New seco-limonoids from Cipadessa baccifera: Isolation, structure determination, synthesis and their antiproliferative activities

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Abstract: A comprehensive reinvestigation of chemical constituents from $CHCl_3$-soluble extract of *Cipadessa baccifera* led to the isolation of two new limonoids **1**, **2** together with six known compounds **3-8**. Their structures were established on the basis of extensive analysis of spectroscopic (IR, MS, 2D NMR) data. Further, a series of cipaferen G (**3**) derivatives were efficiently synthesized utilizing Yamaguchi esterification (2, 4, 6-trichlorobenzoyl chloride, Et_3N, THF, DMAP, toluene) at the C-3 position of the limonoids core, which is being reported for the first time. The anti-proliferative activity of the isolates and the synthetic analogues were studied against HeLa, PANC 1, HepG2, SKNSH, MDA-MB-231 and IMR32 cancer cells using the sulphorodamine B assay. Among the tested compounds, **13d** and **13h** manifested potent activity against IMR32, HepG2 cell lines with GI_{50} 0.013 and 0.01µM, respectively.

Keywords: *Cipadessa baccifera*, limonoids, Yamaguchi esterification, ester derivatives and anti-proliferative activity.

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Graphical Abstract

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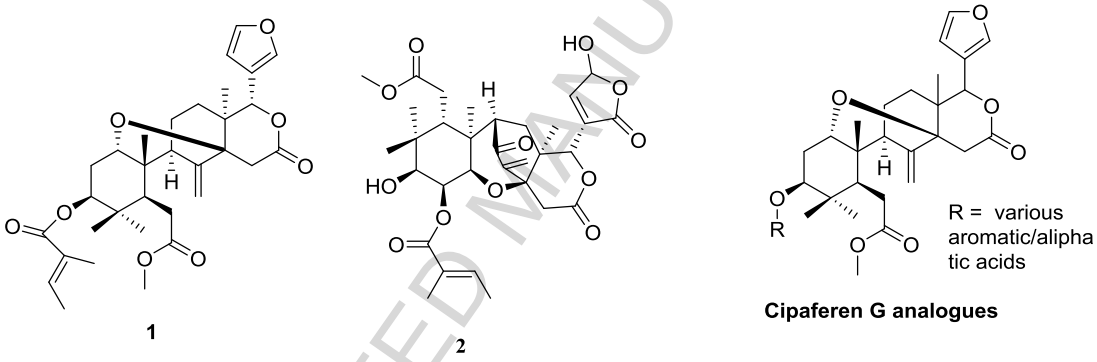

Phytochemical investigation of $CHCl_3$-soluble extract from the seeds of *Cipadessa baccifera* afforded two new limonoids (**1**-**2**) along with the six known compounds (**3**-**8**). Further, a series of ester analogues of cipaferen G (**3)** were prepared using Yamaguchi esterification protocol and screened for their anti-proliferation against panel of cancer cell lines.

1. Introduction

Limonoids, a class of nortriterpenoids from the plants of Meliaceae & Rutaceae families attracted considerable attention from medicinal chemists as well as chemical biologists because of their fascinating structural diversity and important biological activities [1]. *Cipadessa baccifera* is a prominent member of the Meliaceae family, which mainly grows in the tropical areas of Asia and one of the most popular traditional medicines in India for the treatment of dysentery and pruritus [2]. The bark has a bitter taste and its decoction has been utilized to treat dysentery, skin itches and malaria fevers by tribal communities [3]. Previous investigations on the chemical constituents of this plant resulted in isolation of tetranortriterpenoids and terpenoids [4-6].

As part of our continuous endeavors of phytochemical–pharmacological integrated studies on the Indian medicinal plants [7-9], we focused on the investigation of limonoid constituents from plants of the Meliaceae family, and recently reported the isolation and characterization of few new compounds from seeds of *Cipadessa baccifera* [10-11]. In the present work, to obtain the minor components, we decided to increase the amount of plant material and to reinvestigate the chemical constituents of the seed extract. This has resulted in the isolation of two new limonoids (**1, 2**) along with six known compounds (**3**-**8**). The structures of these novel metabolites were established by spectroscopic methods, especially, 2D NMR techniques and mass spectral data. Further, a series of ester derivatives were synthesized utilizing Yamaguchi esterification (2, 4, 6-trichlorobenzoyl chloride, Et_3N, THF, DMAP, toluene) at the C-3 position of the cipaferen G (**3**). All the isolates (**Figure-1**) and analogues were tested for their anti-proliferation activity against the panel of human cancer cell lines. In this paper, we describe the isolation, structural elucidation and biological activities of these novel compounds along with synthesis of analogues.

(Figure-1)

2. Experimental

2.1 General

Optical rotations were measured using a JASCO DIP 300 digital polarimeter and at 1ml cell at 25^{o}C. IR spectra were recorded on a Nicolet-740 spectrometer with KBr pellets. The NMR spectra were recorded on a Bruker FT-300 MHz spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C respectively, using TMS as internal standard. The chemical shifts are expressed as δ values in parts per million (ppm) and the coupling constants (*J*) are given in hertz (Hz). Mass spectra were performed on a LC-MS/MS (Agilent Technologies 6510) Q-TOF Mass spectrometer. The 2D experiments (^{1}H–^{1}H COSY, HSQC, HMBC, NOESY) were performed using standard Bruker microprograms. Column chromatography was performed with silica gel (100–200 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). Semipreparative HPLC was performed on an Agilent 1100 series LC/MSD Trap SL, with a Phenomenex Luna C18 (250x10 mm 10µ) column. Preparative HPLC was performed on a Dionex P680 equipped with PDA detector and Shimadzu PRC-ODS (K) column: Zorbax SB (C18, 9.4 x 50 mm, 5 µ), Analytical TLC was performed on precoated Merck plates (60 F_{254}, 0.2 mm) with the solvent system EtOAc-hexane (50:50), and compounds were viewed under a UV lamp (254 and 365 nm) and sprayed with 10% H_2SO_4, followed by heating.

2.2. Plant material

The seeds of *C. baccifera* were collected from Tirumala forest region, Tirupati, Andhra Pradesh, India. It was authenticated by Dr. K. Madhava Chetty and a voucher specimen (No-10147) was deposited in the herbarium of the Botany department, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

2.3. Extraction and isolation

The seeds of *C. baccifera* (4 kg) were shade dried, powdered, and extracted with $CHCl_3$(8L) at room temperature for 48 h. The resulting $CHCl_3$ extract was evaporated to dryness under reduced pressure, affording syrupy residue (60 g) which was then subjected to column chromatography using silica gel (100-200 mesh) eluting with hexane / EtOAc in the order of increasing polarity to give 70 column fractions. All the column fractions were analyzed by TLC (silica gel 60 F254, EtOAc: hexane, 40:60), and fractions with similar TLC patterns were combined to give six major fractions (F_1, F_2, F_3, F_4, F_5 and F_6). Fraction F_3 was chromatographed on silica gel (100-200 mesh) using hexane-EtOAc (10:0-6:4) to yield compound **3** (500 mg) along with sub fractions B_1 and B_2. Sub fraction B_1 was then purified by preparative TLC with EtOAc: hexane (20:80) to get compound **7** (30 mg) & **8** (2 mg). Sub fraction B_2 was subjected to CC using EtOAc: hexane (4:6) as an eluent to yield compound **2** (1 mg). Repeated purification of fraction F_4 on silica gel (230-400 mesh) yielded compound **1**(2.3 mg). Fraction F_5 was subjected to CC to yield compound **5** (2 mg). Fraction F_6 was subjected to cc to get compound **6** (10 mg).

2.4. Spectral data

2.4.1. Cipaferen N (**1)**

White amorphous powder; $[\alpha]_D^{25}$ -40.5 (*c* 0.09, $CHCl_3$); IR (KBr): ν_{max} 34**4**4, 2973, 1729, 1461cm^{-1}; For ^1H NMR and ^{13}C NMR: see Table1. HRMS (ESI+) *m/z*: calcd for $C_{32}H_{42}O_8Na$: 577.2786; found: 577.2783$[M+Na]^+$.

2.4.2. Cipaferen O (**2)**

Pale yellow gum; $[\alpha]_D^{25}$ - 42.5 (*c* 1, $CHCl_3$); IR (KBr): ν_{max} 3444, 2974, 1710, 1459cm^{-1}; For ^1H NMR and ^{13}C NMR: see Table 1. HRMS (ESI+): calcd for $C_{32}H_{40}O_{12}Na$: 639.2442); found: *m/z* 639.2440 $[M+Na]^+$.

2.5. *Experimental procedure for the synthesis of **9**.*

To a stirred solution of Cipaferen G (0.5g) in EtOH (40 mL) was added 1.5 eq K_2CO_3.This solution was stirred for 12 h at room temperature. After completion of reaction (monitored by TLC), the mixture was concentrated under reduced pressure and re-dissolved in ethyl acetate then washed with water, brine and dried over anhydrous Na_2SO_4.The solvent was removed under reduced pressure to give the residue. The residue was purified by silica gel column chromatography (20% EtOAc in hexane) to afford 3-*O*-deacetylcipaferen G (**9**) as a white solid (65%). HRESIMS, IR, ^{1}H & ^{13}C NMR values of Compound **9** see in supporting information.

2.6. *General Experimental procedure for preparation of compounds **10a-10c & 12***

To a cooled solution of the 3-*O*-deacetylcipaferen G (1 eq) in dry DCM (3 mL) cooled at 0 ºC was added triethylamine (1.5 eq), aliphatic acid chloride (1.2 eq) stirred at room temperature for 4 h. After completion of the reaction (monitored by TLC), reaction mixture was diluted with ethyl acetate (10 mL), washed with brine solution. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate in hexane) to afford the title compounds **10a-10c**. HRESIMS, IR, ^{1}H & ^{13}CNMR values of Compounds ***10a-10c & 12*** see in supporting information.

2.7. *General Experimental procedure for preparation of compounds **11a-11e***

To a solution of the different aromatic acid (1.5 eq) in dry THF (3 mL) cooled at 0 ºC was added triethylamine (20.2 mmol), 2,4,6-trichlorobenzoyl chloride (20.2 mmol) stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and the residue was dissolved in toluene (2 mL). To this mixture at 0 °C was added a solution of DMAP (0.105 mmol) and 3-*O*-deacetylcipaferen G (**9)** (0.015 mg, 0.035 mmol) in toluene (2 mL), and the resulting mixture was stirred for 6-20 h. The reaction mixture was diluted with Ethyl acetate (10

mL), washed with saturated aqueous $NaHCO_3$, then with brine solution. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (Ethyl acetate in Hexane) afforded esters. HRESIMS, IR, ^1H & ^{13}C NMR values of compounds *11a-11e* see in supporting information.

2.8. General Experimental procedures for preparation of compounds ***13a-13i***

To a cooled solution of the compound **12** (1 eq) in dry DMF (3 mL) was added secondary amine (piperazine, 1.2eq) and reaction mixture was stirred at room temperature for 5 h. After completion, the reaction mixture was diluted with ethyl acetate (20 mL), washed with cooled ice and brine solution. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (Ethyl acetate in Hexane) to afford derivatives. HRESIMS, IR, ^1H & ^{13}C NMR values of compounds 13a-13i see in supporting information.

2.9. Cytotoxic activity

All cell lines (***HeLa, PANC 1, MDA MB- 231, IMR 32, HepG$_2$ and SKNSH***) used in this study were procured from the American Type Culture Collection (ATCC), United States. The synthesized test compounds were evaluated for their *in vitro* anti proliferative activity in these six different human cancer cell lines. A protocol of 48 h continuous drug exposure was used, and a SRB cell proliferation assay was used to estimate cell viability or growth. All the cell lines were grown in Dulbecco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO_2 at 37˚C). Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and seeded in 96-well plates in 100µL aliquots at plating densities depending on the doubling time of individual cell lines. The microliter plates were incubated at 37˚C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs and were

incubated for 48 hrs with different doses (0.01, 0.1, 1, 10, 100µM) of the prepared derivatives**.** After 48 hours of incubation at 37˚C, cell monolayers were fixed by the addition of 10% (wt/vol) cold trichloroacetic acid and incubated at 4˚C for 1h and were then stained with 0.057% SRB dissolved in 1% acetic acid for 30 min at room temperature. Unbound SRB was washed with 1% acetic acid. The protein bound dye was dissolved in 10mM Tris base solution for OD determination at 510 nm using a microplate reader (Enspire, Perkin Elmer, USA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50 % (GI_{50}) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from [(Ti-Tz)/Tz] x 100 = -50. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.

2.10. Insect antifeedant activity

Antifeedant activity of the compounds was assessed on *Spodoptera litura*(F.).The experiments were conducted according to the classical no choice leaf disc bioassay described earlier method [21] . To study the antifeedant activity of the test compounds, a small circular disk of 5cm diameter was cut from fresh castor leaves. The leaf discs were treated on their upper surface with individual concentrations of the compounds, and one leaf disk each was transferred to each petri plate of 15cm diameter containing moist filter paper. Control leaf discs were treated with the same volume of the Dimethyl sulphoxide only. In each petri dish, prestarved healthy third instar larvae of *S. litura* were introduced to assess antifeedant activity. Progress of the consumption of the leaf area was measured at 6, 12 and 24h in both treated and control leaf disks. Areas of control and treated leaf discs consumed were measured after 6 h using a leaf area meter (AM-3000, ADC, Bioscientific limited, England).The antifeedant index was then calculated as [(C -T) /(C + T)] x 100]. Where "C" is the area of leaf disc consumed in the control and "T" is the area of leaf discs consumed in the treatment [19-20]. For each concentration, 10 experimental sets were assayed. All tests we replicated three times. The mean of the 10 sets was taken for each compound. Means were subjected to probit analysis [22]. The antifeedant activity was given as percentage for the compounds **1-9, 10a-c, 11a-e, 12** and **13a-i** are included in Table–4.

3. Results and discussion

The concentrated $CHCl_3$- soluble extract of *Cipadessa baccifera* seeds was chromatographed on silica gel, and the resultant fractions were subjected to bioassay for cytotoxic activity against cancer cell lines. Repeated column chromatography of the bioactive fractions resulted in the isolation of nine compounds, out of which compounds **1-2** were new compounds and structures were established using IR, MS, 1D and 2D NMR (HSQC HMBC COSY and NOESY) spectroscopic techniques. The known compounds were identified as, cipaferen E (**4**)[20],

cipaferen F (**5**)[10], and cipaferen I (**6**)[10], 1,4-epoxy-16-hydroxyheneicos-1,3,12,14,18-pentaene (**7**)[3] and 1,4-epoxy-16-hydroxyheneicos- 1,3,12,14-tetraene(**8**) [3] from ^{1}H and ^{13}C NMR spectral data, which were compared with those reported in literature.

Compound **1** was isolated as a white amorphous powder and its molecular formula was established as $C_{32}H_{42}O_8$ based on the quasi-molecular ion peak at *m/z* 577.2783 $[M+Na]^+$ (calcd for $C_{32}H_{42}O_8Na$, 577.2786) in the HRESIMS, requiring twelve degrees of unsaturation. The IR spectrum displayed absorption bands at 1729 cm^{-1}, 1461 cm^{-1} indicating the presence of double bonds and ester functionalities. In the ^{1}H NMR spectrum of **1** (Table 1), a methoxy group [δ_H 3.67 (3H, s)], characteristic proton signals for a β-substituted furan ring [δ_H 6.43 (1H, brs), 7.49 (1H, brs),7.39 (1H, brs)], four methyl groups [δ_H 0.81 (3H, s), 0.87 (3H, s), 0.95 (3H, s) and 0.82 (3H, s)], an exocyclic methylene group [δ_H 5.09 (1H, s), 4.85 (1H, s)] and tigloyl group [(δ_H 1.77(3H, br d), 2.00 (3H, s), and 6.83(1H, m)] were observed.

The ^{13}C NMR spectrum (Table 1), combined with HSQC and DEPT 135^0 spectrum, revealed 28 carbon signals, consisting of three carbonyl, eight methines, five methylenes and six methyls. Aforementioned data suggested that compound **1** was a typical *seco*-limonoid ring as cipaferens and comparison of its ^{1}H and ^{13}C NMR data (**Table 1**) with those of cipaferen G, indicated that the marked difference between these two compounds was a presence of tigloyl group in compound **1** instead of an acetyl group at C-3 (**Table-1 & Figure-1**). The presence of tiglate group in A_1 ring at C-3 was further supported by HMBC spectra, which indicated the correlation between H-3/C-1', H-3'/ C-1', H-4'/ C-1'(δ 167.1) (**Figure-2**). Similarly, β-furyl ring was attached to C-17 in the D-ring which was supported by the HMBC correlations of H-17/C-20, C-21 and C-22. In addition to the above correlations, carbon skeleton suggested several diagnostic correlations very similar to cipaferen G. The relative stereochemistry of the

compound **1** was assumed to be the same as that of known methyl angolensate-type limonoid [10], bearing the same skeleton such as cipaferen G and NOESY (**Figure -2**). Based on these spectral data, the structure of **1** was assigned and trivially named as cipaferen N.

(Figure-2)

Compound **2** was obtained as an white solid, with $[\alpha]_D^{25}$ - 42.5 (*c* 1, chloroform). The HRESIMS displayed quasi-molecular ion peak at *m/z* 639.2440 [M+Na]$^+$ (calcd 639.2442), consistent with a molecular formula of $C_{32}H_{40}O_{12}$ which indicated 13 degrees of unsaturation. The IR spectrum implies the presence of hydroxy and ester functionalities on the basis of the absorption bands at 3444 and 1710 cm^{-1}, respectively. Analysis of the IR,1D and 2D NMR data ($CDCl_3$) for **2** suggested general features for a cipadessin-type limonoids,[11] very similar to that of Cipaferen A, except for the replacement of a β-furyl ring with the γ-hydroxybutenolide group and 2-methylbutanoate group with tiglate group. The presence of γ-hydroxybutenolide ring was characterized by the presence of two broad singlets at δ_H 7.54(H-22) and 6.30 (H-23) in ^{1}H NMR spectrum and the signals at δc 148.96 (C-22) and 98.66 (C-23) in ^{13}C NMR spectrum (Table-1). The appearance of signals at δ_H 6.92 (1H, m, H-3′), 1.84 (3H, s, H_3-4′), and 1.83 (3H, d, *J* = 6.9 Hz, H_3-5′] in the NMR spectrum also provided evidence that compound **2** has tiglate linkage. The key HMBC correlations of H-17/ C-20, C-21 and C-22 confirmed the position of γ-hydroxy butenolide group at C-17. The position of the tiglate group was determined at C-3 on the basis of the HMBC correlations of H-2 (δ_H 4.94, dd, *J* = 11.3) /C-1^1 (δ 169.37), C-3 (δ 80.78), C-4 (δ 41.38), and H-3(δ_H 3.86,1H, br s)/C-28(δ 15.77), and C-29(δ 28.10), respectively. The relative configuration of **2** is also analogous to that of cipaferen A[11] on the basis of similar NMR chemical shifts and NOE data (**Fig-3**). Therefore, the structure of **2** was characterized and trivially named as cipaferen O.

(Figure-3)

Limonoids are typical secondary metabolites which possess various therapeutic effects, such as antitumor, anti-inflammatory, analgesic, antibacterial, antimalarial, antifeedant etc.[1], the chemistry and pharmacology of these compounds have attracted great interest in medicinal chemistry. Unlike the structural modification/simplification studies on triterpenoids [12-15], modification of limonoids has had little attention and, therefore, very little is known with respect to their analogue synthesis and their biological properties. To the best of our knowledge, there are no reports in the literature with regard to the cipaferen G analogues and their activities. The ease of acquiring and high abundance of cipaferen G (**3**) from crude extract and the functionality present on the natural products motivated us to focus on semisynthetic derivatization for the development of more efficacious compounds. In this perspective, we have planned to design and synthesize new class of derivatives and studied their activity. The basic skeleton of cipaferen G contains A, B, C, D rings with an 1,14 oxygen bridge in B ring which joins ring A and C. In the present study, we aimed to explore the C-3 position by replacing acetyl group with various aliphatic and aromatic substituted acids. As shown in scheme-1, initially, cipaferen G (**3**) was subjected to ester hydrolysis with 1.5eq of K_2CO_3 in MeOH under reflux conditions for 12h to yield corresponding secondary hydroxyl compound known as 3-*O*-deacetylcipaferen G (**9**) in 61% yield. In the second step, 3-*O*-deacetylcipaferen G(**9**) was subjected to Yamaguchi esterification [9], protocol (2,4,6-trichlorobenzoyl chloride, Et_3N, THF, DMAP, toluene) using various acid chlorides/acids to yield ester derivatives (**10a-10c, 11a-11e)**. Compounds **12** and **13a-13i** were synthesized from 3-*O*-deacetylcipaferen G (**9**) as outlined in Scheme 1. 3-*O*-deacetylcipaferen G was treated with chloro acetyl chloride in DCM and Et_3N to give **12** which

was coupled with the different piperazines / secondary amines in DMF to afford the target compounds **13a-13i** in good yields (**Scheme-1**). All the reactions proceeded smoothly and yielded the target compounds (**10-13**) in moderate to good yields (65 to 90%) which were shown in experimental section (**Supporting information** & **table - 2**)**.** All the compounds were well characterized by ^{1}H NMR, ^{13}C NMR and ESI HRMS spectral data.

(Scheme-1)

The *in vitro* anti-proliferative activity of isolates and its derivatives were examined against HeLa, PANC1, MDAMB-231, IMR32, HepG2 and SKNSH cell lines using the SRB assay [9]. Doxorubicin was used as the reference standard and the results are summarized in Table 3. The results revealed that some of the synthetic analogues exhibited promising anti-proliferative activity when compared to the parent isolated cipaferen G (**3**). Among derivatives, compounds **10c, 11b, 13b, 13c and 13h** manifested potent activity against HepG2 with GI_{50} value of 0.04 ± 0.01, 0.02 ± 0.01, 0.05 ± 0.02, 0.1±0.06, and 0.01±0.04 µM, respectively. While compounds **13c** and **13d** showed potent activity against **IMR 32** cell line with GI_{50} value of **0.04±0.01, 0.013±0.001** µM. The remaining compounds showed moderate activity against tested cell lines. It is evident from **table-2**, that Methyl, ethyl, benzoyl substitution on piperazines (**13c, 13d and 13h**) and compound (**11b**) which has p-chloro substitution on benzene ring significantly enhanced the activity.

In view of the broad spectrum insecticidal potentials of limonoids, we investigated the insect antifeedant activity of isolated compounds (**1-8**) and derivatives (**9-13**) against tobacco caterpillar (*Spodoptera litura)* using a no-choice laboratory bioassay [19-20]. The dosages of the compounds for our study were fixed at 100 µg/cm^2 and 25µg/cm^2. As shown in **Table-4**, compounds **1-13** exhibited antifeedant activity ranging from 61% to 100% at concentration

100µg/cm^2 and 26% to 76% at concentration 25µg/cm^2. Based on these results we predict the SAR of cipaferen G. Among all isolated and synthetic methyl angolensate type limonoids, compounds **13b, 13c** and **13f** with pyrrolidine, N-substituted piperazine group at C-3, exhibited the best activity with 100% activity at 100 µg / cm^2, suggesting these groups at C-3 enhance antifeedant activity. Moreover, cipaferen G (**3**) gave moderate activity with 81% which was much better than cipaferen E (**4**) and cipaferen F (**5**) with 71% and 73% respectively. This indicated that the insertion of one hydroxyl group into C-2 of compound **1** led to a decrease in activity. Compound **6** showed better activity than cipaferen G, indicating that the presence of γ-hydroxy butenolide group at C-17, enhanced the activity. Also, compound **8** showed 100% activity at 100 µg / cm^2 and 71% at 25µg / cm^2.

4. Conclusion

In conclusion, we have isolated two novel limonoids from the chloroform extract of *Cipadessa baccifera* along with the six known compounds and their structures were established based on the spectroscopic methods. In an attempt to search for novel limonoids with better therapeutic potentials, we prepared cipaferen G derivatives using Yamaguchi esterification protocol and assayed for the ability of isolates and synthetic derivatives against the panel of cancer cell lines. Among the tested derivatives, **10c, 11b, 13b, 13c and 13h** displayed potent activity against HepG2, IMR 32 cell lines. Further, insecticidal activity of the compounds also indicated their potentials. This has laid a solid foundation for further lead optimization of this class of compounds by a systematic chemical modification including the synthesis of water-soluble compounds to improve their overall pharmaceutical properties.

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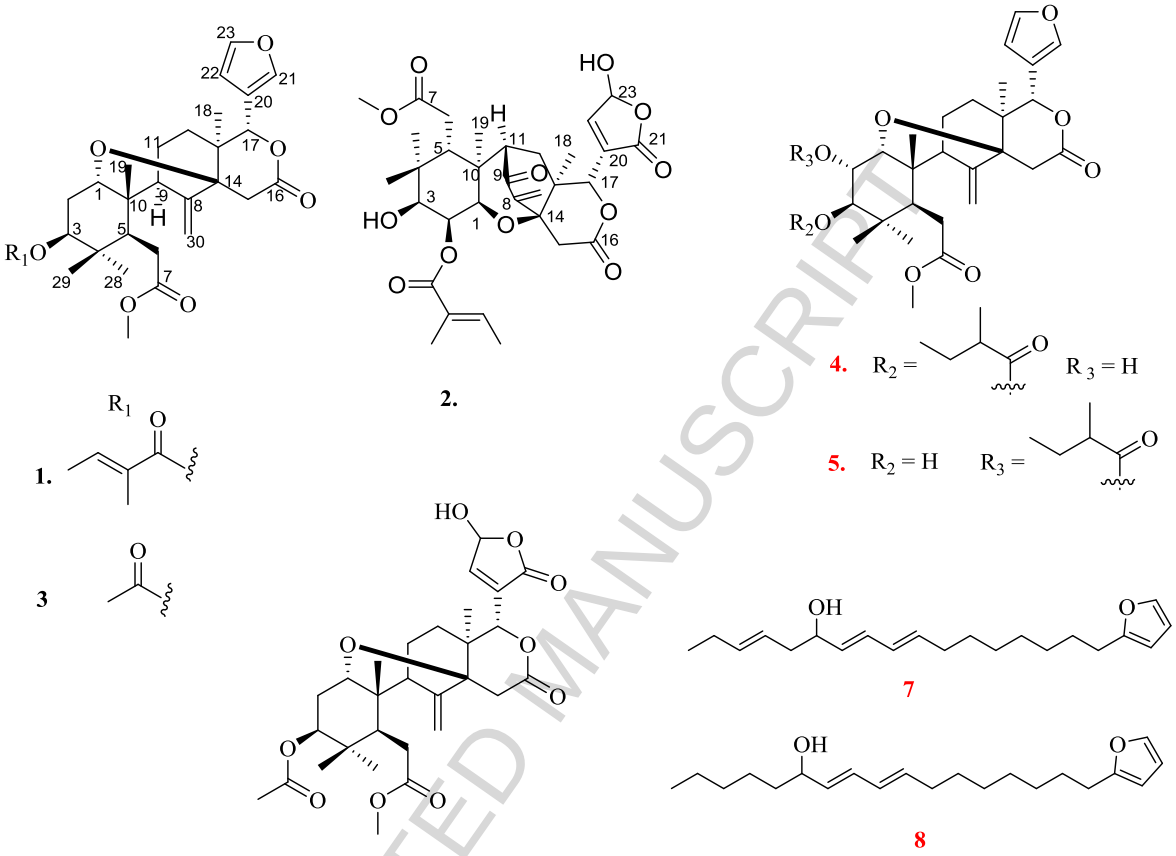


Figure-1: Isolated compounds (**1-8**) from *Cipadessa baccifera*

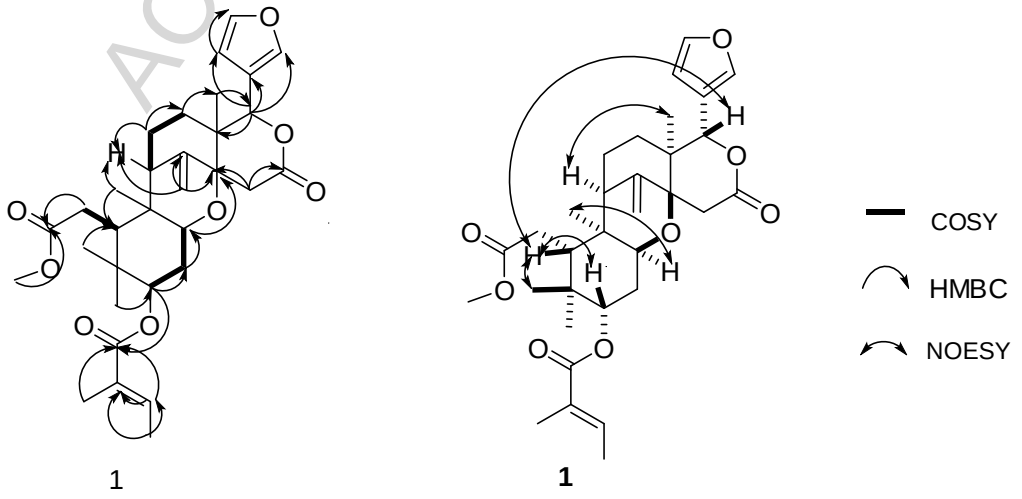


Figure-2: Key HMBC, COSY, NOESY correlations of compound **1**.

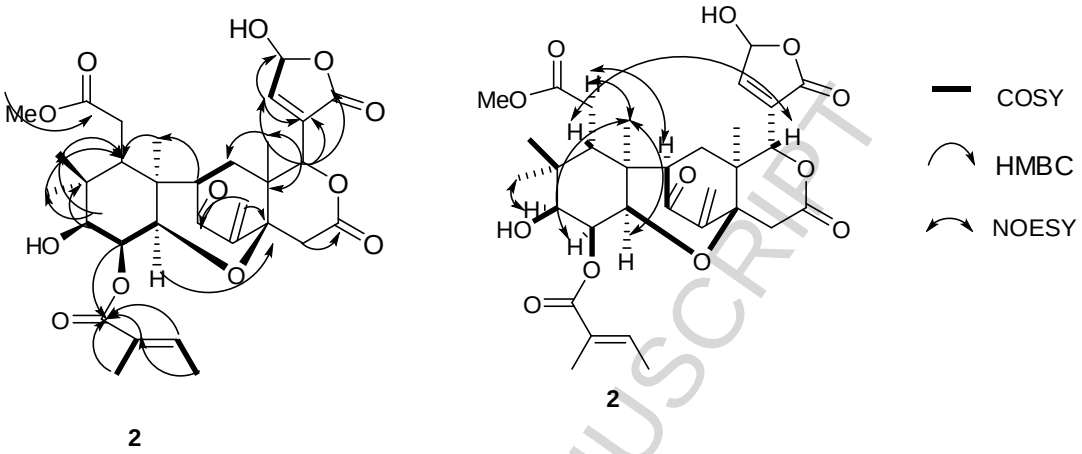
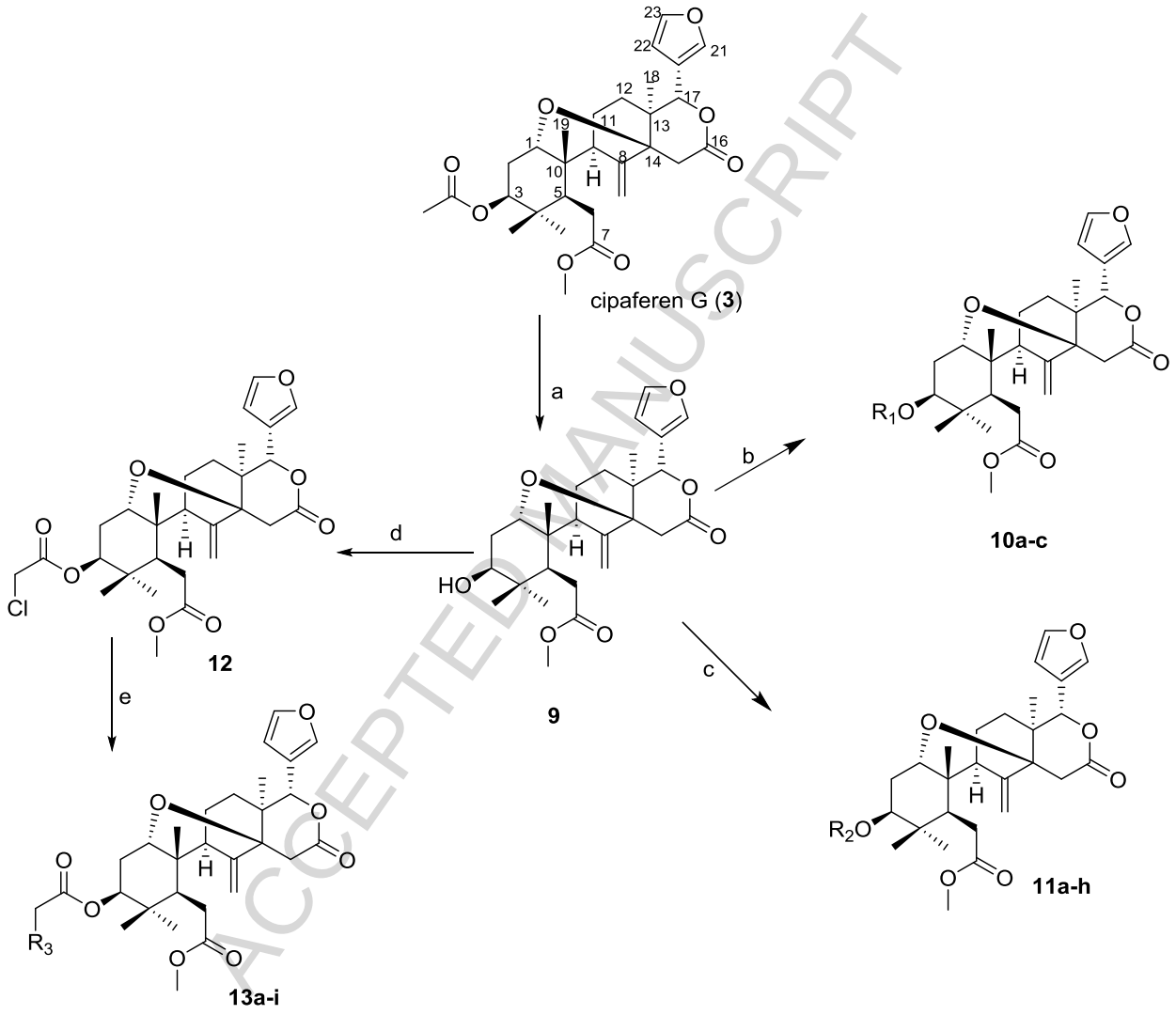


Figure-3: Key HMBC, COSY, NOESY correlations of compound **2**.

Table-1: ^{1}H NMR and ^{13}C NMR data of compound **1** and **2** in $CDCl_3$ (300 MHz, δ in ppm)

Position	Compound **1**		Compound **2**	
	^{1}H NMR (δ in ppm, mult, J)	^{13}C NMR (δ in ppm)	^{1}H NMR (δ in ppm, mult, J)	^{13}C NMR (δ in ppm)
1	3.23 (1H, t, 6.5 &2.8)	74.45	3.69(1H, br s)	73.32
2	1.86 (2H, m)	29.57	4.94(1H, dd, 3.7 & 11.2)	71.30
3	4.98 (1H, dd, 10.8, 5.9)	74.70	3.86(1H, br s)	80.78
4	-------	38.74	------	41.38
5	2.55 (1H, m)	23.85	2.71(1H, m)	41.21
6	2.55 (1H, d, 15.8) & 2.10 (d, 15.8)	32.35	2.01(1H, d, J = 4.9) & 2.4 (1H, d, J = 16.2)	
7	--------	174.23	------	175.0
8	--------	146.41	-----	
9	2.06 (1H, br s)	50.43	------	196.9
10	--------	43.84	-------	45.00
11	2.01 (1H, m) &1.52(1H, m)	29.34	2.40(1H, m)	56.07
12	1.88 (1H, m)&1.16 (1H, m)	41.48	2.70(1H, m) & 1.18(1H, m)	30.13
13	-------	41.48	-------	39.79
14	-------	79.98	-------	81.81
15	2.90 (1H, d, 18.1) & 2.57 (1H, d, 18.1)	33.78	2.91(1H, d, J = 17.2) & 2.69 (1H, d, J =17.2).	37.24
16	----------	170.09	------	167.79
17	5.80 (1H, s)	79.77	5.96(1H, s)	78.08
18	0.87 (3H, s)	13.85	0.95(1H, s)	20.92
19	0.82 (3H, s)	21.90	0.99(1H, s)	18.42
20	---------	120.9	-------	132.5
21	7.49 (1H, br s)	140.88	--------	167.64
22	6.43 (1H, br s)	110.01	7.54(1H, br s)	148.96
23	7.39 (1H, br s)	142.61	6.30(1H, br s)	98.66
28	0.95 (3H, s)	26.90	1.04(1H, s)	15.77
29	0.81 (3H, s)	15.65	1.07(1H, s)	28.10
30	5.09 (1H, s) & 4.85 (1H, s)	110.93	6.60(1H, s) & 5.53(1H, s)	126.04
1'	--------	167.1	------	169.37
2'	-------	111.41	-------	128.08
3'	6.83 (1H, m)	139.32	6.92(1H, m)	139.08
4'	2.00 (3H, s)	27.36	1.84(3H, s)	14.54
5'	1.77 (3H, br d)	14.53	1.83(3H, d, J =6.9)	12.03
OMe	3.67 (3H, s)	51.85	3.86(3H, s)	52.51

Assignments were based on 2D NMR including DQF-COSY, HSQC, HMBC and NOESY. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses. For overlapped signals, only chemical shift values are given



Scheme-1: Reagents and Conditions: (a) 1.3 eq K_2CO_3, EtOH, RT, 12 h, 65%. (b) R-COCl, Et_3N, DCM, 0-RT , 3-5 hr (c) R-COOH, 2, 4, 6-trichlorobenzoylchloride, Et_3N, THF, 0˚C to rt 2 h, then **9**, DMAP, toluene, 0˚C to rt 6-20 h.(d) 1.2 eq Et_3N, DCM, Chloroacetyl chloride, 0 to RT, 60 % (e) 1.3 eq Secondary amine, DMF, RT, 4-6 h.

Table-2: Ester derivatives of cipaferen G

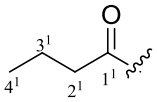
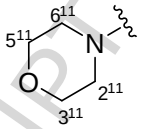
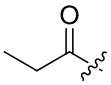
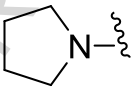
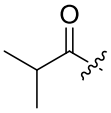
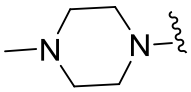
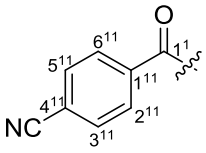
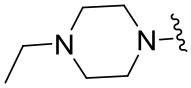
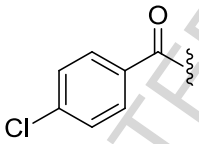
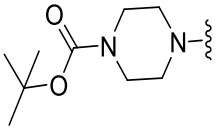
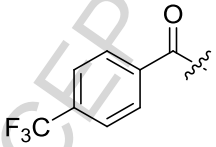
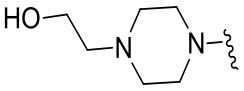
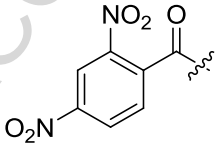
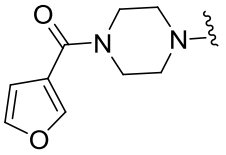
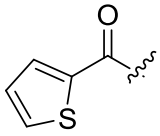
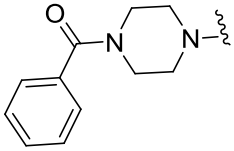
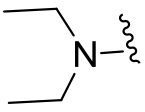
Compounds	R_1/ R_2-Groups	Compounds	R_3-Group
10a		**13a**	
10b		**13b**	
10c		**13c**	
11a		**13d**	
11b		**13e**	
11c		**13f**	
11d		**13g**	
11e		**13h**	
		13i	

Table-3: Antiproliferative activity of cipaferen G and their analogues (GI_{50} in µM).

Compound	HeLa	PANC 1	HepG2	SKNSH	MDA MB 231	IMR 32
3	60.5±1.7	6.8±1.5	>100	1.1±0.2	>100	0.022±0.01
9	16.3±2.2	0.3±0.04	19.5±2.7	>100	>100	>100
10a	2.9±0.3	60.6±8.5	0.3±0.1	>100	>100	0.39±0.09
10b	44.4±4.8	0.3±0.03	>100	86.1±0.8	4.4±0.85	>100
10c	60.6±5.8	4.3±0.3	0.04 ± 0.01	4.0±0.02	9.2±0.61	>100
11a	2.7±0.7	>100	2.8±0.9	>100	>100	0.66 ± 0.06
11b	2.9±0.8	9.8±1.3	0.02 ± 0.01	7.4±1.2	1.1±0.62	0.1 ± 0.05
11c	4.4±1.4	>100	48.5±6.8	65.3±0.9	1.55±0.16	0.56 ± 0.02
11d	8.5±0.5	0.7 ± 0.08	0.2 ± 0.04	7.6±0.6	>100	>100
11e	5.2±0.02	>100	>100	1.3±0.4	1.6±0.37	1.8±0.08
12	34.3±2.7	8.9±0.7	>100	>100	13.0±0.8	>100
13a	9.6±5.1	2.1±0.6	4.7±0.5	>100	15.1±0.9	>100
13b	19.1±1.2	7.4±1.0	0.05 ± 0.02	>100	8.4±0.8	>100
13c	13.3±1.9	7.8±1.1	0.1±0.06	1.9±0.5	2.8±0.65	0.04±0.01
13d	27.4±1.4	0.7 ± 0.3	1.8±0.9	>100	1.1±0.6	0.013±0.001
13e	5.7±1.3	5.3±1.7	1.0±0.1	>100	9.0±1.8	>100
13f	3.0±0.04	>100	0.5±0.1	>100	>100	>100
13g	5.4±0.01	0.3±0.04	0.4±0.1	14.3±1.5	14.4±0.24	0.23±0.06
13h	14.1±1.1	4.3±0.6	0.01±0.04	>100	>100	>100
13i	17.7±2.8	1.7±0.04	2.9±0.2	>100	>100	>100
Doxorubicin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 4:- Antifeedant activity of the compounds

Compound	(Mean ± SEM) Antifeedancy @ 100 µg/cm^2	(Mean ± SEM) Antifeedancy @ 25 µg/cm^2
1	62.48±1.39	28.50±3.60
2	90.32±1.49	59.5±1.60
3	81.06±2.25	51.70±1.90
4	71.07±0.58	32.21±0.29
5	73.43±0.75	30.00±0.80
6	100±0.00	75.9±0.56
7	73.15±1.75	26.7±2.15
8	100±00	71.09±2.05
9	76.50±1.01	26.18±2.48
10a	83.02±1.22	54.95±0.75
10b	84.00±1.19	57.15±0.65
10c	75.05±1.42	46.00±1.40
11a	79.71±1.28	51.0±2.40
11b	73.90±0.66	39.23±0.50
11c	80.74±1.19	43.15±3.30
11d	61.71±1.31	39.10±1.40
11e	73.07±1.65	33.2±2.00
12	81.56±0.97	36.75±1.45
13a	ND	ND
13b	100.00±0.00	72.05±1.15
13c	100.00±0.00	75.30±1.10
13d	ND	ND
13e	63.01±1.43	26.7±0.80
13f	100.00±00	76.20±0.90
13g	ND	ND
13h	79.37±0.97	41.30±0.80
13i	62.48±1.39	28.50±3.60

ND = not determined