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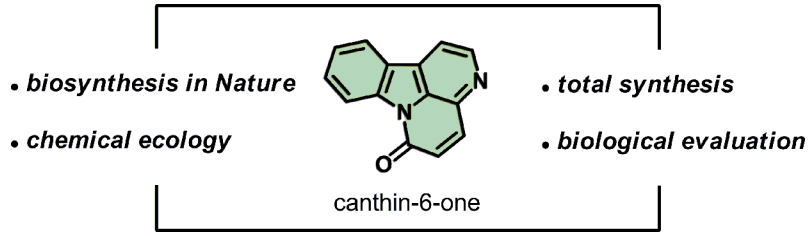

Canthin-6-one, a particular representative β-carboline alkaloid, was targeted for synthesis keeping in mind its biosynthetic origin. In fact, several biosynthetic intermediates were synthesized and nanoparticular mimicry of key steps was also achieved permitting further evaluation of biological properties for this class of alkaloids.

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Solution Phase and Nanoparticular Biosynthetically Inspired Interconnections in the Canthin-6-one β-Carboline Series and Study of Phenotypic Properties on *C. elegans*

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Solution Phase and Nanoparticular Biosynthetically Inspired Interconnections in the Canthin-6-one β-Carboline Series and Study of Phenotypic Properties on *C. elegans*

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Based on the biosynthetic line of canthin-6-one alkaloids from their simple precursors such as tryptamine, the present work is focused on the study of alternative protocol of the Bischler-Napieralski reaction and has led to a full coverage of the different biosynthetic intermediates. Nanoparticles were also prepared as mimics of biosynthetic assembly lines and some interesting biological results in term of chemical ecology are also reported.

Introduction

Canthinones are a subclass of β-carboline alkaloids that possess an additional D-ring. As a result, these compounds display a characteristic tetracyclic core.[1] Canthin-6-one (**1**) (Figure 1) is the lead compound of this family, and was discovered in 1952 by Haynes from *Pentaceras australis* (Rutaceae). The compound was subsequently isolated from many other species.[2] Since then, several dozen derivatives have been found in natural sources, mainly plants of the Rutaceae and Simaroubaceae families, but also from the Malvaceae, Amaranthaceae, Caryophyllaceae, and Zygophyllaceae families.[3] More recently, these natural products have also been found in fungi (*Boletus curtisii* Berk).[4] Centered on the canthin-6-one scaffold, a range of diverse functionalities are encountered in nature characterized principally by oxidation patterns exemplified by **2**, one the most highly oxygenated analogs isolated to date. Diversity is nevertheless moderate as shown by thiomethylated analog **3** and quassidine E (**4**), the most complex canthin-6-one-type alkaloid disclosed to date. Noteworthy is the fact that members of the canthin-6-one class display a broad range of pharmacological activities including antifungal, antiprotozoal, antimalarial and antitumorigenic activities. These traits of the canthin-6-one class have stimulated the synthesis of a large number of analogs.[1b,5–11]

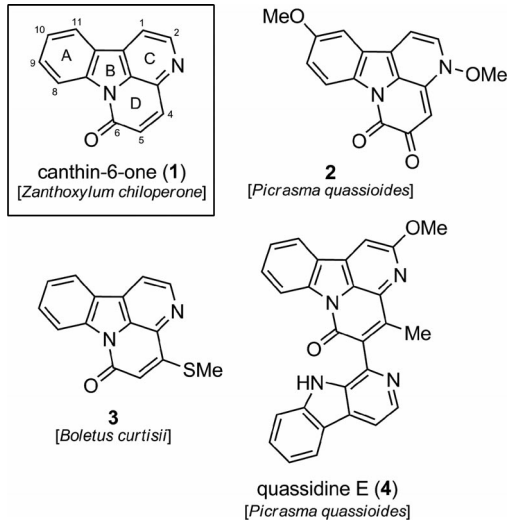

Figure 1. Representative examples of canthin-6-one-type alkaloids.

The biosynthesis of canthinone-type alkaloids was, in part, demonstrated by the Guicciardi group by feeding experiments using cell cultures of *Ailanthus altissima* (Simaroubaceae).[12] A general biosynthetic pathway starting from tryptophan (**5**) is presented in Scheme 1. It features intermediates that were characterized by the authors as products of incorporation of [methylene-^{14}C]-tryptophan (**5**) (see also Scheme 1) and also integrates plausible intermediates.[13] Following the decarboxylation of tryptophan (**5**) into tryptamine (**6**) a first intermediate may plausibly be dihydro-β-carboline-1-propionic acid (**7**) with the non-

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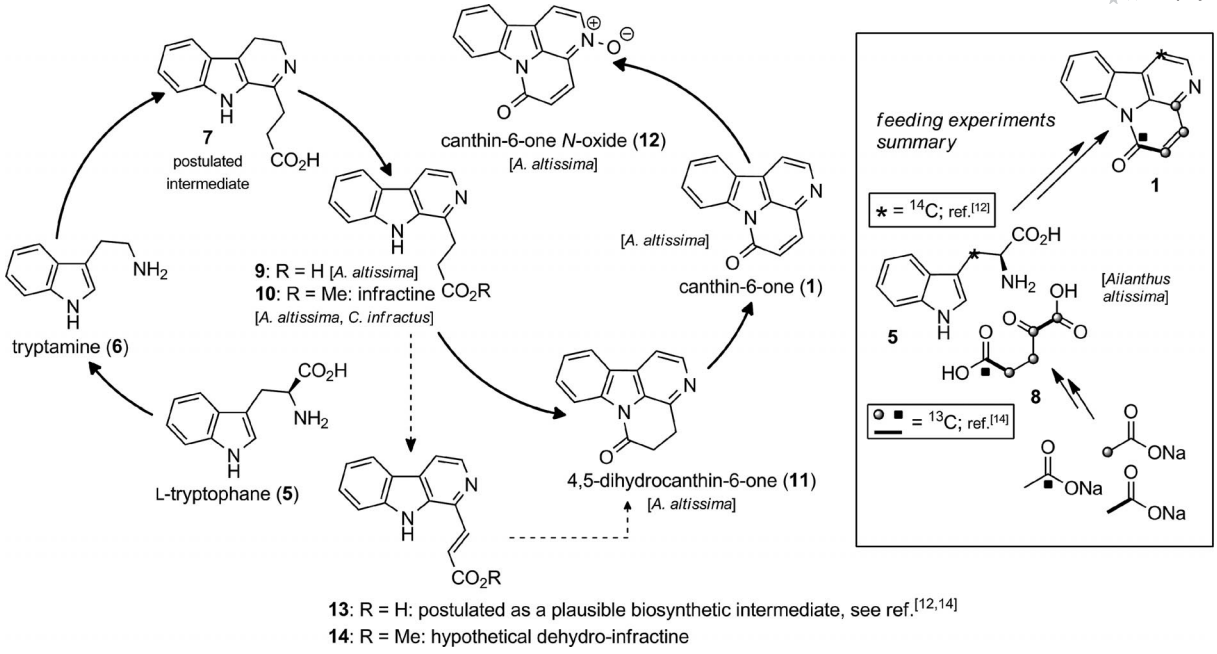


Scheme 1. Biosynthetic machinery leading to canthin-6-one alkaloids and supporting feeding experiments.

tryptaminic carbons originating from ketoglutarate (**8**, Scheme 1). This latter issue was indeed supported by a study from the Arragozzini group a few years later with the incorporation of [^{13}C]-acetate (i.e. [1-^{13}C]-, [2-^{13}C]- and [1,2-^{13}C]-acetate) into canthin-6-one (**1**) (the combined incorporation patterns are presented in Scheme 1).[14] Proceeding through the synthesis, a suitable oxidation may lead to β-carboline-1-propionic acid **9**, (isolated in the course of the feeding experiments) and also known as infractine (**10**) when isolated as a methyl ester; this compound was first isolated from *Cortinarius infractus* and also from *Picrasma quassioides*.[15,16] Tricyclic intermediate **9** could then be transformed into 4,5-dihydrocanthin-6-one (**11**) as the first representative of the canthinone family and which was also characterized from the feeding experiment cultures. Finally, oxidation may occur to yield canthin-6-one (**1**) which in turn may be the substrate for several functionalizations observed in nature as exemplified by the formation of canthin-6-one *N*-oxide (**12**) and as evoked earlier in the introduction (Figure 1). Intermediate **13** was also postulated by Guicciardi et al. as a possible precursor of **1** but was not isolated in the course of feeding experiments. Conversely, corresponding methyl ester **14** could be considered to be hyptothetical dehydroinfractine.[14c,17]

As part of our recent interest in the chemistry of canthin-6-one (**1**) and our interest in the biomimetic synthesis of indole alkaloids, we designed a straightforward pathway to **1** based on the Bischler–Napieralski reaction. In the present publication is reported an in-depth investigation of the experimental conditions of this venerable reaction that led to a general Scheme of selective and efficient transformations tracing all intermediates of the canthin-6-one biosynthetic route including infractine (**10**) and dehydroinfractine (**14**). Chemically speaking, infractine (**10**) synthesis has not been extensively studied. Bracher and Hildebrand published in 1995 the first chemical synthesis of **10**.[18] In their work, they reported the synthesis of the hypothetical dehydroinfractine (**14**) (see Scheme 1) which was then reduced to infractine (**10**). Another synthesis of infractine (**10**) has been reported by Nowak employing the Pictet–Spengler reaction.[19] Also described herein are original results dedicated to nanoparticular mimicry of key biosynthetic steps in the canthinone series,[20a] following a bio-inspired approach.[20b,20c] Finally, we have also performed bioactivity tests of the prepared compounds in the genetically tractable metazoan *Caenorhabditis elegans*.

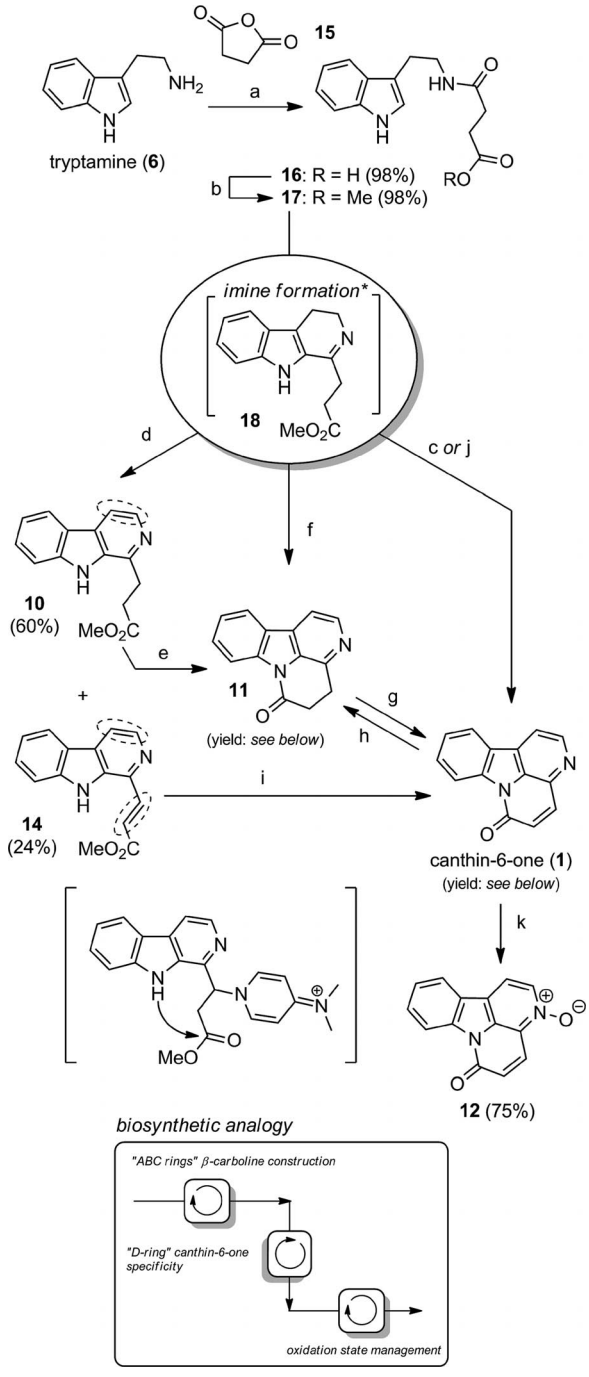
Results and Discussion

Conversion of Tryptamides to Canthinone Skeletons

We recently disclosed a straightforward synthesis of canthin-6-one (**1**) starting from tryptamine (**6**) and exploiting the century old Bischler–Napieralski reaction.[6] In this previous work, tryptamine was first exposed to succinic anhydride (**15**) in CH_2Cl_2 at room temperature, which produced the corresponding amide (**16**). Esterification of tryptamide **16** with Amberlyst H-15® as a catalyst in MeOH produced methyl ester **17** in almost quantitative yield [conditions (a) and (b) in Scheme 2]. The treatment of tryptamide **17** under classical Bischler–Napieralski conditions ($POCl_3$ in benzene) gave previously isolated and characterized[6] but unstable imine **18** (the methyl ester analogue of biosynthetic intermediate **7**, 60% yield after isolation), which was then treated with a strong hindered base such as diazabicycloundecene (DBU) in the presence of air to pleasingly furnish

canthin-6-one (**1**) in a satisfactory overall yield. An efficient protocol was then optimized and consisted of direct treatment of the crude mixture of **18** with DBU. This 3-step strategy from tryptamine also permitted the preparation of several analogues. In the present work, with these optimized conditions in hand, we have turned our attention to circumventing the use of benzene in the Bischler–Napieralski reaction. Although the use of toluene did not give satisfactory yields, we found that the reaction could be performed directly in excess $POCl_3$ (used as the solvent) followed by a DBU-induced final cyclization/spontaneous oxidation cascade [conditions (c) in Scheme 2]. Furthermore, we also envisioned alternative conditions for the Bischler–Napieralski reaction.

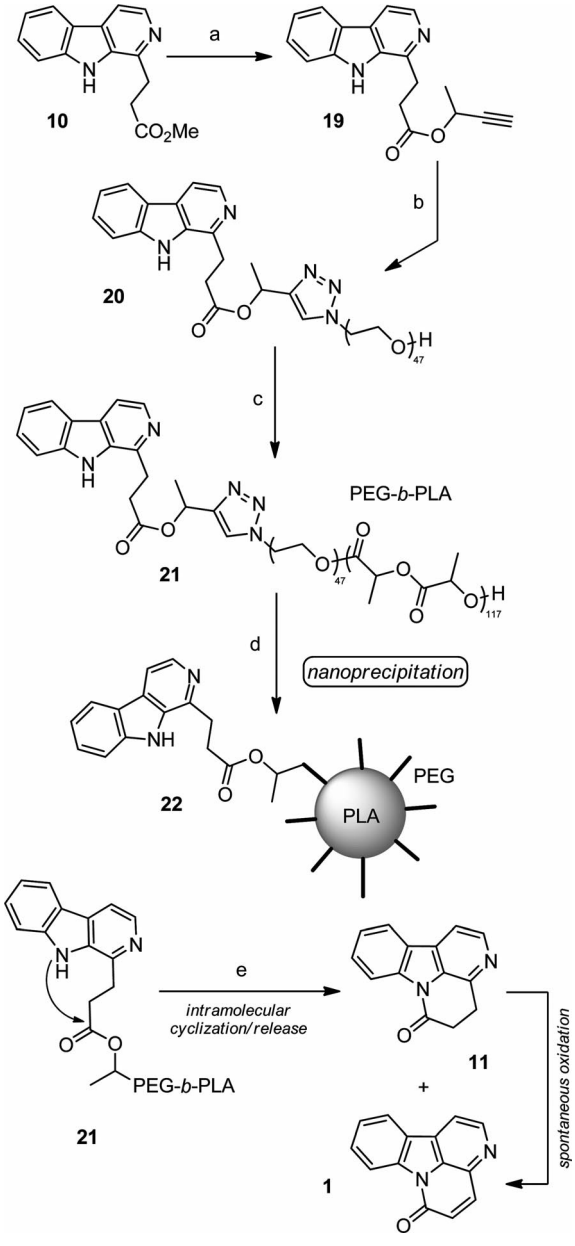
We were particularly interested by conditions recently described by Movassaghi using trifluoromethanesulfonic anhydride (Tf_2O) and 2-chloropyridine (2-ClPyr) as a base additive.[21] We applied such conditions to tryptamide **16** (CH_2Cl_2, –78 °C to room temp., 1.5 equiv. of both Tf_2O and 2-ClPyr) which yielded a mixture of infractine (**10**) (60%) and (*E*)-dehydro-infractine (**14**, 24%) that were separated on a silica gel column. Interestingly, total aromatization of the β-carboline (i.e. oxidation of C-1/C-2 bond of intermediate **18**) was observed, probably due to the excess Tf_2O, as also pointed out by Movassaghi.[22] Partial oxidation of the lateral chain also occurred spontaneously explaining formation of **14** as the *E* isomer[17] (i.e. oxidation of C-4/C-5 bond) as a side product. This oxidation is consistent with oxidation of the C-4/C-5 bond within the canthinone scaffold observed in the previous synthesis described by our group.[6,11b] We then turned our attention to the formation of the additional D-ring of canthin-6-one in different conditions. Dihydrocanthin-6-one (**11**) could be obtained when the aforementioned crude mixture of **10** and **14** was submitted to DBU (3 equiv. in CH_2Cl_2), unchanged amounts of **14** were, of course, also isolated. Dihydrocanthin-6-one (**11**) was oxidized into canthin-6-one (**1**) with DDQ (50% yield) and conversely, **1** could be easily reduced to **11** under catalytic hydrogenation conditions. In the presence of dimethylamino-pyridine (DMAP), dehydro-infractine (**14**) was converted to canthin-6-one (**1**) possibly through the intermediate represented in parentheses in Scheme 2. A good overall yield of canthin-6-one (**1**) (73% starting from 250 mg of **17**) was observed when tryptamide **17** was treated with an excess of cyclodehydrating agents (4 equiv.) followed by treatment of the crude mixture of **10** and **14** with DMAP and air bubbling providing us a good alternative to our previous protocol (reaction conditions j in Scheme 2). A drop in the global yield of canthin-6-one was observed (42%) when carried out on gram scale but could be considered satisfactory in view of the succession of reactions that is performed without recovering any intermediate. The final compound, in terms of both biosynthesis stream and oxidation state, namely canthin-6-one *N*-oxide (**12**), was also prepared by oxidation with *m*-chloroperbenzoic acid (*m*CPBA) in good yield thus finalizing a complete set of reactions relating tryptamide **17**, β-carboline **10**, **14**, **18** and canthinones **1**, **11** and **12**.



Scheme 2. Chemical mapping of canthin-6-one-type alkaloids from tryptamine. Reagent and conditions: (a) Succinic anhydride (1.1 equiv.), CH_2Cl_2, 18 h (98%); (b) Amberlyst H^+ (20% *m*/*m*), MeOH, reflux, 18 h (98%); (c) $POCl_3$ (3 equiv.), C_6H_6, reflux, 16 h then DBU (3 equiv.), CH_2Cl_2, room temp. (see ref 6) or $POCl_3$ (excess), reflux; then DBU (3 equiv.), CH_2Cl_2, room temp.; (d) Tf_2O (1.5 equiv.), 2-ClPyr (1.5 equiv.), CH_2Cl_2, –78 °C, 3 h (**10**: 60% + **14**: 24%); (e) DBU (3 equiv.), CH_2Cl_2, room temp., 18 h; (f) Tf_2O (1.5 equiv.), 2-ClPyr (1.5 equiv.), CH_2Cl_2, –78 °C, 3 h, then DBU (3 equiv.), CH_2Cl_2, room temp., 18 h (61%); (g) DDQ, toluene, room temp., 24 h (55%); (h) H_2, Pd/C (5%), EtOAc, 5 h (90%); (i) DMAP (3 equiv.), CH_2Cl_2, room temp., 24 h; (j) Tf_2O (4 equiv.) 2-ClPyr (4 equiv.), CH_2Cl_2, –78 °C, then DMAP (3 equiv.), air, room temp., 24 h (73%); (k) *m*CPBA (3 equiv.), CH_2Cl_2, room temp., 18 h (75%). *Imine formation was found to occur under conditions c, d, f and j.

Infractine-Functionalized Nanoparticles (10) and Nanoparticle-Supported Biomimetic Synthesis of Canthin-6-one (1)

Our interest in biomimetic chemistry, especially in the field of natural products total synthesis, usually inspires us to closely dissect the intimate biochemical mechanisms of biosynthetic pathways. On the other hand, rapid advancements in nanotechnology also prompted us to design a mimic system that could recreate biosynthetic assembly lines at the surface of nanoparticles.[20b,20c] Our strategy relied on: (i) the covalent linkage of infractine (**10**) by "*click*" chemistry to poly(ethylene glycol); (ii) the use of **10**-PEG-OH as a macroinitiator for ring opening polymerization (ROP) of lactide to form **10**-PEG-*b*-PLA copolymer; (iii) the formation of **10**-PEG-*b*-PLA nanoparticles from self-assembly of **10**-PEG-*b*-PLA in aqueous solution and (iv) the study of a potential biomimetic release of canthin-6-one (**1**) from the nanoparticles. This would involve traceless cyclization/release of dehydrocanthinone followed by spontaneous 4,5-oxidation as a first proof of concept. Infractine (**10**) was first *trans*-esterified with 3-butyn-2-ol in the presence of ytterbium trifluoromethanesulfonate [Yb(OTf_3)] to afford ester **19** (Scheme 3). The copper-catalyzed azide–alkyne cycloaddition (CuAAC) was then selected as the means to functionalize an azidopoly(ethylene glycol) (N_3-PEG-OH).[23] The resulting **10**-PEG-OH (**20**) was characterized by NMR, UV absorbance and size exclusion chromatography (SEC). The analysis of the UV spectrum of **20** showed three peaks (246, 289 and 337 nm) (see Supporting Information). Therefore, for the SEC analysis, the wavelength at 337 nm was chosen to follow the polymer-infractine conjugate so that no interference with the UV signal of toluene could be observed (Figure 2). The chromatogram showed a perfect match between the UV signal of compound **21** and the refractive index signal of the polymer conjugate, thus confirming the grafting of **10**.



Scheme 3. **10**-Functionalized nanoparticles and biomimetic release of **1** and **11**. (a) Yb(Otf)$_3$ (20 mol-%), CH_2Cl_2, reflux, 16 h (27%); (b) 3-butyn-2-ol (excess), N_3-PEG-OH (1 equiv.), CuBr (1 equiv.), PMDTA*, DMF, room temp., 24 h (46%); (c) *d,l*-lactide (excess), Sn(Oct)$_2$ (0.4 equiv.), toluene, 115 °C, 16 h (57%); (d) Self-assembly: AcOEt/H_2O (1:3); (e) DBU (excess), CH_2Cl_2, air, 72 h (identification of **11** and **1** by MS and HPLC-UV studies). *PMDTA = *N*,*N*,*N*′,*N*′′,*N*′′-pentamethyldiethylenetriamine.

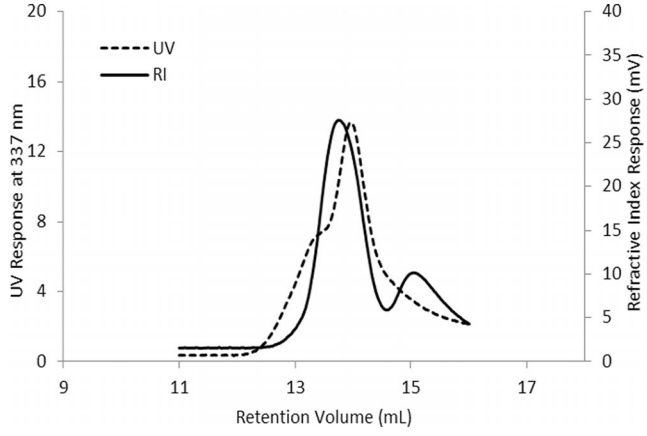


Figure 2. SEC analysis of copolymer **21** with UV (337 nm) and DRI detection. The UV signal is overlaid with the DRI curve, showing thereby that **10** is covalently linked to the PLA-*b*-PEG copolymer.

The NMR analysis of **20** revealed the presence of the characteristic peak of the triazole hydrogen at δ = 7.81 ppm. Moreover, the integration of protons assigned to alkaloid **10** permitted us to calculate a coupling yield of approximately 46%. Compound **20** was then used as a macroinitiator for the ROP of *d,l*-lactide in the presence of Sn(Oct)$_2$ in toluene to afford PLA-*b*-PEG-**10** functional amphiphilic copolymer (**21**). The latter was analyzed by ^{1}H NMR (M_n = 19200 g mol^{-1}), SEC with differential refractive index (DRI) and UV detections at 337 nm (Figure 2). Self-assembly allowed the formation of PLA-*b*-PEG-**10** nanoparticles (**22**) in an AcOEt/H_2O emulsion. Nanoparticles showed a mean

average diameter of 115 nm, a narrow particle size distribution (polydispersity = 0.139), and UV analysis clearly showed a covalent association of **22** (see Supporting Information). As mentioned above, a canthinone release test was performed; **21** was treated with DBU in CH_2Cl_2 with air bubbling, HPLC and MS analysis of the crude mixture demonstrated the release of dehydrocanthin-6-one (**11**) and the spontaneous oxidation of **11** into canthin-6-one (**1**), the last intermediates in the biosynthetic assembly line (see Supporting Information).

Biological Activities on *C. elegans*

The nematode *Caenorhabditis elegans* is probably one of the most popular metazoan model organisms for genetic and phenotypic studies.[24,25] This nematode has been, for example, used before as a model to assay chemicals with antiparasitic properties.[26,27] Two types of tests were performed on wild-type animals: i) coordination abilities of the organisms were evaluated in a performance test that may uncover defects of the nervous system and/or body wall striated muscles (graph A, Figure 3), and ii) growth test as xenobiotic may impair the normal growth of the worms (graph B, Figure 3). To evaluate coordination abilities the number of trashes (body bents) of swimming worms in a biological buffer were counted when incubated with the different compounds prepared and also with albendazole as a positive control. Albendazole is an antiparasitic agent known to interact with tubulin in the cell and to cause a loss of mobility.[28,29] All compounds displayed mild antimobility activity although compounds **11** and **12** reduced the mobility of worms by 50% and 25%, respectively relative to controls. In a similar way, all compounds delayed phenotypic growth. For example, when subjected to compound **1**, 60 animals showed an arrested development in the first larval stage, suggesting that **1** is a fairly strong inhibitor of nematode growth. Ultimately, the fraction of animals that reached adulthood was delayed of approximately 10 h (90 h vs. 80 h for 40% of the animals).

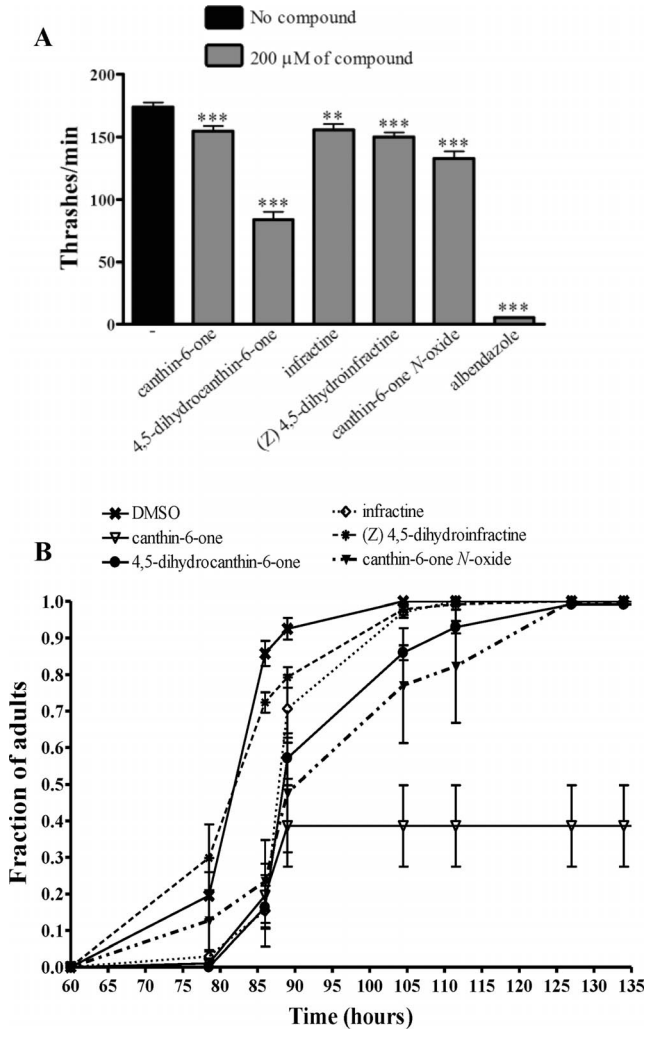


Figure 3. Phenotypic properties of compounds **1**, **10**–**12**, **14** on *C. elegans*. **A**: thrashing assays performed in wild-type animals grown on DMSO (negative control) or the different compounds, albendazole (a strong well-characterised nematicide) was used as a positive control. ***p value* ⩽ *0.001;* ****p value* ⩽ *0.0001*; **B**: growth assays performed on wild-type animals grown in the presence of each of the compounds described in this work, and DMSO as a negative control.

Conclusions

A study of Movassaghi's alternative conditions for the Bischler–Napieralski reaction permitted us to formulate a general strategy for the synthesis of all intermediates in the canthin-6-one biosynthetic pathway and their chemical interconversions. High fidelity to what is known from the natural system, especially through previous feeding experiments, was achieved using our new approach. Furthermore, the first results with a nanoparticular mimic system of the relevant biosynthetic assembly lines have been disclosed and merit further developments. Finally, biological evaluations using *C. elegans* revealed new findings that may contribute to a better understanding of the role of canthin-6-one alkaloids in chemical ecology and may provide new insight into their natural "*raison d'être*" in addition to previously reported biological activities such as their strong antifungal activities.

Experimental Section

General Experimental Procedures: Reactions were monitored by thin-layer chromatography carried out on silica gel plates (Merck TLC Silicagel 60_{F254}) using UV light as visualizing agent and sulfuric vanillin and heat and Dragendorff reagent and heat as developing agents. Merck Silicagel Geduran® Si 60 (particle size 40–63 µm) was used for flash chromatography. NMR spectra were recorded in deuterated solvent with AM-300 (300 MHz) or AM-400 (400 MHz) Bruker spectrometers and calibrated using nondeuterated solvent as an internal reference. The following abbreviations are used to explain the multiplicities: s = singlet; d = doublet, t = triplet; q = quartet; m = multiplet; br. = broad (see Supporting Information for molecule numbering). IR spectra were recorded using a Vector 22 Bruker spectrometer and values are reported in cm^{-1} units. Mass spectra were recorded at the Service d'Analyse des Médicaments et des Métabolites (Université Paris-Sud).

Tryptamide (16): Tryptamine **6** (10 mmol, 1.6 g), was dissolved in dry CH_2Cl_2 (20 mL). Succinic anhydride (1.1 equiv., 11 mmol, 1.1 g) was added slowly. The reaction was stirred at room temp. for 18 h. The resulting precipitate was filtered, washed with a small amount of CH_2Cl_2 and dried to furnish tryptamide (**16**) (2.5 g, 98%), white crystalline powder; R_f = 0.3 (CH_2Cl_2/MeOH, 9:1); m.p. 128–130 °C (CH_2Cl_2). IR (film, CH_2Cl_2): $\tilde{\nu}_{max}$ = 3394, 3271, 2939, 1686, 1638, 1226, 1172, 833, 739, 669, 623 cm^{-1}. ^1H NMR (400 MHz, CD_3OD, 25 °C): δ = 7.54 (d, J = 8 Hz, 1 H, 4-H), 7.31 (d, J = 8 Hz, 1 H, 7-H), 7.10–7.07 (m, 2 H, 2-H, 6-H), 7.0–6.99 (dt, J = 8.0, 0.9 Hz, 1 H, 5-H), 3.45 (t, J = 7.3 Hz, 2 H, 9-H), 2.91 (t, J = 7.3 Hz, 2 H, 8-H), 2.56 (t, J = 6.9 Hz, 2 H, 13-H), 2.42 (t, J = 6.9 Hz, 2 H, 12-H) ppm. ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 174.2 (14-C), 171.1 (11-C), 136.6 (b-C), 127.6 (a-C), 122.9 (2-C), 121.2 (6-C), 118.8 (5-C), 118.5 (4-C), 112.2 (3-C), 111.7 (7-C), 40.8 (9-C), 30.5 (12-C), 29.6 (13-C), 25.5 (8-C) ppm. ESIMS m/z 283 $[M + Na]^+$. HRESIMS m/z 283.1053 (calcd. for $C_{14}H_{16}N_2O_3Na$, 283.1059).

Methyl Ester 17: Tryptamide **16** (7.6 mmol, 2 g), was dissolved in MeOH (60 mL). Catalytic Amberlyst H-15® (20% w/w, 200 mg) was added and the solution was heated under reflux for 18 h. The solution was then filtered and the solvent was removed under reduced pressure. The crude product was crystallized from diisopropyl ether to furnish **17** (2.0 g, 98%), white crystalline powder; m.p. 101–102 °C (CH_2Cl_2); R_f = 0.6 (CH_2Cl_2/MeOH, 9:1). IR (film, CH_2Cl_2): $\tilde{\nu}_{max}$ = 3336, 3282, 2862, 1721, 1651, 1538, 1258, 1221, 1188, 936, 800 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.30 (br., 1 H, -NH), 7.58 (d, J = 7.8 Hz, 1 H, 4-H), 7.35 (d, J = 7.8 Hz, 1 H, 7-H), 7.18 (t, J = 7.8 Hz, 1 H, 6-H), 7.10 (t, J = 7.8 Hz, 1 H, 5-H), 7.04 (s, 1 H, 2-H), 5.30 (s, 1 H, -NH), 3.64 (s, 3 H, -OMe), 3.57 (t, J = 6.5 Hz, 2 H, 9-H), 2.95 (t, J = 6.5 Hz, 2 H, 8-H), 2.63 (t, J = 6.8 Hz, 2 H, 13-H), 2.39 (t, J = 6.8 Hz, 2 H, 12-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 173.4 (14-C), 172.7 (11-C), 136.6 (b-C), 127.3 (a-C), 122.1 (2-C), 121.8 (6-C), 120.8 (5-C), 117.8 (4-C), 111.7 (3-C), 110.7 (7-C), 57.0 (-OMe), 40.0 (9-C), 29.9 (12-C), 28.7 (13-C), 24.7 (8-C) ppm. ESIMS m/z 297 $[M + Na]^+$. HRESIMS m/z 297.1216 (calcd. for $C_{15}H_{18}N_2O_3Na$, 297.1215).

Infractine (10) and 4,5-Dehydroinfractine (14): Trifluoromethanesulfonic anhydride (1.5 equiv., 1.37 mmol, 0.23 mL) was added over 1 min to a stirred mixture of **17** (0.91 mmol, 250 mg) and 2-chloropyridine (1.5 equiv., 1.37 mmol, 0.13 mL) in CH_2Cl_2 (10 mL) at –78 °C. After 5 min, the reaction mixture was brought to 0 °C for 5 min and then to room temp. After 3 h, the reaction was quenched with a saturated $NaHCO_3$ solution (10 mL), and the aqueous fraction was washed successively with dichloromethane (2× 30 mL), dried with Na_2SO_4 and concentrated under reduced pressure. The crude reaction mixture was either used in the next reaction without further purification or purified by flash chromatography (cyclohexane/EtOAc, 6:4) to furnish **10** (142 mg, 60%) and **14** (57 mg, 24%). **10**: brown oil; R_f = 0.6 (cyclohexane/EtOAc, 7:3). IR (film, CH_2Cl_2): $\tilde{\nu}_{max}$ = 2850, 1698, 1632, 1182, 1040, 748 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.25 (br., 1 H, -NH), 8.4 (d, J = 5.2 Hz, 1 H, 2-H), 8.13 (d, J = 8.0 Hz, 1 H, 11-H), 7.85 (d, J = 5.2 Hz, 1 H, 1-H), 7.57 (m, 2 H, 8-H, 9-H), 7.30 (m, 1 H, 10-H), 3.68 (s, 3 H, -OMe), 3.47 (t, J = 6.8 Hz, 2 H, 4-H), 3.03 (t, J = 6.4 Hz, 2 H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 174.98 (6-C), 143.93 (3b-C), 140.50 (7a-C), 138.76 (2-C), 134.63 (3a-C), 129.02 (11b-C), 128.29 (8-C), 122.01 (11a-C), 121.72 (11-C), 119.99 (10-C), 113.25 (1-C), 111.85 (9-C), 52.0 (-OMe), 32.70 (5-C), 28.85 (4-C) ppm. ESIMS m/z 255 $[M + H]^+$. HRESIMS m/z 255.1123 (calcd. for $C_{15}H_{15}N_2O_2Na$, 255.1123). **14**: brown oil; R_f = 0.7 (cyclohexane/EtOAc, 7:3). IR (film, CH_2Cl_2): $\tilde{\nu}_{max}$ = 2850, 1698, 1632, 1182, 1040, 748 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.98 (br., 1 H, -NH), 8.51 (d, J = 5.2 Hz, 1 H, 2-H), 8.17 (d, J = 15.2 Hz, 1 H, 4-H), 8.11 (d, J = 8.0 Hz, 1 H, 11-H), 7.96 (d, J = 4.8 Hz, 1 H, 1-H), 7.56 (m, 2 H, 9-H, 8-H), 7.32 (t, J = 7.6 Hz, 1 H, 10-H), 7.18 (d, J = 15.2 Hz, 1 H, 5-H), 3.84 (s, 3 H, -OMe) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 167.76 (6-C), 140.64 (7a-C), 139.75 (2-C), 138.77 (4-C), 136.24 (3b-C), 135.39 (3a-C), 130.86 (11b-C), 129.30 (8-C), 122.02 (11-C), 121.78 (5-C), 121.62 (11a-C), 120.86 (10-C), 116.06 (1-C), 111.90 (9-C), 52.14 (-OMe) ppm. ESIMS m/z 253 $[M + H]^+$. HRESIMS m/z 253.0976 (calcd. for $C_{15}H_{13}N_2O_2$, 253.0972).

4,5-Dihydrocanthin-6-one (11): The preceeding crude mixture was dissolved in CH_2Cl_2 (30 mL) and DBU (3 equiv., 2.73 mmol, 0.4 mL) was added, the solution was then stirred at room temp. overnight. The reaction was quenched with a saturated $NaHCO_3$ solution (10 mL), and the aqueous fraction was washed successively with CH_2Cl_2 (2× 30 mL), dried with Na_2SO_4 and concentrated under reduced pressure. The mixture was purified by flash chromatography (cyclohexane/EtOAc, 6:4) to furnish **11** (126 mg, 61%). Alternatively, to a solution of **1** (0.09 mmol, 20 mg) in EtOAc (2 mL) was added 5% Pd/C (1 mg). The reaction mixture was stirred under an atmosphere of H_2. An additional amount of 5% Pd/C (1 mg) was added to the reaction mixture every hour until completion of the reaction as determined by TLC; 4 additions were needed). The reaction mixture was filtered thought a plug of silica gel and concentrated under reduced pressure. The crude material was column chromatographed (cyclohexane/EtOAc, 6:4) to yield **11** (18 mg, 90%), pale yellow crystalline powder; m.p. 161–163 °C (CH_2Cl_2); R_f = 0.4 (Cyclohexane/EtOAc, 7:3). IR (film, CH_2Cl_2): $\tilde{\nu}_{max}$ = 1673, 1434, 1141, 841, 793, 746 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.52 (m, 2 H, 8-H, 2-H), 8.05 (d, J = 7.6 Hz, 1 H, 11-H), 7.73 (d, J = 5.6 Hz,1 H, 1-H), 7.66 (t, J = 8.4 Hz, 1 H, 9-H), 7.48 (t, J = 7.6 Hz, 1 H, 10-H), 3.49 (t, J = 7.6 Hz, 2 H, 4-H), 3.22 (t, J = 7.6 Hz, 2 H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 166.85 (6-C), 144.03 (2-C), 142.74 (3b-C), 137.89 (7a-C), 135.41 (11b-C), 130.25 (9-C), 129.67 (3a-C), 124.707 (10-C), 124.51 (11a-C), 122.17 (11-C), 116.81 (8-C), 113.40 (1-C), 33.33 (5-C), 27.65 (4-C) ppm. ESIMS m/z 223 $[M + H]^+$. HRESIMS m/z 223.0869 (calcd. for $C_{14}H_{11}N_2O$, 223.0866).

Canthin-6-one (1): Trifluoromethanesulfonic anhydride (4 equiv., 3.64 mmol, 0.62 mL) was added over 1 min to a stirred mixture of **17** (0.91 mmol, 250 mg) and of 2-chloropyridine (4 equiv., 3.64 mmol, 0.34 mL) in CH_2Cl_2 (10 mL) at –78 °C with air bubbling in the reaction vessel. After 5 min, the reaction mixture was brought to 0 °C for 5 min and then to room temp. After 5 h, the reaction was diluted with an aqueous saturated solution of $NaHCO_3$ (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (2× 30 mL), then dried with Na_2SO_4 and concentrated under reduced pressure. The crude reaction was dissolved in toluene (30 mL) and DMAP (3 equiv., 2.73 mmol, 333.5 mg) was added. The solution was then stirred with air bubbling, at room temp. for 1 d. The reaction was diluted with an aqueous saturated solution of $NaHCO_3$ (10 mL), the aqueous layer was extracted with CH_2Cl_2 (2× 30 mL), dried with Na_2SO_4 and concentrated under reduced pressure. The crude mixture was then purified by flash chromatography (cyclohexane/EtOAc, 7:3) to furnish **1** (150 mg, 73%). Alternatively, to a solution of **11** (0.09 mmol, 20 mg) in toluene (2 mL) DDQ was added (0.09 mmol, 20.5 mg). The reaction mixture was stirred 1 d at room temp., filtered through a plug of silica gel and purified by flash chromatography (cyclohexane/EtOAc, 7:3) to furnish **1** (11 mg, 55%), white crystalline powder; m.p. 161–163 °C (CH_2Cl_2); R_f = 0.6 (CH_2Cl_2/MeOH, 9:1). IR (film, CH_2Cl_2): $\tilde{\nu}_{max}$ = 1673, 1434, 1141, 841, 793, 746 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.78 (d, J = 5.0 Hz, 1 H, 2-H), 8.63

(d, J = 8.0 Hz, 1 H, 8-H), 8.05 (d, J = 8.0 Hz, 1 H, 11-H), 8.0 (d, J = 9.8 Hz, 1 H, 4-H), 7.91 (d, J = 5.0 Hz, 1 H, 1-H), 7.72 (t, J = 8.0 Hz, 1 H, 9-H), 7.50 (t, J = 8.0 Hz, 1 H, 10-H), 6.95 (d, J = 9.8 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 159.32 (6-C), 145.69 (2-C), 139.45 (4-C), 139.06 (7a-C), 136.09 (3a-C), 132.25 (3b-C), 130.69 (9-C), 130.09 (11b-C), 128.77 (5-C), 125.48 (10-C), 124.23 (11a-C), 122.47 (11-C), 117.12 (8-C), 116.18 (1-C) ppm. ESIMS m/z 243 $[M + Na]^+$.

Canthin-6-one *N*-Oxide (12): To a solution of **1** (50 mg, 0.2 mmol), in CH_2Cl_2 (35 mL), and the system was cooled to 5 °C. *m*CPBA (3 equiv., 0.6 mmol, 103 mg) was added, and the solution was then stirred at room temp. for 18 h. The reaction was quenched with H_2O, and organic fractions were washed successively with an aqueous saturated solution of $NaHCO_3$ (5× 40 mL) and then a saturated NaCl solution (2× 30 mL). The organic fraction was dried with Na_2SO_4, solvent was removed under reduced pressure and the crude mixture was purified by flash chromatography (CH_2Cl_2/MeOH, 99:1) to furnish **12** (40 mg, 75%), yellow powder; m.p. 243–245 °C (CH_2Cl_2/MeOH); R_f = 0.2 (CH_2Cl_2/MeOH, 9:1). IR (film, CH_2Cl_2): $\tilde{\nu}_{max}$ = 1677, 1432, 1329, 1299, 1140, 826, 779, 749 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.65 (d, J = 8.0 Hz, 1 H, 8-H), 8.38 (d, J = 10.0 Hz,1 H, 4-H), 8.34 (d, J = 6.6 Hz, 1 H, 2-H), 7.99 (d, J = 8.0 Hz, 1 H, 11-H), 7.82 (d, J = 6.6 Hz,1 H, 1-H), 7.64 (t, J = 8.0 Hz, 1 H, 9-H), 7.53 (t, J = 8.0 Hz, 1 H, 10-H), 6.92 (d, J = 10.0 Hz, 1 H, 5-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 174.98 (6-C), 140.0 (7a-C), 134.0 (3b-C), 133.0 (4-C), 132.1 (11b-C), 130.0 (9-C), 129.6 (10-C), 129.1 (3a-C), 128.4 (2-C), 126.3 (5-C), 123.7 (11a-C), 121.8 (11-C), 117.7 (8-C), 117.4 (1-C) ppm. ESIMS m/z 237 $[M + H]^+$.

Infractine Derivative 19: 3-butyn-2-ol (20 equiv., 3.9 mmol, 0.31 mL) was added over 1 min to a stirred mixture of **10** (0.19 mmol, 50 mg) and ytterbium trifluoromethanesulfonate (20 mol-%, 0.78 mmol, 483.79 mg) at 80 °C overnight. The cooled solution was then diluted with a saturated aqueous solution of $NaHCO_3$ (10 mL) and the aqueous phase extracted with CH_2Cl_2 (2× 30 mL), the combined organic layers were dried with Na_2SO_4 and concentrated under reduced pressure. The crude mixture was then purified by flash chromatography (cyclohexane/EtOAc, 1:1) to furnish **19** (15 mg, 27%), orange crystalline powder; m.p. 161–163 °C (CH_2Cl_2); R_f = 0.3 (CH_2Cl_2/MeOH, 9:1). IR (film, CH_2Cl_2): $\tilde{\nu}_{max}$ = 1673, 1434, 1141, 841, 793, 746 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.93 (br., 1 H, -NH); 8.37 (d, J = 3.9 Hz, 1 H, 2-H); 8.10 (d, J = 6.0 Hz, 1 H, 11-H); 7.82 (d, J = 3.9 Hz, 1 H, 1-H); 7.55–7.53 (m, 2 H, 8, 9-H); 7.28–7.26 (m, 1 H, 10-H); 5.37 (q, J = 5.1, J = 1.5 Hz, 1 H, 12-H); 3.49–3.39 (m, 2 H, 4-H); 3.0 (t, J = 5.1 Hz, 2 H, 5-H); 2.34 (d, J = 2 Hz, 1 H, 14-H); 1.41 (d, J = 5.1 Hz, 3 H, 15-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 173.12 (6-C); 143.38 (3b-C); 140.06 (7a-C); 138.49 (2-C); 134.18 (3a-C); 128.71 (11b-C); 127.94 (8-C); 121.65 (11a-C); 121.35 (11-C); 119.67 (10-C); 112.89 (1-C); 111.48 (9-C); 81.53 (13-C); 72.67 (14-C); 60.25 (12-C); 32.77 (5-C); 28.44 (4-C); 20.69 (15-C) ppm. ESIMS m/z 293 $[M + H]^+$. HRESIMS m/z 292.1215 (calcd. for $C_{18}H_{16}N_2O_2$, 292.1212).

PEG-Infractine (20): To a previously degassed solution of azido PEG 2000[30] (0.019 mmol, 38 mg) and **19** (1.1 equiv., 6.15 mg) in 1 mL of anhydrous DMF was added, with a syringe, a degassed solution of CuBr (1 equiv., 0.019 mmol, 2.73 mg) and PMDETA (2 equiv., 0.038 mmol, 7.93 µL) in anhydrous DMF (1.1 mL). The reaction mixture was stirred for 1 d at room temp. and then concentrated under reduced pressure. CH_2Cl_2 (2 mL) was then added and the solution was filtered first through an alumina column and then through a 0.22 µm PTFE filter disc. The solution was finally reduced to a volume of 1 mL and precipitated into a solution of cold Et_2O (30 mL). This process was repeated once and the final precipitate was dried overnight under reduced pressure. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 9.73 (s, 1 H), 8.34 (m, 1 H), 8.08 (d, J = 7.9 Hz, 1 H), 7.81 (s, 1 H), 7.58 (d, J = 7.9 Hz, 1 H), 7.57 (m, 1 H), 7.50 (t, J = 7.4 Hz, 1 H), 7.24 (t, J = 7.4 Hz, 1 H), 6.04 (q, J = 6.4 Hz, 1 H), 4.42 (t, J = 5.0 Hz, 2 H), 3.73–3.33 (m, 182 H), 2.99 (t, J = 6.3 Hz, 2 H), 2.63 (m, 2 H), 2.12 (s, 1 H), 1.57 (d, J = 6.5 Hz, 3 H) ppm.

PLA-*b*-PEG-Infractine (21): To a mixture of **20** (M_n = 2406 g mol^{-1}, 8.3 µmol, 20 mg) and D,L-lactide (2.08 mmol, 300 mg) was added, in dry conditions, a solution of $Sn(Oct)_2$ (3.9 µmol, 1.6 mg) in anhydrous toluene (1 mL). The reaction mixture was degassed by bubbling of argon for 10 min and then stirred at 115 °C overnight. The reaction was stopped at 57% of conversion. The toluene was removed under reduced pressure and the obtained product was dissolved in a minimum amount of CH_2Cl_2 and further precipitated in Et_2O. The precipitate was then dissolved in a minimum amount of THF and further precipitated in water and subsequently freeze-dried overnight to yield a light brown powder. SEC was performed at 30 °C with two columns [Polymer Laboratories (PL-gel MIXED-D; 300× 7.5 mm; bead diameter: 5 µm; linear part: 400–4× 10^5 g mol^{-1})] and a DRI detector (Spectrasystem RI-150 from Thermo Electron Corp.) eluted with $CHCl_3$ (flow rate of 1 mL min^{-1}, toluene was used as a flow-rate marker). The calibration curve was based on poly(methyl methacrylate) (PMMA) standards (peak molar masses, M_p = 625–625 500 g mol^{-1}) from Polymer Laboratories. This technique allowed M_n (the number-average molar mass), M_w (the weight-average molar mass) and M_w/M_n (the dispersity, $Đ$) to be determined.

Nanoparticle Formation (22): PLA-PEG-Infractine **21** (10.34 mg) was dissolved in AcOEt (1.3 mL). The organic phase was mixed with water (3.3 mL) containing Pluronic® (0.5 wt.-%). The mixture was vigorously shaken with a vortex shaker for 1 min and the resulting emulsion was sonicated for 3 min with a probe. The organic phase was then removed under reduced pressure and nanoparticles **22** were ultracentrifuged at 30000 rpm for 30 min. Finally, the nanoparticles were resuspended in water (3 mL) and filtered through a 1 µm glass filter disc and analyzed (D_z = 114.8 nm, PDI = 0.139).

Infractine Relesease Test: Compound **21** (2.54× 10^{-4} mmol, 5 mg) was dissolved in CH_2Cl_2 (500 µL) and DBU (5 equiv., 1.23× 10^{-3} mmol, 0.18 µL from a 1% solution in CH_2Cl_2) was added, the solution was stirred at room temp. 1 d. The resulting solution was studied by MS and a m/z 266 was found corresponding to the adduct formed between 4,5-dihydrocanthin-6-one **11** and ethylene oxide; the momomer of PEG, this kind of adducts appears frequently in MS studies involving PEG. The spontaneous oxidation of 4,5-dihydrocanthin-6-one (**11**) to canthin-6-one (**1**) was found to occur in the presence of air. After 72 h a sample of the reaction medium was taken for MS analysis and **1** and **11** were identified. A sample of the mixture was then collected, concentrated under reduced pressure for MS and HPLC analysis [Waters 150 mm×4.6 mm Sunfire C_{18} column 1100 series HPLC, 1100 series LC/MSD spectrometer, elution/H_2O+0.1% formic acid/CH_3CN (1:1), UV detection at 230 nm, injected volume: 5 µL]. Peak identification was performed by comparison of the retention time, the UV absorption, and the mass spectrum with a reference sample of natural **1**. The results were in agreement with data available for **1**.

Nematode Handling and Behavioural Assays: Worms were cultured according to standard techniques and media.[31] Worms were grown and assayed always at 20 °C. To culture worms in the presence of

compounds, solid Nematode Growth Medium (NGM) was prepared and compounds were added at the adequate concentration when the media was at 50 °C. For growth assays between 20 and 40 animals were cultured in Petri dishes from the larval stage L1, and were scored every 8 or 12 h to check for larval and adult individuals. We considered time point 0 the moment of hatch from embryo to the first larval stage, L1. We assayed more than 150 animals per condition and per experiment. Mobility assays were performed in liquid media M9.[31] Worms were grown in solid media in the presence of drugs, and they were transferred to liquid media, allowed to swim for 2–5 min and then thrashes over 30 sec were scored with the aid of a dissecting microscope, as described elsewhere.[32] We assayed more than 30 animals per condition. Statistical analysis was performed using Graphpad Prism® software (GraphPad Software, Inc. La Jolla, USA), to compare averages among different populations.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of **1**, **10**, **11**, **14**, **19** and **20**, complementary nanoparticles and biological studies data.

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