

Antibacterial Activity of Synthetic Precursors of Podophyllotoxin

N. Nanjundaswamy¹, S. Satish², K. M. Lokanatha Rai¹, S. Shashikanth¹, K. A. Raveesha²

¹Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore, India;

²Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore, India

ABSTRACT

Precursors of podophyllotoxin were synthesized and screened for their antibacterial activity. The results proved that ethyl-2-(3'-methyl-4'-methoxybenzoyl)-3-(4'' methoxyphenol)-cyclopropane-1-carboxylic acid and Ethyl-2-(3'-methyl-4'-methoxybenzoyl)-3-(3'', 4''-dimethoxyphenyl)-cyclopropane-carboxylic acid have significant antibacterial activity against *Citrobacter* sp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sonnei* and *Streptococcus faecalis*. The activity is lower than Ciprofloxacin and equal to Gentamicin and more than Penicillin and Streptomycin.

Keywords: podophyllotoxin; antibacterial activity; human pathogenic

INTRODUCTION

Podophyllotoxin (Fig. 1) and several of its analogs are being used as cytotoxic spindle poisons and antitumor agents at clinical levels (1, 2). Recently Lee et al. (3) discovered that some modified derivatives of podophyllotoxin possess an anti HIV property. Most of these compounds contain a transfused highly strained γ -lactone system (4), a feature that correlates with the smooth Isomerisation of podophyllotoxin to its thermodynamically stable epimer, picropodophyllin. The biological activity of *cis* analogs are very much low than that of the *trans* isomer (5). Literature survey on the structure and activity relationship among the podophyllin derivatives revealed efforts have not been made towards the study of the other biological activities in general and antibacterial activity in particular. Literature studies (6) also reveal that prythroid derivatives (Fig. 2) possessing cyclopropane ring system show strong insecticidal property.

These results prompted us to synthesize the molecules from cyclopropane ring system, which can serve as a synthetic precursor (Fig. 3) for the synthesis of podophyllotoxin derivatives and evaluated their antibactericidal potential against some important human pathogenic bacteria.

MATERIALS AND METHODS

The following compounds (3a-, 3b-, 3c-, 3d- and 3e) were synthesized by cyclopropanation of chalcones using the literature method (7).

3a. 2-(3'-methyl-4'-methoxybenzoyl)-3-phenyl-cyclopropane-1-carboxylic acid.

3b. 2-(3'-methyl-4'-methoxybenzoyl)-3-(4''-methoxy-

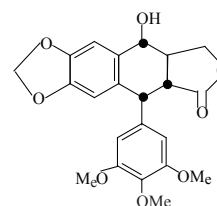
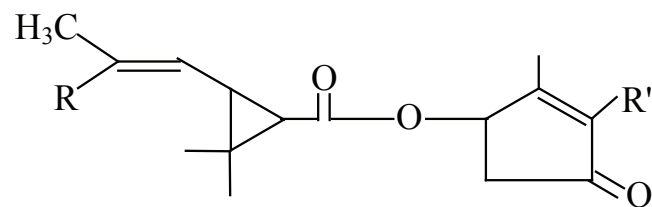


Figure 1. Chemical structure of podophyllotoxin.

Corresponding author: K. M. Lokanatha Rai, Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore, India. E-mail: kmlrai@yahoo.com.



	R	R'
Pyrethrin – I	CH ₃	-CH ₂ CH = CH CH = CH ₂
Pyrethrin – II	COOCH ₃	-CH ₂ CH = CH CH = CH ₂
Sinerin – I	CH ₃	-CH ₂ – CH = CH = CH ₃
Sinerin – II	COOCH ₃	-CH ₂ – CH = CH – CH ₃
Jasmolin – II	COOCl ₃	-CH ₂ CH = CH
Allethrin	CH ₃	-CH ₂ CH = CH ₂

Figure 2. Chemical structures of pyrethroid derivatives tested in the current study.

phenyl)-cyclopropane-1-carboxylic acid.

3c. 2-(3'-methyl-4'-methoxybenzoyl)-3-(3'',4''-dimethoxyphenyl)-cyclopropane-1-carboxylic acid.

3d. 2-(3'-methyl-4'-methoxybenzoyl)-3-(3'',4'',5''-trimethoxyphenyl)-cyclopropane-1-carboxylic acid.

3e. Ethyl-2-(3'-methyl-4'-methoxybenzoyl)-3-(5''-nitrophenyl)-cyclopropane-1-carboxylic acid.

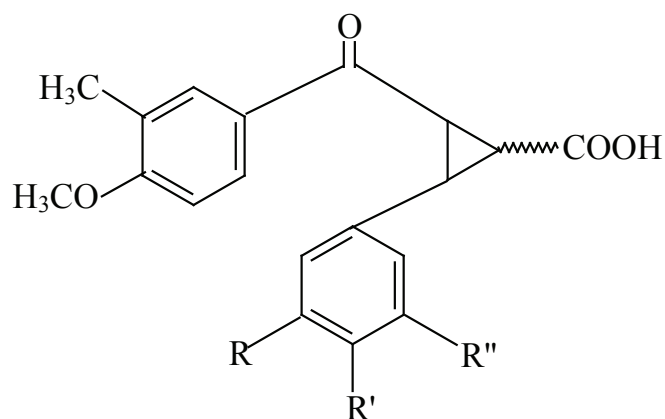
Bacterial cultures

Citrobacter sp., *Escherchia coli* (MTCC443), *Klebsiella pneumoniae* (MTCC109), *Pseudomonas aeruginosa* (MTCC1688), *Salmonella typhi* (MTCC733), *Shigella sonnei* (MTCC2957) and *Streptococcus faecalis* (MTCC459) obtained from Microbiology laboratory, Government Medical College, Mysore, India served as test organisms for antibacterial activity assay.

Antibacterial assay

Preparation of nutrient broth. Nutrient broth was prepared by dissolving peptic digest of animal tissue (0.5%), yeast extract (0.15%), beef extract (0.15%) and sodium chloride (0.50%) in distilled water (100ml). The pH of solution was adjusted to 7.4 by adding sodium hydroxide solution (4%) and the resulting solution was autoclaved for 20 minutes at 15 psi.

Preparation of sub cultures (Inoculums). One day prior to the experiment, the cultures of *Citrobacter* sp., *Escherichia coli*, *Klebsiella-penmoniae* sp., *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sonnei*, and *Strep-*



- R = R' = R'' = H
- R = R'' = H, R' = OM_c
- R = H, R'=R''= OM_c
- R = R' = R'' = OM_c
- R' = NO₂, R' = R' = H

Figure 3. Molecules based on the cyclopropane ring system used in the current study as the synthetic precursor for synthesis of podophyllotoxin derivatives.

tococcus faecalis was inoculated to nutrient broth and incubated over night at 37°C.

Preparation of Nutrient agar medium. Nutrient agar medium was prepared by dissolving peptic digest of animal tissue (0.5%), yeast extract (0.15%), beef meet extract (0.15%), sodium chloride (0.5%) in distilled water (100 ml). The pH of the solution was adjusted to 7.2 by adding 4% aqueous sodium hydroxide solution and agar (1.5%) was added and autoclaved for 20 minutes at 15 psi.

Preparation of test sample solution. Each test sample (5 mg) was dissolved in ethyl alcohol (5 ml) and 0.01 ml of this solution (10 µg) was used for testing.

Method of testing. Antibacterial activity was determined by cup diffusion method on nutrient agar medium. The sterile medium (20 ml) was poured into 9 cm petri-plates. The medium was allowed to cool in a sterile condition and plates were then inoculated with 1×10^5 cfu cultures of test bacteria. The concentration of bacterial cells in the suspension was adjusted to minimum of 1×10^5 cfu/ml in nutrient broth solution. By punching the agar container with sterile cork borer and scooping out the punched part agar cup of 5 mm diameter were made.

Test solution (0.01 ml) containing 10 µg of the test compounds were placed in each cup, ethyl alcohol (10 µl) served as control. The plates were left to stay for an hour in order to facilitate the diffusion of the drug solution. Then the plates were incubated at 37°C for 24 hours. The

zones of inhibition if any against the test bacteria were measured in mm. Ciprofloxacin, Gentamicin, Penicillin G and Streptomycin were used for comparison.

Minimal Inhibitory Concentration (MIC). MIC was determined by broth dilution methods. For broth dilution tests, 0.1ml of standardized suspension of test bacteria (10^5 CFU/ml) was added to each tube containing different concentrations of the podophyllotoxin derivatives and antibiotics (05-30 μ l/ml) incubated for 24hours at 37°C. The lowest concentration of the tube that did not show any visible growth by macroscopic evaluation was considered as the MIC.

RESULTS

The antibacterial activity of all the synthetic derivatives and the antibiotics against all the test bacteria is presented in table 1.

The three derivatives namely 3a, 3d, 3e and antibiotic penicillin G did not reveal any antibacterial activity, whereas the other two derivatives namely 3b and 3c recorded significant activity against all the test bacteria. Streptomycin was active only against *E. coli* and *Klebsiella pneumoniae*. Ciprofloxacin and Gentamicin were active against all the test bacteria.

Antibacterial activity was maximum against *Shigella sonnei* (13.33 mm) and was minimum against *Streptococcus faecalis* (9.83 mm) in 3b derivative. In case of 3c derivative the antibacterial activity was more against *Klebsiella pneumoniae* (12.16 mm) and less against *E. coli* (10.66 mm). Antibiotic Streptomycin recorded higher activity in comparison with the derivatives 3b and 3c against *E. coli*. *Salmonella typhi* (17.75 mm) was highly susceptible to Gentamicin followed by *Shigella sonnei* (15.25 mm) and

Citrobacter sp. (12.68 mm). The zone of inhibition was highest in case of *Pseudomonas aeruginosa* (34.25 mm) against Ciprofloxacin followed by *Shigella sonnei* (21.75 mm) *Salmonella typhi* (20.25 mm) and *Klebsiella pneumoniae* (20.25 mm). Lowest inhibitory activity was observed against *E. coli* and *Streptococcus faecalis*.

Minimal inhibitory concentration of all the five derivatives of Podophyllotoxin and antibiotics tested against the test bacteria are present in table 2. It is observed that among the antibiotic tested, Ciprofloxacin was highly active with low MIC of 10 μ l against *Citrobacter* sp., *Klebsiella pneumoniae*, *Shigella sonnei* and *Salmonella typhi*. Gentamicin also recorded low MIC value of 10 μ l against *Salmonella typhi*. Streptomycin was active only against *E. coli* and *Klebsiella pneumoniae*, whereas penicillin was not active against all the test bacteria. Among the synthetic derivatives 3b and 3c were active against all the test bacteria with MIC ranging between 15 -25 μ l where as 3a, 3d and 3e did not show any activity against all the test bacteria.

The MIC values of Ethyl-2-(3'-methyl-4'-methoxybenzoyl)-3-(4''methoxyphenyl)-cyclopropane-1-carboxylic acid and Ethyl-2-(3'-methyl-4'-methoxybenzoyl)-3-(3'',4''-dimethoxyphenyl)-cyclopropane-carboxylic acid was almost equal to that of Gentamicin and slightly higher than that of Ciprofloxacin against the test bacteria. Further it is observed that 3b and 3c derivatives were better antibacterially active than penicillin and streptomycin.

DISCUSSION

Continuous and frequent use of antibiotics to manage human disease has caused bacteria to acquire resistance against the commonly used therapeutic agents (9). Con-

Table 1. Antibacterial activity of podophyllotoxin derivatives and antibiotics

Sl. No.	Bacteria	3a	3b	3c	3d	3e	C	G	P	S
1.	<i>Citrobacter</i> sp.	0.00	11.66 \pm 0.24	11.00 \pm 0.36	0.00	0.00	19.62 \pm 0.18	12.68 \pm 0.22	0.00	0.00
2.	<i>Escherichia coli</i>	0.00	10.33 \pm 0.23	10.66 \pm 0.24	0.00	0.00	10.00 \pm 0.25	10.25 \pm 0.25	0.00	13.50 \pm 0.36
3.	<i>Klebsiella pneumoniae</i>	0.00	11.66 \pm 0.24	12.16 \pm 0.51	0.00	0.00	20.25 \pm 0.16	11.75 \pm 0.25	0.00	09.25 \pm 0.16
4.	<i>Pseudomonas aeruginosa</i>	0.00	11.00 \pm 0.73	11.33 \pm 0.24	0.00	0.00	34.25 \pm 0.16	12.63 \pm 0.18	0.00	0.00
5.	<i>Salmonella typhi</i>	0.00	11.66 \pm 0.24	12.00 \pm 0.00	0.00	0.00	20.25 \pm 0.16	17.75 \pm 0.25	0.00	0.00
6.	<i>Shigella sonnei</i>	0.00	13.33 \pm 0.24	11.66 \pm 0.24	0.00	0.00	21.75 \pm 0.16	15.25 \pm 0.25	0.00	0.00
7.	<i>Streptococcus faecalis</i>	0.00	09.83 \pm 0.15	11.50 \pm 0.27	0.00	0.00	10.00 \pm 0.25	12.50 \pm 0.18	0.00	0.00

Values given are mean \pm standard error of 3 replicates. ($p < 0.05$).
C, Ciprofloxacin; G, Gentamicin; P, Penicillin; S, Streptomycin.

Table 2. Minimal inhibitory concentration of podophyllotoxin derivatives and antibiotics

Sl. No.	Bacteria	3a	3b	3c	3d	3e	C	G	P	S
1.	<i>Citrobacter</i> sp.	—	20	20	—	—	10	20	—	—
2.	<i>Escherichia coli</i>	—	20	20	—	—	20	20	—	15
3.	<i>Klebsiella pneumoniae</i>	—	20	15	—	—	10	20	—	25
4.	<i>Pseudomonas aeruginosa</i>	—	20	20	—	—	05	20	—	—
5.	<i>Salmonella typhi</i>	—	20	20	—	—	10	10	—	—
6.	<i>Shigella sonnei</i>	—	15	20	—	—	10	15	—	—
7.	<i>Streptococcus fecalis</i>	—	25	20	—	—	20	20	—	—

Values given are mean of 3 replicates.

C, Ciprofloxacin; G, Gentamicin; P, Penicillin; S, Streptomycin.

sequently the problem of microbial resistance is growing and the need to search for alternative newer molecules is increasing. Hence there is a continuous need to evaluate for newer molecules that are antibacterially active. Hence in the present study all the five newly synthesized derivatives (3a-3e) of podophyllotoxin and their analogues that are already in use as antitumor agent (1, 2), and also are known to possess anti HIV property (3) were evaluated for antibacterial activity. None of the earlier evaluation have demonstrated the antibacterial potential of the five synthesized derivatives tested in the present investigation. The present investigation has demonstrated clearly the antibacterial potential of 3b and 3c derivatives for the first time also and demonstrated the inefficacy of 3a, 3d and 3e.

The comparative evaluation of the synthetic derivatives with that of the commonly used antibiotics reveal that Ethyl-2-(3'-methyl-4'-methoxybenzoyl)-3-(4''methoxyphenyl)-cyclopropane-1-carboxylic acid and Ethyl-2-(3'-methyl-4'-methoxybenzoyl)-3-(3'',4''-dimethoxyphenyl)-cyclopropane-carboxylic acid were active against many penicillin and streptomycin resistant bacteria. It is also observed that the activity of these two derivatives was also equal to that of a broad-spectrum antibiotic gentamicin, however the activity was lower than that of ciprofloxacin. The results of the present investigations suggest that 3b and 3c are important derivatives for further investigations

for the therapeutic value in managing diseases caused by the test bacteria based on toxicological and pharmacological study.

REFERENCES

- Jordain, I. "Podophyllotoxin is anticancer band on natural products and models". Academic press, Inc., New York, 1980; 319.
- Hartwell, J.L. and Schrecker, A.W., Chemistry of Podophyllotoxin, Fortschr. Chem. Org. Natsrofte, 1958; 15: 93.
- C.T.L. Lee, V.C.K. Lin, S.X. Zhang, X.K. Zhu, D. Van Vliet, H. Hu, S.A. Beers, Z.Q. Wang, L.M. Cosentino, S.L. Morris Natschke, et al. Anti-AIDS agents. 29. Anti-HIV activity of modified podophyllotoxin derivatives. Bio.Org. and Med. Chem. Lett., 1997; 7: No. 22, 1997; 2897.
- Watter J. Gensler, C.D. Gatsonis; "The podophyllotoxin-picropodophyllin equilibrium". J. Org. Chem., 1966; 31(10): 3224.
- Schreier, E.G., Synthesis and biological activity of compounds related to podophyllotoxin, 152th National meeting of American Chemical Society, New York, Sept. 1966; Abs. p.34.
- Elliot, M, Future use of natural and synthetic perethroids in "The future of insecticides", Eds. Metcalf RC and McKelvey Jr. J.J. 1976; 6: 163-194.
- Lokanath Rai K.M., Anjanamurthy C, and Radha Krishna P.M., Synth. Commun. 1990; 20: 1273.
- Anon. Pharmacopocia of India (The Indian Pharmacopocia), 3rd edn. Government of India, New Delhi, 1996, Ministry of Health and Family Welfare.
- Cohen, M.L. Epidemiology and drug resistance: implications for a post-antimicrobial era. Science, 1992; 257: 1050.