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ANTIBACTERIAL ACTIVITY OF 1-METHYL-7-METHOXY-β-CARBOLINE AND ITS PHENACYL AND COUMARINE ANALOGUES

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Antibacterial activity of 1-methyl-7-methoxy-β-carboline (harmaline) and its phenacyl and coumarine analogues 1-(3-nitro-phenyl)-(2-(7-Methoxy-1-methyl-1,3,4,9-tetrahydro-β-carbolin-2-yl)-ethanone(II), 1-(3,4-Dihydroxy-phenyl)-2-(7-methoxy-1-methyl-1,3,4,9-tetrahydro-β-carbolin-2-yl)-ethanone(III) 7-(methoxy-β-carboline),15-24, dehydro(19,20-dimethoxy)coumarine (IV), 7-(methoxy-β-carboline)15-24,dehydro(20-methoxy)coumarine (V) were studied by disc diffusion method. All compounds were tested against three Gram positive and four Gram-negative bacteria. Parent compound showed good activity. All compounds revealed better results against Gram positive as compared to Gram-negative bacteria. 1-(3,4-dihydroxy-phenyl)-2-(7-methoxy-1-methyl-1,3,4,9-tetrahydro-β-carbolin-2-yl)-ethanone (III) was found most potent compound showing broad spectrum activity when compared with all synthesized analogues. Coumarine analogues showed more or less same activity indicating that number and position of methoxy groups are not important regarding antimicrobial activity.

Keywords: Harmaline, β-carboline, antibacterial, phenacyl, coumarine.

INTRODUCTION

The challenge associated with fighting bacterial infections has become an increasingly complex one, partly because of the fast development of resistance to the classic antibiotics and also due to the changing nature of the infections observed in the elderly and other immunocompromised patients. 1-methyl-7-methoxy-β-carboline (harmaline) is an important alkaloid present in plant *Peganum harmala*. Extracts isolated from different parts of the plant has varied application in the folk medicine and used in the treatment of various diseases (Chopra, 1958; Pelletier, 1970 and Dymock, 1980). Harmaline and other beta carboline containing compounds isolated from different sources, and they exhibited antimicrobial activity (Aassila, 2003, Schupp, 2003 and Rao, 1999). In the course of microbial transformation of the antitumor compounds, it has been found that iso-alpha-carbolines and their certain derivatives undergo N-1 methylation by Kitasatosporia setae. The resulting products, iso-alpha-carbolines exhibited antibacterial and antifungal properties (Peczynska-Czoch, 1986]. 2,3-Benzo-alpha-carboline, 7,8-benzo-alpha-carboline and their 4-methyl derivatives were subjected to microbial conversion yielding corresponding benzo-alpha-iso-carbolines. All obtained products showed significant antimicrobial and cytotoxic properties (Peczynska-Czoch, 1987).

MATERIALS AND METHOD

Compounds and solvents

Parent compound; 1-methyl-7-methoxy-β-carboline (harmaline) (I), derivatives (II-V) and DMSO (2%).

Medium

The antimicrobial screening was performed using Muller-Hington Agaar, (MHA). 25 ml dispensed of 25ml give plates with internal diameter of 9 and 15cm respectively. Plates stored at 4-8^{o}C.

Test microorganism

Gram positive, like *Bacillus subtilis, Corneybacterium diphteriae, Staphylococcus aureus* and Gram-negative, like *Escherichia coli, Shigella dysenteriae, Salmonella typhi* and *Klebsiella pneumoniae* were used for antimicrobial activity.

Preparation of inoculum

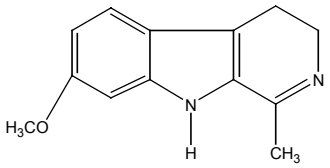
All microorganisms were cultured overnight at 37^{o}C in the trypticase soy broth and used as inoculum. The turbidity of the suspension was adjusted to the McFarland 0.5 turbidity standard.

Antibacterial disc diffusion method and minimum inhibition concentration

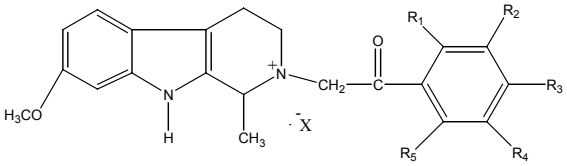
Antibacterial activity of synthesized compounds was investigated by disc diffusion method (Bauer *et al.,* 1966). Parent compound, 1-methyl-7-methoxy-β-carboline (harmaline) (I) and its derivatives (II-V) were dissolved in DMSO (2%) to obtain concentration of 10mg/ml. The solutions (0.02ml) were impregnated on sterile paper disc of 6mm diameter and discs were let to dry to remove any residual solvent which might interfere with the determination. The solvent control (DMSO (2%) did not show any antimicrobial activity).

Seeded agar plates were prepared and inoculated with 0.1ml of inoculum, discs were then placed on the seeded agar plates. Plates were incubated at 35^{o}C for 18-20 hours. The

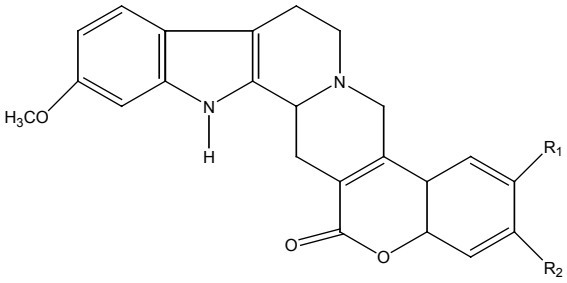
zones of growth inhibition around the disc were measured after 18-20 hours of incubation. Experiment was performed in duplicate.



(I)



(II) R_1=H R_2=NO_2 R_3=H R_4=H R_5=H X=Br
(III) R_1=H R_2=OH R_3=OH R_4=H R_5=H X=Cl



(IV) R_1=OCH_3 R_2=OCH_3
(V) R_1=H R_2=OCH_3

RESULTS AND DISCUSSION

The results of the antimicrobial screening of 1-methyl-7-methoxy-β-carboline (Harmaline) and its analogues (II-V) are shown in table.

The antimicrobial activity was evaluated by disc diffusion method. All compounds were tested against three Gram positive and four Gram- negative bacterial strains.

According to the table parent compound (I) showed good activity against all tested Gram-positive and Gram-negative bacteria. It displayed much more potent activity against *Bacillus subtilis,* and *Klebsiella pneumoniae* when compared with its derivatives.

Encouraging results were obtained when derivatives of harmaline were tested. Compound II, IV and V were found active against all tested Gram-positive microorganisms with more or less same inhibition zones against *Bacillus subtilis,* and *Staphylococcus* while against*, Corneybacterium diphtheriae,* these compounds displayed better results with better Inhibition zones.

Compound III was also found active against all tested Gram-positive bacteria except *Corneybacterium diphtheriae.* This compound showed same or better inhibition zone values when compared to other synthesized analogues.

Interestingly compound III was found inactive against *Corneybacterium diphtheriae* is structurally similar with compound II with only difference of substitution of NO_2 and dihydroxyl groups at the phenyl ring in compound II and III respectively. Results of compound II and III indicating that hydroxyl functional groups making the compound (III) inactive against *Corneybacterium diphtheriae.*

Compound IV and V are also structurally similar compounds and they showed almost same activity indicating that, number of methoxy groups in the compounds are not interfering in the antimicrobial activity.

Regarding the activity of synthesized compounds against Gram negative strains, again compound III was found active against all tested Gram negative microorganisms with good inhibiting zone value. Structurally similar compound II was inactive against *Escherichia coli,* and *Shigella dysenteriae,* while showing good activity against*, Salmonella typhi* and *Klebsiella pneumoniae.* These results indicated that presence of NO_2 group making the compound inactive against *Escherichia coli,* and *Shigella dysenteriae,* while, giving good results against *Salmonella typhi* and *Klebsiella pneumoniae* but not good enough as shown by compound III having hydroxyl functional groups at 3-C and 4-C in phenyl ring, showing that presence of hydroxyl groups are not only responsible for the antimicrobial activity, but also showing good effect indicating by greater inhibition zone.

Compound IV and V again displayed same activity as they were found active against *Escherichia coli,* and *Shigella dysenteriae,* with more or less same inhibition zone and against *Salmonella typhi* and *Klebsiella pneumoniae* both the compounds were found without any antimicrobial activity. Results again suggesting regarding their activity against tested Gram negative microorganisms that number of methoxy groups in the compound playing no role.

Compound III was found effective against all strains except*, Corneybacterium diphteriae.* This compound not only found active but screening clearly indicated that this compound has more powerful activity when compared with other derivatives.

Comparing the activity of derivatives with parent compound it can be seen that derivatives did not show better activity

Table
Antimicrobial activity of 1-methyl-7-methoxy-β-carboline (Harmaline) (I)
and its derivatives (II-V) by disc diffusion method

Microorganisms	Values of diameters of inhibition zones in mm				
Gram positive	I	II	III	IV	V
Bacillus subtilis	20	08	12	8	10
Corneybacterium diphteriae	08	10	-	11	12
Staphylococcus aureus	12	08	12	08	09
Gram negative					
Escherichia coli	10	-	08	10	08
Shigella dysenteriae	08	-	08	08	08
Salmonella typhi	11	10	12	-	-
Klebsiella pneumoniae	17	09	12	-	-

Experiment was performed in duplicate.

than the parent compound and it can be suggested that the position of different functional groups present in phenacyl moiety or the whole phenacyl moiety with parent compound is not playing any role to increase antibacterial activity when compared with parent compound. But when compared two phenacyl derivatives hydroxyl derivative was found better than NO_2 derivative. Coumarins derivatives when compared with parent compound did not show better activity revealing that attachment of coumarine moiety is not making any difference in the antibacterial activity of parent molecule.

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