GAVIN PUBLISHERS

Archives of Natural and Medicinal Chemistry

Suryachandra RR, et al. Nat Med Chem 2017-ANMC-103.

Review Article

Synthesis of Potent Cytotoxic Analogs of Boswellic Acids

Suryachandra Rao Ravada, Lakshmareddy Emani, Machi Raju Garaga, Trimurtulu Golakoti*

Laila Impex R & D Center, Jawahar autonagar, Vijayawada, Andhra Pradesh, India

***Corresponding author:** Trimurtulu Golakoti. Laila Impex R&D Center, Jawahar autonagar, Vijayawada, India Tel: +91-866-6636666; E-mail: research@lailanutra.com

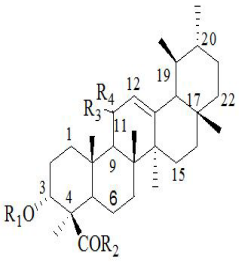
Citation: Suryachandra RR, Lakshmareddy E, Machi Raju G, Trimurtulu G (2017) Synthesis of potent cytotoxic analogs of boswellic acids. Arch Nat Med Chem 2017-ANMC-103.

Received Date: 4 April, 2017; **Accepted Date:** 17 April, 2016; **Published Date:** 24 April, 2017

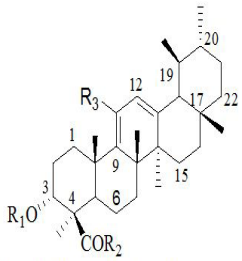
Abstract

Synthesis of a new group of boswellic acid analogs (3-9) has been accomplished through modification of enone functional group of 11-keto-β-boswellic acid (KBA, 1) and 3-O-acetyl-11-keto-β-boswellic acid (AKBA, 2) to chloro-diene moiety. The structures of the analogs were confirmed by using 1H, 13C NMR and mass spectral data. These compounds exhibited potent brine shrimp lethality and strong cytotoxicity against human cancer cell lines. The analogue 5 was found to be the most potent among the group as indicated by its efficacy against brine shrimp and MCF-7 cancer cell line with half inhibitory concentration (IC_{50}) values of 3.88 and 13.05 µM respectively.

Key words: Boswellic acid; AKBA; 3-O-acetyl-11-chloro-9-ene boswellic acid; cytotoxicity; anti-inflammatory activity General structure:



1. R_1 = H, R_2 = OH, R_3,R_4 = O
2. R_1 = Ac, R_2 = OH, R_3,R_4 = O
8. R_1 = H, R_2 = OH, R_3(or) R_4 = OH



3. R_1 = H, R_2 = OH, R_3 =Cl
4. R_1 = Ac, R_2 = OH, R_3 = Cl
5. R_1 = Ac, R_2 = $NHNH_2$, R_3 = Cl
6. R_1 = Ac, R_2 = $NHCH_2CH_2NH_2$, R_3 = Cl
7. R_1 = Ac, R_2 = $NH(CH_2CH_2)_2NH_2$, R_3 = Cl
8a. R_1 = H, R_2 = OH, R_3 = H
9. R_1 = Ac, R_2 = OH, R_3 = H

Introduction

The gum resin and extracts of the gum resin of *Boswellia serrata* are widely known in Indian Ayurvedic System of Medicine as treatments for various inflammatory diseases [1-3]. The major phytochemical constituents of gum resin are triterpenic acids called boswellic acids, which include β-boswellic acid (BA), 11-keto-β-boswellic acid (KBA, 1), 3-O-acetyl-β-boswellic acid and 3-O-acetyl-11-keto-β-boswellic acid (AKBA, 2) [4]. Boswellic acids are non-redox type and non-competitive selective inhibitors of 5-lipoxigenase [5-7]. In addition, boswelic acids exhibited chemo-preventive and anti-cancer properties [8]. Semi-synthetic analogs of boswellic acids have also shown promising anticancer activity [9]. Boswellic acids induce apoptosis in leukemic cells [10-12]. A few synthetic analogs of boswellic acids having amine functional group in place of acid moiety also exhibited apoptic activities [13]. AKBA and KBA are known to be the most potent biologically among its congeners. The review of therapeutic potential of boswellic acids has been reported recently [14]. Synthesis of 3-O-acetyl-11-keto-beta-boswellic acid analogs and their anti-inflammatory and anti-cancer activities have been also reported [15]. The pharmacokinetic studies established that AKBA is bio-available. Hence, boswellic acids have lot of potential for development as anti-cancer agents.

Result and discussion

A few semi synthetic analogs of AKBA were developed with modifications done in variations functional groups. However, the 11-keto function has not been properly explored to generate potential new analogs of AKBA. Organochloro compounds, such as vancomycine, loratadine, setraline etc, are well known to be used

as important medicines to combat different diseases. This observation encouraged the authors to prepare some chloro analogs of boswellic acids. Hence, the authors of present investigation were interested on the development of new analogs of AKBA through modification of 11-keto function (more specifically to convert the enone functional group to chlorodiene moiety).

As per classical chemistry, the displacement of carbonyl function by gem-dichloride function is generally achieved in the presence of phosphorous pentachloride [16]. The said displacement reaction in conjunction with E_2 elimination in presence of an inorganic base is employed to prepare alkenyl halides, allenes and alkynes. Cycloalkenylhalides can also be formed using a similar approach, but formation of cycloalkynes and cycloallenes can be a difficult task due to ring strain. The enone function in 3-O-acetyl-11-keto-βλ-boswellic acid (AKBA, 2) has been found to be resistant towards many chemical reagents, such as peroxy acids and reducing agents. As part of developing new analogs of AKBA, the authors intended to explore the feasibility of chlorinating the 11-keto group of AKBA. To achieve this objective, AKBA was dissolved in DMF and treated with three equivalents of PCl5 at room temperature. The TLC monitoring of the reaction mixture showed complete consumption of AKBA and appearance of a prominent new TLC spot. The product was originally perceived to be 3-O-acetyl-11, 11-dichloro-β-boswellic acid. However, the chromatographic purification of the reaction mixture yielded pure compound (4), which showed λ_{max} at 248 nm in the UV spectrum. The characteristic 12-H proton (δ 5.57) of the enone moiety of AKBA was absent in the 1H NMR spectrum of 4. Instead, a sharp singlet was observed at δ 5.40. Careful analysis of 1H NMR and 13C NMR spectral data revealed that the structure of the new analog is 3-O-acetyl-11-chloro-9-ene boswellic acid (4). It is presumed to be de-hydrochlorination product of initially formed dichloro precursor, wherein the transformation could have been facilitated by the acedic proton α to the gem-dichloro function. Similar treatment of 11-ketoboswellic acid (KBA) under identical conditions yielded 11-chloro-9 -ene boswellic acid (3). The chlorodiene compounds 3 and 4 were evaluated for their anti-tumor activity in comparison with AKBA (2) in breast cancer cell line MDA-MB-231. MBA-MD-231 is a triple negative breast cancer cell line, which has high propensity to develop metastasis. Human epidermal growth factor receptor (Her 2) tumors of this type are highly aggressive, resistant to chemotherapy and prognosis is very poor. The natural compound AKBA has shown potent cytotoxicity against MBA-MD-231 with an IC_{50} value of 55.26μM. Interestingly, the chlorodiene compounds both 3 and 4 showed significantly better efficacy when compared to AKBA with IC_{50} values of 42.85 and 35.72μM respectively. This unexpected outcome encouraged the authors, to select compound 4 as a lead compound and to conduct further analog development through modification of other key functional group, i.e. an acid function. The acid function was converted to an amide moiety using the following strategy [17].

Compound 4 was treated with thionylchloride at reflux temperature to obtain its acid chloride. Without purification, the crude acid chloride was subjected to amidification separately with diamino compounds selected from hydrazine, 1,2-diaminoethane and piperazine in THF at ambient temperature and each of the corresponding crude reaction mixtures were subjected to column chromatography over silica gel using hexane/ethyl acetate mixture as eluents to yield the amide compounds 5, 6 and 7 respectively in 60-70% over all yield. The structures of the analogs were confirmed by using ^{1}H, ^{13}C NMR, mass and IR spectra. For comparison, 3-O-acetyl-9-ene-β-boswellic acid (9) was prepared by subjecting KBA to reduction using excess lithium aluminum hydride followed by purification and acetylation of the intermediate 9-ene-β-boswellic acid (8a) with pyridine/acetic anhydride. During the reduction step 11-hydroxy-β-boswellic acid (8) was also formed as one of the product.

All the analogs (3 to 9) were tested *in vitro* for brine shrimp lethality and cytotoxicity against breast cancer cell lines, MCF-7 and MDA-MB-231. The Brine shrimp lethality has been shown to have correlation to the cytotoxicity by a comparative proliferation study in 9KB and 9PS cells [18-19]. The Brine shrimp lethality has been evaluated in comparison with a positive standard podophyllotoxin. The amides of chlorodiene showed potent brine shrimp lethality. The efficacies shown by the analogs 5, 6 and 7 in brine shrimp assay are superior as indicated by their IC_{50} values 3.88, 3.69 and 4.01μM respectively, when compared to that exhibited by the positive control podophyllotoxin (IC_{50}: 8.15μM). MCF-7 is an estrogen positive human breast adenocarcinoma cell line. The chlorodienes 3 and 4 exhibited relatively poor cytotoxicities against MCF-7 cell with IC_{50} values 70.57 and 60.89 μM respectively when compared to that of AKBA (IC_{50}: 49.52 μM), but the amide analogs 5, 6 and 7 of AKBA showed significantly improved efficacy with IC_{50} values at 13.05, 27.18, and 19.84 μM respectively. In addition to the chlorodiene compounds 3 and 4, the amide analogs 5, 6 and 7 also showed superior cytotoxic activity, when compared with AKBA in MDA-MB-231 cell line as summarized Table 1, with ethylenediamine amide being the most potent of all analogs with an IC_{50} value of 20.89μM. The analogs 3-O-acetyl-9-ene-μ-boswellic acid (9) and 11-hydroxy-μ-boswellic acid (8) showed inferior activity (IC_{50} values > 100 μM). The improved efficacy for chloro analog 4 in conjunction with complete lack of efficacy for its closest cousin 8 indicates potential contribution of chloride group to the efficacy. Over all the chlorodiene analogs and their amides may offer potential benefit against the solid tumors, more particularly against estrogen negative adenocarcinoma of breast, which justifies the need for their further development as anticancer therapies.

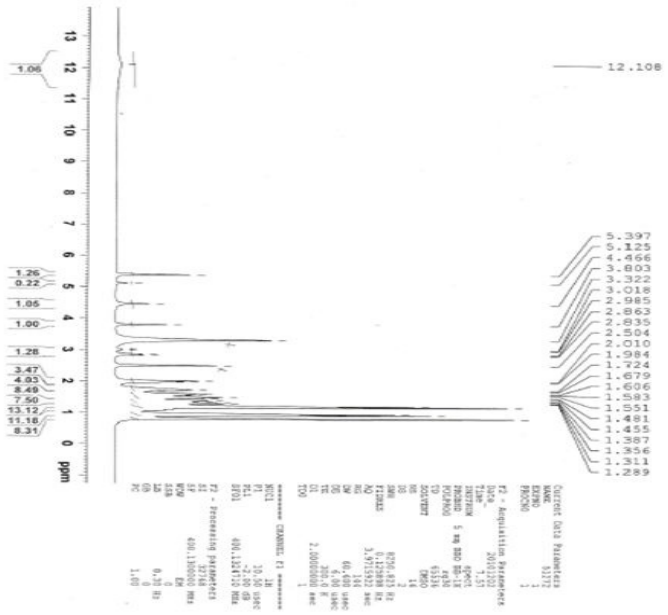


Figure S 01: ^{1}H NMR spectrum of 11-chloro-9-ene-boswellic acid (**3**)

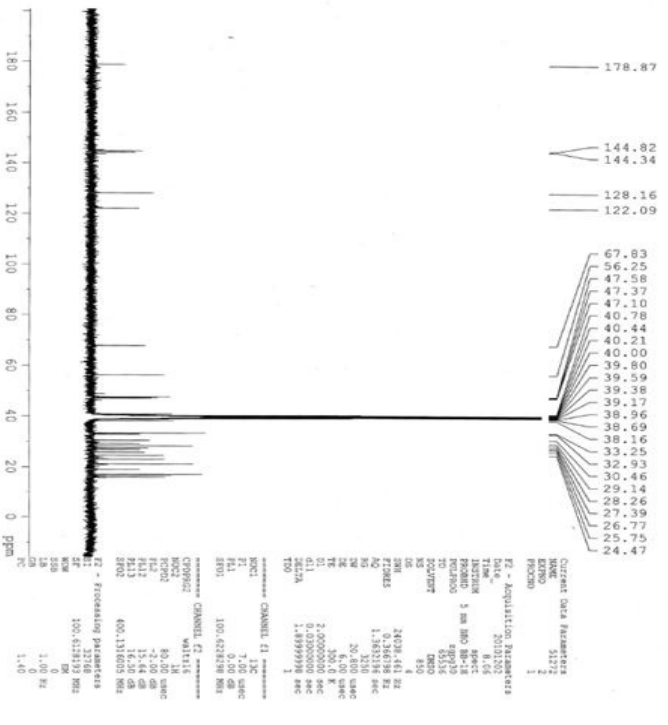


Figure S 02: ^{13}C NMR spectrum of 11-chloro-9-ene-boswellic acid (**3**)

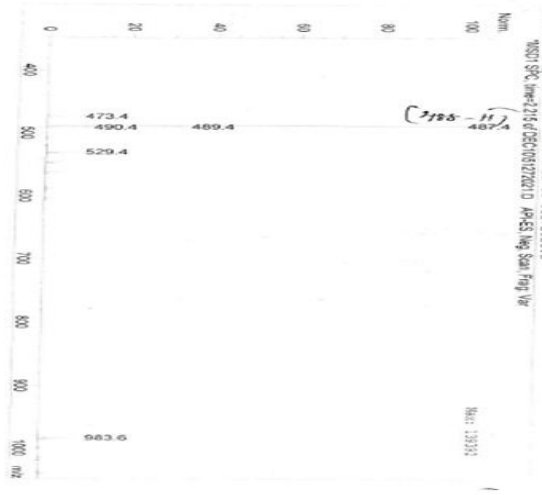


Figure S 03: Mass spectrum of 11-chloro-9-ene-boswellic acid (**3**)

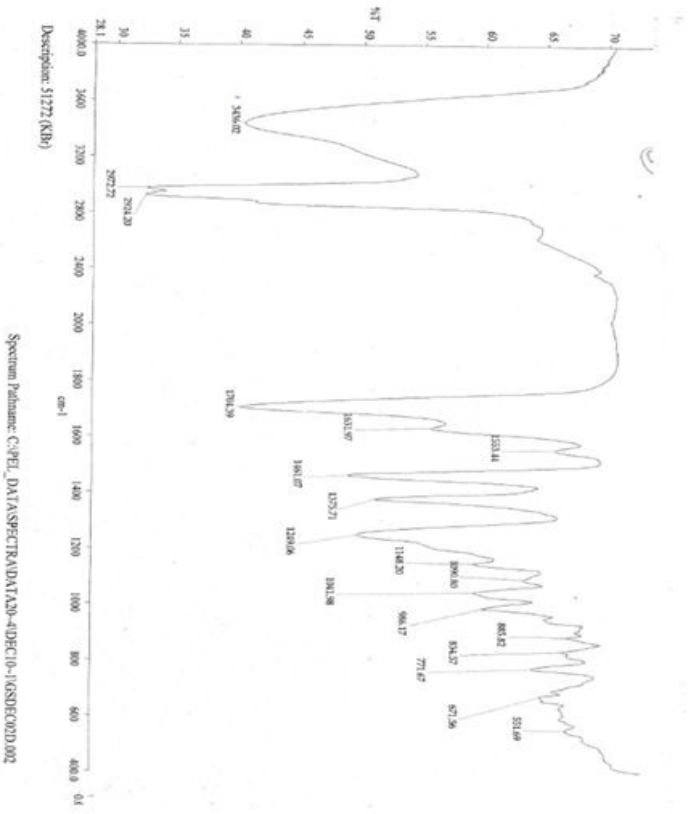


Figure S 04: IR spectrum of 11-chloro-9-ene-boswellic acid (**3**)

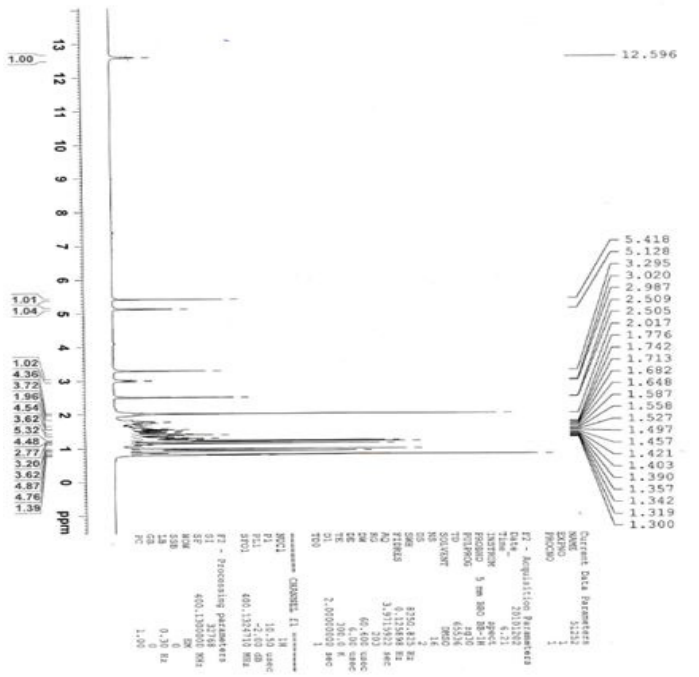


Figure S 05: ^{1}H NMR spectrum of 3-O-acetyl-11-chloro-9-ene-boswellic acid (4)

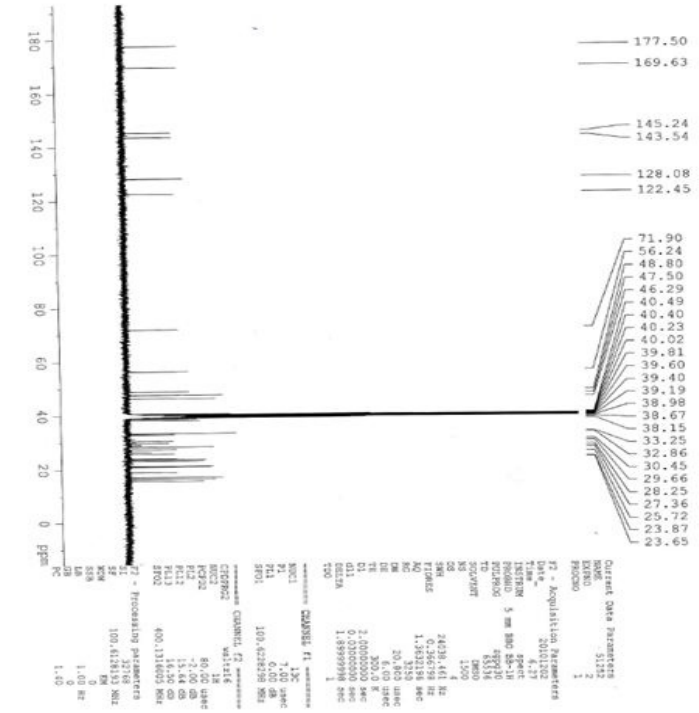


Figure S 06: 13C NMR spectrum of 3-O-acetyl-11-chloro-9-ene-boswellic acid (4)

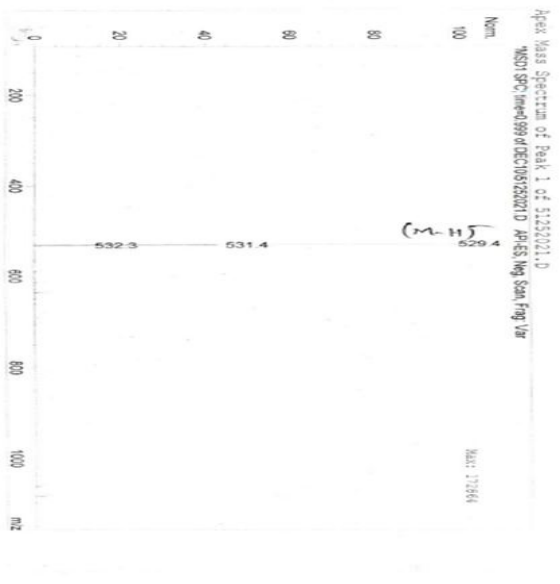


Figure S 07: Mass spectrum of 3-O-acetyl-11-chloro-9-ene-boswellic acid (4)

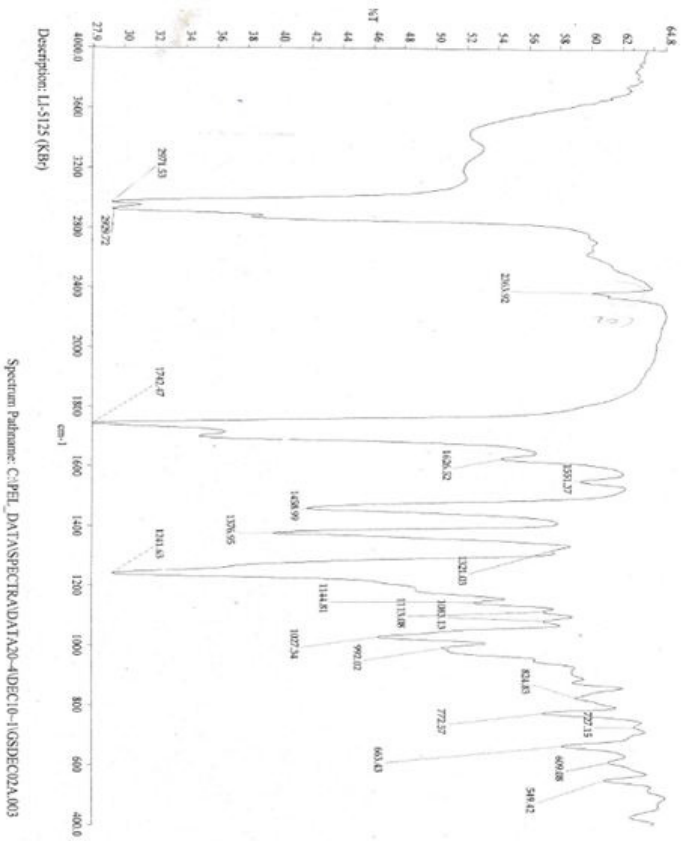


Figure S 08: IR spectrum of 3-O-acetyl-11-chloro-9-ene-boswellic acid (4)

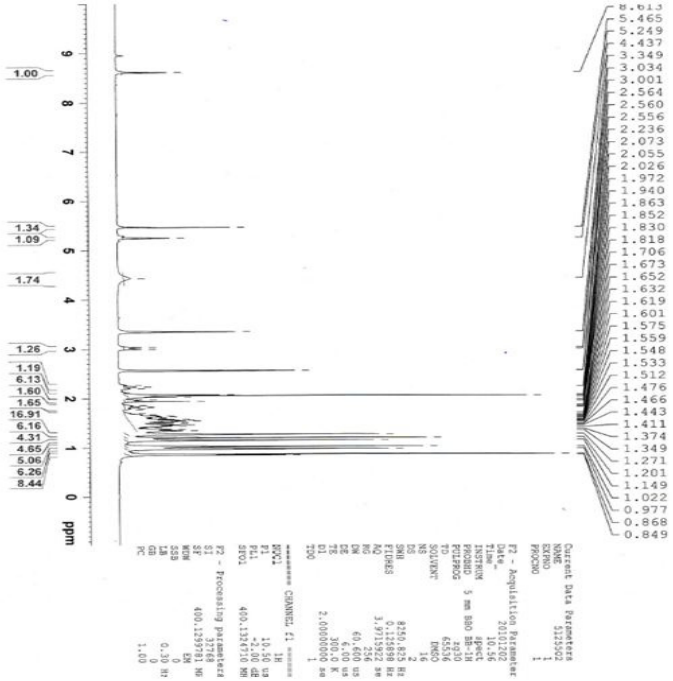


Figure S 09: ^{1}H NMR spectrum of hydrazine amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**5**)

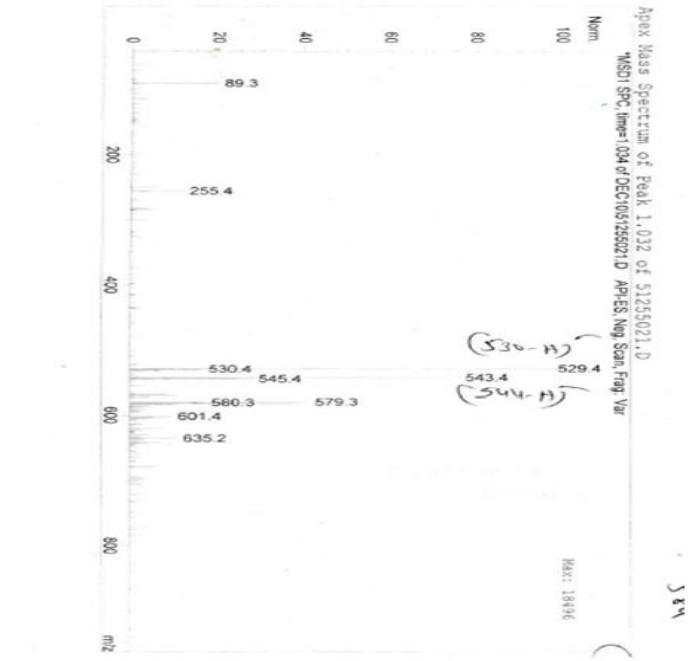


Figure S 11: Mass spectrum of hydrazine amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**5**)

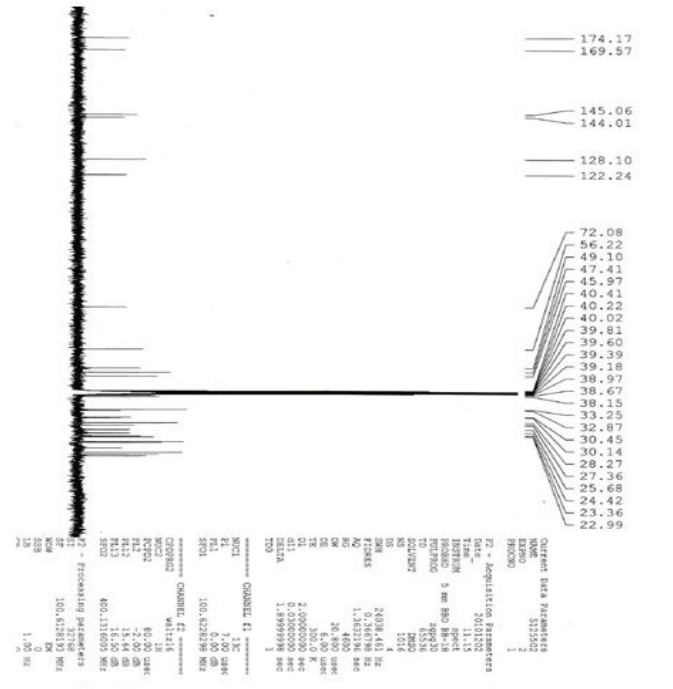


Figure S 10: ^{13}C NMR spectrum of hydrazine amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**5**)

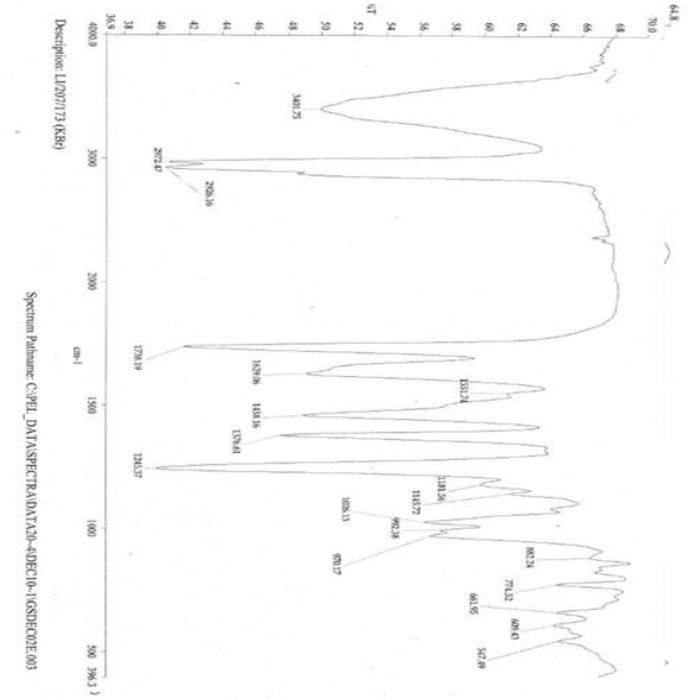


Figure S 12: IR spectrum of hydrazine amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**5**)

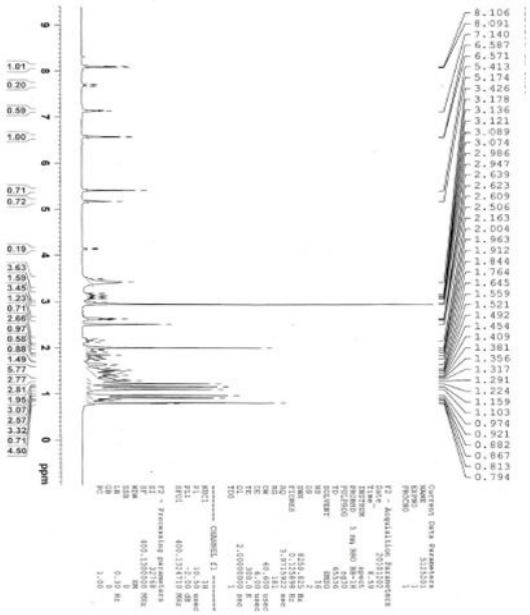


Figure S 13: ^{1}H NMR spectrum of 1,2-diaminoethane amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**6**)

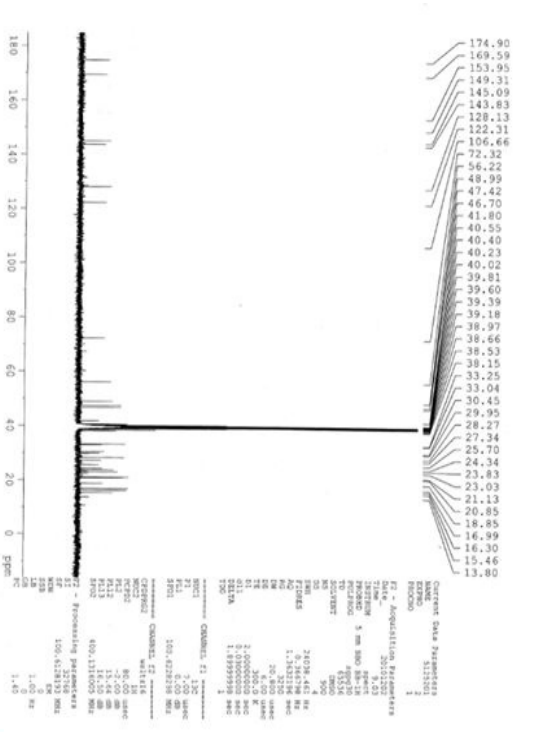


Figure S 14: ^{13}C NMR spectrum of 1,2-diaminoethane amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**6**)

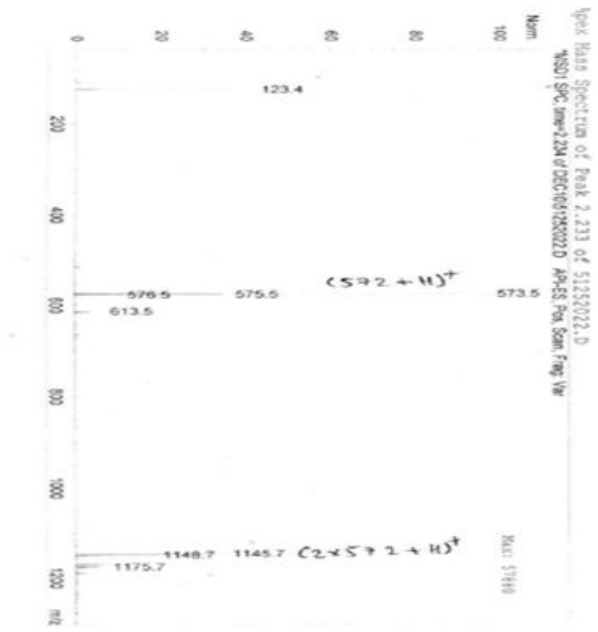


Figure S 15: Mass spectrum of 1,2-diaminoethane amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (6)

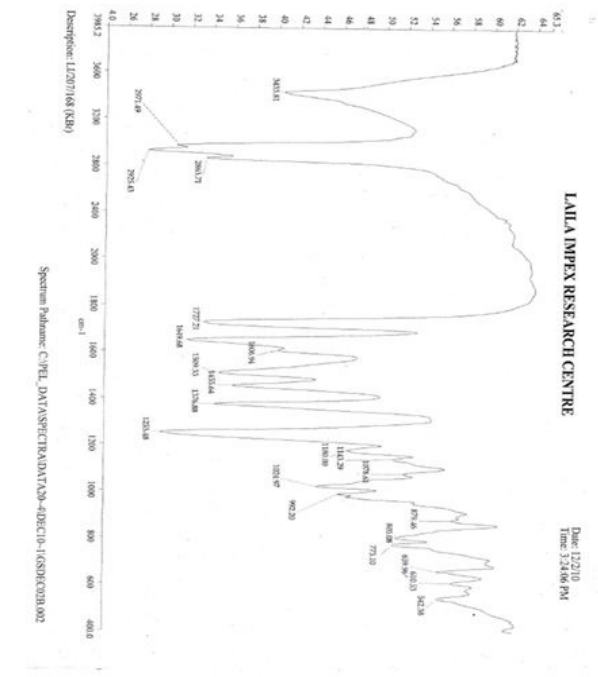


Figure S 16: IR spectrum of 1,2-diaminoethane amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**6**)

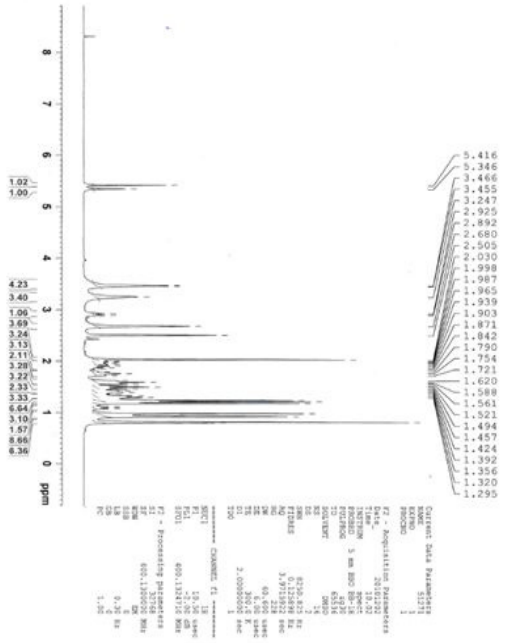


Figure S 17: ^{1}H NMR spectrum of piperazine amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**7**)

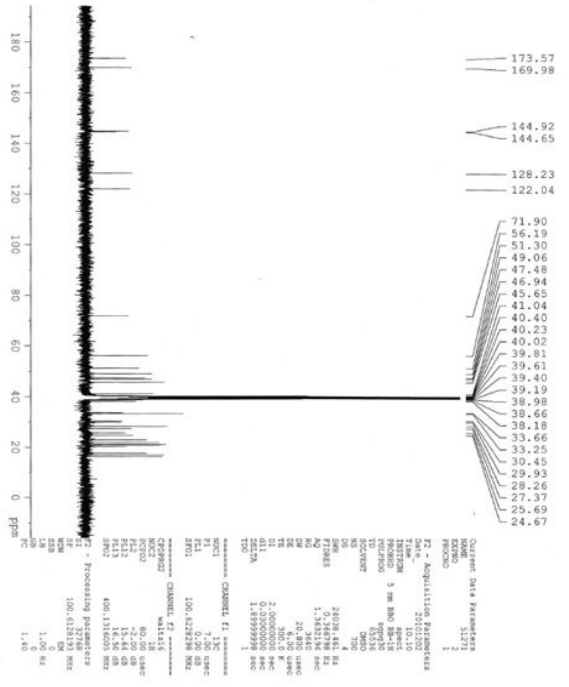


Figure S 18: ^{13}C NMR spectrum of piperazine amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**7**)

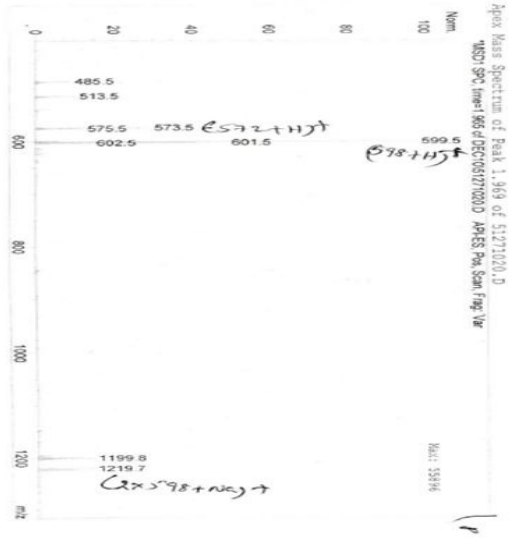


Figure S 19: Mass spectrum of piperazine amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**7**)

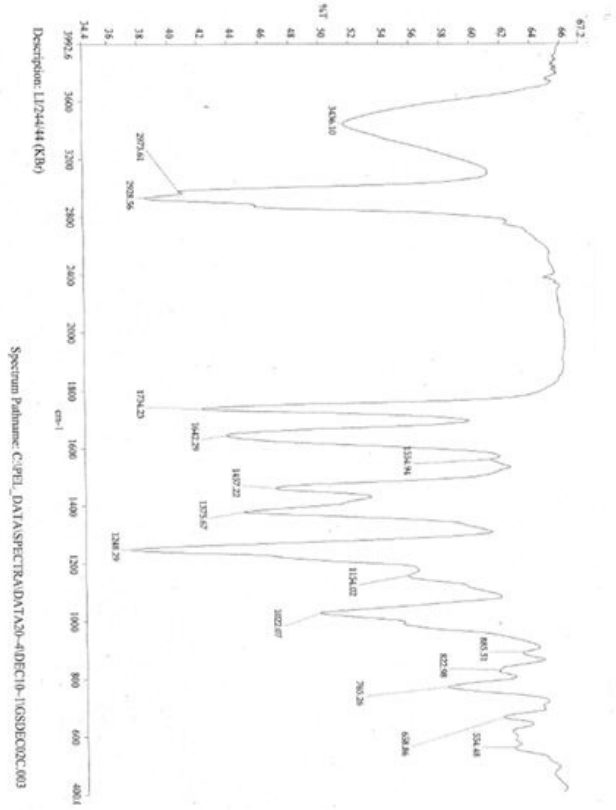


Figure S 20: IR spectrum of piperazine amide of 3-O-acetyl-11-chloro-9-ene-boswellic acid (**7**)

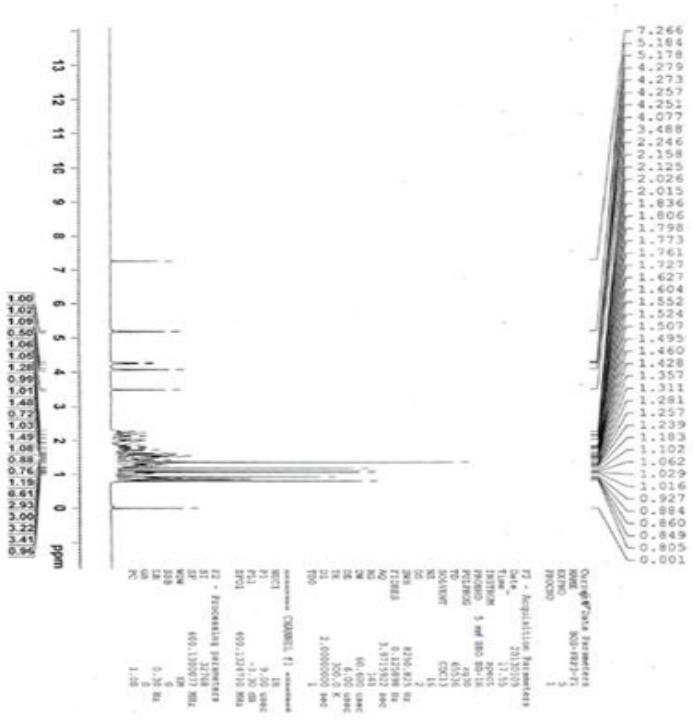


Figure S 21: ^{1}H NMR spectrum of 11-hydroxy-β-boswellic acid (**8**)

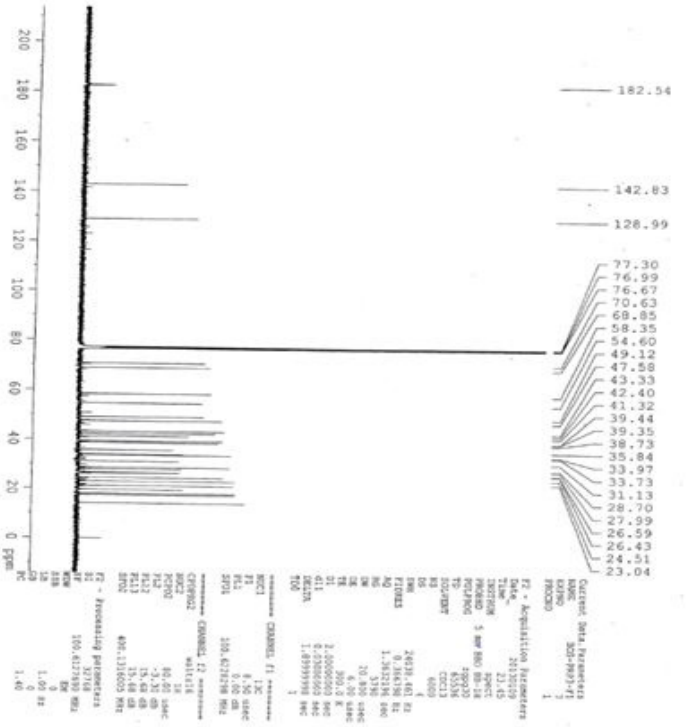


Figure S 22: ^{13}C NMR spectrum of 11-hydroxy-β-boswellicacid(**8**)

Experimental

General experimental procedures:

IR spectra were recorded on Perkin-Elmer (model spectrum BX) FT-IR instrument using KBr disc. The 1H NMR spectra were recorded on BrukerAvance AV 400 MHz Spectrometer and ^{13}C NMR spectra were recorded on BrukerAvance AV 100 MHz Spectrometer. Mass studies were performed on LC-MS system equipped with Agilent 1100 series LC/ MSD detector and 1100 series Agilent HPLC pump. Normal phase silica gel (ACME, 100-200 mesh) was used for column chromatography. Silica gel pre-coated plates (AlugramSil G/UV_{254}) were used for thin layer chromatography. Brine shrimp (*Artemiasalina* Cysts) eggs were obtained from Argent Chemical Laboratories, Redmond (USA). The solvents and other chemicals used were of LR grade and were procured from Qualigens Fine Chemicals, Mumbai (India).

Procedure for preparation of chlorodiene 3 and 4

11-Keto-β-boswellic acid (1) or 3-O-acetyl-11-keto-β-boswellic acid (2,0.01mol) was dissolved in dimethylformamide (30 ml) and treated slowly with phosphorous pentachloride (0.03 mol) portion wise for 30 min. After completion of addition, the stirring of the reaction mixture was continued for two hours at ambient temperature. Following the completion of reaction, the mixture was poured into ice water and the mixture was extracted with ethyl acetate (3×150 mL). The organic layer was washed with water followed by brine solution and dried over sodium sulphate. The solution was concentrated under vacuum and the crude residue was subjected to column chromatography using hexane /ethyl acetate mixtures as eluents. The fractions were eluted with 20-25% ethyl acetate/hexane mixtures were monitored by TLC and those containing the pure compound containing fractions were combined and concentrated to yield 11-chloro-9-ene boswellic acid (3) or 3-O-acetyl-11-chloro-9-ene boswellic acid (4) from the reaction of KBA (1) or AKBA (2) respectively.

Compound 3

Yield: 3.67g (75%); 1H NMR (d_6DMSO, 400 MHz): δ 12.1 (1H, brs, COOH), 5.40 (1H, s, H-12), 4.46 (1H, s, OH), 3.80 (1H, s, H-3), 2.84 (1H, d, J = 11.2 Hz), 2.03-1.99 (3H, m), 1.20 (3H, s), 1.17 (3H, s), 0.95 (3H, s), 0.92 (6H, s), 0.81(6H, s); ^{13}C NMR (d_6DMSO, 100 MHz): δ 178.9, 144.8, 144.3, 128.2, 122.1, 67.8, 56.3, 47.6, 47.4, 47.1, 40.8, 40.4, 38.7, 38.2, 33.3, 32.9, 30.46, 29.14, 28.26, 27.4, 26.8, 25.8, 24.5, 22.9, 21.1, 18.9, 17.0, 16.4, 15.8; IR (KBr, ν_{max}): 3436.02, 2972.72, 2924.2, 1704.39, 1631.97, 1461.07, 1375.71, 1249.06, 1041.98 cm-1; LC-MS: m/z 487.4 (M-H)- negative ion mode.

Compound 4:

Yield: 4.29g (81%); ^{1}H NMR (d_6DMSO, 400 MHz): δ 12.59

(1H, s, COOH), 5.42 (1H, s, H-12), 5.13 (1H, s, H-3), 2.99-2.96 (1H, m), 2.01(3H, s, COCH3) 1.85- 1.64 (4H, m), 1.60 - 1.45(6H, m), 1.42 - 1.26 (H, m), 1.22 (3H, s), 1.20 (3H, s), 1.14(3H, s), 0.98 (3H, s), 0.92 (3H, s), 0.81(3H, s), 0.80 (3H, d, J = 6.4 Hz); ^{13}C NMR (d_6DMSO, 100 MHz): δ 177.5, 169.6, 145.2, 143.5, 128.1, 122.5, 71.9, 56.2, 48.8, 47.5, 46.3, 40.5, 40.4, 38.7, 38.2, 33.3, 32.8, 30.5, 29.7, 28.3, 27.4, 26.3, 25.7, 23.8, 23.6, 23.0, 21.1, 20.8, 18.8, 16.9, 16.3, 15.6; IR(KBr, ν_{max}): 2971.53, 2929.72, 1742.47, 1458.99, 1376.95, 1241.61, 1027.34, 992.02 cm-1; LC-MS: m/z 529.4 (M-H)- negative ion mode.

Procedure for preparation of 3-O-acetyl-11-chloro-9-ene boswellic acid amides (5-7)

3-O-acetyl-11-chloro-9-ene boswellic acid (4,0.53g, 0.001 mol) was taken into a around bottom flask and treated with thionyl chloride (0.5 mL, 0.006 mol). The reaction mixture was heated at 70˚C for one hour. Excess thionyl chloride from the reaction mixture was stripped off under high vacuum and the residue was subjected to vacuum drying. The crude acid chloride of 4 was dissolved in THF (5mL) and then treated separately with one of the diamines selected from hydrazine, 1,2-diaminoethane and piperazine (0.0025 mol) under stirring at ambient temperature for 3h. The progress of the reaction was monitored over TLC and after completion of reaction, the reaction mixture was poured in ice-cold water and the mixture was extracted with ethyl acetate (3×100 mL). The combined organic layer was washed with water followed by brine and dried over sodium sulphate. The solution was concentrated under vacuum and each of the crude products was subjected to column chromatography using hexane/ethyl acetate mixtures as eluents. The fractions were eluted with 30-35% ethyl acetate/hexane were monitored and the pure fractions were combined to obtain compounds 5, 6 or 7 respectively from the reaction of hydrazine,1,2-diaminoethane or piperazine with acid chloride.

Compound 5

Yield: 0.38 g (70%); ^{1}H NMR (d_6DMSO, 400 MHz): δ 8.61 (1H, s, CONH), 5.46 (1H, s, H-12), 5.25 (1H, s, H-3), 4.43 (2H, brs, NH-NH_2), 3.02 (1H, d, J = 13.2 Hz), 2.26-2.20 (1H, m), 2.05 (3H, s, COCH3), 2.04-2.02 (2H, m), 1.97-1.94 (2H, m), 1.86 - 1.78 (2H, m), 1.70 - 1.44 (14H, m), 1.41 - 1.37 (2H, m), 1.27 (3H, s), 1.20 (3H, s), 1.15 (3H, s), 1.02 (3H, s), 0.98 (3H, s), 0.87 (3H, s), 0.85 (3H, d, J = 7.6 Hz); ^{13}C NMR (d6DMSO, 100 MHz): δ 174.2, 169.6, 145.0, 144.0, 128.1, 122.2, 72.1, 56.2, 49.1, 47.4, 46.0, 40.4, 40.2, 38.9, 38.7, 38.1, 33.3, 32.9, 30.4, 30.1, 28.3, 27.4, 25.7, 24.5, 23.4, 23.0, 21.1, 20.9, 18.8, 17.0, 16.3, 15.7; IR (KBr, ν_{max}): 3401.75, 2972.47, 2926.16, 1738.19, 1629.06, 1458.16, 1376.61, 1245.37, 1026.13, 970.17 cm^{-1}; LC-MS: m/z 543.4 (M-H)- negative ion mode.

Compound 6

Yield: 0.35 g (62%); ^{1}H NMR (d_6DMSO, 400 MHz): δ 7.14 (1H, t, CONHCH2), 5.41 (1H, s, H-12), 5.17 (1H, s, H-3), 3.49-3.47 (2H, m), 3.18-3.07 (2H, m), 2.62 (2H, t, J = 6.0 Hz, NH_2-CH_2), 2.20-2.12 (1H, m), 2.00 (3H, s, COCH3) 1.95 - 1.91 (1H, m), 1.84 (1H, s), 1.81-1.73 (2H, m), 1.64 - 1.62 (2H, m), 1.55 - 1.29 (10H, m), 1.22 (3H, s), 1.19 (3H, s), 1.10 (3H, s), 0.97 (3H, s), 0.92 (3H, s), 0.87 (1H, m), 0.81 (3H, s), 0.80 (3H, d, J = 6.8 Hz); ^{13}C NMR (d_6DMSO, 100 MHz): δ 174.9, 169.6, 145.1, 143.8, 128.1, 122.3, 72.3, 56.2, 49.0, 47.4, 46.7, 41.8, 40.6, 40.4, 38.6, 38.5, 38.2, 33.3, 33.0, 30.5, 29.9, 28.3, 27.4, 25.7, 24.3, 23.8, 23.0, 22.3, 21.1, 20.9, 18.8, 16.9, 16.3, 15.5; IR (KBr, ν_{max}): 3433.81, 2971.49, 2925.43, 2863.71, 1727.21, 1649.68, 1509.33, 1455.64, 1376.88, 1253.48, 1024.97, 992.20 cm-1; LC-MS: m/z 573.5 (M+H)+, positive ion mode.

Compound 7

Yield: 0.36g (60%); ^{1}H NMR (d_6DMSO, 400 MHz): δ 5.42 (1H, s, H-12), 5.35 (1H, s, H-3), 3.46 (4H, d, J = 4.4 Hz, N(CH_2)2), 2.91 (1H, d, J = 13.2 Hz), 2.68 (4H,s), 2.03 (3H, s, COCH3), 1.99 - 1.84 (3H, m), 1.79-1.72 (2H, m), 1.62 - 1.56 (3H, m), 1.52 - 1.45 (4H, m), 1.42-1.35 (2H, m), 1.32-1.29 (3H, m), 1.23 (3H, s), 1.21 (3H, s), 1.18 (3H, s),1.11-1.07 (2H, m), 0.97 (3H, s), 0.92 (3H, s), 0.82 (3H, s), 0.81 (3H, d, J = 6.0 Hz); ^{13}C NMR (d6DMSO, 100 MHz): δ 173.6, 169.9, 144.9, 144.7, 128.2, 122.0, 71.9, 56.2, 51.3, 49.1, 47.5, 46.9, 45.7, 41.0, 40.4, 38.7, 38.2, 33.7, 33.3, 33.0, 30.6, 29.9, 28.3, 27.4, 25.7, 24.7, 22.9, 21.8, 21.4, 20.8, 20.2, 17.7, 17.0, 16.4, 15.5; IR (KBr, ν_{max}): 3436.10, 2973.61, 2928.56, 1734.23, 1642.29, 1457.22, 1375.67, 1248.29, 1022.07 cm^{-1}; LC-MS: m/z 599.5 $(M+H)^+$, positive ion mode.

9-Ene-β-boswellic acid (8a) and 11-hydroxy-β-boswellic acid (8)

Lithium aluminum hydride (90 mg, 2.36m.mol) was dispersed in THF and cooled in ice water bath. A solution of 11-keto-β-boswellic acid (300 mg, 0.64 m.mol) in THF (2 ml) was slowly added to the above dispersion and the stirring continued in the ice water bath for 2h. The reaction mixture was diluted with ethyl acetate (2 ml) and after 5 minutes it was poured in to ice water. The mixture was carefully neutralized with 1 N HCl and extracted with ethyl acetate (3 × 25 ml). The combined organic layer was washed with water, brine, dried over Na_2SO_4 and evaporated. The residue was purified on silica column using hexane and ethyl acetate mixtures. The fractions eluted with 25% ethyl acetate/hexane mixture yielded 9-ene-ß-boswellic acid (8a,150 mg), 11-hydroxy-β-boswellic acid (8,70 mg).

11-hydroxy-β-boswellic acid (8)

Melting point: 152 - 160°C, 1HNMR ($CDCl_3$): δ 5.18 (1H,

d; J = 2.4 Hz, 12-H), 4.26 1H, dd; J = 9.1 and 2.9 Hz, 11-H), 4.08 (1H, br s), 2.19 - 2.31 (1H, m), 2.15 (1H, brd; J = 13.3), 1.96 - 2.08 (1H, m), 1.36 (3H, s, -CH_3), 1.18 (3H, s, -CH_3), 1.10 (3H, s, -CH_3), 1.07 (3H, s, -CH_3), 0.93 (3H, s, -CH_3), 0.85 (3H, s, -CH_3), 0.80 (3H, d; J = 5.6 Hz, -CH3); 13C NMR ($CDCl_3$, 100 MHz): δ 182.5, 142.8, 128.9, 70.6, 68.8, 58.4, 54.6, 49.1, 47.6, 43.3, 42.4, 41.3, 39.4, 39.3, 35.8, 33.9, 33.7, 31.1, 28.7, 27.9, 26.6, 26.4, 24.5, 23.0, 21.3, 19.6, 18.0, 17.5, 14.2; IR (KBr, ν_{max}): 3390, 2921, 2860, 1696, 1451, 1379, 1246, 1001 cm-1. LC-MS: m/z 471 (M-H)-, negative ion mode

9-ene-ß-boswellic acid (8a)

Melting point: 186 - 190°C, 1HNMR (CDCl3): δ 5.65 (1H, d, J = 5.6Hz), 5.47 (1H, d, J = 5.6Hz), 3.83 (1H, brs, 3-H), 2.14 - 2.21 (1H, m), 1.96 - 2.03 (1H, m), 1.27 (3H, s, -CH3), 1.20 (3H, s, -CH_3), 1.12 (3H, s, -CH_3), 0.95(3H, s, -CH3), 0.93 (3H, s, -CH_3), 0.87 (3H, s, -CH3), 0.81 (3H, d; J = 6.4Hz, -CH3); 13C NMR ($CDCl_3$, 100 MHz): δ 179.2, 152.7, 141.3, 123.5, 116.1, 73.3, 57.2, 47.1, 46.9, 43.4, 41.2, 40.7, 39.3, 39.0, 38.8, 33.7, 33.2, 31.7, 31.2, 29.9, 28.7, 28.6, 26.2, 24.4, 23.4, 23.3, 21.5, 21.5, 21.2, 19.7; IR (KBr, ν_{max}): 3390, 2921, 2860, 1696, 1451, 1379, 1246, 1001 cm-1. LC-MS: m/z 453 (M-H)-, negative ion mode.

Acetylation of 9-ene-ß-boswellic acid (8a): The compound 8a (40 mg) was dissolved in pyridine (0.1 ml) and treated with acetic anhydride (0.1ml) at room temperature. After 3 h, the mixture was poured into ice water (10 ml) and extracted with ethyl acetate (20 ml). The organic layer was washed with brine, dried over Na2SO4 and evaporated to obtain 3-acetoxy-9-ene-boswellic acid (9). Yield : 31 mg; Melting point: 170-176°C, 1HNMR ($CDCl_3$): δ 5.65(1H, d, J = 5.6 Hz), 5.47(1H, d, J = 5.6Hz), 5.31 (1H, brs, 3-H), 2.14 - 2.21 (1H, m), 2.06 (3H, s), 1.96-2.03 (1H, m), 1.27 (3H, s, -CH_3), 1.20 (3H, s,-CH_3), 1.12 (3H, s, -CH3), 0.95(3H, s -CH_3), 0.93 (3H, s, -CH3), 0.87 (3H, s,-CH3), 0.81 (3H, d; J = 6.4Hz,-CH3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.3, 170.2, 152.5, 141.6, 123.1, 116.6, 72.9, 57.4, 47.5, 46.9, 43.4, 41.4, 40.7, 39.5, 39.1, 39.0, 33.7, 33.2, 31.9, 31.2, 29.7, 28.7, 28.3, 26.2, 24.4, 23.7, 23.3, 21.8, 21.5, 21.2, 19.6, 17.4; IR (KBr, ν_{max}): 3390, 2921, 2860, 1696, 1451, 1379, 1246, 1001 cm^{-1}. LC-MS: m/z 495.4 (M-H)-, negative ion mode.

Brine Shrimp Lethality Bioassay

Brine shrimp (*Artemia salina*) nauplii were hatched using brine shrimp eggs [20]. In a conical shaped vessel (1L), filled with sterile artificial sea water (prepared using sea salt 38g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48h. After hatching, 10 nauplii were drawn through a pipette and placed in each vial containing 4.5 mL brine solution and treated with various concentrations of test substances (2-9) and the final volume was made up to 5 mL using brine solution. The cultures were maintained at 37°C for 24h under incandescent lamps. The surviving larvae were counted. Each experiment was conducted along with control (vehicle treated), at various concentrations of the test substance with each set containing 6 tubes and mean of the results were noted in the table. The percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. The LC50 values were obtained from the plot drawn containing concentration (μM) verses percentage inhibition. Podophyllotoxin was used as a positive control. The results are summarized in (Table 1).

Determination of cytotoxicity using MTT assay

The 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [21] was employed to evaluate the cytotoxicity of the boswellic acid analogs. The cell lines human breast adrenocarcinoma cell line (MCF-7) and breast cancer cell line (MDA-MB-231) were obtained from American Type Culture Collection (Rockville, MD), and cultured with complete RPMI-1640 medium (Gibco BRL) supplemented with 10% (V/V) heat-inactivated fetal calf serum, antibiotics, L-glutamine. The cell numbers were determined with a hemocytometer, and viabilities were assessed by try pan-blue dye exclusion. The known amount of test compounds (2-9) were dissolved in a few drops of DMSO and diluted with water to make the concentration of DMSO less than 0.1%. Various concentrations of test compounds were added to the test cultures ($2x10^6$ cells/mL) grown in a 96-well plate and the drug treated cultures were incubated for 24h. The control cultures were exposed to the solvent carrier only. MTT is a pale yellow substance that was reduced by living cells to yield a dark blue formazan product. This process requires active mitochondria only and dead cells, even the freshly dead ones do not significant reduce the amount of MTT. MTT in PBS (0.1 mg) was added into each well and then incubated at 37°C for 4h. The MTT formazan [1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan] crystals formed due to dye reduction by viable cells were dissolved using acidified isopropanol (0.1N HCl) and mixed at room temperature. After 20 min, index of cell viability was calculated by measuring the optical density (OD) of color produced by MTT dye reduction with a microplate reader (BIO-RAD, model 3550, USA) at 570 nm ($OD_{570-620}$) and expressing it as percentage of control. The mean OD of the content of four wells was used for assessing the cell viability. Viable cells (%) = [(total cells-dead cells)/total cells] × 100%. The IC50 values were obtained from the plot drawn containing concentration (μM) verses percentage inhibition. The data is summarized in (Table 1).

S. no	Compound	IC_{50}/µM		
		Brine shrimp	MCF-7	MDA-MB-231
1	2	6.24	49.52	55.26
2	3	42.46	70.57	42.85
3	4	39.21	60.89	35.72
4	5	3.88	13.05	31.75
5	6	3.69	27.18	20.89
6	7	4.01	19.84	25.74
7	8	>100	>100	>100
8	9	>100	>100	>100
9	Podophyllo -toxin	8.15	-	-
10	Tamoxifen*	-	17.49	12.37

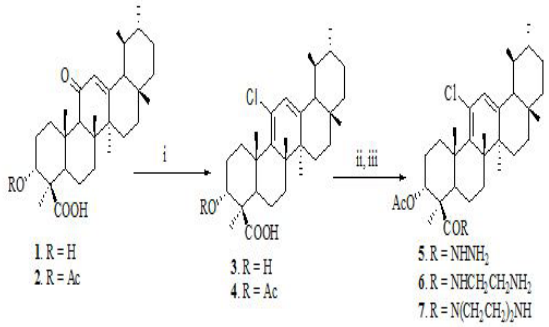
Table 1: In vitro activities of compounds 2-9

*The IC50 values for this positive control (tamoxifen) are obtained from literature [22] for comparison.

Conclusion

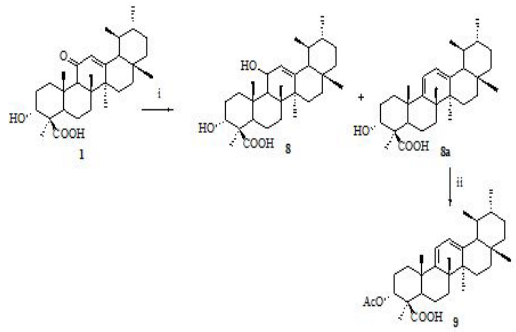
Boswellic acids are indicated for the wide range of disease indications especially inflammation and cancer. Five new analogs of AKBA with chlorodiene functionality were synthesized and their cytotoxicity was evaluated. All the chlorodiene analogs and their amides offer potential benefit against the solid tumors, more particularly against estrogen negative adenocarcinoma of breast. This work justifies the need for further exploration of Boswellic acid analogs as anticancer agents.

Scheme-1



Reagents & conditions: i). PCl5, DMF, RT, 2h, 75-81%; ii). SOCl2,70˚C, 1h; iii). Amines, THF, RT, 3h, 60-70%.

Scheme-2



Reagents & conditions: i). LAH, THF; ii). Acetic anhydride, pyridine.

References

1. Kirtikar KR, Basu, BD (1935) Indian medicinal plants. Latter EB, Caius Jf 2nd edition 0-42, Vivelt Vihar, Delhi, India.
2. Chatterjee GK, Pal SD (1984) Indian Drugs 21: 431.
3. Ammon HP, Safayhi H, Mack T, Sabieraj J (1993) Mechanism of anti inflammatory actions of curcumine and boswellic acids. J Ethnopharmacol 38: 113-119.
4. Mahajan B, Sethi VK, Taneja SC, Dhar KL (1995) Two triterpenoids from Boswellia serrata gum resin. Phytochemistry 39: 453-455.
5. Safayhi H, Meck T, Sabieraj J, Anazodo MI, Subramannian LR, et al. (1992) Boswellic acids: novel, specific, nonredox inhibitors of 5-lipoxygenase. J Pharmacol Exp Ther 261: 1143-1146.
6. Schweizer S, Von Brocke AF, Boden SE, Bayer E, Ammon HP, et al. (2000) Workup-dependent formation of 5-lipoxygenase inhibitory boswellic acid analogues. J Nat Prod 63: 1058-1061.
7. Safayhi H, Sailer ER, Ammon HP (1995) Mechanism of 5-lipoxygenase inhibition by acetyl-11-keto-beta-boswellic acid. Mol Pharmacol 47: 1212-1216.
8. Han R (1994) Highlight on the studies of anticancer drugs derived from plants in china. Stem cells 12: 53-63.
9. Shashi B, Kumar A, Malik F, Andotra SS, Sethi VK, et al. (2007) Reactive oxygen species generation and mitochondrial dysfunction in the apoptotic cell death of human myeloid leukemia HL-60 cells by a dietary compound withaferin A with concomitant protection by N-acetyl cysteine. Apoptosis 12: 2115-2133.
10. Hoernlein RF, Orlikowsky T, Zehrer C, Niethammer D, Sailer ER, et al. (1999) Acetyl-11-keto-beta-boswellic acid induces apoptosis in HL-60 and CCRF-CEM cells and inhibits topoisomerase I. J Pharmacol Exp Ther 288: 613-619.
11. Jing Y, Nakajo S, Xia L, Nakaya K, Fang Q, et al. (1999) Boswellic acid acetate induces differentiation and apoptosis in leukemia cell lines. Leuk Res 23: 43-50.
12. Shao Y, Ho CT, Chin CK, Badmaev V, Ma W, et al. (1998) Inhibitory activity of boswellic acids from Boswellia serrata against human leukemia HL-60 cells in culture. Planta Med 64: 328-331.

13. Shah BA, Kumar A, Gupta P, Sharma M, Sethi VK, et al. (2007) Cytotoxic and apoptotic activities of novel amino analogues of boswellic acids. Bioorganic and Medicinal chemistry letters 17: 6411-6416.

14. Hussain H, Al-Harrasi A, Csuk R, Shamraiz U, Green IR, et al. (2017) Therapeutic potential of boswellic acids: a patent review (1990-2015). Expert Opinion on Therapeutic Patents 27: 81-90.

15. Ganga Raju G, Rama Raju G, Venkata Subbaraju G, Trimurtulu G, Krishanu S (2014) Analogs of 3-O-acetyl-11-keto-beta-boswellic acid.

16. Melvin SN, Louis LW (1959) Concerning the Mechanism of the Reaction of Phosphorus Pentachloride with Ketones. J Am Chem Soc 81: 4300-4303.

17. Mahajan SS, Mahalakshmi A (2006) Synthesis of 2-amino-5-chlorobenzonitrile. Indian journal of chemistry 45B: 1299-1300.

18. McLaughlin JL, Chang C-J, Smith DL (1993) Simple bench-top bioassays (brine shrimp and potato discs) for the discovery of plant anti tumor compounds : Review of recent progress. Human Medicinal Agents from plants. American Chemical Society 112-134.

19. McLaughlin JL, Rogers LL, Jon E. Anderson (1998) The use of biological assays to evaluate botanicals. Drug information Journal 32: 513-524.

20. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, et al. (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 45: 31-34.

21. Tian Q, Miller EG, Ahmad H, Tang L, Patil BS (2001) Differential inhibition of human cancer cell proliferation by citrus limonoids. Nutritional Cancer 40: 180-184.

22. Fita A, Goua M, Wahle KW, Schofield AC, Hutcheon AW, et al. (2007) Potentiation of the anti-tumour effect of Docetaxel by conjugated linoleic acids (CLAs) in breast cancer cells in vitro. Prostaglandins Leukot Essent Fatty Acids 77: 87-96.