

Synthesis and biological evaluation of natural *Lachnophyllum* methyl ester, *Lachnophyllum* lactone and synthetic analogs thereof

Kodjo Adande,^[a,b] Oudjaniyobi Simalou,^[b] Juline Ardanuy,^[a] Kodjo Eloh^[c], Chérine Mehalla^[d], Patricia Constant^[d], Isabelle Fabing,^[a] Yves Génisson,^{*[a]} Stéphanie Ballereau^{*[a]}

^[a] Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique (SPCMIB), Université de Toulouse, CNRS, Université Toulouse III – Paul Sabatier (UT3), 118 route de Narbonne, 31062, Toulouse Cedex 9

^[b] Laboratoire de chimie organique et des substances naturelles (Lab COSNat), Département de Chimie, Faculté Des Sciences, Université de Lomé, Lomé, Togo

^[c] Laboratoire de Chimie Organique et des Sciences de l'Environnement (LaCOSE), Département de Chimie, Faculté Des Sciences et Techniques, Université de Kara, Kara, Togo

^[d] Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, UPS, 31077, Toulouse, France

Abstract

Z-*Lachnophyllum* methyl ester and (4*Z*)-*Lachnophyllum* lactone were recently identified as major components in essential oils and extracts of *Conyza bonariensis* from Togo. Extended biological evaluation of these acetylenic compounds was however hampered by the reduced amounts isolated. A synthetic route was designed affording access to larger quantities of these two natural products as well as to original non-natural analogs with the prospect of exploring for the first time the structure-activity relationships in this series. Using LC/MS analysis, synthetic samples allowed confirming the presence of the two previously isolated natural products in plant extracts obtained by accelerated solvent extraction technique. The nematocidal activity of synthesized compounds confirmed the potency of the natural products, which remain the most active among all analogs tested. The synthesized compounds were also assessed against *Leishmania infantum* axenic amastigotes and *Mycobacterium tuberculosis* H₃₇Rv pathogenic strain. *Z*-*Lachnophyllum* methyl ester, (4*Z*)-*Lachnophyllum* lactone and lactone analogs exhibited the strongest antileishmanial potency. As expected, a longer alkyl chain was necessary to observe significant antimycobacterial activity. The lactone analog bearing a C10 lipophilic appendage displayed the highest antimycobacterial potency. The notable activities of lactones, naturally occurring or analogs, either nematocidal, antileishmanial or antimycobacterial were compared to their cytotoxicity for mammalian cells and revealed moderate selectivity index values. In this regard, the innocuous *Z*-*Lachnophyllum* methyl ester and analogs open up more promising perspectives for the discovery of bioactive agents to protect both agricultural crops and human health.

Introduction

Among the numerous plants traditionally used in herbal medicine, *Conyza* plant species of the Asteraceae family are known for their various pharmacological applications ranging from the treatment of malaria to toothache.¹ The chemical composition of essential oils obtained from *Conyza* species collected in various regions of the world such as Africa, South America or Mediterranean sea area have been extensively studied.² In addition to known flavonoids or terpenoids, enyne derivatives were also identified as components of *Conyza* species essential oil extracts.³⁻⁶

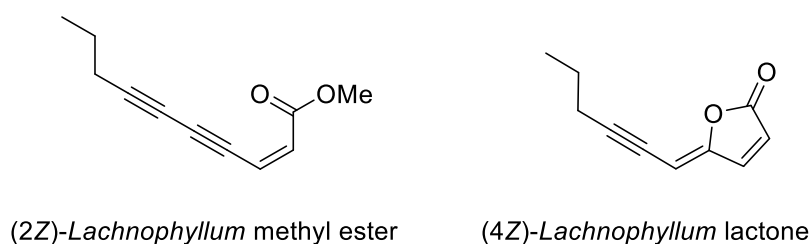


Fig. 1

We recently isolated (2Z)-Lachnophyllum methyl ester and (4Z)-Lachnophyllum lactone (Fig. 1) as major components in extracts of *Conyza bonariensis*.⁷ The quantities obtained either from essential oils or solvent extraction were however too low to explore further the biological activities of these acetylenic compounds. To overcome this limitation, we developed a synthetic access to these natural compounds. The designed synthetic route affords also original analogs to extend the structure-activity relationship of this family of compounds.

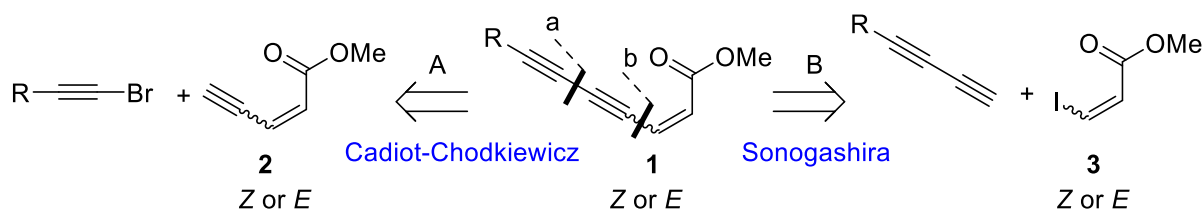
Results and discussion

Synthesis

Despite the numerous publications describing the isolation of *Lachnophyllum* esters and lactone from plants, mostly from *Conyza* species,² there were, until the work of Soriano *et al.* published during this study,⁸ only two articles reporting the chemical synthesis of these natural compounds. The synthesis of the *Lachnophyllum* lactone as a mixture of *E* and *Z* stereoisomers starting from 2-trimethylsiloxyfuran was described in 1981⁹ whereas the (2*E*) *Lachnophyllum* ethyl ester was mentioned as an intermediate in a synthetic study published in 1987.¹⁰ We thus designed a synthetic route allowing not only to afford larger quantities of natural *Lachnophyllum* methyl ester and lactone but also to access non-natural analogs for biological evaluation.

Synthesis of Lachnophyllum esters and analogs (1)

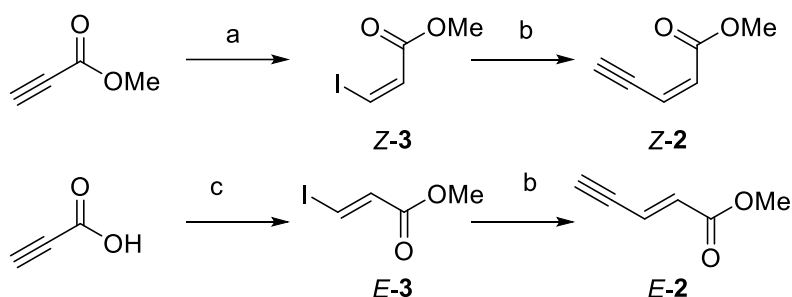
The retrosynthetic routes for the acetylenic esters **1** was envisioned according to two alternative disconnections (A and B) as depicted in Scheme 1.



Scheme 1. Retrosynthetic route to *Lachnophyllum* methyl esters and analogs

According to route A, a Cadiot-Chodkiewicz coupling between methyl pent-2-en-4-ynoate (**2**) and an alkyne bromide leads to the methyl hepta-2-en-4,6-diyynoate motif, characteristic of the *Lachnophyllum* esters. This sequence has already been validated for the synthesis of natural epoxypolyynes.^{11,12} Alternatively, the more convergent route B relies on a Sonogashira coupling of methyl 3-iodoacrylate (**3**) with a terminal 1,3-diyne. These two synthetic pathways potentially allow the synthesis of the two stereoisomers (*Z* or *E*) of *Lachnophyllum* esters as well as synthetic analogs.

The commercially available methyl 3-iodoacrylates (*Z*-**3** and *E*-**3**) can also be readily prepared in the laboratory.^{13,14} The methyl pent-2-en-4-ynoates (*Z*-**2** and *E*-**2**) were obtained from the corresponding vinyl iodides (*Z*-**3** and *E*-**3** respectively) by means of a Sonogashira coupling with TMS-acetylene followed by the cleavage of the trimethylsilyl protecting group (Scheme 2).¹³

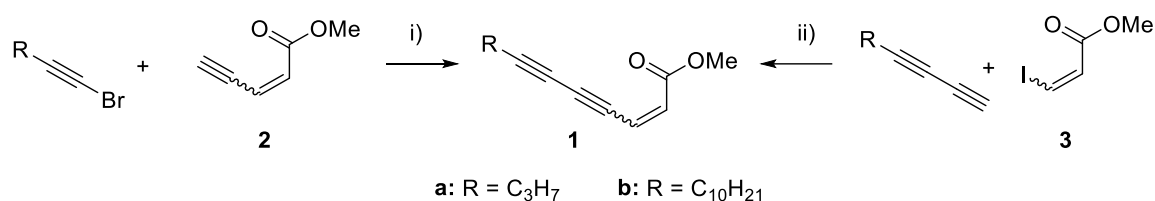


Scheme 2. Synthesis of methyl 3-iodoacrylates *Z*-**3** and *E*-**3** and methyl pent-2-en-4-ynoates *Z*-**2** and *E*-**2**. *Reagents and conditions:* a) NaI, AcOH, 70 °C, 84%; b) i) TMS-C≡CH, PdCl₂(PPh₃)₂, CuI, Et₃N, THF, 0 °C to rt; ii) TBAF, THF, 0 °C to rt, 68% over two steps for *Z*-**2**, 76% over two steps for *E*-**2**; c) i) HI, H₂O, reflux, ii) H₂SO₄, MeOH, reflux, 74% over two steps.

In order to compare the two envisioned alternative reaction sequences, we initially synthesized the naturally occurring (*Z*) *Lachnophyllum* methyl ester and its (*E*) isomer according to routes A and B (Scheme 3). Thus, 1-bromopent-1-yne was engaged in a Cadiot-Chodkiewicz coupling with methyl (*Z*)-pent-2-en-4-ynoate (*Z*-**2**) to deliver (*2Z*)-*Lachnophyllum* methyl ester (*Z*-**1a**) in 38% yield (Table 1, entry 1). The same reaction with methyl (*E*)-pent-2-en-4-ynoate (*E*-**2**) gave (*2E*)-*Lachnophyllum* methyl ester (*E*-**1a**) in 25% yield (Table 1, entry 2). According to route B, the (*2Z*)-*Lachnophyllum* methyl ester (*Z*-**1a**) was obtained from the Sonogashira coupling of hepta-1,3-diyne¹⁵ with methyl (*Z*)-3-iodoacrylate (*Z*-**3**) in 70% yield (Table 1, entry 5). Similarly, (*2E*)-*Lachnophyllum* methyl ester (*E*-**1a**) resulted from the same reaction with methyl (*E*)-3-iodoacrylate (*E*-**3**) in a 54% yield (Table 1, entry 6).

In addition of being more convergent, the route B, relying on the Sonogashira coupling, is thus more efficient than the Cadiot-Chodkiewicz coupling-based route A for the synthesis of both

(*Z*) and (*E*) isomers of *Lachnophyllum* methyl esters. This trend was confirmed during the synthesis of a long chain analog of natural *Lachnophyllum* methyl esters. The compound *Z-1b* was thus obtained in 40% yield from 1-bromododec-1-yne¹⁶ according to route A (Table 1, entry 3) versus 82% yield for the reaction with tetradeca-1,3-diyne¹⁷ in route B (Table 1, entry 7). The same gap in efficiency was observed for the synthesis of *E-1b* (Table 1, entry 7 vs entry 8).



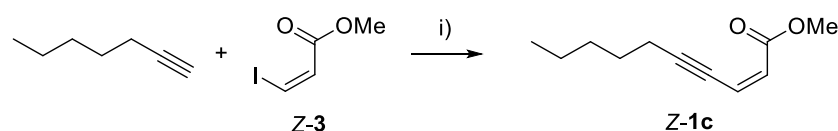
Scheme 3. Synthesis of *Lachnophyllum* methyl esters *Z-1a* and *E-1a* and analogs *Z-1b* and *E-1b*. Reagents and conditions: i) Cadiot-Chodkiewicz coupling: CuCl, NH₂OH·HCl, *n*BuNH₂, THF, 0 °C to rt; ii) Sonogashira coupling: PdCl₂(PPh₃)₂, CuI, Et₃N, THF, 40 °C overnight.

Entry	Transformation (Route)	Substrates	Product	Yield (%)	
1	Cadiot-Chodkiewicz coupling (Route A)	C ₃ H ₇ C≡CBr	<i>Z-2</i>	<i>Z-1a</i>	38
2			<i>E-2</i>	<i>E-1a</i>	25
3		C ₁₀ H ₂₁ C≡CBr	<i>Z-2</i>	<i>Z-1b</i>	40
4			<i>E-2</i>	<i>E-1b</i>	55
5	Sonogashira coupling (Route B)	C ₃ H ₇ C≡C-C≡CH	<i>Z-3</i>	<i>Z-1a</i>	71
6			<i>E-3</i>	<i>E-1a</i>	54
7		C ₁₀ H ₂₁ C≡C-C≡CH	<i>Z-3</i>	<i>Z-1b</i>	82
8			<i>E-3</i>	<i>E-1b</i>	81

Table 1. Synthesis of *Lachnophyllum* methyl esters *Z-1a* and *E-1a* and analogs *Z-1b* and *E-1b*

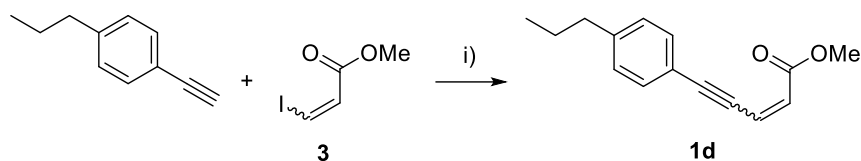
In addition of being the most effective tested routes for the synthesis of natural *Lachnophyllum* methyl esters, the Sonogashira coupling with building block **3** also offers an efficient access to various analogs. Indeed, it can be used not only with diynes as depicted in Scheme 1, but also with other alkynes such as alkyl or aromatic ones. To exemplify this flexibility, the synthesis of saturated analog *Z-1c* (Scheme 4) as well as analogs *Z-1d* and *E-1d* where one of the triple bond is replaced by an aromatic ring was planned (Scheme 5). This replacement of a triple bond by a bioisosteric aromatic ring proved already to be advantageous in another polyacetylenic lipid family.¹⁸

Methyl (*Z*)-dec-2-en-4-ynoate (*Z-1c*) was obtained by the coupling of (*Z*)-3-iodoacrylate (*Z-3*) with hept-1-yne with 67% yield (Scheme 4).



Scheme 4. Synthesis of analogs *Z-1c*. Reagents and conditions: i) PdCl₂(PPh₃)₂, CuI, Et₃N, THF, 0 °C to rt, 67%.

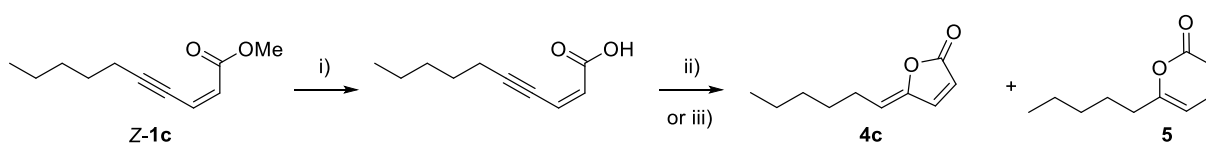
Coupling of (*Z*)-3-iodoacrylate (**Z-3**) (or (*E*)-3-iodoacrylate (**E-3**)) with 1-ethynyl-4-propylbenzene furnished compound **Z-1d** in 89 % yield (respectively **E-1d** with the same yield) (Scheme 5).



Scheme 5. Synthesis of analogs **Z-1d** and **E-1d**. *Reagents and conditions:* i) PdCl₂(PPh₃)₂, CuI, Et₃N, THF, 40 °C overnight, **Z-1d**, 89 %, **E-1d**, 89 %.

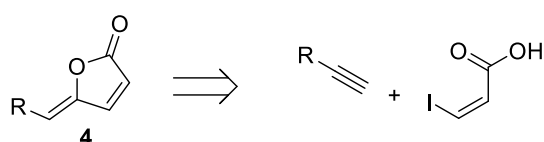
Synthesis of (*4Z*)-Lachnophyllum lactone and analogs (**4**)

With the *Lachnophyllum* esters in hand, our first plan to reach the corresponding lactones was to proceed to the 5-exo-dig cyclization of a (*Z*)-2-en-4-ynoic acid precursor,¹⁹ readily accessible from the corresponding methyl esters. We chose to use methyl (*Z*)-dec-2-en-4-ynoate (**Z-1c**) as a model compound to test this pathway since the regioselective lactonization of (*Z*)-alk-2-en-4-ynoic acids with diverse Ag(I) salts was already described in the literature.²⁰⁻²² After saponification of the methyl ester **Z-1c** under standard conditions, (*Z*)-dec-2-en-4-ynoic acid was engaged in the cyclisation step (Scheme 6). A mixture of (*Z*)-5-hexylidenefuran-2(*5H*)-one (**4c**) and 6-pentyl-2*H*-pyran-2-one (**5**) was however systematically obtained with 55-62% yields either using silver iodide at 100 °C²⁰ or silver carbonate at room temperature.²²



Scheme 6. Preparation and subsequent lactonization of (*Z*)-dec-2-en-4-ynoic acid. *Reagents and conditions:* i) NaOH, MeOH/H₂O (8:2), 80 °C, 88%; ii) AgI (0.1 eq.), DMF, 100 °C, **4c** : **5** = 6 : 4, 62%; iii) Ag₂CO₃ (0.1 eq.), DMF, rt, **4c** : **5** = 8 : 2, 55%.

This route, lacking of selectivity in our hands, was anticipated to reveal even more intricate with (*Z*)-alk-2-en-4,6-diynoic acid. We thus chose to explore a potentially more robust pathway based on a palladium-catalyzed tandem alkyne–alkene cross coupling/lactonization process (Scheme 7).²³ This one-pot access to γ -ylidene butenolides was, first described starting from (*Z*)-3-bromopropenoic acid^{24, 25} to notably afford access to (*Z*)-5-benzylidenefuran-2(*5H*)-one²⁴ (Scheme 7, R = Ph) or the naturally occurring (+)-goniobutenolide A.²⁵ It was later on developed with (*Z*)-3-iodopropenoic acid for the synthesis of natural products xerulin^{15, 26} and freelingyne²⁷ as well as related synthetic compounds.^{28, 29}

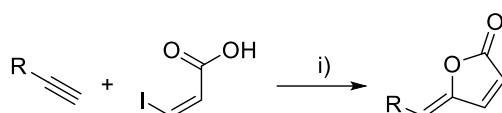


Scheme 7. Retrosynthetic route to *Lachnophyllum* lactone and analogs.

As a model reaction, (*Z*)-3-iodoacrylic acid³⁰ was engaged in the Pd-catalyzed tandem coupling/cyclization reaction with 1-heptyne (Scheme 8 and Table 2, entry 3). (*Z*)-5-

Hexylidenefuran-2(5*H*)-one (**4c**) was obtained as the sole product in a 66% yield upon smooth heating overnight in the presence of PdCl₂(PPh₃)₂, CuI and Et₃N in CH₃CN.

Encouraged by this result, we envisioned to target the (4*Z*)-*Lachnophyllum* lactone using this palladium-catalyzed tandem process. Several precedents were described for the preparation of (poly)unsaturated γ -alkyldene butenolides from enyne precursors,^{15, 26-29} but no example making use of a terminal diyne was known. Gratifyingly, the reaction of (*Z*)-3-iodopropenoic acid with hepta-1,3-diyne in the same conditions delivered the natural unsaturated lactone **4a** (Table 2, entry 1) in 46% yield. Simultaneously to our work, this pathway was used for the gram scale synthesis of (4*Z*)-*Lachnophyllum* lactone.⁸

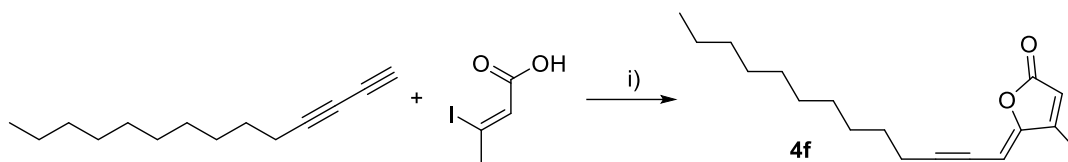


Scheme 8. Synthesis of (4*Z*)-*Lachnophyllum* lactone and analogs. *Reagents and conditions:* i) PdCl₂(PPh₃)₂, CuI, PPh₃ (when R was an aromatic ring), Et₃N, CH₃CN, 40 °C overnight.

Entry	Alkyne	Lactone	Yield (%)
1		4a	46
2		4b	73
3		4c	66
4		4d	41
5		4e	33

Table 2. Synthesis of (4*Z*)-*Lachnophyllum* lactone **4a** and analogs **4b**, **4c**, **4d** and **4e**

As for *Lachnophyllum* esters, we applied this reaction to the synthesis of analogs of natural lactone **4a**. The longer alkyl chain analog **4b** (Table 2, entry 2) was obtained from tetradeca-1,3-diyne in a 73% yield. Reaction between 1-ethynyl-4-propylbenzene and (*Z*)-3-iodopropenoic acid gave the analog **4d** (Table 2, entry 4) wherein the triple bond is replaced by an aromatic ring. In this case, the yield could be optimized by addition of 0.1 equivalent of triphenylphosphine to the reaction mixture.¹⁹ This synthetic pathway gave also access to a functionalized aromatic analog **4e** (Table 2, entry 5) with 5-ethynyl-1,3-difluoro-2-(heptyloxy)benzene³¹ in a 33% yield. Finally, we also varied the iodopropenoic acid, using (*Z*)-3-iodobut-2-enoic acid³² which by reaction under the same coupling/cyclisation conditions with tetradeca-1,3-diyne gave compound **4f** in a 35% yield (Scheme 9).



Scheme 9. Synthesis of (4Z)-*Lachnophyllum* lactone analog **4f**. Reagents and conditions: i) $\text{PdCl}_2(\text{PPh}_3)_2$, CuI, Et_3N , CH_3CN , 40°C overnight, 35%.

Extraction optimization and HPLC analyses

The extraction methods used previously to isolate (2Z)-*Lachnophyllum* methyl ester and (4Z)-*Lachnophyllum* lactone from extracts of *Conyza bonariensis*⁷ are very solvent consuming, require the use of toxic solvents such as chloroform, hexane and methanol, and lead to very low extraction yields (0.34%). We thus tried to optimize the extraction method to improve the yield, the environmental impact and the safety of the process. Traditional Soxhlet, ultrasonic and supercritical carbon dioxide extraction techniques were carried out on roots of *Conyza bonariensis* using different solvents or co-solvents such as heptane, methyl tert-butyl ether and ethanol. However, the masses extracted were low, with extraction yields remaining inferior to 1%. Moreover, these techniques require long extraction time (between 10 and 12 h) and hundreds of milliliters of solvent are necessary. To overcome this problem, accelerated solvent extraction (ASE) was therefore preferentially used. Automating the extraction of 7 g of powdered plant virtually eliminates the variability of manual sample preparation, guaranteeing uniform and reproducible chromatographic analyses. The ASE system (Dionex™ ASE™ 350) automates extraction and filtration (10 μm) steps, requiring only 15 minutes (3 cycles of 5 minutes) and 100 mL of solvent. The yield of this ASE extractions was 0.55% for methyl tert-butyl ether (MTBE), 1.22% for heptane and 4.61% for ethanol.

Analyses of the three fractions were carried out in UHPLC-PDA-MS on a reverse-phase Acquity Premier BEH C18 1.7 μm (2.1x100) mm column (see SI). The heptanoic fraction appeared to be the richest in terms of compounds extracted and contained (4Z)-*Lachnophyllum* lactone and (2Z)-*Lachnophyllum* methyl ester (retention time 7.048 and 8.160 min respectively, Fig. 2 B) as the hexanoic fraction previously issued from the classical liquid extraction (Fig. 2 A).⁷ Despite many efforts, attempts to fractionate this heptanoic extract on a semi-preparative scale were unsuccessful. This strengthens the relevance of our synthesis route to access these natural compounds.

In addition to NMR analyses, UHPLC-PDA-MS was also used to confirm the purity of the synthesized compounds **Z-1a** and **4a** and their identity with the natural compounds found in the plant extracts. The same analytical method was used for all samples and the chromatograms were compared (Fig. 2). Compounds **4a** (Fig. 2 C) and **Z-1a** (Fig. 2 D) emerge at retention times of 7.014 min and 8.050 min respectively, identical to those present in the extracts as confirmed by extracted MS. In addition, such comparison with synthetic samples also confirmed that, despite similar retention time, the major product at 8.894 min in the heptanoic fraction (Fig. 2 B) or at 8.883 in the hexanoic fraction (Fig. 2 A) is not (2E)-*Lachnophyllum* methyl ester **E-1a** (retention time 8.793 min) according to MS analysis (see SI).

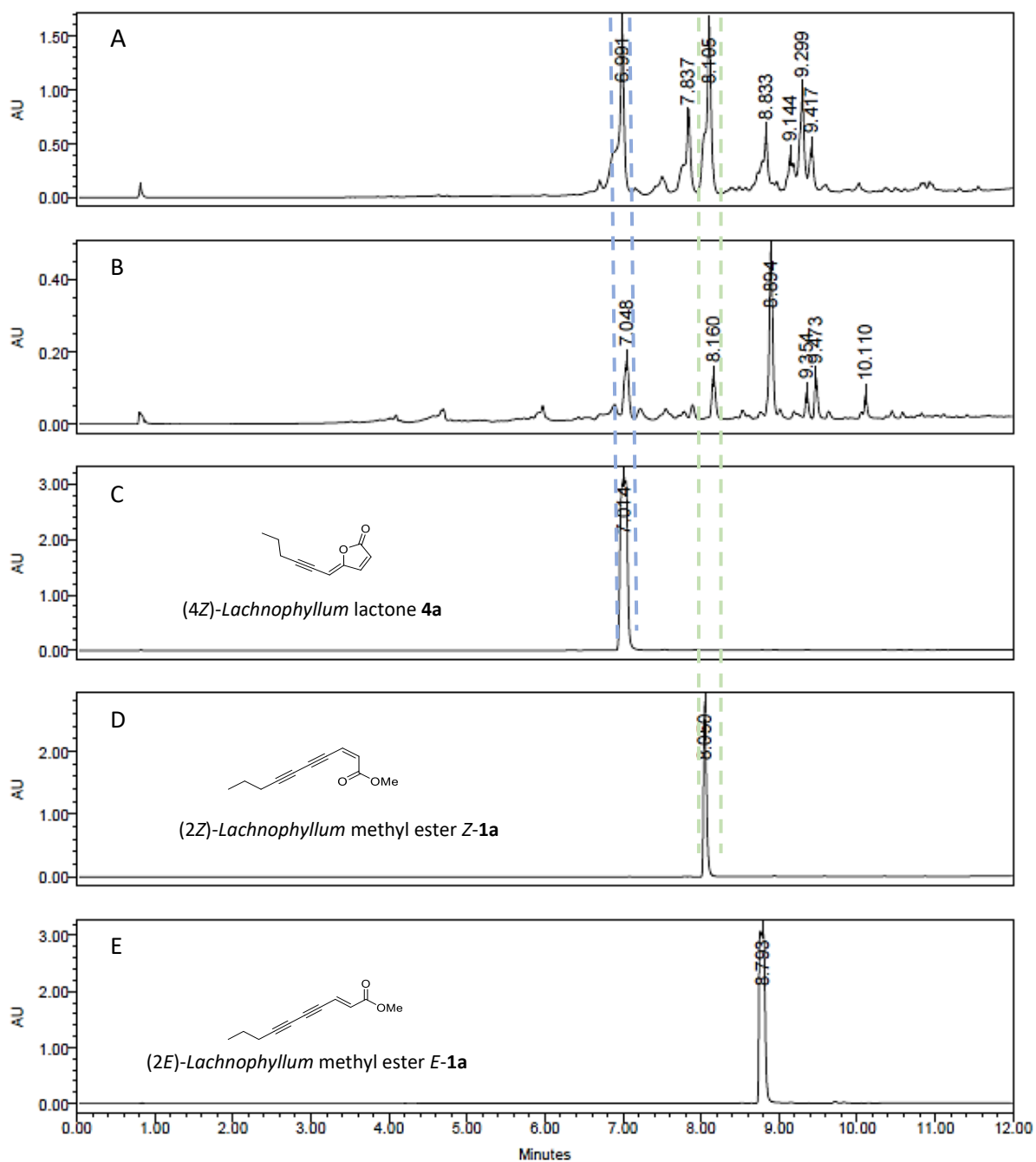


Fig. 2. Analytical chromatograms Acquity Premier BEH C18 1.7 μm (2.1x100) mm column, $\text{H}_2\text{O}/\text{CH}_3\text{CN}$, 0.1% HCOOH (95:5 to 0:100, 0.3 mL/min, 40 $^\circ\text{C}$, UV detection at 254 nm). **A.** Hexanoic extract obtained according Adande *et al.*⁷; **B.** Heptanoic extract obtained by ASE; **C.** Compound **4a**; **D.** Compound **Z-1a**; **E.** Compound **E-1a**.

Biological evaluation

Many biological evaluations have been carried out on plant extracts or essential oils containing *Lachnophyllum* ester or lactone. These studies identified a wide range of biological activities including antitumor^{33, 34} and antimicrobial³⁵⁻³⁷ for the (2Z)-methyl ester and allelopathic^{4, 38-40} and antifungal^{8, 41-44} for the lactone. We also recently evidenced an insecticidal and nematicidal activities of different extracts of *Conyza bonariensis* of which (2Z)-*Lachnophyllum* methyl ester and (4Z)-*Lachnophyllum* lactone were the major components.⁷

The literature indicates that the two closely related natural products *Matricaria* esters and lactone ($\Delta^{8,9}$ unsaturated analogs, Fig. 3) possess weak antifungal,^{42, 43} antimycobacterial activities⁴⁵ and that (2*E*,8*Z*)-*Matricaria* methyl ester exhibits a promising antileishmanial potency.⁴⁶

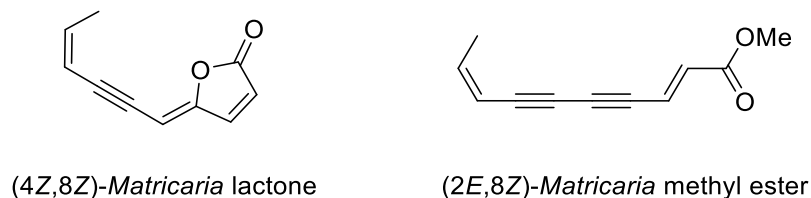


Fig. 3

Despite the numerous biological activities demonstrated for *Lachnophyllum* ester or lactone, to the best of our knowledge, no studies on their antitubercular nor antileishmanial activities have been reported so far. This prompted us to evaluate the *in vitro* potency of naturally occurring *Lachnophyllum* methyl esters and lactone against the associated pathogens *Mycobacterium tuberculosis* and *Leishmania*. The cytotoxicity towards African Green Monkey kidney mammalian cell lines (VERO) was also evaluated in order to determine their selectivity in favor of the mycobacterial or protozoan organisms. All the results of these different biological evaluations are brought together in Table 3.

Nematicidal activity

With several hundred milligrams of pure synthetic samples of the two natural compounds in hand, we firstly reproduced the assay ran previously on essential oils⁷ to confirm that the nematicidal activity is due to the two main identified components.

Nematicidal activity of natural compounds (*Z*-**1a**, *E*-**1a**, **4a**) and analogs (*Z*-**1b**, *E*-**1b**, *Z*-**1c**, *Z*-**1d**, **4b**, **4c**) was evaluated on second stage juveniles J2 of *Meloidogyne incognita*. Natural compounds, *Lachnophyllum* lactone (**4a**), *E*-*Lachnophyllum* methyl ester (*E*-**1a**) and *Z*-*Lachnophyllum* methyl ester (*Z*-**1a**), showed nematicidal activity with IC₅₀ under 100 mg/mL (Table 3), with lactone **4a** being the most effective with an IC₅₀ = 18.9 mg/L. *Lachnophyllum* lactone (**4a**) has been widely identified in Asteraceae family plants and several studies report its herbicidal activity on *Lemna paucicostata*,³⁸ bio-fungicide and bio-preservative on orange fruits.^{8, 44} A diethyl ether extract from roots of *C. bonariensis* containing a high level of *Lachnophyllum* lactone also demonstrated nematicidal activity⁷ on *M. incognita*. Somehow, the Michael acceptor behavior of the lactone, likely to be associated to its bioactivity, should be more pronounced than that of the esters. *E*-**1a** and *Z*-**1a** isomers were approximately 3 times less potent than the lactone, the *trans* isomer being slightly more effective than the *cis* one. The same trend was reported when (4*Z*) and (4*E*) *Matricaria* lactones were tested against weed growth.⁴⁰ Furthermore, nematicidal activity of synthetic sample of *Z*-**1a** (IC₅₀ = 75.3 mg/L) was similar to that of the sample of natural product isolated from *C. bonariensis* (IC₅₀ = 78.6 mg/L),⁷ highlighting the relevance of the synthetic approach to confirm the nematicidal activity of this compounds.

Compound		Nematicidal activity	Antileishmanial activity		Antimycobacterial activity			Cytotoxicity (VERO cells)	
	Structure	IC ₅₀ (mg/L)	EC ₅₀ (μM)	EC ₅₀ (mg/L)	MIC (μM)	IC ₅₀ (μM)	IC ₅₀ (mg/L)	CC ₅₀ (μM)	CC ₅₀ (mg/L)
Z-1a		75.3 ± 18.9	22.4 ± 2.7	4.0 ± 0.5	>10	ND		>250	>44
E-1a		56.9 ± 16.2	133 ± 6.8	23.4 ± 2.1	ND	ND		ND	
Z-1b		>200	61.2 ± 2.1	16.8 ± 0.7	2.5	1.11	0.30	>250	>68
E-1b		>200	ND		5	1.48	0.41	>250	>68
Z-1c		152 ± 47.3	77.8 ± 3.6	14.0 ± 0.9	>10	ND		>250	>45
Z-1d		>200	56.1 ± 2.3	12.8 ± 0.7	ND	ND		>250	>57
4a		18.9 ± 12.3	2.8 ± 0.6	0.4 ± 0.1	>10	ND		23.1 ± 0.9	3.7 ± 0.1
4b		>200	9.7 ± 1.4	2.5 ± 0.4	1.25	0.44	0.11	18.5 ± 1.2	4.8 ± 0.3
4c		110 ± 42.2	9.6 ± 1.0	1.6 ± 0.2	>10	ND		43.9 ± 2.5	7.3 ± 0.4
4d		ND	36.2 ± 1.3	7.8 ± 0.3	ND	ND		85.6 ± 3.2	18.3 ± 0.7
4e		ND	ND		5	1.6	0.52	18.4 ± 1.6	5.9 ± 0.5
4f		ND	ND		5	2.3	0.63	124.4 ± 2.4	34.1 ± 0.7

Table 3. Nematicidal, antileishmanial, antimycobacterial activities and cytotoxicity of synthetic *Lachnophyllum* compounds and analogs.

Nematicidal activities of the natural products synthetic analogs were assessed to explore the structure-activity relationship in this series. Three groups of compounds were evaluated, namely long-chain, saturated and aromatic analogs. None of the long-chain analogs (**Z-1b**, **E-1b** and **4b**) showed any biological activity on the phytoparasitic nematodes *M. incognita* with IC₅₀ > 200 mg/L. Short chain organic compounds may induce more damage to cell membranes that allow leakage of K⁺ ions causing osmotic stress to nematodes.^{47, 48} In addition to the impact of chain length on nematicidal activity, the influence of compounds unsaturation was also observed. Saturated analogs **Z-1c** and **4c** were approximately 2 to 6 times less effective against nematodes than their unsaturated analogs, with IC₅₀ values of 151.9 mg/L and 110.0 mg/L, respectively. It can be suggested that conjugation of the Michael acceptor system of *Lachnophyllum* natural products could enhance its electrophilicity. The conjugation of acetylenic bonds with other functions and unsaturations could also increase biological activity of compounds.⁴⁹ The aromatic analog **Z-1d** of *Lachnophyllum* ester did not show significant nematicidal activity with an IC₅₀ > 200 mg/L. Overall, the most potent compounds tested in

this study demonstrated nematicidal activities on phytoparasitic nematodes in the same range as that of commercial synthetic nematocides such as abamectin ($IC_{50} = 2.1 \text{ mg/L}$).⁵⁰ Interestingly, some SAR trends observed in this nematicidal evaluations, highlighting the importance for bioactivity of the lactone moiety and of the conjugated triple acetylenic units, were also shared with other biological studies of this work.

Antileishmanial activity

Antileishmanial activity of synthetic samples of natural products (**Z-1a**, **E-1a**, **4a**) and analogs thereof (**Z-1b**, **Z-1c**, **Z-1d**, **4b**, **4c**, **4d**) was evaluated on *Leishmania infantum* axenic amastigotes. Natural compounds exhibited some degree of antileishmanial activity: *Z-Lachnophyllum* methyl ester (**Z-1a**) was almost 6 times more potent than the *E* isomer (**E-1a**) ($EC_{50} = 22.4 \text{ }\mu\text{M}$ and $132.6 \text{ }\mu\text{M}$ respectively) indicating that the *Z* configuration have a favorable effect on the activity, while *Lachnophyllum* lactone (**4a**) exhibited the best activity with an $EC_{50} = 2.8 \text{ }\mu\text{M}$. However, none of the chemical modifications carried out increased the antileishmanial potency compared to the natural *Z-Lachnophyllum* methyl ester (**Z-1a**) since for all the *Z*-methyl esters analogs tested (**Z-1b**, **Z-1c**, **Z-1d**) the EC_{50} were 3 times higher than that of the natural **Z-1a**. The replacement of a triple bond by a benzene ring (**Z-1d**, $EC_{50} = 56.1 \text{ }\mu\text{M}$) or the lengthening of the alkyl chain (**Z-1b**, $EC_{50} = 61.2 \text{ }\mu\text{M}$) have a comparable effect on the antileishmanial activity whereas the saturation of an acetylenic bond (**Z-1c**, $EC_{50} = 77.8 \text{ }\mu\text{M}$) is the most detrimental.

As mentioned earlier, the (4*Z*)-*Lachnophyllum* lactone (**4a**) was 8 times more potent against *Leishmania infantum* axenic amastigotes than the corresponding methyl ester **Z-1a**. This trend was also observed for the other tested lactones: lactone **4b** ($EC_{50} = 9.7 \text{ }\mu\text{M}$) was more than 6 times more potent than ester **Z-1b** while lactone **1c** ($EC_{50} = 9.6 \text{ }\mu\text{M}$) was almost 8 times more potent than ester **Z-1c**. The gap in potency between the lactone **4d** ($EC_{50} = 36.2 \text{ }\mu\text{M}$) and corresponding ester **Z-1d** embedding a phenyl group was less prominent with only a 1.5-fold difference in favor of the lactone.

The naturally occurring (4*Z*)-*Lachnophyllum* lactone (**4a**) demonstrated the best antileishmanial activity among synthetic samples of natural products and analogs tested. Even if it remained lower than that of the reference molecule amphotericin b ($EC_{50} = 0.07 \text{ }\mu\text{M}$), this activity was better than the one reported for (2*E*,8*Z*)-*Matricaria* methyl ester ($EC_{50} = 61.2 \text{ }\mu\text{M}$).⁴⁶ However, the selectivity index with respect to the cytotoxicity on mammalian cells was modest ($SI = 8$) as otherwise observed for (2*E*,8*Z*)-*Matricaria* methyl ester.⁴⁶ From this point of view, the methyl esters **Z-1a**, **Z-1b**, **Z-1c** and **Z-1d** are more promising for developing new anti-leishmanial drugs as they do not present any detectable cytotoxicity against VERO cells at up to $250 \text{ }\mu\text{M}$ concentration while their anti-leishmanial activity remains significant.

Antimycobacterial activity

Antimycobacterial activity of *Lachnophyllum* methyl ester **Z-1a** and analogs **Z-1b**, **E-1b** and **Z-1c** were evaluated on pathogenic *Mycobacterium tuberculosis* strain H₃₇Rv using a colorimetric micro assay based on the reduction of MTT to formazan by metabolically active bacteria. The naturally occurring compound **Z-1a** and its partially saturated analog **Z-1c** did not display significant antimycobacterial activity with $MIC > 10 \text{ }\mu\text{M}$. By increasing the chain

length and therefore the compound lipophilicity, an expected gain in antimycobacterial activity was observed with MIC of 2.5 μM and 5 μM for analogs **Z-1b** and **E-1b** respectively.

In the lactone series, the same tendency was observed. The naturally occurring lactone **4a** and its partially saturated analog **4c** were inactive with MIC > 10 μM . Considering the favorable influence of the length chain on antimycobacterial activity, we also evaluated the lipophilic lactones **4b**, **4e** and **4f**. Compound **4b** proved to be the most active of all tested compounds with a MIC down to 1.25 μM . This potency compared favorably with that of ciprofloxacin, a drug used as reference in the assay (MIC = 2.5 μM). Adding a methyl group on the lactone lead to a 4-fold decrease in activity with compound **4f** exhibiting a MIC of 5 μM . The data is in agreement with the putative Michael acceptor behavior of these compounds. Besides, the addition in the alkyl chain of a fluorinated aromatic ring gives a similar loss of activity with compound **4e** presenting a MIC = 5 μM .

Considering the cytotoxicity towards mammalian cells (*vide infra*), lactone **4b** with an CC_{50} = 18.5 μM presented a moderate selectivity index of 15. Nevertheless, the methyl esters **Z-1b** and **E-1b**, devoid of cytotoxicity at a concentration up to 250 μM , were more promising compounds despite not having the highest activity against *Mycobacterium tuberculosis*.

Cytotoxicity towards VERO Cells

The active compounds were also evaluated on normal eukaryotic VERO cells in an MTT assay to determinate their selectivity index. The difference between methyl esters (**Z-1a**, **Z-1b**, **Z-1c** and **Z-1d**) and the lactones (**4a**, **4b**, **4c** and **4d**) was unequivocal: esters did not present any cytotoxicity for VERO cells below concentration of 250 μM while lactones showed CC_{50} values ranging from 85.6 to 18.5 μM . It should be noted that introduction of a methyl group on the lactone ring led to a drop in cytotoxicity since methylated analog **4f** was almost 7 times less cytotoxic than **4b**.

Conclusion

In the present paper, we have developed a synthetic route allowing us to access to two naturally occurring compounds, *Z-Lachnophyllum* methyl ester and (4*Z*)-*Lachnophyllum* lactone, that we recently identified as major components in essential oils and extracts of *Conyza bonariensis*. A nematicidal activity was evidenced for the plant extracts suggesting that these two naturally occurring compounds could be responsible of this biological property but the quantities obtained from the plant were too low to validate this hypothesis.

Lachnophyllum methyl esters (*Z* and *E*) were synthesized through a Sonogashira coupling between methyl 3-iodoacrylates and hepta-1,3-diyne whereas the access to (4*Z*)-*Lachnophyllum* lactone resulted from a palladium-catalyzed tandem alkyne–alkene cross coupling/lactonization process with (*Z*)-3-iodoacrylic acid and hepta-1,3-diyne. Varying the alkyne partner in these two reactions yielded analogs of the naturally occurring compounds.

The synthesis allowed us to obtain sufficient quantity to evaluate not only nematicidal but also antileishmanial and antimycobacterial activities of naturally occurring compounds and analogs. We could thus confirm the nematicidal activity of (4*Z*)-*Lachnophyllum* lactone, which remain the most active compound among all tested analogs. Compared to synthetic analogs,

the naturally occurring compounds (*Z*-**1a**, *E*-**1a**, and **4a**) were more active against nematodes. Regarding the antileishmanial activity, naturally occurring compounds *Z*-**1a** and **4a** proved to be the most active and, overall, lactone analogs show lower EC₅₀ than corresponding esters. As expected, longer alkyl chain (compared to the naturally occurring compounds) were necessary to observe significant antimycobacterial activity. The lactone analog **4b** presenting a C10 lipophilic chain was the most antimycobacterial compound. The notable activities of lactones, naturally occurring or analogs, either nematicidal, antileishmanial or antimycobacterial should be compared to the relative high cytotoxicity of these compounds for mammalian cells, conferring them a low selectivity index. From this point of view, *Z*-*Lachnophyllum* methyl ester and analogs are more promising since, despite more moderate biological activities their innocuity toward mammalian cells suggest a greater potential in this series. These results open up perspectives in the discovery of new bioactive agents to protect both agricultural crops and human health.

Experimental Section

General synthetic methods

All reagents were obtained from commercial suppliers and used without any further purification. If not specified, reactions were run under nitrogen atmosphere in oven-dried glassware. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents. Toluene, dichloromethane (DCM), tetrahydrofuran (THF), dimethylformamide (DMF) and diethyl ether (Et₂O) were obtained by filtration through a drying column on a filtration system. Thin-layer chromatography (TLC) analyses were performed on precoated, aluminum-backed silica gel (Merck 60 F254). Visualization of the developed chromatogram was performed by UV light (254 nm) and using 10% phosphomolybdic acid in EtOH, or aqueous potassium permanganate (KMnO₄) stain. Flash chromatography columns were performed using flash silica gel (SDS 35-70 μm). Nuclear magnetic resonance spectra were recorded on a Bruker Advance 300 or 400 or 500 MHz spectrometer. Chemical shifts for ¹H NMR spectra are given in parts per million (ppm) with the residual solvent resonance as the reference CHCl₃ (δ = 7.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet and br = broad), coupling constant in Hz and integration. Chemical shifts for ¹³C NMR spectra are given in ppm using the central peak of CDCl₃ (δ = 77.16 ppm) as the reference. All ¹³C NMR spectra were obtained with complete proton decoupling. Infrared analyses were run on a Thermo-Nicolet Diamond ATR (4 cm⁻¹ of resolution, 16 scans) equipped with a DTGS detector and are reported in reciprocal centimeters (cm⁻¹). High-resolution mass spectrometry (HRMS) was performed on a Thermo-Finnigan MAT 95 XL instrument. Mass spectrometry *m/z* values are given in Dalton units.

Synthesis of *Lachnophyllum* esters and analogs (1)

General procedure A: Cadot-Chodkiewicz coupling

CuCl (0.03 eq.) was added to aqueous solution 30% of n-butylamine (1.5 eq.) at 0°C. After stirring for 5 min, hydroxylamine hydrochloride (0.02 eq.) was added, causing the

disappearance of the blue color of solution. Enyne precursor (1 eq.) in THF (0.6 M) was then added and the mixture was stirred at 0 °C for 15 min. A solution of the bromoalkyne (1.2 eq.) in THF (0.6 M) was added dropwise, and the resulting mixture was allowed to warm to room temperature overnight (TLC monitoring). The mixture was quenched with sat. NH₄Cl aqueous solution and the aqueous layer was extracted with diethyl ether. The combined organic layers were dried with MgSO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography.

General procedure B: Sonogashira coupling

To a stirred solution of PdCl₂(PPh₃)₂ (0.05 eq.), CuI (0.05 eq.) in degassed triethylamine (0.7 M), under nitrogen, was added alkene iodide (1 eq.). Terminal alkyne (1.5 eq.) was then slowly added dropwise and the solution was stirred at 40 °C overnight. After cooling to room temperature, the mixture was quenched with HCl aqueous solution (1M) and extracted with diethyl ether (3x). The combined organic layers were dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel

(2Z)-Lachnophyllum methyl ester (Z-1a) (or methyl (Z)-deca-2-en-4,6-diyanoate)

Following to general procedure A using terminal alkyne **Z-2**¹³ (0.25 g, 2.31 mmol), 1-bromopent-1-yne (0.4 g, 2.72 mmol), CuCl (6.9 mg, 0.069 mmol) and hydroxylamine hydrochloride (3.4 mg, 0.048 mmol), 30% of n-butylamine (5 mL). The resulting residue was purified by silica gel chromatography using 5% of diethyl ether in pentane to give **Z-1a** as a yellow solid (157.4 mg, 38% yield).

Following the general procedure B using iodoalkene **Z-3**¹³ (50 mg, 0.24 mmol), hepta-1,3-diyne (32.5 mg, 0.35 mmol), PdCl₂(PPh₃)₂ (8.5 mg, 0.012 mmol), CuI (2.3 mg, 0.012 mmol). The crude product was purified using 5% diethyl ether in pentane to give **Z-1a** as a yellow solid (29.7 mg, 70% yield).

¹H NMR (300 MHz, CDCl₃) δ (ppm) 6.23 (d, J = 11.4 Hz, 1H); 6.20 (dt, J = 11.7, 1.0 Hz, 1H); 3.79 (s, 3H); 2.36 (td, J = 7.0, 0.9 Hz, 2H); 1.69-1.53 (m, 2H); 1.02 (t, J = 7.4 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 164.8, 130.7, 122.6, 90.1, 86.6, 70.9, 65.2, 51.6, 21.8, 21.6, 13.5. HRMS (DCI-CH₄): calcd for C₁₁H₁₃O₂ [M+H]⁺: 177.0916 m/z, found: 177.0910 m/z. FTIR: 3091.3, 3026.1, 2959.1, 2933.4, 2904.9, 2873.3, 2226.0, 1714.8, 1600.1.

(2E)-Lachnophyllum methyl ester (E-1a) (or methyl (E)-deca-2-en-4,6-diyanoate)

Following to general procedure A using terminal alkyne **E-2**¹³ (100 mg, 0.91 mmol), 1-bromopent-1-yne (160 mg, 1.09 mmol), CuCl (2.67 mg, 0.03 mmol) and hydroxylamine hydrochloride (1.32 mg, 0.019 mmol), 30% of n-butylamine (1.5 mL). The resulting residue was purified by silica gel chromatography using 5% of diethyl ether in pentane to give **E-1a** as yellow solid (40.7 mg, γ = 25%).

Following the general procedure B using iodoalkene **E-3**¹⁴ (150 mg, 0.71 mmol), hepta-1,3-diyne (98 mg, 1.06 mmol), PdCl₂(PPh₃)₂ (25 mg, 0.036 mmol), CuI (6.8 mg, 0.036 mmol). The crude product was purified using 5% diethyl ether in pentane to give **E-1a** as a yellow solid (67 mg, 54% yield).

¹H NMR (300 MHz, CDCl₃) δ (ppm) 6.78 (dt, J = 15.8, 1.1 Hz, 1H); 6.32 (d, J = 15.9 Hz, 1H); 3.78 (s, 3H); 2.36 (td, J = 7.0, 1.1 Hz, 2H); 1.61 (h, J = 7.3 Hz, 2H); 1.03 (t, J = 7.4 Hz, 1H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 166.0, 132.0, 124.6, 89.1, 83.3, 71.3, 64.9, 51.9, 21.62, 13.5. HRMS

(DCI-CH₄): calcd for C₁₁H₁₃O₂ [M+H]⁺: 177.0916 *m/z*, found: 177.0913 *m/z*. **FTIR**: 3069.3, 2965.2, 2934.6, 2232.9, 2141.3, 1725.1, 1614.0.

Methyl (Z)-heptadeca-2-en-4,6-diynoate (Z-1b)

Following to general procedure A using terminal alkyne Z-**2**¹³ (125 mg, 1.13 mmol), 1-bromododec-1-yne (330 mg, 1.36 mmol), CuCl (3.4 mg, 0.034 mmol) and hydroxylamine hydrochloride (1.58 mg, 0.023 mmol), 30% of n-butylamine (2.5 mL). The resulting residue was purified by silica gel chromatography using 5% of diethyl ether in pentane to give Z-**1b** as yellow solid (126 mg, 40% yield).

Following the general procedure B using iodoalkene Z-**3**¹³ (50 mg, 0.24 mmol), tetradeca-1,3-diyne (68 mg, 0.36 mmol), PdCl₂(PPh₃)₂ (8.4 mg, 0.012 mmol), CuI (2.3 mg, 0.012 mmol). The crude product was purified using 5% diethyl ether in pentane to give Z-**1b** (54 mg, 82% yield). **¹H NMR** (300 MHz, CDCl₃) δ (ppm) 6.24 (d, *J* = 11.4 Hz, 1H); 6.19 (dt, *J* = 11.3, 0.9 Hz, 1H); 3.80 (s, 1H); 2.38 (td, *J* = 7.0, 1.0 Hz, 2H); 1.63-1.51 (m, 2H); 1.48-1.19 (m, 14H); 0.96-0.85 (m, 3H). **¹³C NMR** (75 MHz, CDCl₃) δ (ppm) 164.8, 130.7, 122.6, 90.4, 86.7, 70.8, 65.1, 51.6, 31.9, 29.6, 29.5, 29.3, 29.1, 28.9, 28.1, 22.7, 19.8, 14.1. **HRMS** (DCI-CH₄): calcd for C₁₈H₂₇O₂ [M+H]⁺: 275.2011 *m/z*, found: 275.2008 *m/z*. **FTIR**: 3096.0, 3037.5, 2953.8, 2922.6, 2852.4, 2223.3, 1716.8, 1600.4.

Methyl (E)-heptadeca-2-en-4,6-diynoate (E-1b)

Following to general procedure A using terminal alkyne E-**2**¹³ (73 mg, 0.64 mmol), 1-bromododec-1-yne (188 mg, 0.77 mmol), CuCl (1.9 mg, 0.02 mmol) and hydroxylamine hydrochloride (0.9 mg, 0.013 mmol), 30% of n-butylamine (1.1 mL). The resulting residue was purified by silica gel chromatography using 5% of diethyl ether in pentane to give E-**1b** as yellow solid (99 mg, 55% yield).

Following the general procedure B using iodoalkene E-**3**¹⁴ (50 mg, 0.24 mmol), tetradeca-1,3-diyne (68 mg, 0.36 mmol), PdCl₂(PPh₃)₂ (8.3 mg, 0.012 mmol), CuI (2.3 mg, 0.012 mmol). The crude product was purified using 5% diethyl ether in pentane to give E-**1b** (53 mg, 81% yield). **¹H NMR** (300 MHz, CDCl₃) δ (ppm) 6.78 (dt, *J* = 15.8, 1.1 Hz, 1H); 6.32 (d, *J* = 15.9 Hz, 1H); 3.78 (s, 3H); 2.38 (td, *J* = 7.0, 1.1 Hz, 2H); 1.67-1.50 (m, 2H); 1.47-1.15 (m, 14H); 0.92-0.80 (m, 3H). **¹³C NMR** (75 MHz, CDCl₃) δ (ppm) 166.0, 132.0, 124.5, 89.3, 83.4, 71.3, 64.8, 51.9, 31.9, 29.5, 29.4, 29.3, 29.1, 28.8, 28.0, 22.7, 19.7, 14.1. **HRMS** (DCI-CH₄): calcd for C₁₈H₂₇O₂ [M+H]⁺: 275.2011 *m/z*, found: 275.2014 *m/z*. **FTIR**: 2957.8, 2926.0, 2854.6, 2231.3, 2141.5, 1786.6, 1727.0, 1614.0.

Methyl (Z)-dec-2-en-4-ynoate (Z-1c)

Following the general procedure B at 0 °C to rt using iodoalkene Z-**3**¹³ (500 mg, 2.35 mmol), hept-1-yne (720 μL, 2.82 mmol), PdCl₂(PPh₃)₂ (30 mg, 0.042 mmol, 0.017 eq.), CuI (8 mg, 0.042 mmol, 0.017 eq.). The crude product was purified using 10% diethyl ether in pentane to give Z-**1c** (282 mg, 67% yield).

¹H NMR (300 MHz, CDCl₃) δ (ppm) 6.18 (dt, *J* = 11.4, 2.4 Hz, 1H); 6.05 (dt, *J* = 11.4, 0.7 Hz, 1H); 3.77 (s, 3H), 2.46 (tdd, *J* = 7.2, 2.4, 0.7 Hz, 2H); 1.70-1.52 (m, 2H); 1.62 (q, *J* = 7.6 Hz, 2H); 1.51-1.18 (m, 4H); 0.92 (t, *J* = 6.5 Hz, 3H). **¹³C NMR** (75 MHz, CDCl₃) δ (ppm) 165.4, 126.9, 124.3, 104.5, 51.4, 31.1, 28.1, 22.2, 20.1, 13.9. **HRMS** (DCI-CH₄): calcd for C₁₁H₁₇O₂ [M+H]⁺: 181.1229 *m/z*, found: 181.1233 *m/z*. **FTIR**: 2954.7, 2932.3, 2860.1, 2207.6, 1731.6, 1716.9, 1610.7.

Methyl (Z)-5-(4-propylphenyl)pent-2-en-4-ynoate (Z-1d)

Following the general procedure B using iodoalkene **Z-3**¹³ (83 mg, 0.390 mmol), 1-ethynyl-4-propylbenzene (84 mg, 0.581 mmol), PdCl₂(PPh₃)₂ (13.6 mg, 0.019 mmol), CuI (3.6 mg, 0.019 mmol). The crude product was purified using 5% diethyl ether in pentane to give ester **Z-1d** (79 mg, 89% yield) as yellow oil.

¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.45 (d, *J* = 8.2 Hz, 2H); 7.15 (d, *J* = 8.2 Hz, 2H); 6.37 (d, *J* = 11.4 Hz, 1H); 6.12 (d, *J* = 11.4 Hz, 1H); 3.80 (s, 3H); 2.59 (d, *J* = 7.6 Hz, 2H); 1.71-1.55 (m, 2H); 0.93 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 165.3, 144.4, 132.1, 128.6, 127.2, 123.4, 119.8, 102.0, 86.0, 51.5, 38.0, 24.3, 13.7. HRMS (DCI-CH₄): calcd for C₁₅H₁₇O₂ [M+H]⁺: 229.1229 *m/z*, found: 229.1216 *m/z*. FTIR: 3028.0, 2958.5, 2931.0, 2871.5, 2199.8, 2180.2, 1727.3, 1713.7, 1613.1, 1599.3.

Methyl (E)-5-(4-propylphenyl)pent-2-en-4-ynoate (E-1d)

Following the general procedure B using iodoalkene **E-3**¹⁴ (49.0 mg, 0.231 mmol), 1-ethynyl-4-propylbenzene (50 mg, 0.347 mmol), PdCl₂(PPh₃)₂ (8.1 mg, 0.011 mmol), CuI (2.2 mg, 0.011 mmol). The crude product was purified using 5% diethyl ether in pentane to give ester **E-1d** (46.9 mg, 89% yield) as yellow solid.

¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.39 (d, *J* = 8.2 Hz, 2H); 7.15 (d, *J* = 8.1 Hz, 2H); 6.99 (d, *J* = 15.8 Hz, 1H); 6.29 (d, *J* = 15.8 Hz, 1H); 3.78 (s, 3H); 2.59 (t, *J* = 7.6 Hz, 2H); 1.72-1.56 (m, 2H); 0.95 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 166.5, 144.5, 131.9, 129.1, 128.7, 125.6, 119.3, 99, 85.9, 51.8, 38, 24.3, 13.7. HRMS (DCI-CH₄): calcd for C₁₅H₁₇O₂ [M+H]⁺: 229.1229 *m/z*, found: 229.1226 *m/z*. FTIR: 3077.9, 3033.1, 2955.3, 2928.4, 2869.2, 2195.0, 1710.84, 1621.7, 1601.7.

Synthesis of (4Z)-Lachnophyllum lactone and analogs (4)

General procedure C: Sonogashira coupling/lactonization

A solution of terminal alkyne (1 eq.) in CH₃CN (1 M) was added at room temperature, under nitrogen, to a stirred mixture vinyl iodide (1 eq.), degassed Et₃N (27 eq.), CuI (0.05 eq.) and PdCl₂P(Ph₃)₂ (0.025 eq.). PPh₃ (0.1 eq.) was only used for the synthesis of aromatic analogues. After was stirring at 40 °C overnight, the mixture was quenched with sat. NH₄Cl aqueous solution (10 mL), and extracted with diethyl ether (3x10 mL). The combined organic layers were washed with water (3x10 mL), dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel using 10% diethyl ether in pentane as eluant to gives lactone compounds.

(4Z)-Lachnophyllum lactone (4a) (or (Z)-5-(hex-2-yn-1-ylidene)furan-2(5H)-one)

Following the general procedure C using hepta-1,3-diyne (75 mg, 0.76 mmol), (Z)-3-iodoacrylic acid (100 mg, 0.505 mmol), PdCl₂P(Ph₃)₂ (8.9 mg, 0.013 mmol), CuI (4.8 mg, 0.025 mmol), Et₃N (2 mL, 13.6 mmol). The lactone **4a** was obtained as a yellow oil (37.2 mg, 46 % yield).

¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.37 (d, *J* = 5.4 Hz, 1H); 6.20 (dd, *J* = 5.4, 0.9 Hz, 1H); 5.30 (td, *J* = 2.6, 0.9 Hz, 1H); 2.40 (td, *J* = 7.0, 2.5 Hz, 2H); 1.59 (h, *J* = 7.2 Hz, 2H); 1.00 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 168.9, 156.1, 142.7, 120.1, 104.6, 95.1, 74.8, 22.1, 21.8, 13.5. HRMS (DCI-CH₄): calcd for C₁₀H₁₁O₂ [M+H]⁺: 163.0759 *m/z*, found: 163.0752 *m/z*. FTIR: 3138.5, 3106.6, 3046.3, 2964.8, 2934.3, 2873.4, 2211.0, 1780.9, 1752.3.

(Z)-5-(Tridec-2-yn-1-ylidene)furan-2(5H)-one (4b)

Following the general procedure C using tetradeca-1,3-diyne (115 mg, 0.616 mmol), (Z)-3-iodoacrylic acid (80 mg, 0.404 mmol), PdCl₂P(Ph₃)₂ (7.1 mg, 0.01 mmol), CuI (3.8 mg, 0.02 mmol), Et₃N (1.5 mL, 10.9 mmol). The lactone **4b** was obtained as a yellow oil (76.9 mg, 73% yield).

¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.36 (d, *J* = 5.2 Hz, 1H); 6.21 (dd, *J* = 5.4, 0.8 Hz, 1H); 5.30 (t, *J* = 2.6 Hz, 1H); 2.44 (td, *J* = 7.1, 2.5 Hz, 2H); 1.57 (q, *J* = 7.1 Hz, 2H); 1.47-1.34 (m, 2H); 1.34-1.20 (m, 14H); 0.92-0.83 (m, 3H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 169.0, 156.2, 142.8, 120.3, 105.0, 95.3, 74.8, 32.0, 29.7, 29.6, 29.4, 29.2, 29.1, 28.5, 22.8, 20.3, 14.2. HRMS (DCI-CH₄): calcd for C₁₇H₂₅O₂ [M+H]⁺: 261.1855 *m/z*, found: 261.1848 *m/z*. FTIR: 3066.3, 3049.1, 2953.6, 2922.9, 2849.9, 2868.9, 2209.2, 1787.3, 1759.1.

(Z)-5-Hexylidenefuran-2(5H)-one (4c)

Following the general procedure C using hept-1-yne (170 mg, 1.33 mmol), (Z)-3-iodoacrylic acid (176 mg, 0.89 mmol), PdCl₂P(Ph₃)₂ (15.6 mg, 0.022 mmol), CuI (8.5 mg, 0.045 mmol), Et₃N (3.4 mL, 24.03 mmol). The lactone **4c** was obtained as a yellow oil (98 mg, 66 % yield).

¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.32 (d, *J* = 5.4 Hz, 1H); 6.12 (d, *J* = 5.4 Hz, 1H); 5.29 (t, *J* = 8.0 Hz, 1H); 2.38 (q, *J* = 7.6 Hz, 2H); 1.54-1.38 (m, 2H); 1.36-1.26 (m, 4H); 0.92-0.80 (m, 3H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 170.2, 149.7, 143.7, 118.9, 117.9, 31.4, 28.6, 26.4, 22.4, 14.0. HRMS (DCI-CH₄): calcd for C₁₀H₁₅O₂ [M+H]⁺: 167.1072 *m/z*, found: 167.1070 *m/z*. FTIR: 3110.0, 2860.3, 2957.3, 2930.2, 2873.5, 1780.1, 1745.8.

(Z)-5-(4-Propylbenzylidene)furan-2(5H)-one (4d)

Following the general procedure C using 1-ethynyl-4-propylbenzene (81.8 mg, 5.68 mmol), (Z)-3-iodoacrylic acid (75 mg, 0.38 mmol), PdCl₂P(Ph₃)₂ (13.3 mg, 0.019 mmol), CuI (3.6 mg, 0.019 mmol), PPh₃ (9.9 mg, 0.038 mmol), Et₃N (1.4 mL, 10.26 mmol). The lactone **4d** was obtained as a yellow solid (76.9 mg, 41% yield).

¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.71 (d, *J* = 8.1 Hz, 2H), 7.48 (d, *J* = 5.4 Hz, 1H), 7.21 (d, *J* = 8.1 Hz, 2H), 6.19 (dd, *J* = 5.4, 0.8 Hz, 1H), 6.02 (s, 1H), 2.61 (t, *J* = 7.6 Hz, 2H), 1.65 (h, *J* = 7.4 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 170.5, 148.1, 145.4, 144.7, 130.9, 130.5, 129.2, 117.7, 114.6, 38.1, 24.4, 13.9. HRMS (DCI-CH₄): calcd for C₁₄H₁₅O₂ [M+H]⁺: 215.1072 *m/z*, found: 215.1064 *m/z*. FTIR: 3106.5, 3022.2, 2960.2, 2930.5, 1786.6, 1748.1, 1608.8, 1550.0.

(Z)-5-(3,5-Difluoro-4-(heptyloxy)benzylidene)furan-2(5H)-one (4e)

Following the general procedure C using 5-ethynyl-1,3-difluoro-2-(heptyloxy)benzene (see SI) (50 mg, 0.251 mmol), (Z)-3-iodoacrylic acid (95.6 mg, 0.38 mmol), PdCl₂P(Ph₃)₂ (8.8 mg, 0.012 mmol), CuI (2.4 mg, 0.012 mmol), PPh₃ (6.6 mg, 0.025 mmol), Et₃N (0.94 mL, 6.78 mmol). The lactone **4e** was obtained as a yellow solid (27 mg, 33% yield).

¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.47 (d, *J* = 5.4 Hz, 1H), 7.41-7.29 (m, 2H), 6.24 (dd, *J* = 5.4, 0.8 Hz, 1H), 5.87 (s, 1H), 4.19 (t, *J* = 6.7 Hz, 2H), 1.76 (p, *J* = 6.8 Hz, 2H), 1.53-1.40 (m, 2H), 1.40-1.19 (m, 6H), 0.92-0.85 (m, 2H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 169.7, 155.8 (dd, *J* = 247.9, 6.6 Hz), 148.9, 145.1, 136.7 (t, *J* = 14.1 Hz), 127.4 (t, *J* = 9.7 Hz), 118.8, 114.6-114.1 (m), 111.8 (t, *J* = 3.0 Hz), 74.9 (t, *J* = 3.3 Hz), 31.9, 30.1, 29.1, 25.7, 22.7, 14.2. ¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ (ppm) -127.5. HRMS (DCI-CH₄): calcd for C₁₈H₂₁O₃F₂ [M+H]⁺: 323.1459 *m/z*,

found: 323.1451 *m/z*. **FTIR**: 3127.3, 3112.4, 2952.3, 2927.1, 2896.8, 1773.4, 1744.1, 1515.9, 1440.6.

(Z)-4-Methyl-5-(tridec-2-yn-1-ylidene)furan-2(5H)-one (4f)

Following the general procedure C using tetradeca-1,3-diyne (134.4 mg, 0.707 mmol), (Z)-3-iodobut-2-enoic acid³² (100 mg, 0.471 mmol), PdCl₂P(Ph₃)₂ (16.6 mg, 0.024 mmol), CuI (4.5 mg, 0.024 mmol), Et₃N (3 mL, 19.09 mmol). The lactone **4f** was obtained as a yellow powder (58.6 mg, 35% yield).

¹H NMR (300 MHz, CDCl₃) δ (ppm) 5.95 (s, 1H), 5.31 (dt, *J* = 2.6, 1.5 Hz, 1H), 2.43 (td, *J* = 7.1, 2.5 Hz, 2H), 2.14 (d, *J* = 1.4 Hz, 3H), 1.68-1.49 (m, 2H), 1.49-1.39 (m, 2H), 1.37-1.16 (m, 14H), 0.92-0.81 (m, 3H). **¹³C NMR** (75 MHz, CDCl₃) δ (ppm) 168.4, 157.3, 153.9, 117.0, 104.0, 91.6, 74.4, 32.0, 29.7, 29.6, 29.4, 29.2, 29.0, 28.6, 22.8, 20.2, 14.2, 11.6. **HRMS** (DCI-CH₄): calcd for C₁₈H₂₇O₂ [M+H]⁺: 275.2011 *m/z*, found: 275.2011 *m/z*. **FTIR**: 3046.9, 2925.4, 2854.3, 2212.2, 1782.5, 1754.2, 1641.0, 1605.7.

Accelerated Solvent Extraction ASE conditions

Accelerated solvent extraction (ASE) has been done on 7 g of powdered roots of the plant. The accelerated solvent extraction system (Dionex™ ASE™ 350) automates extraction and filtration (10 μm). Each sample was extracted for 15 minutes (3 cycles of 5 minutes) with 3 x 34 mL of solvent at 100 °C and 117 bars. Samples were extracted with MTBE, heptane or ethanol. Each fraction was analyzed by UHPLC-PDA-MS.

UHPLC-PDA-MS analysis conditions

All analyses of sample were carried out by reverse-phase UHPLC-PDA-MS on an Acquity Premier BEH C18 1.7 μm (2.1x100) mm column. Water with 0.1% HCOOH and acetonitrile with 0.1% HCOOH were used as solvents A and B respectively at a flow rate of 0.3 mL/min at 40°C. The gradient was generic, starting at 5% B and remaining there for the first minute, gradually increasing to 100% B over 10 minutes, then remaining there for 2 minutes before returning to the initial conditions. 2 detection modes were used, the UV detector at 254 nm and the single quadrupole positive and negative electrospray mass detector between 100 and 950 Daltons.

Nematicidal assay

Nematicidal tests were conducted according to Adande, K. *et al.*⁷ and references in. *Meloidogyne incognita* Nematode species used for test were obtained from tomato (*Solanum lycopersicum* L.) roots harvested in a greenhouse. The highly susceptible to root-knot nematodes of cultivar *cv Belladonna* were infested by this nematode population. Experimental plants maintained in the greenhouse at a temperature of 25 to 28 °C, 60% humidity, and a 16-hour photoperiod, were matured in plastic pots (18 cm diameter) filled with a 10:1 (V/V) mixture of peat and perlite. The 40 days plants were uprooted, while the free-soiled roots after washing process were cut into 2 cm pieces. A sodium hypochlorite procedure facilitated nematode eggs extraction,⁵¹ and second-stage larvae (J2) were obtained using the modified Bearmann method at 28 °C. All J2 that hatched within the first 3 days were discarded, and subsequent generations were collected and used for biological test.⁵²

The nematicidal activities of synthesized natural compounds and their analogues were assessed by IC₅₀ value which indicate the loss of mobility in second-stage juveniles (J2). Stock solutions of the test samples were prepared in dimethyl sulfoxide (DMSO). The final test

solutions were obtained by diluting the stock solutions with water containing the surfactant polysorbate 20 (Tween-20). After preliminary tests, the DMSO and Tween-20 concentrations in each testing well never exceeded 1.0% v/v and 0.3% v/v respectively. In these concentrations, the mobility of nematodes was preserved compared to nematodes maintained in pure water.⁵³ More, 96-well microplate was used for the tests. Each test well contained 200 µL of solution with 25-30 J2s of the nematode. After 72 hours, the mortality of the nematodes was examined under a reversed microscope (Zeiss, Germany). Each test was repeated 4 times while the whole experiment was repeated at least twice at different time points. The naturally dead nematodes (less than 5% of the total number of J2s) observed in the control group consisting of water, Tween 20, and DMSO were discarded for dead J2s percentages calculation. The correction was made according to Schneider-Orelli formula⁵⁴ below:

$$\text{Corrected \%} = \frac{\text{mortality \% in treatment} - \text{mortality \% in control}}{100 - \text{mortality \% in control}} \times 100$$

The corrected rates of mortality of J2 treated with tested compound were subjected to nonlinear analysis using the logistic equation proposed by Seefeldt *et al.*⁵⁵

$$Y = C + \frac{D - C}{1 + e^{b \log x - \log IC_{50}}}$$

C = the lower limit, D = the slope at the IC₅₀, IC₅₀ = the test compounds required for 50% death/immotility). In the regression equation, Independent variable (x) was the test compounds concentration (% w/v) and the immotile J2 (percentage increase over water control) was the dependent variable (y). The mean value of the six replicates per compound concentration and immersion period was used to calculate the IC₅₀ value.

Antileishmanial activity on *L. infantum* axenic amastigotes

L. infantum promastigotes (MHOM/MA/67/ITMAP-263, CNR Leishmania, Montpellier, France, expressing luciferase activity) were cultivated in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS), 2 mM L-glutamine and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) and harvested in logarithmic phase of growth by centrifugation at 900 g for 10 min. The supernatant was removed carefully and was replaced by the same volume of RPMI 1640 complete medium at pH 5.4 and incubated for 24 h at 24 °C. The acidified promastigotes were then incubated for 24 h at 37 °C in a ventilated flask to transform acidified promastigotes into axenic amastigotes. The effects of the tested compounds on the growth of *L. infantum* axenic amastigotes were assessed as follows. *L. infantum* amastigotes were incubated at a density of 2 × 10⁶ parasites/mL in sterile 96-well plates with various concentrations of compounds dissolved in DMSO (final concentration 0.5% v/v), in duplicate. Appropriate controls, DMSO and amphotericin B, were added to each set of experiments. After a 48 h incubation period at 37 °C, each plate-well was then microscopically-examined to detect any precipitate formation. To estimate the luciferase activity of axenic amastigotes, 80 µL of each to white 96-well plates, Steady Glow[®] reagent (Promega) was added according to manufacturer's instructions, and plates were incubated for 2 min. The luminescence was measured in a FLUOstar Omega microplate reader (BMG Labtech). Efficient concentration 50% (EC₅₀) was defined as the concentration of drug required to inhibit by 50% the metabolic

activity of *L. infantum* amastigotes compared to control. EC₅₀ values were calculated by non-linear regression analysis processed on dose response curves, using TableCurve 2D V5 software.

Mycobacterial growth inhibition assays

The susceptibility of *Mycobacterium tuberculosis* strain H₃₇Rv to all synthesized compounds was evaluated using a colorimetric micro assay based on the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) to formazan by metabolically active bacteria. Briefly, serial twofold dilutions of each compound solubilized in DMSO were prepared in 7H9 broth (Difco Middlebrook 7H9 broth, Becton Dickinson and Company, complemented with glycerol (4g/L)) using 96-well microtiter plates and 100 µL of *M. tuberculosis* H₃₇Rv suspension in 7H9 broth were added to each well (OD₆₀₀ 0.05). After 6 days incubation, MTT was added (50 µL, 1 mg/mL in 7H9 broth). After 24 h incubation, 50 µL of SDS 20% were added to each well. The optical densities were measured at 570 nm with a microplate reader Expert Plus (ASYS HITECH, Austria). The absorbance value for untreated bacilli was set as 100% growth control. The MIC from at least three independent experiments was determined as the lowest concentration of compound that inhibited bacterial growth; dose-response curves based a non-linear regression were fitted using the GraphPad Prism software. When indicated, the selectivity index defined as the ratio between CC₅₀ on VERO cells and MIC obtained on *Mycobacterium tuberculosis* was calculated.

Cytotoxicity evaluation on VERO cell line

The evaluation of the tested molecules cytotoxicity by MTT assay was done on the VERO cell line. Briefly, cells (5.10⁴ cells/mL) in 100 µL of complete medium, [MEM supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine and antibiotics (100U/mL penicillin and 100 µg/mL streptomycin)] were seeded into each well of 96-well plates and incubated at 37 °C in a humidified 5% CO₂ with 95% air atmosphere. After a 24 h incubation, 100 µL of medium with various product concentrations and appropriate controls were added and the plates were incubated for 72 h at 37 °C. Each plate-well was then microscope-examined for detecting possible precipitate formation before the medium was aspirated from the wells. 100 µL of MTT solution (0.5 mg/mL in complete MEM) were then added to each well. Cells were incubated for 1 h at 37 °C. After this time, the MTT solution was removed and DMSO (100 µL/well) was added to dissolve the resulting formazan crystals. Plates were shaken vigorously (300 rpm) for 5 min. The absorbance was measured at 570 nm with a microplate spectrophotometer (BIOTEK Eon). DMSO was used as blank. CC₅₀ were calculated by non-linear regression analysis processed on dose–response curves, using TableCurve 2D V5 software.

Data availability

The data supporting this article have been included as part of the Supplementary Information

Acknowledgements

Authors are grateful to Erasmus+ program KA171 - Mobility of higher education students and staff for financing the mobility of Kodjo Adande. We thank the Integrated Screening Platform of Toulouse (PICT, IBISA) for providing access to UPLC Acquity equipment from Waters, and Institut de Chimie de Toulouse ICT-UAR 2599 (Université de Toulouse, CNRS, Toulouse, France, <https://ict.cnrs.fr>) for providing access to Autopurification preparative HPLC equipment from Waters. We also thank Emmanuel DESMARTIN of Toulouse White Biotechnology (UMS INRAE / INSA / CNRS) for his valuable help with Accelerated Solvent Extractor, as well as Audrey Brault from ThermoFisher Scientific. The cytotoxicity assays on VERO cells were performed by Ms. Sandra Bourgeade-Delmas, manager of the "Plateau technique de Biologie Cellulaire" at Pharma-Dev, UMR152 IRD/UT3.

References

1. S. Opiyo, *IOSR-JAC*, 2023, **16**, 36-48.
2. S. Opiyo, P. Njoroge and K. Muna, *IOSR-JAC*, 2023, **16**, 61-71.
3. J. F. Sanz and J. A. Marco, *Liebigs Ann. Chem.*, 1991, **1991**, 399-400.
4. O. Tzakou, A. Gani, G. Economou and A. Yannitsaros, *J. Essent. Oil Res.*, 2004, **16**, 425-428.
5. L. C. A. Barbosa, V. F. Paula, A. S. Azevedo, E. A. M. Silva and E. A. Nascimento, *Flavour Fragr J.*, 2005, **20**, 39-41.
6. O. Tzakou, C. Vagias, A. Gani and A. Yannitsaros, *Flavour Fragr J.*, 2005, **20**, 425-428.
7. K. Adande, K. Elo, O. Simalou, M.-F. Bakaï and P. Caboni, *Am. J. Anal. Chem.*, 2023, **14**, 95-120.
8. G. Soriano, D. Arnodo, M. Masi, M. Fernández-Aparicio, B. B. Landa, C. Olivares-García, A. Cimmino and C. Prandi, *J. Agric. Food. Chem.*, 2024, **72**, 4737-4746.
9. M. Asaoka, N. Yanagida, K. Ishibashi and H. Takei, *Tetrahedron Lett.*, 1981, **22**, 4269-4270.
10. A. Carpita, D. Neri and R. Rossi, *Gazz. Chim. Ital.*, 1987, **117**, 481-489.
11. D. Grandjean, P. Pale and J. Chucho, *Tetrahedron Lett.*, 1992, **33**, 5355-5358.
12. D. Grandjean, P. Pale and J. Chucho, *Tetrahedron*, 1993, **49**, 5225-5236.
13. S. Garrais, J. Turkington and W. P. D. Goldring, *Tetrahedron*, 2009, **65**, 8418-8427.
14. K. S. Madden, H. R. E. Jokhoo, F. D. Conradi, J. P. Knowles, C. W. Mullineaux and A. Whiting, *Org. Biomol. Chem.*, 2019, **17**, 3752-3759.
15. V. Fiandanese, D. Bottalico, G. Marchese and A. Punzi, *Tetrahedron*, 2004, **60**, 11421-11425.
16. V. Le Fouler, G. Duret, P. Bissere and N. Blanchard, *Tetrahedron Lett.*, 2018, **59**, 3349-3352.
17. M. Bourkhis, H. Gaspard, P. Rullière, D. K. C. de Almeida, D. Listunov, E. Joly, R. Abderrahim, M. C. de Mattos, M. C. F. de Oliveira, V. Maraval, R. Chauvin and Y. Génisson, *ChemMedChem*, 2018, **13**, 1124-1130.
18. M. Bossuat, P. Rullière, N. Preuilh, A. Peixoto, E. Joly, J.-G. Gomez, M. Bourkhis, F. Rodriguez, F. Gonçalves, I. Fabing, H. Gaspard, V. Bernardes-Génisson, V. Maraval, S. Ballereau, R. Chauvin, S. Britton and Y. Génisson, *J. Med. Chem.*, 2023, **66**, 13918-13945.
19. M. Kitora and E.-i. Negishi, *Synthesis*, 1997, **1997**, 121-128.
20. Y. Ogawa, M. Maruno and T. Wakamatsu, *Heterocycles*, 1995, **41**, 2587-2599.
21. R. Rossi, F. Bellina, A. Catanese, L. Mannina and D. Valensin, *Tetrahedron*, 2000, **56**, 479-487.
22. L. Anastasia, C. Xu and E.-i. Negishi, *Tetrahedron Lett.*, 2002, **43**, 5673-5676.
23. M. V. N. De Souza, *Mini-Rev. Org. Chem.*, 2005, **2**, 139-145.
24. X. Lu, X. Huang and S. Ma, *Tetrahedron Lett.*, 1993, **34**, 5963-5966.
25. M. Kitora and E.-i. Negishi, *Tetrahedron Lett.*, 1996, **37**, 9041-9042.
26. E.-i. Negishi, A. Alimardanov and C. Xu, *Org. Lett.*, 2000, **2**, 65-67.
27. F. Liu and E.-i. Negishi, *J. Org. Chem.*, 1997, **62**, 8591-8594.
28. V. Fiandanese, D. Bottalico and G. Marchese, *Tetrahedron*, 2001, **57**, 10213-10218.

29. D. Rambabu, S. Bhavani, K. S. Nalivela, S. Mukherjee, M. V. B. Rao and M. Pal, *Tetrahedron Lett.*, 2013, **54**, 2151-2155.
30. T. Zoller and D. Uguen, *Tetrahedron Lett.*, 1998, **39**, 6719-6720.
31. Q. Zhang, P. Prins, S. C. Jones, S. Barlow, T. Kondo, Z. An, L. D. A. Siebbeles and S. R. Marder, *Org. Lett.*, 2005, **7**, 5019-5022.
32. S. Inack-Ngi, R. Rahmani, L. Commeiras, G. Chouraqui, J. Thibonnet, A. Duchêne, M. Abarbri and J.-L. Parrain, *Adv. Synth. Catal.*, 2009, **351**, 779-788.
33. R. C. Ferreira, S. S. Duarte, V. M. de Sousa, R. R. M. de Souza, K. K. G. Marques, R. A. de Abrantes, Y. M. do Nascimento, N. F. de Sousa, M. T. Scotti, L. Scotti, J. F. Tavares, J. C. R. Gonçalves, M. S. da Silva and M. V. Sobral, *Pharmaceuticals*, 2023, **16**, 1553.
34. R. C. Ferreira, Y. M. do Nascimento, P. B. de Araújo Loureiro, R. X. Martins, M. E. de Souza Maia, D. F. Farias, J. F. Tavares, J. C. R. Gonçalves, M. S. da Silva and M. V. Sobral, *Biomolecules*, 2023, **13**, 1439.
35. P. Satyal, B. K. Chhetri, N. S. Dosoky, S. Shrestha, A. Poudel and W. N. Setzer, *Nat. Prod. Commun.*, 2015, **10**, 1934578X1501001028.
36. F. Ayaz, N. Küçükboyacı and B. Demirci, *J. Essent. Oil Res.*, 2017, **29**, 336-343.
37. V. Kumar, C. S. Mathela, G. Tewari, A. Panwar and V. Pandey, *Indian J. Nat. Prod. Resour.*, 2017, **8**, 63-68.
38. S. C. N. Queiroz, C. L. Cantrell, S. O. Duke, D. E. Wedge, V. K. Nandula, R. M. Moraes and A. L. Cerdeira, *J. Agric. Food. Chem.*, 2012, **60**, 5893-5898.
39. M. Fernández-Aparicio, G. Soriano, M. Masi, P. Carretero, S. Vilariño-Rodríguez and A. Cimmino, *Agriculture*, 2022, **12**, 790.
40. A. C. Peralta, G. Soriano, J. G. Zorrilla, M. Masi, A. Cimmino and M. Fernández-Aparicio, *Molecules*, 2022, **27**, 7421.
41. L. Rahalison, M. Benathan, M. Monod, E. Frenk, M. P. Gupta, P. N. Solis, N. Fuzzati and K. Hostettmann, *Planta Med.*, 1995, **61**, 360-362.
42. G. Vidari, S. Abdo, G. Gilardoni, A. Ciapessoni, M. Gusmeroli and G. Zanoni, *Fitoterapia*, 2006, **77**, 318-320.
43. R. S. Porto, S. Rath and S. C. N. Queiroz, *J. Braz. Chem. Soc.*, 2017, **28**, 913-919.
44. D. Terao, S. C. N. Queiroz and A. d. H. N. Maia, *Journal of Phytopathology*, 2022, **170**, 158-166.
45. T. S. Lu, C. L. Cantrell, S. L. Robbs, S. G. Franzblau and N. H. Fischer, *Planta Med.*, 1998, **64**, 665-667.
46. S. Chandra Pandey, D. S. Dhami, A. Jha, G. Chandra Shah, A. Kumar and M. Samant, *ACS Omega*, 2019, **4**, 14640-14649.
47. N. Togashi, A. Shiraishi, M. Nishizaka, K. Matsuoka, K. Endo, H. Hamashima and Y. Inoue, *Molecules*, 2007, **12**, 139-148.
48. K. Elo, M. Demurtas, A. Deplano, A. Ngoutane Mfopa, A. Murgia, A. Maxia, V. Onnis and P. Caboni, *J. Agric. Food. Chem.*, 2015, **63**, 9970-9976.
49. D. A. Konovalov, *Pharm. Chem. J.*, 2014, **48**, 613-631.
50. K. Elo, M. Demurtas, M. G. Mura, A. Deplano, V. Onnis, N. Sasanelli, A. Maxia and P. Caboni, *J. Agric. Food. Chem.*, 2016, **64**, 4876-4881.
51. R. S. Hussey and K. R. Barker, *Plant dis. rep.*, 1973, **57**, 1025-1028.
52. Coyne, D. L.; Nicol, J.M; Claudius-Cole. Les nématodes des plantes : Un guide pratique des techniques de terrain et de laboratoire. Secrétariat SP-IPM, Institut International d'Agriculture Tropicale (IITA), Cotonou, Benin 2010
53. P. Caboni, N. G. Ntalli, N. Aissani, I. Cavoski and A. Angioni, *J. Agric. Food. Chem.*, 2012, **60**, 1146-1151.
54. Püntener, W.; Zahner, O. Manual for Field Trials in Plant Protection, 2nd ed., rev.enl.; CIBA-GEIGY documenta; Ciba-Geigy: Basle, Switzerland, 1981.
55. S. S. Seefeldt, J. E. Jensen and E. P. Fuerst, *Weed Technol.*, 1995, **9**, 218-227.

