Vol 1 | Issue 1 | Jul –Dec 2010 | 35-38.

Research Article

Anticancer and Antimicrobial Activity of Embelin Derivatives

KANTHAM SRINIVAS *

[1]Department of Pharmaceutical Chemistry, Sri Rajya Laxmi (SRL) Institute of Pharmaceutical Sciences, Kummarigudem Road, Madikonda, Kazipet, Warangal, Andhra Pradesh, India- 506003

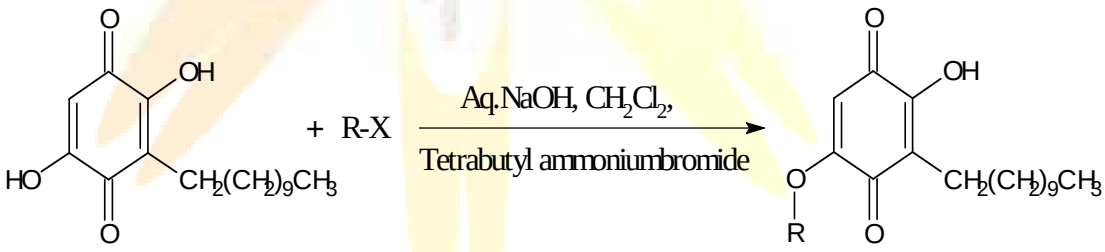
ABSTRACT

Embelin is isolated from berries of *embelia ribes* using hexane and crystallized from benzene. Embelin derivatives, 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones (IIa-f) were synthesized from Embelin by phase transfer catalyst (PTC) process. All the title compounds (IIa-f) were screened for anticancer activity using HBL-100 cell lines by MTT method and antibacterial activity against *B. subtiliis*, *S.aureus*, *E.coli* and *P.vulgaris*. The structures of newly synthesized compounds were established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data.

Key words: Anticancer activity, antibacterial activity and Embelin

INTRODUCTION

Ayurveda is a 5000-year-old "science of long life" that prescribes certain herbal preparations for the prevention and treatment of disease. Identifying the chemical compounds in these herbal preparations and the molecular targets of those compounds helps validate the use of these ancient medicines. The fruit of the *Embelia ribes* Burm plant (Myrsinaceae) (called false black pepper in English, Vidanda in Sanskrit, and Babrang in Hindi languages) has been used to treat fever, inflammatory diseases, and a variety of gastrointestinal ailments for thousands of years [1]. More than 4 decades ago, the active component from this plant was isolated and named embelin [2] and later chemically synthesized [3]. Embelin has been shown to have antitumor, anti-inflammatory, and analgesic properties [4], and it has been shown to decrease testosterone levels In view of these, a project was undertaken to synthesize a new series of 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones and to evaluate new compounds for screen for anticancer activity by the MTT method [5] and antibacterial activity by cup plate method [6]. Synthesis of title compounds was shown in Scheme 1.



I
Embelin

IIa-f

Scheme 1: Synthesis of 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones

*Corresponding Author Kantham Srinivas E mail: kanthamsrinivas@gmail.com

MATERIALS AND METHODS

Melting points were determined in open capillary tubes, using Toshniwal melting point apparatus and are uncorrected. IR spectra were recorded on Perkin – Elmer spectrum BX-I series, FT IR spectrophotometer using KBr discs. PMR spectra were recorded on Brucker spectrospin 400 MHz spectrophotometer using TMS as an internal standard. Purity was checked by TLC using

TLC aluminum sheets silica gel 60, supplied by E.Merk, Mumbai, India. The physical constants, yield and analytical data of 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-dione IIa–f are given in table 1.

Extraction and Isolation of Embelin:

The berries of *embelia ribes* (2 kg) were purchased from the local market. They were dried, size reduced and passed through 20 mesh. 2 kg of powdered *embelia berries* were soaked in n-hexane for 7 days. The level of n-hexane was maintained 1 inch above powdered *emdelica berries*. The contents were then referred for 2 hours. It is filtered at the vaccum pump while it is hot, upon cooling Embelin precipitates out, which was filtered and filtrate was again used to soak the material for second extraction for another week, the process is repeated.

The crude Embelin obtained from the extraction was crystallized from benzene. The filtrate of second extraction was concentrated and the product was also recrystalized from Benzene. The recrystalization process was repeated until golden crystals of Embelin (32g) were observed m.p. 142-144^0 C.

Reaction of Embelin with Alkyl halides Under "PTC" conditions:

General Procedure:

The Embelin-5-O-Alkyl Ethers were prepared by vigorously stirring a mixture of Embelin (0.297 gms, 0.001 mole), aqueous sodium hydroxide (1.5 times the Embelin), alkylating agent (excess 2-3 times of the Embelin) and Tetrabutyl Ammonium bromide (catalytic amount) in Dichloromethane (DCM) for 3-20 hr at room temperature. The organic layer is separated, washed with Ammonia solution (2%) and then with sodium hydroxide solution (2N), saturated sodium chloride, dried and solvent was evaporated to get oily product. The product displayed two spots on TLC, it was chromato graphed over oxalic acid coated silicagel using solvents of increasing polarity from hexane through ethyl estate. The fractions were monitored through TLC.

After performing column chromatography orange coloured oily compounds were obtained as a product. This did not solidify even on standing at the ordinary temperature for a month. But on cooling in a refrigerator for a long time the compounds were become as semi solid mass [1].

2,5-dihydroxy-3-undecylcyclohexa-2,5-diene-1,4-dione (I)

IR (KBr) (cm $^{-1}$): 1619.98(C=O), 3320.15 (OH), 3336.09(OH),1170 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.8-1.0 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1-2.3 (t, 2H,CH_2(allylic)), 6.0 (S, 2H,2 OH), 7.3 (S,1H,Ar-H). LC-MS (m/z): 279.06 (M+1).

2-hydroxy-5-methoxy-3-undecylcyclohexa-2,5-diene-1,4-dione (II a)

IR (KBr) (cm $^{-1}$): 1615.83 (C=O), 3310.25 (OH), 1195.94 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 Hz), δ (ppm): 0.8-1.0 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1-2.3 (t, 2H, CH_2(allylic)), 6.0 (S,H,OH), 7.3 (S, 1H, Ar-H) 3.4-3.5 (t, 3H, CH_3). LC-MS (m/z): 305.11 (M+1).

5-ethoxy-2-hydroxy-3-undecylcyclohexa-2,5-diene-1,4-dione(II b)

IR (KBr) (cm $^{-1}$): 1619.98(C=O), 3320.15 (OH), 1170 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 3.3-4.5 (q, 2H, OCH2), 0.8-0.9 (t, 3H, CH_3), 1.2-1.3 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1-2.3 (t, 2H, CH_2 (allylic)), 6.0 (S, H, OH), 7.3 (S, 1H, Ar-H). LC-MS (m/z): 326.13 (M+1).

2-hydroxy-5-propoxy-3-undecylcyclohexa-2,5-diene-1,4-dione (II c)

IR (KBr) (cm $^{-1}$): 1661.32 (C=O), 3319.98 (OH), 1221.15 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): . 3.2-3.4 (t, 2H, OCH2), 1.60-1.7 (m, 2H, CH2), 0.9-1 (t,3H,CH3), 0.8-1.0 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1 - 2.3 (t, 2 H, CH_2 (allylic)), 6.0 (S, H, OH), 7.3 (S, 1H, Ar-H). LC-MS (m/z): 339.83 (M+1).

5-butoxy-2- hydroxy-3-undecyl benzo-1,4-quinone (II d)

IR (KBr) (cm $^{-1}$): 1658(C=O), 3320.17 (OH), 1200 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 3.3-3.4 (t,2H, OCH2), 1.60-.1.71(m, 4H, $2CH_2$), 0.86 - 0.92 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1- 2.3 (t, 2H, CH_2 (allylic)), 6.0 (S, H, OH), 7.3 (S, H, Ar-H). LC-MS (m/z): 354.16 (M+1).

5-allyloxy-2- hydroxy-3-undecyl benzo-1,4-quinone (II e)

IR (KBr) (cm $^{-1}$): 1670(C=O), 3402 (OH), 1150(C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 5.9-6.0 (m, H, CH=), 3.4-3.6 (d, 2H, OCH_2), 5.2-5.3 (t, 2H,=CH_2),1.5 (S, 18H,

$(CH_2)_9$), 2.1-2.3 (t,2H, CH_2 (allylic)), 6.0 (S,H,OH),7.3 (S,1H, Ar-H). LC-MS (m/z): 338.10 (M+1).

5-benzyloxy-2- hydroxy-3-undecyl benzo-1,4-quinone (II f)

IR (KBr) (cm $^{-1}$): 1713(C=O), 3390(OH), 1170(C-O-C). ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 3.2 (s, 2H, OCH_2), 1.5 (S, 18H,$(CH_2)_9$), 2.1 - 2.3 (t,2H,CH_2 (allylic)),6.0(S,H,OH),7.2-7.4(m,6H,Ar-H). LC-MS (m/z): 388.10 (M+1).

Antimicrobial Activity

The antimicrobial activity of all the newly synthesized compounds were determined by well plate method in nutrient agar (Hi-Media) was used for antibacterial activity. The antibacterial activity of the test compounds was assayed against *Bacillus subtilis*, *Staphylococcus aureus* (gram – positive) and *Escherichia coli* and *Proteus vulgaris* (gram – negative) by CUP-plate method.

The compounds were tested at a concentration of a 100 µg/ml were prepared in dimethyl formamide (DMF). The Petri dishes used for antibacterial screening were incubated at 37 ± 1º for 24 h; the diameters of zone of inhibition (mm) surrounding each of the wells were recorded. The results were compared with Ampicillin of a 50 µg/ml concentration and the screening results were presented in Table 2.

Anticancer Activity

Embelin derivatives, 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones (IIa-f) were subjected to *in vitro* MTT [3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium Bromide] assay to detect cytotoxic antitumor property and *in vivo* test using tumor mouse model to detect noncytotoxic antitumor property were used. MTT assay was used for *in vitro* cytotoxicity test and was performed as per the method of Alley *et al*[x]. Cells were harvested from experimental-phase maintenance cultures. Four hundred cells were counted by trypan blue exclusion and dispensed within triplicate 96-well culture plates in 100 µl volumes for each venom concentration. The assay at each concentration was repeated twice. The cell proliferation activity was qualified on HBL-100 (ICLC NO. HTL 00004)- breast myoepithelial tumor cell line, by using Cisplatin as a standard. The results are represented in Table.2.

Table 1: Physical data of 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones

Compound	R	Mol. Formula	Melting Point (^{0}C)	R_f	Yield (%)
I	H	$C_{17}H_{26}O_4$	142-144	0.51	60
IIa	$-CH_3$	$C_{18}H_{28}O_4$	165-168	0.52	72
IIb	$-CH_2-CH_3$	$C_{19}H_{30}O_4$	172-176	0.58	68
IIc	$-CH_2-CH_2-CH_3$	$C_{20}H_{32}O_4$	128-132	0.63	74
IId	$-CH_2-CH_2-CH_2-CH_3$	$C_{21}H_{34}O_4$	126-128	0.66	78
IIe	$-CH_2-CH = CH_2$	$C_{20}H_{30}O_4$	169-173	0.64	66
IIf	$-CH_2-C_6H_5$	$C_{24}H_{32}O_4$	112-116	0.68	58

Table 2: Anticancer and antibacterial activity of 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones (IIa-f)

Compound	Cytotoxic activity IC_{50} (µM)	Antibacterial activity (Zone of inhibition in mm)			
		B. Subtilis	*S. aureus*	*E. coli*	*P. vulgaris*
IIa	48	16	10	--	06
IIb	51	08	10	10	11
IIc	77	20	18	16	15
IId	112	15	08	12	10
IIe	101	10	10	11	09
IIf	31	15	17	14	12
Cisplatin	25	NA	NA	NA	NA
Ampicillin	NA	22	20	18	17

RESULTS AND DISCUSSION

The title compounds were obtained in good yields and purity. All the test compounds at the conc. of 20 µg/ml, 80 µg/ml, 100 µg/ml and 200 µg/ml were taken to evaluate the anticancer activity against HBL-100 cell lines and the results are presented as IC_{50} values. All the compounds showed anticancer activity in the range of 31 µM to 171 µM. The structure activity studies reveal that among the test compounds, the compound IIf with benzyloxy substitution at C-5 position showed relatively high degree of anticancer activity with IC_{50} of 31 µM. The compounds, IIb IIa, IIc were next in the order of anticancer activity with IC_{50} values of 48 µM and 51 µM, 77 µM respectively. The results are statistically significant and the activity of the compounds is compared with the standard Cisplatin.

The test compounds showed mild antibacterial activity at the concentration of 100 µg/disc against gram-positive organism (*B. subtilis, S. aureus*) and gram negative (*E. coli, P. vulgaris*) organisms. The compound IIb was more active among all the test compounds followed by compound IIe, IId, IIa.

ACKNOWLEDGMENTS

The authors are thankful to the Director and Principal, Sri Rajya Laxmi (SRL) Institute of Pharmaceutical Sciences, Warangal, Kakatiya University and IICT- Hyderabad, for providing laboratory facilities and financial support.

REFERENCES

1. Gupta OP, Ali MM, Ray Ghatak BJ, Atal CK. Some pharmacological investigations of embelin and its semisynthetic derivatives. *Indian J Physiol Pharmacol.* 21, 1977, 31–39.
2. Du YC and Wie JS. Study of vermifuges. I. Isolation of embelin from the fruit of mugua-wha (*Embelia oblongifolia* Hemsl). *Yao Xue Xue Bao*. 10, 1963, 578–580.
3. Dallacker F and Lohnert G. Derivatives of methylenedioxybenzene. 35. A novel synthesis of 3,6 dihydroxy-2-ethyl-1,4-benzoquinone, embelin, vilangin, rapanone, dihydromaesaquinone, bhogatin, spinulosin and oosporein. *Chem Ber*. 105, 1972, 614–624.
4. Chitra M, Sukumar E, Suja V, Devi CS. Antitumor, anti-inflammatory and analgesic property of embelin, a plant product. *Chemotherapy*. 40, 1994, 109–113.
5. Krief P, Saint-Ruf C, Bracke M, Boucheix C, Billard C, Billard M, et al . Acquisition of tumorigenic potential in the human myoepithelial HBL 100 cell line is associated with decreased expression of HLA class I, class II and integrin beta 3 and increased expression of C-myc. *Int J Cancer*. 43, 1989, 658-64.
6. Seely HW, Van Demark PJ. Microbes in action: A laboratory manual of Microbiology, Mumbai: DB Taraporewala Sons and Co, 1975, 55.