–COOH was absent which confirms that –COOHp was involved in complexation. From physical & spectral characterization it

was confirmed that –COOH group of CA was involved in complexation.

% inhibition of **BSN-I** against *Aspergillus flavus* is more than CA i.e cholic acid & is slightly less against *Aspergillus niger*

than CA, at 10 µg/ml,20 µg/ml,50 µg/ml & 100 µg/ml.% inhibition of **CN-II** against *Aspergillus flavus* is slightly less than

CA i.e cholic acid & is more against *Aspergillus niger* than CA acid , at 10 µg/ml,20 µg/ml,50 µg/ml & 100 µg/ml.%

inhibition of **ZA-III** against *Aspergillus flavus* is equal or more than CA i.e cholic acid & is more against *Aspergillus*

niger than CA, at 10 µg/ml, 20 µg/ml,50 µg/ml & 100 µg/ml.% inhibition of **MCL-IV** against *Aspergillus flavus* is equal

or more than CA i.e cholic acid & is more against *Aspergillus niger* than CA,at 10 µg/ml,20 µg/ml,50 µg/ml & 100 µg/ml.

% inhibition of **NA-V** against *Aspergillus flavus* is more as well as less than CA i.e cholic acid at 10 µg/ml and equal or

more at 20 µg/ml ,50 µg/ml & 100 µg/ml & is more against *Aspergillus niger* than CA , at 10 µg/ml,20 µg/ml, 50 µg/ml &

100 µg/ml.% inhibition of **MA-VI** against *Aspergillus flavus* is more than CA i.e cholic acid & is more or equal against

Aspergillus niger than CA, at 10 µg/ml,20 µg/ml,50 µg/ml & 100 µg/ml.

4.Conclusion

Organometallic complexes of Cholic acid exhibits more antifungal activity than Cholic acid alone i.e they exhibit synergistic antifungal activity.

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