Trends in Applied Sciences Research

ISSN 1819-3579

Academic
Journals Inc.

www.academicjournals.com

Trends in Applied Sciences Research 1 (5): 529-533, 2006
ISSN 1819-3579

Water Soluble Amide Derivatives of Polyene Antibiotic and their Antifungal Activity

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Abstract: Until recently the incidence of life-threatening fungal infections was considered to be too low to warrant extensive research, however over the last two decades infections caused by fungi have emerged as a growing threat to human health and there are studies indicating that the situation will become worse in the near future. Polyene antibiotics like nystatin and amphotericin B are widely employed for antifungal activities. Nystatin is used to clear candidosis and amphotericin B is useful in the treatment of deep-seated mycoses. Other polyene antibiotics like primaricin, hamycin, trichomycinare and candicidin have been used to a limited extent, for the treatment of localized mycotic infections. However, the polyene antibiotics especially aureofungin are less extensively used for the therapeutic treatment due to their toxicity and particularly less water solubility. The solubility of these antibiotics may play an important role in the enhancement of their therapeutic activity, keeping this view in mind, we have prepared water soluble amide derivatives of aureofungin, seven derivatives have been prepared and six of them were found to be active as antifungal agents when tested against *Candida albicans*. The structures of the derivatives were confirmed by UV and IR spectroscopy. The Minimum Inhibition Concentration (MIC) was found to be 1000 μg mL^{-1}.

Key words: Antifungal, aureofungin, *Candida albicans*, MIC, polyene

Introduction

Over the last thirty years, the frequency of life-threatening fungal infections have increased dramatically, particularly among cancer, diabetic and immunocompromised patients (Zotchev, 2003). As many as 30% of fungal infections are leading to deaths (Ablordeppey *et al*., 1999). Several factors have contributed to this rise: improved recognition and diagnosis of fungal infections; prolonged survival of patients with defects in their host defense mechanisms; more invasive surgical procedures; the use of prosthetic devices and indwelling catheters; increased administration of parenteral nutrition; development of resistance fungal strains to currently available drugs; the increase in the number of patients contracting AIDS and the use of peritoneal dialysis and hemodialysis (Gallis *et al*., 1990).

Polyene antibiotics remains the drug of choice for life-threatening fungal infections (Arathoon, 2001). Among the polyene antibiotics, Amphotericin B (AmB) and Nystatin (Nys), remain the most widely employed for therapeutic purposes for both pre-systemic and systemic fungal infections (Heimenz and Walsh, 1996). Nystatin and amphotericin B are used to clear candidosis (Mandell and Petr, 1996). Deep seated systematic mycoses are treated with amphotericin B and successful results have been obtained in histoplasmosis, coccidiodomycosis, blastomycosis, cryptococcal meningitis and disseminated candidosis (Heimenz and Walsh, 1998). The other polyenes such as pimaricin, hamycin, trichomycin and candicidin have been used to a limited extent for the treatment of localized mycotic infections (Georgopapadakou and Walsh, 1994).

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Utilization of polyene antibiotics implies severe side effects that have restricted their use as antifungal agents, they can cause unpleasant side effects including chills, fever, lowering of blood pressure and even kidney damage (Gulati *et al*., 1998). The side effects of therapy can mimic the clinical appearance of serious systemic infection, complicating patient management.

The major difficulty associated with polyene antibiotics is poor water solubility (Gates and Pinney, 1993) at neutral pH. The toxic effects and poor water solubility limits the use of these class of antibiotic in the therapy. The concentrated efforts are being made to improve the water solubility. Thus, the selective toxicity and increased water solubility is the target of many research methods (Falk *et al*., 1999).

Aureofungin are heptane macrolide antibiotics and they are produced by *Streptomyces cinnamoneus* and possesses excellent antifungal activity. An aureofungin is used to control the growth of fungal infections in plants and various diseases in crops but it cannot be used for clinical purposes in animals due to its toxicity and low water solubility. The chemical modifications of amphotericin to various water soluble derivatives have resulted in low toxicity and it is useful in clinical application (Naik *et al*., 2001). Hence, the aim of the present study was to prepare different amide derivatives of aureofungin and evaluation for their solubility and antifungal activity which will be beneficial for exploring the clinical effectiveness of aureofungin.

Materials and Methods

General

All melting points are uncorrected and reported in °C. All solvents were distilled and dried before use. IR spectra were recorded in (cm^{-1}) on Perkin Elmer FTIR in KBr. The UV spectra was recorded on Schimadzu UV Visible spectrophotometer (UV300).

Silica gel used for TLC was more than 200 mesh. Organic extracts were washed with brine and water successively and dried over anhydrous sodium sulphate before evaporation of solvents. Aureofungin was a generous gift from Hindustan Antibiotics Ltd., Pimpri, Pune.

Preparation of Amide Derivatives of Aureofungin

In general 500 mg (0.44 mM) of aureofungin was suspended in 20 mL of dry DMF, stirred at room temperature and treated with 10 mM of triethyl amine, 10 mM of appropriate amide group containing compound and 10 mM of diphenyl phosphorylazide (DPPA). The course of reaction was followed by means of TLC on silica gel using $CHCl_3$: MeOH: H_2O, 10:6:1 (v/v/v) solvent system. After completion of reaction the crude product was precipitated with 300 mL of dry ether, centrifuged, dissolved in 1-butanol. After evaporation of 1-butanol, the product was again precipitated with dry ether, centrifuged, washed three times with ether and dried in vacuum. The purity of the prepared derivatives (Jarzebski *et al*., 1981) were monitored by TLC.

Reactions Involved in the Synthesis of Amide Derivatives

The carboxyl group of aureofungin was converted to seven different amide derivatives (R' = substituted amines) with use of DPPA in presence of DMF and triethylamine

$$\text{R-COOH} + \text{R'-NH}_2 \xrightarrow[\text{DMF}]{\text{DPPA}} \text{R-CONHR'}$$

R = aureofungin moiety

1) R' = -$NHCONHNH_2$ 2) R' = -$NHNHCONC_5H_5$

3) R' = -$NHCONH_2$ 4) R' = -$NHCONCH_3COCH_3$

5) R' = -$NHCONHCH_2COOH$

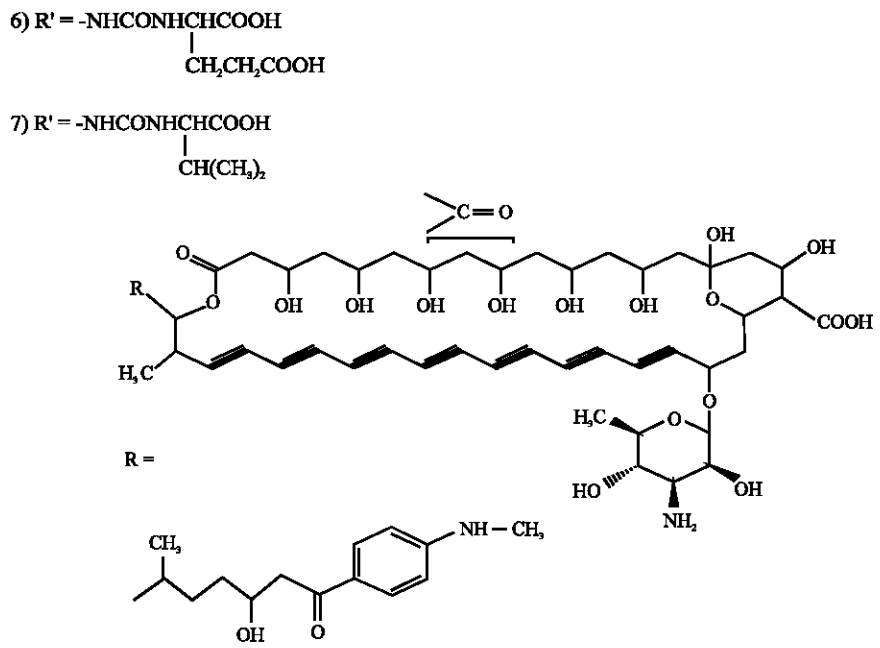

Fig. 1: Structure of aureofungin

Table1: R_f values and water solubility of amides of aureofungin

Compound	R_f values $CHCl_3$: MeOH: H_2O (10:6:1)	Solubility mg mL^{-1} (pH = 7)
1	0.67	0.01
2	0.76	25
3	0.60	5
4	0.59	1
5	0.60	1.9
6	0.74	2
7	0.57	0.02

Table 2: Antifungal activity of amides of aureofungin

Compound	Zone of inhibition (mm) for 1 mg mL^{-1} solution
1	-
2	15
3	14
4	13
5	13
6	15
7	15

Solubility of Amides of Aureofungin in Water at Neutral pH

The solubility was checked for all the derivatives at neutral pH in distilled water. To determine solubility a fix quantity of sample was taken and volume of water increased till clear solution is obtained. The solubility was confirmed by turbidemetric assay. The solubility of amides of aureofungin in mg mL^{-1} is given in Table 1. The successful result for all derivatives were obtained. The enhanced water solubility is observed under same conditions.

Antifungal Activity

An antifungal activity of aureofungin was tested on *Candida albicans*. The antifungal activity was determined by cup and plate method (Bhat *et al*., 2001) and inhibition zones shown by different amide

Table 3: Spectroscopic data of amide derivative of aureofungin

Compound	UV (nm)	IR (cm^{-1})
1	235, 306, 362, 372, 405	3405, 2360, 1666, 1451, 1070, 630
2	230, 270, 338, 362, 375, 406	3389, 2134, 1635, 1457, 1024, 369
3	233, 275, 340, 363, 380, 405	3389, 2134, 2036, 1598, 1401, 1022, 639
4	235, 279, 344, 362, 376, 406	3389, 2149, 2037, 1597, 1404, 1048, 639
5	235, 279, 343, 362, 380, 406	3390, 2135, 2037, 1597, 1404, 1048, 639
6	235, 278, 343, 362, 378, 406	3388, 2150, 2036, 1596, 1406, 1055, 638
7	235, 278, 343, 362, 375, 406	3383, 2077, 1698, 1627, 1428, 1029, 623

derivatives were measured. Each experiment was repeated thrice and the mean values for zone of inhibition is reported in Table 2. The minimum inhibition concentration was 1000 μg mL^{-1}. All the aureofungin derivatives except Amide-1 showed excellent antifungal activity as shown in Table 2.

Results and Discussion

The UV absorption spectra were recorded in distilled methanol. All the amide derivatives showed a sharp band at 405-406 nm as reported for parent compound (May Dean, 1976 ; Deshpande and Narshimachari, 1969). Little variation is observed in the position of other bands, but the spectra maintained the same pattern as reported except for amide-1, where the absorption peak at 380 nm is not well distinguished from other peaks. The absorption peak located at this value indicated the presence of cis double bond in the heptaene chromophore. The intensity of peaks steadily increases from lower to higher values upto 380 nm indicating the presence of cis double bond. Thus, the absorption spectra confirmed the presence of conjugated heptaene of modified aureofungin. The absorption data of amide is given in Table 3. The IR spectra are dominated by broad hydroxyl absorption centered at 3388-3405 cm^{-1} indicating the presence of large number of hydroxyl groups. The carbonyl stretching frequencies are not well resolved due to overlapping of bands. A strong band at 638-639 cm^{-1} is observed for amide derivatives except for amide-1. Several other bands in the region of 1540-1600 cm^{-1} are observed due to conjugated polyene chromophore. Thus, UV and IR spectrums of amide derivatives of aureofungin shows that the structure of parent molecule remain intact during modification.

Aureofungin is insoluble in water at neutral pH. The solubility in water is the main hurdle in the clinical application of these antifungal agents. The newly prepared amide derivatives of aureofungin has resulted in improved biological and physicochemical properties. The solubility of the amide derivatives has increases significantly as represented in the Table 1. The free carboxyl group and amino group of polyene macrolide have no significant activity. The carboxyl group activating agent DPPA is used for the preparation of amide derivatives of aureofungin and successful results were obtained without modifying the other functionalities in the parent molecule. The solubility in water at neutral pH is enhanced for all the amide derivatives of aureofungin. The antifungal activity is retained in all the modified derivatives of aureofungin. It has been observed that the improvement of selective toxicity is related to the absence of a free carboxyl group in the modified antibiotic molecule. Seven amide derivatives of the aureofungin, except amide-1 were found to be active as antifungal agents. The MIC was found to be 1 mg mL^{-1}.

Conclusions

The complex structural and physicochemical properties of the polyene antibiotics offers a great challenge for their clinical use. The solubility of these antibiotics always limits their utility and results either in the toxicity and unwanted effects. The chemical modification of the aureofungin to their amide derivatives has resulted in the increased solubility and even the antifungal activity was

also retained, which indicate that the synthesized amide derivatives will be useful in their actual clinical application. The further toxicity and formulation studies on the amide derivatives will explore their exact clinical role.

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