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Synthesis and biological evaluation of some novel analogue of Boswellic acid derivatives

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Abstract

In the present study, some novel derivatives of boswellic acid were synthesized were which include the synthesis of its amino acid conjugates using methyl esters of various amino acids. These amino acid conjugates work like guided missiles and have a role in right cell recognition. All the synthesized compounds were identified by spectral data of NMR. The compounds were screened for anticancer, antibacterial and antifungal activities and appreciable anticancer activity was observed.

Keywords: Anti-inflammatory, anticancer, neurotransmitter, antioxidant.

Introduction

Persual of literature on pharmacological studies reported for boswellic acids reveals that these compounds have anti-inflammatory and anticancer activities. Amino acid conjugates of boswellic acid were prepared which work like guided missiles. They have a role in right cell recognition. Thus, the activity of boswellic acid is enhanced. These amino acid conjugates breakdown in the body into an active moiety 'boswellic acid' and amino acids. Amino acids are essential components of body. Phenylalanine gets converted into tyrosine which is used to synthesize two important neurotransmitters-dopamine and norepinephrine. Tryptophan is required for the production of niacin (vitamin B_3). It is used by the human body to produce serotonin, a neurotransmitter that is important for normal nerve and brain functions. Cysteine is a component of the antioxidant gluthione. It is useful to detoxify the body from harmful toxins and help protect the brain and liver from damage from alcohol, drugs, etc.

Materials and Method

IR data were recorded in KBr disks on Perkin Elmer (spectrum 100) FTIR spectrophotometer and ^1H NMR spectra on Bruker (dpx 200) spectrometer. Acid chlorides were obtained by reacting acetyl boswellic acid with thionyl chloride in accordance with procedure as reported in the literature (Furniss et al. 1998).

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Chemistry

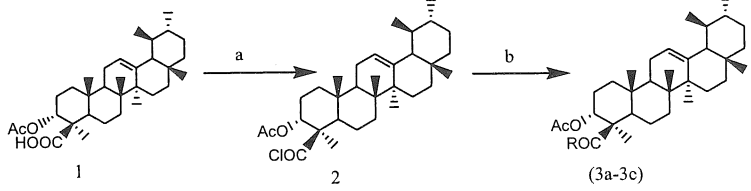
Synthesis of acid chloride of 3-acetyl-β-boswellic acid (2)

Thionyl chloride (6 mmol) was added to a solution of 3-acetyl-β-boswellic acid (1) (0.5-0.8 mmol), in dry dichloromethane (DCM) and stirred for 1 h at 60 °C. The completion of the reaction was monitored through TLC and the reaction mixture was then dried on rotary evaporator to afford the acid chloride of 3-acetyl-β-boswellic acid (2).

Synthesis of amino acid conjugates (3a-3e)

To a stirred solution of (2) in DCM, amino ethyl ester (1.5 mmol), hydroxybenzotriazole (HOBT) (1 eqv.) and triethylamine (0.3 ml) were added and the solution was kept on overnight stirring. The completion of the reaction was monitored through TLC. The reaction was quenched with water, extracted with DCM and concentrated using vacuum distillation to afford the crude product. The crude product was subjected to column chromatography over silica gel (60-120 mesh size) and the pure product was eluted using gradient of petroleum ether and ethyl acetate (90:10). The formation of the product was further confirmed through TLC.

SCHEME 1

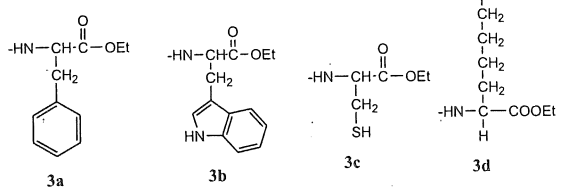


1 2 (3a-3c)

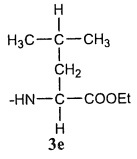
Reagents:

(a) $SOCl_2/(COCl)_2$
(b) amino ethyl ester (phenylalanine, tryptophan, cysteine, lysine, leucine), hydroxybenzotriazole & triethylamine.

R=



3a 3b 3c 3d



3e

IR (KBr ν cm–1) and ^{1}H NMR ($CDCl_3$, δ ppm)

3a. FTIR (cm^{-1}): 3280 (N-H *str*), 2998.4 (C-H *str*), 1524 (Aromatic, C=C *str* or N-H bend), 1675 (Alkene, C=C *str*), 1687 (Amide, C=O *str*), 1267.9 (C-O *str*), 1720 (Ketone, C=O *str*), 1465 (-CH_2 bend), 1416 (-CH_3 bend). ^{1}H NMR (ppm): 0.9-1.9 (43H, m, -CH, -CH_2, -CH_3), 2.3 (3H, s, -$COCH_3$), 4.97 (1H, t, -C=C-), 4.32 (1H, t, -CH-), 7.5 (1H, d, -CONH), 4.7 (1H, q, -CH-), 2.7 (2H, d, -CH_2), 3.87 (2H, q, -OCH_2), 3.1 (3H, t, -CH_3), 7.1-7.3 (5H, m, aromatic protons).

3b. FTIR (cm^{-1}): 3273.4 (N-H *str*), 3000.6 (C-H *str*), 1535 (Aromatic, C=C *str* or N-H bend), 1638 (Alkene, C=C *str*), 1677 (Amide, C=O *str*), 1100 (C-O *str*), 1709 (Ketone, C=O *str*), 1214 (C-N *str*), 1465 (-CH_2 bend), 1390 (-CH_3 bend). ^{1}H NMR (ppm): 0.8-1.9 (43H, m, -CH, -CH_2, -CH_3), 2.2 (3H, s, -$COCH_3$), 4.72 (1H, t, -C=C-), 4.1 (1H, t, -CH-), 7.7 (1H, d, -CONH), 4.6 (1H, q, -CH-), 2.8 (2H, d, -CH_2), 3.8 (2H, q, -OCH_2), 3.0 (3H, t, -CH_3), 7.1-7.3 (4H, m, aromatic protons), 6.7 (1H, d, -CH neighboring –NH), 9.8 (1H, d, -NH).

3c. FTIR (cm^{-1}): 3150 (N-H *str*), 2970 (C-H *str*), 1590 (Aromatic, C=C *str* or N-H bend), 1675.8 (Alkene, C=C *str*), 1689 (Amide, C=O *str*), 1225 (C-O *str*), 1712 (Ketone, C=O *str*), 2550 (S-H *str*), 1463 (-CH_2 bend), 1391 (-CH_3 bend). ^{1}H NMR (ppm): 0.8-1.88 (43H, m, -CH, -CH_2, -CH_3), 2.08 (3H, s, -$COCH_3$), 4.8 (1H, t, -C=C-), 4.2 (1H, t, -CH-), 7.7 (1H, d, -CONH-), 5.2 (1H, q, -CH-), 1.75 (1H, t, -SH), 3.9 (2H, q, -OCH_2), 3.1 (3H, t, -CH_3).

3d. FTIR (cm^{-1}): 3298 (N-H *str*), 2834 (C-H *str*), 1610 (Aromatic, C=C *str* or N-H bend), 1663.6 (Alkene, C=C *str*), 1692 (Amide, C=O *str*), 1289 (C-O *str*), 1723.6 (Ketone, C=O *str*), 1289 (C-N *str*), 1464 (-CH_2 bend), 1385.5 (-CH_3 bend). ^{1}H NMR (ppm): 0.7-1.9 (49H, m, -CH, -CH_2, -CH_3), 2.17 (3H, s, -$COCH_3$), 4.78 (1H, t, -C=C-), 4.2 (1H, t, -CH-), 7.61 (1H, d, -CONH), 4.7 (1H, q, -CH-), 2.3 (2H, q, -NH_2), 3.6 (2H, q, -OCH_2), 2.97 (3H, t, -CH_3), 2.7 (2H, m, -CH_2).

3e: FTIR (cm^{-1}): 3217.4 (N-H *str*), 2935 (C-H *str*), 1585 (Aromatic, C=C *str* or N-H bend), 1612 (Alkene, C=C *str*), 1688 (Amide, C=O *str*), 1298 (C-O *str*), 1717 (Ketone, C=O *str*), 1465 (-CH_2 bend), 1380 (-CH_3 bend). ^{1}H NMR (ppm): 0.8-1.98 (52H, m, -CH, -CH_2, -CH_3), 2.3 (3H, s, -$COCH_3$), 4.8 (1H, t, -C=C-), 4.3 (1H, t, -CH-), 7.8 (1H, d, -CONH), 4.5 (1H, t, -CH-), 4.1 (2H, q, -OCH_2), 2.7 (3H, t, -CH_3).

Pharmacology

Anticancer activity

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay utilizes a color reaction as a measure of viable cells. The assay is dependent on the cellular reduction of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazolium salt to a blue formazan product by the mitochondrial dehydrogenase of viable cells/metabolically active cells. The intensity of blue colored formazan produced active cells. The intensity of blue colored formazan produced is directly proportional to the cell viability.

The cells from a particular cell line when in log phase of growth are trypsinized, counted in a haemocytometer and adjusted to appropriate density in a suitable medium and then inoculated in 96-well plates. The cells are treated with various concentrations (in replicates) of drugs for specified duration i.e. 1 to 4 days, after which MTT dye is added in each well and plates are incubated at 37°C for 4 h in a CO_2 incubator. The plates are then taken out of incubator and dark blue colored formazan crystals are

$$\% \text{ cell viability} = \frac{\text{OD of treated cells} * 100}{\text{OD of control cells}}$$

thoroughly dissolved in DMSO at room temperature. The plates are then read on an ELISA reader at 570 nm. The percent cell viability with respect to control is calculated using the formula,

The results are given in Table 1.

Table 1. Primary screening: cytotoxicity against human cell lines

Cell line type		Colon			Breast
Codes	Conc.	HCT-15	HT-29	502713	MCF-7
		% Growth Inhibition			
3a	$1*10^{-5}$M	6	23	48	32
3b	$1*10^{-5}$M	4	6	19	23
3c	$1*10^{-5}$M	11	2	36	24
3d	$1*10^{-5}$M	14	7	22	18
3e	$1*10^{-5}$M	9	4	14	17

Antimicrobial activity: All the newly synthesized compounds were screened for antimicrobial activity against both gram positive *S.aureus* and gram negative *E.coli* bacteria and antifungal activity against *C.albicans* and *A.flavus* according to cup plate method (Vagdevi et al. 2006) at a concentration of 62.5 µg/ml and 125 µg/ml. Streptomycin and Gresofulvin were used as standard for comparison of antibacterial and antifungal activity (Kumar 1996). Indian Pharmacopoeia, 1996).Solvent dimethyl formamide (DMF) was used as control. The results of screening are given in Table 2.

Table 2. Primary screening: Samples tested at 62.5 µg/ml and 125 µg/ml concentration for antibacterial and antifungal activity.

S. No	Code	Antimicrobial activity (µg/ml)			
		Antibacterial		Antifungal	
		Gram +ve	Gram –ve	*Candida*	*Aspergillus*
1.	3a	inactive	inactive	inactive	Inactive
2.	3b	inactive	inactive	inactive	Inactive
3.	3c	inactive	inactive	inactive	Inactive
4.	3d	inactive	inactive	inactive	Inactive
5.	3e	inactive	inactive	inactive	Inactive

Result and Discussion

3-acetyl-β-boswellic acid was reacted with thionyl chloride to afford acid chloride. To a stirred solution of acid chloride was added amino methyl ester and HOBT which acts as a coupling agent. The products formed are depicted in Scheme I. Pharmacological activity is depicted in Table 1 and 2. Amino acid conjugates thus formed showed increased anticancer activity and were found inactive when tested for antibacterial and antifungal activity.

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