Physicochemical and Biological Evaluation of Erythromycin Nicotinate, a New Derivative of Erythromycin

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A new water-soluble derivative of erythromycin, erythromycin nicotinate, was prepared and the physicochemical properties and biological activities were evaluated. The derivative has also good solubility in organic solvents. The partition coefficient value indicates its possible good penetration *in vivo*. Antimicrobial potency is 856 μg/mg and *in vitro* antibacterial spectrum is similar to that of erythromycin base. The LD_{50} value of the new derivative in mice by intraperitoneal route is 612 mg/kg. The present study indicates that the new derivative is a potential alternative to the existing water soluble derivatives.

Erythromycin, a remarkably nontoxic macrolide antibiotic, is in clinical practice since its discovery in 1952[1]. It is bitter in taste, slightly soluble in water (2 mg/ml) and unstable at gastric pH[2]. Erythromycin is absorbed adequately from oral administration but absorption is iregular[3]. Attempts have been made from time to time to prepare and evaluate new derivatives of the parent structure, erythromycin, in order to overcome the problems such as solubility, taste, gastric instability associated with the basic structure and optimize absorption[4-18]. In the present study a new water-soluble derivative of erythromycin, erythromycin nicotinate, was prepared and evaluated.

Erythromycin base (a gift sample from Pradeep Drug Company Ltd., Chennai), nicotinic acid (Sigma Aldrich), culture media (Hi Media), brain heart infusion agar (Hi Media) and other materials of pharmaceutical grade were used in the present investigation.

Erythromycin nicotinate was prepared by the method of Dutta and Basu[18,19] by reacting stoichiometric quantities of erythromycin base with nicotinic acid. The derivative, a salt, was recovered by lyophilisation. Melting point, solubility, partition coefficient, optical rotation, pH of aqueous solution, IR and NMR spectroscopic investigation were carried out. The solubility of erythromycin nicotinate in some pharmaceutical solvents by the method of Marsh and Weiss[20], partition coefficient in octanol-water and ether-water solvent systems, optical rotation of 1% w/v solution in 90% v/v ethanol in a polarimeter (Advance Research Institute, Chennai, Model 96034), pH of 1% w/v solution in freshly prepared glass distilled water using a pH meter (Systronics, model 335) were determined. The IR and NMR spectra of the derivative were recorded.

The *in vitro* antimicrobial potency was determined by the method of Grove and Randall[21] using Sarcina lutea ATCC 9341 as test organism. The *in vitro* antibacterial spectrum was determined by agar dilution test[22] using brain heart infusion agar medium and the minimum inhibitory concentrations were calculated.

The acute toxicity test of Litchfield and Wilcoxon[23,24] was used to determine the LD_{50} value of the new derivative. Male Swiss mice weighing between 20-25 g kept on standard pellet food and maintained under standard condition in the central animal house were used. Animals kept on overnight fasting with water *ad libitum* were injected intraperitoneally with a solution of erythromycin nicotinate in propylene glycol-water (1:1) mixture. At each dose level the experimental animals were divided into two groups while one group received the new derivative and the other received vehicle only as control.

The new derivative, erythromycin nicotinate, is a white amorphous powder with bitter taste. Its melting point is 113-

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TABLE 1: SOLUBILITY OF ERYTHROMYCIN DERIVATIVE AND ERYTHROMYCIN BASE AT ROOM TEMPERATURE 30°.

Solvent	Erythromycin nicotinate (mg/ml)	Erythromycin base (mg/ml)
Water	> 20	2.1
Chloroform	> 20	> 20
Propylene glycol	> 20	> 20
Methanol	> 20	> 20
Ethanol	> 20	> 20

115°. The solubility data obtained for erythromycin nicotinate and the base are given in Table 1. The partition coefficient of the derivative in octanol-water and ether-water is 0.816 and 0.644, respectively. The specific rotation computed from the measured optical rotation is -0.142 and -0.588 for erythromycin nicotinate and erythromycin base, respectively. The pH of 1% w/v aqueous solution is 6.54.

The *in vitro* antimicrobial potency of erythromycin nicotinate was found to be 856 μg/mg (assuming base potency as 1000 μg/mg). The *in vitro* antibacterial spectra of erythromycin nicotinate and erythromycin base are given in Table 2. The LD_{50} value of erythromycin nicotinate in mice by intraperitoneal route was found to be 612 mg/kg.

The appearance of an additional band in IR spectroscopy around 1600 cm^{-1} confirms the formation of quaternary ammonium type salt of erythromycin. The four additional peaks in the range of 7.53 to 8.95 ppm in the NMR of erythromycin nicotinate are due to the four protons present in the pyridine ring of nicotinic acid. The IR and NMR spectra confirm the formation of new compound.

The physicochemical properties such as melting point, partition coefficient, pH of 1% w/v solution, specific rotation, IR and NMR spectra are characteristics of the new derivative. Erythromycin nicotinate has good solubility both in water and organic solvents. Thus it is suitable for parenteral administration in the form of an aqueous solution. It has reasonably good partitioning in organic phase of octanol-water and ether-water systems that indicate that it is likely to have good distribution *in vivo*.

The antimicrobial potency of erythromycin nicotinate 856 μg/mg (assuming base potency as 1000 μg/mg) is comparatively higher than the reported potency of many existing derivatives in the market (viz. erythromycin glucoheptonate, USP, 600 μg/mg; sterile erythromycin lactobionate, USP, 525 μg/mg). The *in vitro* antibacterial spectrum of erythromycin nicotinate is similar to those of erythromycin base and the minimum inhibitory concentrations are similar to those of other derivatives reported earher[13-16].

The LD_{50} value of erythromycin nicotinate in mice (612 mg/kg) is well above the therapeutic dose (base equivalent therapeutic dose of eiythromycin nicotinate is 8.34 mg/kg con-esponding to 7.14 mg/kg i.e. 500 mg/70 kg dose of erythromycin base). Thus the toxicity is found to be lower and the toxicity due to over dose, if any, is unlikely to be serious.

TABLE 2: *IN VITRO* MINIMUM INHIBITORY CONCENTRATION.

Organism	MIC (μg/ml)	
	Erythromycin base	*Erythromycin nicotinate
Bacillus pumilus NCIM 2327	0.3	0.3
Streptococcus faecalis NCIM 2080	0.2	0.1
Bacillus Subtilis NCIM 2063	0.1	0.1
Pseudomonas aeruginosa NCIM 2036	0.5	0.5
Escherichia coli NCIM 2065	10	50
Staphylococcus aureus NCIM 2079	0.5	0.5
Proteus mirabilis NCIM 2387	> 100	> 100

* Base equivalent concentration, NCIM stands for national collection of industrial microogranisms, and MIC represents minimum inhibitory concentration.

The physicochemical and biological evaluation of erythromycin nicotinate showed promising results for potential use and further studies on animals and human beings be undertaken to prove its suitability for clinical use.

REFERENCES

1. Laurence, D.R. and Bennett, P.N., In; Clinical Pharmacology, 6th Edn., ELBS, Edinburgh, 1987, 230.
2. Garrod, L.P., Lambert, H.P. and Grady, P.O., In; Antibiotics and Chemotherapy, 4th Edn, Churchill Livingstone, Edinburgh, 1973.
3. Josselyn, L.E. and Sylvester, J.C., **Antibiot. Chemother.,** 1952, 3, 63.
4. Kirby, W.M.M., Maple, F.M. and Leary, B.O., **Antibiot. Chemother.,** 1953, 3, 437.
5. Griffith, R.S. **Antibiot. Anna.,** 1955, 269.
6. Walasek, O.F., **U.S. patent No.** 2,881,163,1959.
7. Stephens, V.C., Conine, J.W. and Murphy, H.W., **J. Amer. Pharm. Assn.,** 1959, 48, 620.
8. Bray and Stephens, **U.S. patent No.** 3,000, 874, 1961.
9. Shepher, J.T., **U.S. patent No.** 2,852, 429, 1958.
10. Hoffhine, C.E. (Jr.), **U.S. patent No.** 2,761, 859, 1956.
11. Stephens, V.C., **U.S. patent No.** 2,993, 833, 1961.
12. Stephens, V.C. and Conine, J.W., **Antibiot. Ann.,** 1958-1959, 346.
13. Goswami, B.B., Manna, P.K. and Basu, S.K., **Arzneim. Forsch.,** 1995, 45, 813.
14. Manna, P.K., Basu, S.K. and Goswami, B.B., **Drug Develop. Ind. Pharm.,** 1995, 21, 2097.
15. Mohanta, G.P. and Basu, S.K., **Indian Drugs,** 1995, 32, 190.
16. Manna, P.K. and Basu, S.K., **Drug Develop. Ind. Pharm.,** 1998, 24, 879.
17. Manna, P.K. and Basu, S.K., **Drug Develop. Ind. Pharm.,** 1999, 25, 391.
18. Dutta, S.K. and Basu, S.K., **Indian patent No.** 142, 584, 1977.
19. Dutta, S.K. and Basu, S.K., **U.S. patent No.** 4,137, 379,1979.
20. Marsh, J.R. and Weiss, P.G., **J. Assoc. Anal. Chem.,** 1967, 50, 457.
21. Grove, D.C. and Randall, W.A., In; Assay Methods of Antibiotics-A laboratoly Manual, Medical Encyclopedia, New York, 1955.
22. Hale, L.J. and Inkely, G.W., **Lab. Pract.,** 1965, 14, 452.
23. Litchfield, J.T. (Jr). and Wilcoxon, F., **J. Pharmacol. Expt. Ther.,** 1949, 96, 99.
24. Ghosh, M.N., In; Fundamental of Experimental Pharmacology, Scientific Book Agency, Calcutta, 1971, 112.

Spectrophotometric Estimation of Itraconazole in Pharmaceutical Formulations

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Two simple spectrophotometric methods (A and B) have been developed for the determination of itraconazole in pure and in its pharmaceutical formulations. Method A is based on the formation of blood red colored complex with ferric chloride and 1,10-phenanthroline having absorption maximum at 520 nm, where as in method B, itraconazole forms a green colored complex with ferric chloride and MBTH reagent exhibiting maximum absorption at 630 nm . The chromogens obey Beer's law in the concentration ranges of 1.2 to 7.5 μg/ml and 2.5 to 20 μg/ml for methods A and B, respectively. The results obtained are reproducible and are statistically validated.

Itraconazole (ITCZ) is a broad-spectrum triazole antifungal agent used to treat fungal infections. It acts by inhibiting fungal cytochrome P-450 and sterol C-14 α-demethylation that results in inhibition of ergosterol synthesis, and chemically it is 4-[4-[4-[4-[(2-(2A-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-ylmethoxy]-phenyl]-1-piperazinyl] phenyl]-2,4-dihydro-2-(1-

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