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Tryptamine-derived alkaloids from Annonaceae exerting neurotrophin-like properties on primary dopaminergic neurons

Fanny Schmidt [a,b,*], Gael Le Douaron [a], Pierre Champy [a], Majid Amar [a,b], Blandine Séon-Méniel [a], Rita Raisman-Vozari [b], Bruno Figadère [a,*]

[a] Centre National de la Recherche Scientifique, Laboratoire de Pharmacognosie, UMR 8076 BioCIS, Faculté de Pharmacie, Université Paris-Sud 11, 5 rue J.B. Clément 92296 Châtenay-Malabry Cedex, France
[b] Unité Mixte de Recherche, Institut National de la Santé et de la Recherche Médicale, UMR 975 Université Pierre et Marie Curie Paris-6, Centre de Recherche de L'institut du Cerveau et de la Moëlle Épinière, Bât. Pharmacie, Hôpital de la Salpêtrière, 47 boulevard de l'Hôpital, 75013 Paris, France

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ABSTRACT

N-fatty acyl tryptamines constitute a scarce group of natural compounds mainly encountered in Annonaceous plants. No biological activity was reported so far for these rare molecules. This study investigated the neurotrophic properties of these natural tryptaminic derivatives on dopaminergic (DA) neurons in primary mesencephalic cultures. A structure–activity relationships study led us to precise the role of a nitrogen atom into the aliphatic chain conferring to the compounds a combined neuroprotective and neuritogenic activity in the nanomolar range. The potent antioxidant activity of these natural products seems to be involved in part of their mechanism of action. This study provides the first description of natural neurotrophin mimetics present in Annonaceae extracts, and led to the biological characterization of compounds, which present a potential interest in neurodegenerative diseases such as Parkinson's disease.

1. Introduction

N-Fatty acyl tryptamines constitute a scarce group of natural compounds which were recently discovered in several unrelated higher plants taxa. They derive from an indole-ethylamine, amidified with an aliphatic side chain of various lengths (C-9 to C-23) originating from a fatty acid. Most were isolated from the seeds of Annonaceae sp., the family being known for its isoquinolines contents but containing only few indolic alkaloids.[1] In this family, retrieved *N*-saturated fatty acyl derivatives are either tyramine (*Annona cherimola*, *A. atemoya*),[2–4] tryptamine (*A. atemoya*, *A. muricata*,[5] *Rollinia mucosa*)[6,7] or 4-OH-tryptamine (*A. atemoya*)[3] derived. Three analogs (scorodocarpines) were isolated from an Olacaceae (*Scorodocarpus borneensis*), two of them being mono-unsaturated derivatives (C-20, $\Delta_{1:12}$, C-22, $\Delta_{1:14}$).[8] Nevertheless, such compounds have been proposed as markers of cocoa nut-shell traces in cocoa derived products (*Theobroma cacao*, Sterculiaceae/Malvaceae), docosanoyl-2-(3-indolyl)ethylamide and tetracosanoyl-2-(3-indolyl)ethylamide being the major representatives.[9] It is noteworthy that all these compounds are isolated as mixtures (with an exception[3]) and in low amounts, which could explain why no biological activity was described to date for this class of chemicals.

Over the past decade, efforts were made to develop neuronal regenerative processes for central nervous system (CNS) neurodegenerative disorders. Mainly characterized by a progressive degeneration of dopaminergic (DA) neurons involved in the nigrostriatal transmission pathway,[10,11] Parkinson's disease (PD) is the second most predominant neurodegenerative affection of the CNS.[12] Parkinsonian symptoms, including principally motor impairments,[13] can be improved by drugs reinstating the deficiency of the DA

Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); BBB, blood–brain barrier; CNS, central nervous system; DA, dopamine or dopaminergic; DIV, day in vitro; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; FI, fluorescence intensity; HOBt, hydroxybenzotriazole; IC_{50}, inhibitory concentration; PBS, Phosphate-buffered saline; PD, Parkinson's disease; QSAR, quantitative structure–activity relationships; ROS, reactive oxygen species; SAR, structure–activity relationships; TH, tyrosine hydroxylase.

* Corresponding authors. Tel.: +33 142162211; fax: +33 144243658 (F.S.); tel.: +33 146835592; fax: +33 146835399 (B.F.).
E-mail addresses: fanny.schmidt@gmail.com (F. Schmidt), gael.le-douaron@u-psud.fr (G.L. Douaron), pierre.champy@u-psud.fr (P. Champy), majid.amar@u-psud.fr (M. Amar), blandine.seon@u-psud.fr (B. Séon-Méniel), rita.raisman@upmc.fr (R. Raisman-Vozari), bruno.figadere@u-psud.fr (B. Figadère).

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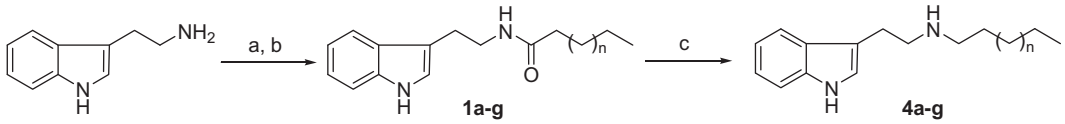


Scheme 1. Reagents and conditions: (a) HOOC–CH_2–$(CH_2)_n$–CH_2–CH_3 (1 equiv), (Cl–C=O)$_2$ (6 equiv), DMF (cat), CH_2Cl_2, rt, 2 h; (b) Et_3N (1 equiv), CH_2Cl_2, rt, 3 h; (c) $LiAlH_4$ (8 equiv), THF, 0 °C then reflux, 8 h. n = 9, 11, 12, 13, 14, 16, 19.

transmission. Neuronal degeneration is not delayed by these treatments, and the ultimate progression of the disease is dramatically of fatal issue. In this context, preservation and stimulation of DA neurons need to be provided with innovative therapies, such as delivery of neurotrophin,[14,15] and of potent neuronal growth factors insuring neuronal maturation during brain development. In spite of their impressive effect in cellular[16–19] and animal[20–24] models of PD, recombinant neurotrophins attempts of use as therapeutic agents, led to disappointing clinical trials.[25–28] This failure is mostly associated with the high liability of these polypeptides[29] and their inability to cross the BBB, imposing either intra-cerebral injections coupled to peripheral side effects or viral and cellular vectors.[30–33]

Our work is based on the discovery of low molecular weight compounds possessing neurotrophic effects on DA neurons with the ultimate aim of avoiding the drawbacks inherent to the therapeutic use of recombinant neurotrophins. In a previous study, we reported the neurotrophic activity of quinoline-derived compounds presenting a long aliphatic lateral chain.[34] These neurotrophin mimetics are able to promote neuritogenesis and survival of dopaminergic (DA) neurons in mesencephalic primary cultures in the nanomolar range.

Tryptaminic alkaloids isolated from Annonaceae or other plant sources share structural analogies with (i) melatonin (*N*-(2-(5-methoxy-1*H*-indol-3-yl)acetamide), a neurotransmitter shown to possess neuroprotective activities on DA neurons[35,36], and (ii) long aliphatic chain-containing neurotrophic compounds.[37,38] Given these structural observations, natural tryptamine-derived alkaloids appear to be good candidates as neurotrophin mimetics.

In this study, we synthesized natural amidic tryptaminic derivatives, and investigated the neurotrophic activity of these compounds on DA neurons in mesencephalic cultures in a validated system of progressive and spontaneous death of DA neurons. Further structure–activity relationships (SAR) studies were focused on the reduction of the amide function, the hydroxylation of the lateral chain terminal position and the use of saturated and unsaturated fatty acids as precursors for the synthesis of non-natural analogs to explore the influence of chain length and rigidity. Considerations emanating from SAR led us to measure antioxidant activities of lead compounds through chemical and biological ways, as part of their mechanism of action.

2. Discussion

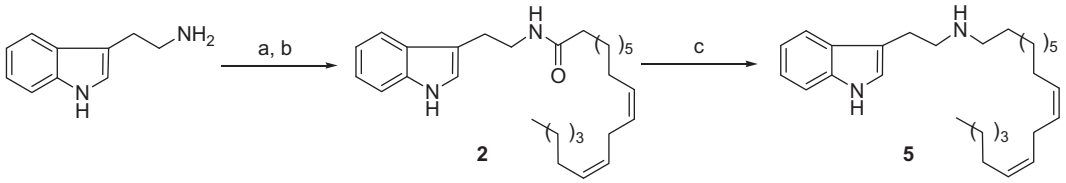
2.1. Chemical syntheses

Tryptamine was easily coupled to increasing length saturated fatty acids previously converted by oxalyl chloride treatment into their respective acid chlorides to give *N*-acyltryptamines **1a**–**g** including some natural compounds (n = 12 (**1c**), 14 (**1e**), 16 (**1f**), 19 (**1g**)). *N*-Acyltryptamines **1a**–**g** were then reduced into their corresponding amines **4a**–**g** in good yields by using $LiAlH_4$ under strong conditions (Scheme 1). One unsaturated analog was synthesized by coupling *all-cis* linoleic acid to tryptamine, through the same way, leading to compound **2** in good yields. Reduction of compound **2** amide function into amine by $LiAlH_4$ led to compound **5** (Scheme 2). One ω-hydroxylated analog **3** was prepared through tryptamine acylation with 15-hydroxypentadecanoic acid using the common peptide cross coupling agents 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and *N*-hydroxybenzotriazole (HOBt). Further reduction of compound **3** using $LiAlH_4$ led to compound **6** in high yields (Scheme 3). Spectroscopic data of all synthesized compounds are available in Section 4.

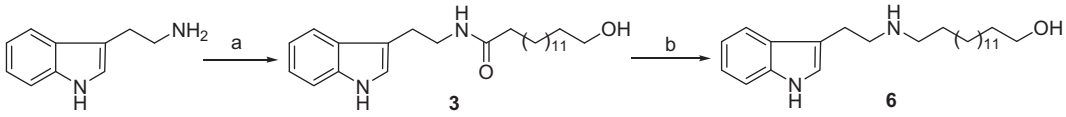
2.2. Combined neuroprotective and neuritogenic effects of the tryptamine-derived compounds on DA neurons

The capacity of each synthesized compound to protect DA neurons from death and induce fiber sprouting was assessed in rat primary mesencephalic cultures. This model involves a progressive and spontaneous degeneration of DA neurons specifically. Cultures were maintained for eight days in the presence or absence of the different tested compounds, and then tyrosine hydroxylase (TH) was immunolabeled to allow the analysis of DA neurons. Neuroprotection was assessed by TH immunopositive (TH^+) neurons counting. Neuritogenesis, expressed as total neurite length per DA neuron, was quantified using image analysis software on at least 100 neurons randomly photographed per condition. Results are merged in Table 1. Appropriate comparisons, described below, allowed drawing of SARs merged into a two-dimensional diagram (Fig. 1).

Compounds **1a–g**, **2**, **3**, **4a–g**, **5**, **6** were preliminary tested at 1, 10 and 100 nM. Data were acquired at 10 nM, concentration leading to the optimal activity. At lower concentration the tryptaminic analogs were inefficient while at higher concentration a toxic effect was observed (data not shown). At 10 nM, all tryptaminic derivatives exert a neuritogenic activity. Amide function-containing analogs **1a–g**, **2** and **3** possess the only capacity to induce neuronal sprouting. When reduced into amines, dual activity is observed without significant change in neuritogenic activity (**1c** vs **4c**, **1e** vs **4e**, **1g** vs **4g**, **2** vs **5**, **3** vs **6**, P_P <0.05), underlining a crucial role of the amine function. Interestingly, compounds possessing a 16 carbon chain length (n = 12) exert the most potent neurotrophic (neuroprotective/neuritogenic, respectively) effect in both the amide (**1c:** 121.3%/168.6%) or amine (**4c:** 131.9%/197.0%) series, while the activity of shorter compounds is weaker (**1a** (n = 9): 98.5%/126.2%; **4a**: 92.8%/121.2%). Similar results had been previously described with stem cell fate modulators presenting a related scaffold.[38] This supports the hypothesis that the introduction of lateral aliphatic chains could be involved in the neuritogenic effect that occurred in the presence of these compounds. Interestingly it appears that the presence of unsaturations into the aliphatic chain is not a prerequisite for both activities, since the introduction of a linoleic acid-derived lateral chain did not modify neuritogenic and neuroprotective activities in both amide or amine series compared to their 18-carbon length saturated analogs (**2** (111.4%/154.0%) vs **1e** (110.0%/149.9%), **5** (137.5%/156.7%) vs **4e** (129.9%/157.3%), non-significant). The presence of a terminal hydroxyl on the lateral chain increases both activities in the amine series only (**3** (108.0%/159.3%) vs **1b** (99.3%/139.0%), nonsignificant; **6** (129.4%/160.8%) vs **4b** (106.0%/132.7%), $P_{P/N}$ <0.05). This result is in accordance with previous studies underlining the benefit of the hydroxyl group in compounds without intra-chain functions in neurotrophic process.[38] Interestingly, the introduction of an amide or an amine function into the aliphatic moiety seems to modulate the benefit provided by the hydroxyl group. Structure–activity

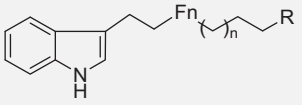


Scheme 2. Reagents and conditions: (a) HOOC–$(CH_2)_7$–CH≡CH–CH_2–CH≡CH–$(CH_2)_4$–CH_3 (1 equiv), (Cl–C=O)$_2$ (6 equiv), DMF (cat), CH_2Cl_2, rt, 2 h; (b) Et_3N (1 equiv), CH_2Cl_2, rt, 3 h; (c) $LiAlH_4$ (8 equiv), THF, 0 °C then reflux 8 h.



Scheme 3. Reagents and conditions: (a) HOOC–CH_2–$(CH_2)_n$–CH_2–CH_2–OH (1 equiv), EDC (2 equiv), HOBt (1.5 equiv), Et_3N (3 equiv), CH_2Cl_2, rt, 5 h; (b) $LiAlH_4$ (8 equiv), THF, 0 °C then reflux, 8 h.

Table 1
Neuroprotective and neuritogenic activities of tryptamine-derived compounds on mesencephalic DA neurons



Compound (10 nM)	Fn	*n*	R	% Relative to control ± SEM: TH^+ neurons[a]	% Relative to control ± SEM: Length/TH^+ neuron[b]
CTL				100 ± 1.9	100 ± 2.9
dbc-AMP (200 μM)				149.9 ± 3.6	333.1 ± 8.7**
1a	NH–C=O	9	CH_3	98.5 ± 1.9	126.2± 6.0*
1b	NH–C=O	11	CH_3	99.3 ± 2.7	139.0 ± 11.3*
1c	NH–C=O	12	CH_3	121.3 ± 3.2**	168.6 ± 11.5**
1d	NH–C=O	13	CH_3	106.0 ± 3.8	136.0 ± 5.4*
1e	NH–C=O	14	CH_3	110.0 ± 5.3	149.9 ± 11.4**
1f	NH–C=O	16	CH_3	121.8 ± 2.0**	147.4 ± 7.0**
1g	NH–C=O	19	CH_3	114.0 ± 4.0	145.4 ± 10.0**
2	NH–C=O	5	Δ[c]	111.4 ± 3.4	154.0 ± 6.2**
3	NH–C=O	11	OH	108.0 ± 3.6	159.3 ± 5.8**
4a	NH–CH_2	9	CH_3	92.8 ± 8.7	121.2 ± 9.9
4b	NH–CH_2	11	CH_3	106.0 ± 8.2	132.7 ± 1.8*
4c	NH–CH_2	12	CH_3	131.9 ± 4.7**	197.0 ± 14.0**
4d	NH–CH_2	13	CH_3	110.6 ± 4.5	139.0 ± 4.6*
4e	NH–CH_2	14	CH_3	129.9 ± 3.5**	157.3 ± 10.3**
4f	NH–CH_2	16	CH_3	128.9 ± 3.4**	132.6 ± 6.2*
4g	NH–CH_2	19	CH_3	129.6 ± 3.5**	141.2 ± 5.0**
5	NH–CH_2	5	Δ[c]	137.5 ± 8.0**	156.7 ± 6.7**
6	NH–CH_2	11	OH	129.4 ± 5.0**	160.8 ± 13.8**

[a] Number of TH^+ neurons per well as a percentage of untreated cultures expressed as the mean ± SEM of at least three independent experiments performed in triplicate.
[b] Neurite length per TH^+ neuron as a percentage of untreated neurons measured on at least 100 neurons per condition using an image analyzer (Neurite Outgrowth, Explora Nova, France) and expressed as the mean ± SEM of three independent experiments performed in triplicate.
[c] Δ = [CH=CH–CH_2–CH=CH–$(CH_2)_4$–CH_3]. Statistical analysis was performed by one-way ANOVA followed by Bonferroni's or Dunnett's *post hoc* test *$P_{P/N}$ <0.05 **$P_{P/N}$ <0.001 compound versus CTRL. P_P and P_N are used to design respective *P* values for neuroprotection and neuritogenesis.

relationships within the compound collection are presented in a two-dimensional diagram (Fig. 1).

Further investigations were carried out with compounds **4c**, **5** and **6**, which showed the best activity for both neuroprotection and neuritogenesis (Fig. 2). In particular, given the antioxidative properties of the melatonine moiety, the antioxidative capacity of the tryptamine-derived compounds **4c**, **5** and **6** was assessed and compared to that of amides **1c**, **2** and **3**.

2.3. Antioxidant activity

Since neuroprotective effects could be linked to antioxidative activities, the antioxidant properties of the tryptamine-derived compounds were assessed following two methods: the ABTS competition assay and the cellular incorporation of dihydrorhodamine-123.

2.3.1. Free-radical scavenging capacity

The free-radical scavenging capacity was performed in the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) competition assay. In the presence of hydroxyl radicals, ABTS is oxidized to the stable ABTS radical cation ($ABTS^{+\cdot}$) absorbing at 450 nm. During the test, hydroxyl radicals are generated through the Fenton reaction by homolytic rupture of hydrogen peroxide in the presence of iron salts.

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\cdot} + HO^- + Fe^{3+}$$

$$ABTS + HO^{\cdot} \rightarrow ABTS^{\cdot} + HO^-$$

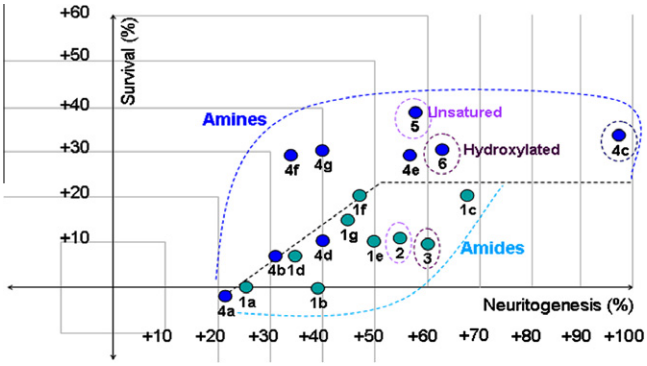


Figure 1. Structure–activity relationships in tryptamine-derived compounds induced DA neurons neuritogenesis and survival. Compounds were tested at 10 nM concentration. Combined neuroprotective and neuritogenic activities were reported from Table 1 in a two-dimensional representation showing two main groups of compounds exhibiting or not both activities in different ratios. This diagram allows us to bring in light compound **4c** exerting the highest combined activities as lead compound of the series.

Table 2
Free-radical scavenging activity assessment by ABTS competition assay

Compound	IC_{50} ± SEM[a] (mM)
Trolox®	0.55 ± 0.02
1a	>1[c]
1c	>1
1g	ND[b]
3	>1
4a	0.81 ± 0.14
4c	0.62 ± 0.05
4g	0.67 ± 0.01
5	0.34 ± 0.01
6	0.73 ± 0.01

[a] IC_{50} expressed as the mean ± SEM of at least three independent experiments.
[b] ND: not detected.
[c] >1: Activity detected; experimental data are missing for an exact determination of the IC_{50} value. IC_{50} are determined by optical density measurement at 450 nm on a 1 mM to 10 nM dilution ranges of samples.

A competition for hydroxyl radicals scavenging occurs between ABTS and the tested compound resulting in a decrease of absorbance at 450 nm by inhibition of $ABTS^{+\cdot}$ formation. IC_{50} can be measured for each compound as the concentration leading to 50% of inhibition of $ABTS^{+\cdot}$ formation. Results are presented in Table 2 and compared to 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, or Trolox® as reference.

N-Acyltryptaminic derivatives **1a**, **1c**, **1g** and **3** possess a weak detectable antioxidant activity only, upper than 1 mM. On the opposite, *N*-alkyltryptaminic analogs (**4a**, **4c**, **4g**, **6**) exert a strong free-radical scavenging ability, with an IC_{50} close to that of Trolox®. These results precise the significant role of the amine function inside the aliphatic chain. Surprisingly, unsaturations seem to improve the antioxidant activity leading to an IC_{50} lower than that of Trolox® for compound **5**.

2.3.2. Antioxidative properties in mesencephalic neuronal cultures

So as to assess these results, a more physiological model was then used: the production of intraneuronal reactive oxygen species (ROS) was quantified in DIV 8 mesencephalic cultures by incorporation of dihydrorhodamine-123, a nonfluorescent precursor of rhodamine. The amount of dihydrorhodamine-123 oxidized into rhodamine by ROS is proportional to their intraneuronal production. A decrease in fluorescence intensity (FI) per neuron in cultures treated with one compound reflects its antioxidant capacity. Mesencephalic cultures were treated with tryptaminic derivatives, at 10 nM until DIV 8, as for neurotrophic effect assessment. The fluorescent signal was monitored by epifluorescence microscopy and quantified by computer-assisted image analysis. Results, compared to Trolox® as reference, are presented in Table 3 and illustrated in Figure 3.

N-Acyltryptaminic **1c**, **2**, **3** and *N*-alkyltryptaminic derivatives **4c**, **5**, **6** induce a significant decrease in fluorescence intensity revealing their antioxidant activity. In this assay, *N*-acyltryptaminic analogs exert a significant impact on ROS production. Non-surprisingly, *N*-alkyltryptaminic analogs possess a strong antioxidant capacity, decreasing fluorescence intensity in the same range as Trolox® does. It is noteworthy that *N*-acyl and *N*-alkyltryptamine derivatives exert their activity in cultures at 10 nM, that is, 1000 times more potent than Trolox®. This result supports once more the role of the amine function into the lateral chain. Note that neither the introduction of unsaturations into the aliphatic moiety nor the presence of a terminal aliphatic hydroxyl significantly improved the antioxidant capacity in this model.

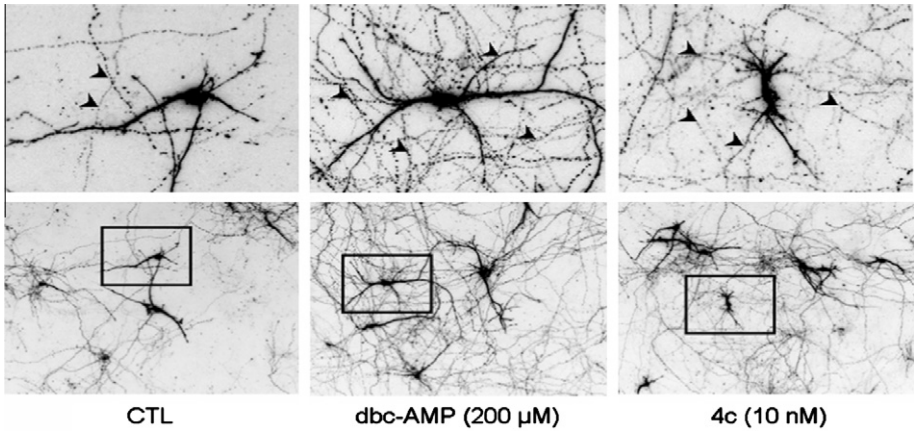


Figure 2. 4c-induced neurotrophic effect on mesencephalic DA neurons assessed via tyrosine hydroxylase immunolabeling. Compound **4c** increased the number and the length of DA neurons processes compared to non-treated cultures (CTL). Dbc-AMP at 200 µM was used as a positive control. Images were acquired with an inverted fluorescent microscope coupled to a digital camera. ➤ Points out the density of the TH immunopositive fibers network. Higher concentrations led to a toxic effect (data not illustrated).

Table 3
Reactive oxygen species production assessment by dihydrorhodamine-123 incorporation into mesencephalic cultures

Compound (10 nM)	FI/neuron ± SEM[a] (%)
CTL	100.0 ± 3.3
Trolox® (10 μM)	44.0 ± 2.8**
1c	55.9 ± 3.0**
2	56.5 ± 2.7**
3	60.9 ± 6.4**
4c	47.8 ± 4.7**
5	44.0 ± 3.6**
6	43.7 ± 5.2**

[a] Fluorescence intensity per mesencephalic neuron expressed as the mean ± SEM of at least three independent experiments. Mesencephalic cultures were treated by 10 nM of each compound. **P <0.001 versus CTL. Statistical analysis was performed by one-way ANOVA followed by Bonferroni's *post hoc* test.

These results provide evidence that these tryptaminic derivatives exert a strong antioxidative capacity in mesencephalic cultures. The ABTS competition assay revealed that this property is exerted either through direct hydroxyl radical scavenging activity or by complexation of ferrous ions (Fe^{2+}), with an IC_{50} equivalent to that of Trolox®. Melatonin analogs without the 5-MeO moiety have been previously described for their capacity to scavenge free radicals supporting the observation that our 5-*H*-tryptaminic derivatives are sensitive to radicals scavenging.[39] However, the fact that the potency of the antioxidant capacity is thousand times increased in biological conditions suggests the involvement of additional processes besides the direct radical scavenging property, including potentially iron scavenging, up-regulation of detoxifying enzymes, or down-regulation of ROS production, for example, at the mitochondrial level. Such activities should be further investigated.

Interestingly, the antioxidant capacity of the tryptaminic derivatives correlates with their neuroprotective activity but not with their neuritogenic properties. This relationship suggests that the neurotrophic effect mimicked by these compounds occurs at least by two distinct mechanisms. Further studies are needed two elucidate the cellular signaling pathways involved in these neuritogenic effects. Such mechanism could implicate ERK1/2 phosphorylation as previously described with similar neurotrophin-like compounds.[34]

2.3.3. In vivo blood–brain barrier penetration

The ability of compound **4c** to cross the blood–brain barrier (BBB) was assessed in vivo after per os administration (150 mg/kg) to 5 months years old Male RjOrl:SWISS mice coupled to a detection by mass spectrometry (MS) (after perfusion, freezing, pulverization and extraction of brain tissues with methanol) and is illustrated in Figure 4. A significant amount (21.80 ng/mL ± 0.99 SEM) of compound **4c** was detected into the brain tissues 3 h after administration, showing the capacity of compound **4c** to cross the BBB. It is noteworthy that the acute per os administration of compound **4c** to mice did not produce any abnormal behavior during the time lapse after administration.

Given that long aliphatic chain-containing compounds are generally very lipophilic, chemical properties of compound **4c** including log *P*, polar surface area (PSA), aqueous solubility were estimated and measured. These parameters entering the 'Lipinski's rule of five' and most of one-dimensional PSA/log *P*-based QSARs methods are commonly evaluated to predict BBB penetration of compounds.[40] Prediction of blood–brain barrier penetration uses a comparison of compound **4c** physicochemical properties and those expected for successful CNS drugs.[40] Data are merged in Table 4. Not surprisingly, log *P* estimation reaches a value of 7.0, which is attempted regarding to the fatty acid-derived chemical scaffold but much higher than what is generally observed for CNS drugs. It is interesting to note that compound **4c** does not satisfy the entire set of basic physicochemical rules predicting his BBB penetration. However, we demonstrated that a significant amount of compound **4c** is found inside cerebral tissues after administration to mice.

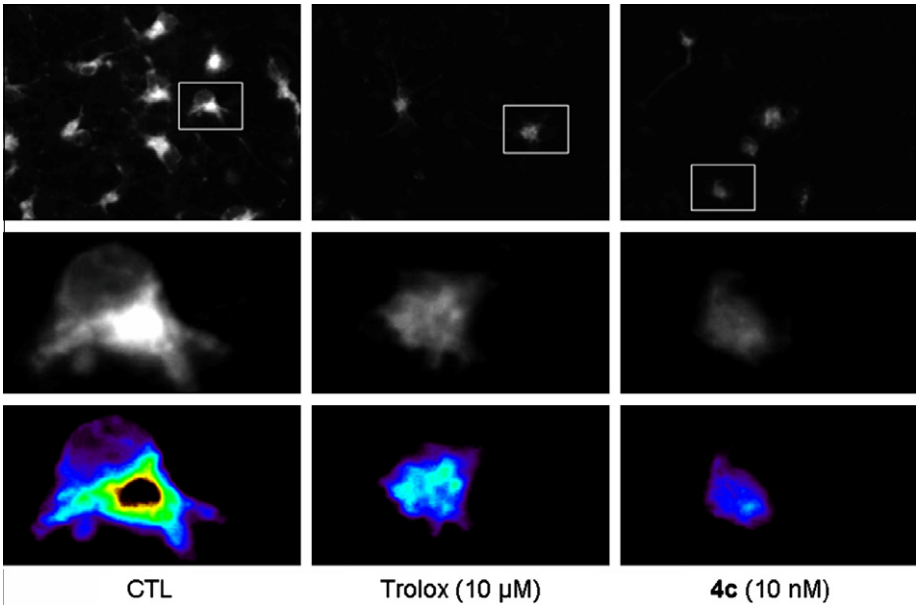


Figure 3. Compound **4c** decreases neuronal reactive oxygen species production. ROS production was measured by using dihydrorhodamine-123 (DHR-123) in mesencephalic cultures. Mean fluorescence intensity (FI) per cell was quantified then normalized to the untreated control (CTL) and represents the average of at least three assays expressed as mean ± SEM. Trolox® at 10 μM was used as positive control. Compound **4c** at 10 nM exerts a dramatic decrease of the FI compared to non-treated cultures. Images were acquired with an inverted fluorescent microscope coupled to a digital camera and colored with the Photoshop gradient map tool. FI was measured by the image analyzer software Simple PCI.

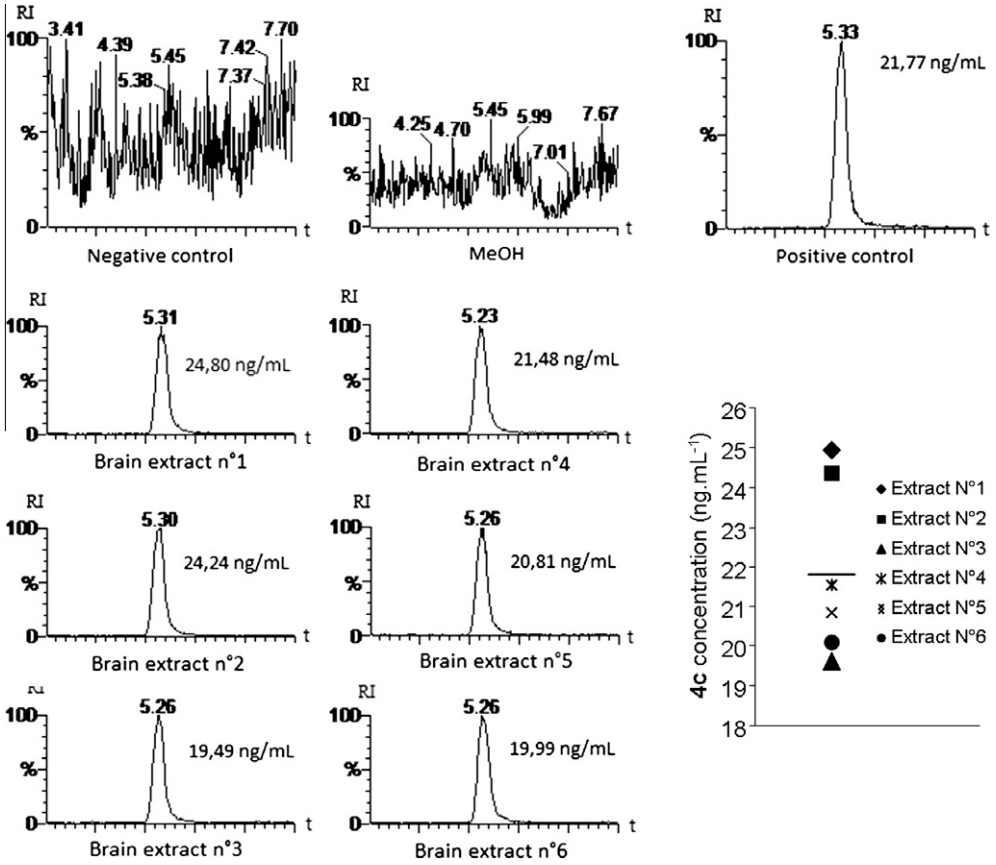


Figure 4. HPLC/MS—measurement of compound **4c** in brain extracts. Chromatograms of the extracts (*x*-axis: times (min); *y*-axis: relative intensity (%)). Concentrations calculated from standard solutions of compound **4c**. Negative control: extract of a brain of vehicle-treated mouse. Positive control: extract of a brain of vehicle-treated mouse supplemented with 0.01 μg of compound **4c** hydrochloride salt. High purity MeOH was used as HPLC solvent. Compound **4c** penetrates the mouse cerebral tissues after oral administration of 150 mg/kg once per day during five days.

Table 4
Prediction of the ability to cross the BBB of CNS drugs using QSARs

Attributes	Successful CNS Drugs[a]	Compound **4c**
Potent activity	Low to subnanomolar	10 nM
Selectivity	High	Unknown
Molecular weight	<450 g mol^{-1}	384 g mol^{-1}
Log *P*	<5	7[b]
H-bond donor	<3	2
H-bond acceptor	<7	2
Rotatable bonds	<8	18
pK_a	7.5–10.5 (avoid acid)	Basic (not determined)
Polar surface area (PSA)	<60–70 Å	28 Å
Aqueous solubility	>60 μg mL^{-1}	>385 μg mL^{-1}[c]

[a] See Ref. 40.
[b] Calculated via ChemDraw ultra 8.0.
[c] In water with 1% ethanol.

3. Methods

3.1. Biological studies

3.1.1. Primary mesencephalic cultures

Animals were treated in accordance with the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals (U.S. National Institutes of Health). The embryos were removed at embryonic day 15.5 from pregnant Sprague–Dawley rats (Elevage Janvier, Le Genest St. Isle, France) that had been anesthetized, then decerebrated. Ventral mesencephalons were dissected and collected. Cell suspensions, prepared by mechanical trituration without proteolytic enzymes, were plated in 48 multiwell culture plates (Costar, Corning Inc., NY) precoated overnight with 1 mg/mL polyethylenimine (Sigma, Saint Quentin Fallavier, France) in borate buffer, pH 8.3. The cells were then maintained for maturation and differentiation in Neurobasal culture medium (Gibco, Invitrogen, Scotland, UK) supplemented with 1%$_{v:v}$ B27 (Gibco, Invitrogen, Scotland, UK), 4 mM glutamine (Glutamax, Gibco, Invitrogen, Scotland, UK), 100 U/mL penicillin (Gibco, Invitrogen, Scotland, UK), and 100 μg/mL streptomycin (Gibco, Invitrogen, Scotland, UK). Cultures were treated at DIV 1 and every four days 300 μL of culture medium were replaced by medium supplemented with treatments.

3.1.2. Immunofluorescence staining

Tyrosine hydroxylase (TH) immunofluorescence was used to detect DA neurons. After 12 min fixation with a 4% formaldehyde solution in Dulbecco's phosphate-buffered saline (PBS), cells were washed three times with PBS and then incubated in PBS$^+$ (PBS containing 0.2% Triton X-100, 10% fetal bovine serum (Sigma, Saint Louis, MO) and 0.01% thimerozal (Sigma, Saint Quentin Fallavier, France) for 1 h. The cells were further incubated overnight at 4 °C with a rabbit anti-TH polyclonal antibody (1:500, Pel Freez, Paris, France). Subsequent incubations were performed, at room temperature, with a secondary anti-rabbit IgG cyanin 3 conjugate (1:500, Jackson immunoresearch Laboratories, Newmarket, UK). Images were acquired with Simple-PCI software from C-Imaging Systems using an inverted fluorescent microscope (TE-300, Nikon, Tokyo, Japan) coupled to an ORCA-ER digital camera from Hamamatsu (Bridgewater, NJ).

3.1.3. Survival quantification

Tyrosine hydroxylase (TH) immunocytochemistry was used to quantify the survival of DA neurons. Ten randomly chosen fields per well were counted. Results were expressed in percentage of untreated cultures. Each value represents the mean ± SEM from three wells per condition of three independent experiments.

3.1.4. Neuritogenesis quantification

Neurite length per cell was quantified using the image analysis software Neurite Outgrowth (Explora Nova, La Rochelle, France) on at least 100 neurons randomly chosen per condition. Results were expressed as percentage of untreated cultures. Each value represents the mean ± SEM from three wells per condition of three independent experiments.

3.1.5. ABTS competition assay

In each well of a 96-well plate were successively introduced 180 μL of an ethanol–water (50:50) mixture, 30 μL of a 1 mM ABTS aqueous solution, 30 μL of a 0.5 mM $FeSO_4$ aqueous solution and 30 μL of each sample dilution comprised between 1 mM and 10 nM (10 times dilution range). Plates were placed for 10 min under stirring at room temperature then 30 μL of a 100 mM H_2O_2 aqueous solution were added per well. Plates were incubated under stirring at 37 °C for one hour. The free-radical scavenging activity was quantified by the decolourization of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) at 450 nm. IC_{50} values were calculated as the concentration required for a 50% diminution of the $ABTS^{+\cdot}$ formed.

3.1.6. Reactive oxygen species measurement

Mesencephalic cultures were maintained and treated as described before in 4-chamber glass microscope slides. At DIV 8, cultures were treated then exposed for 30 min to 50 μM DHR-123 in darkness at 37 °C, then were washed four times with fresh culture medium. The fourth time the medium was replaced by fresh medium devoid of phenol red supplemented or not with tested compound at 10 nM or Trolox® at 10 μM. The fluorescent signal, visualized by epifluorescence microscopy was quantified on 10 images per well acquired with Simple-PCI software from C-Imaging Systems using an inverted fluorescent microscope (TE-300, Nikon, Tokyo, Japan) coupled to an ORCA-ER digital camera from Hamamatsu (Bridgewater, NJ).

3.1.7. Statistical analysis

Comparisons between two groups were performed with Student's *t* test. Multiple comparisons against a single reference group were made by one-way analysis of variance (ANOVA) followed by Dunnett's or Bonferroni's test. SEM values were derived from at least three values per condition of three independent experiments.

3.2. BBB penetration of compound 4c

3.2.1. Subjects and dosing

Five-week males RjOrl:SWISS mice (R. Janvier, France), weighting 20–24 g on arrival, were housed and maintained at a constant temperature (22 ± 1 °C) and in a humidity-controlled (55 ± 20%) environment. A 12 h/12 h light–dark cycle was kept constant, with lights turned on at 08:00 a.m. During the acclimatization period (3 days) and throughout all the study, the mice had free access to food and water. Animal protocols were performed in accordance with the National Institutes of Health Guide for the Care and Use of laboratory Animals. Hydrochloride salt of compound **4c** was prepared in vehicle (0.5% Tween80 + 95.5% carboxy-methyl cellulose at 1% in saline solution). During five days, compound **4c** was orally administrated to six mice at a dose of 150 mg/kg per day (10 mL/kg) except the last day where mice received only one administration. Behavior, food intake and body-weight of mice were controlled each day and compared with the control group. Between 3 and 6 h after the last administration, mice were anesthetized with a solution of pentobarbital sodium 6% and then perfused through the ascending aorta with an anticoagulant saline solution, to remove traces of blood from the brain. Brains were extracted and cerebellum, brainstem and olfactory bulb were removed. Brains were then sliced into three parts (the right hemisphere was cut into two parts, and the left hemisphere was kept intact). Samples were then frozen in isopentane for one minute at −30 °C, allowing further storage at −80 °C.

3.2.2. Mouse brain tissue samples and standards preparation

Mouse brain samples were weighted, homogenized in methanol (100 mg of tissue/mL) then mixed by sonication. The mixture was centrifuged (27,138*g*, 4 °C) for 10 min. The supernatant, diluted onefold in methanol, was transferred into HPLC vials for subsequent analysis of compound **4c** levels by HPLC–MS/MS. Brains of vehicle-treated mice (control) were used for both positive and negative controls. As positive control, the supernatant was supplemented with 0.01 μg (10 μL at 1 μg/mL) of compound **4c** hydrochloride salt then diluted onefold in methanol before being transferred into HPLC vial. All the samples were kept at 4 °C before and during the analysis. Analyses were performed the same day, under the same conditions.

3.2.3. Instrumentation and conditions for the analysis of com pound 4c

The HPLC system consisted of a Dionex Ultimate 3000 pump equipped with a Dionex WPS-3000PL autosampler automatic injector. The mass spectrometer used was a Water-Micromass Quattro Ultima with electrospray interface and a triple quadrupole analyzer. Data acquisition and analysis were performed using Masslynx 3.5. Liquid chromatography was performed in isocratic mode with an XBridge™ (Waters) C18 150 × 2.1 nm with particle size of 3.5 μm column. The mobile phase was composed by an aqueous phase A/organic phase B 3/97 mixture. Phase A was composed of 0.2% triethylamine in water (v/v) (pH 11.5). Phase B consisted of 0.2% triethylamine in methanol (v/v). The flow rate was fixed at 0.2 mL/min; volumes of 10 μL were injected. The mass spectrometer was interfaced with the HPLC system using an ESI electrospray ion source. A remote valve is programmed to send the first 3 min of the chromatography to waste in order to avoid salts accumulation in the ESI capillary. The capillary voltage was set to 3000 V and the cone voltage to 50 V; the source temperature was set to 120 °C; the desolvation gas (N_2) was set at a flow of 463 L/h and temperature of 350 °C. Fullscan and product ion mass spectra for compound **4c** were previously obtained in positive ion mode by direct introduction. Parameters were optimized to obtain the maximum compound **4c** pseudo-molecular ion *m*/*z* 385.66 intensity. The collision was performed with argon gas and the energy was set to 22 eV in MS/MS mode to choose the quantification mass transition: *m*/*z* 385.66→254.23 and 385.66→144.17. Quantification was performed in Multiple Reaction Monitoring (MRM) mode with this mass transition, with external standard range at seven different concentrations (0.5 μg/mL; 0.1 μg/mL; 20 ng/mL; 4 ng/mL; 0.8 ng/mL; 0.16 ng/mL and 0.08 ng/mL).

3.2.4. Toxicity of compound 4c

Along the treatment, there were no indications of any adverse effects, due to either vehicle or compound **4c**-containing vehicle administration. Behavior, food intake and body-weight changes were similar among the two groups.

3.2.5. Linearity, limit of detection and quantitation

Studies of the effect on HPLC peak area as a function of compound **4c** concentration were carried out in the range 0.16–500.0 ng/mL with an R^2 of 0.9997. The limit of detection was found to be 0.08 ng/mL with a *S/N* ratio 3:1. The retention time of compound **4c** was 5.3 min; the total run time was set at 8 min.

3.2.6. Measurement of compound 4c in brain extracts

Compound **4c** is not detectable in extract of the brain of vehicle-treated animals and was quantified at 21.77 ng/mL in the positive control, which was unexpectedly the value found for the compound **4c**-treated mice brains. Indeed, compound **4c** was quantified in the extracts of compound **4c**-treated mice brains at the average dose of 21.80 ng/mL ± 0.99 Standard Error of the Mean ($n = 6$) (SEM).

3.3. Chemistry

3.3.1. Spectroscopic analysis

Tetrahydrofuran was distilled over sodium/ benzophenone under nitrogen prior to use. Dichloromethane and triethylamine were distilled over calcium hydride. All reactions involving moisture-sensitive reactants were performed under nitrogen atmosphere using oven-dried glassware. Routine monitoring of reactions was performed using Riedel-deHaën, S, 0.063 mm, 0.032 silica gel plates, which were dipped in a *o*-vanillin in $EtOH/H_2SO_4$ (95:5) and then heated to allow revelation. Flash chromatography was performed using Riedel-deHaën, S, 0.063 mm, 0.032 silica under moderate pressure with the appropriate solvent giving us a migrate front (R_f) in the range of 0.2–0.3. ^{1}H NMR spectra were recorded on BRUKER AR 200 P (200 MHz) and AM-400 (400 MHz) spectrometers as solutions in $CDCl_3$. Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane (TMS) and are referenced to $CHCl_3$ (7.26 ppm) as internal standard. ^{13}C NMR spectra were recorded on BRUKER AR 200 P (50 MHz) and AM-400 (100 MHz) spectrometers as solutions in $CDCl_3$. Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane (TMS) and are referenced to $CDCl_3$ (77.0 ppm) as internal standard. The attribution of the different carbons (C, CH, CH_2, CH_3) was determined by ^{13}C to ^{1}H polarization transfer (*J* modulation). Infrared spectra (IR, cm^{-1}) were measured on a BRUKER vector 22 FT spectrometer. Mass spectra (MS) were measured on a Nermag-Sidar R10-10C spectrometer with a quadripolar filter. All products where obtained with a purity >95% as determined through HPLC analysis.

3.3.2. General procedure for syntheses of (1a–g, 2)

To a solution of carboxylic acid (2 mmol, 1 equiv) in dichloromethane (10 mL) under nitrogen at 0 °C were added three drops of dried DMF and oxalyl chloride (1.04 mL, 12 mmol, 6 equiv). The mixture was stirred for 1 h at 0 °C. Solvent and residual oxalyl chloride were removed in vacuo at 50 °C. The intermediate acid chloride so obtained was then dissolved in dichloromethane (5 mL). To a solution of tryptamine (160 mg, 1 mmol, 1 equiv) in dichloromethane (10 mL) under nitrogen at 0 °C was added dropwise triethylamine (0.55 mL, 2 mmol, 2 equiv) and the solution of acid chloride previously synthesized. The mixture was stirred for 2 h at room temperature then quenched by adding water (10 mL) and extracted three times with dichloromethane (3 × 30 mL). Combined organic layers were then dried over $MgSO_4$, filtered and concentrated in vacuo. A flash chromatography on silica gel with a mixture of cyclohexane and ethyl acetate (80:20) led to the desired products.

3.3.2.1. *N*-(2-(1*H*-Indol-3-yl)ethyl)tridecanamide (1a). Compound **1a** (360 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 0.88 (t, *J* = 6.8 Hz, 3H), 1.25 (m, 17H), 1.58 (m, 5H), 2.09 (t, *J* = 7.2 Hz, 2H), 2.98 (t, *J* = 6.8 Hz, 2H), 3.59 (t, *J* = 6.8 Hz, 1H), 3.62 (t, *J* = 6.8 Hz, 1H), 5.48 (s, 1H), 7.04 (d, *J* = 1.6 Hz, 1H), 7.13 (t, *J* = 7.2 Hz, 1H), 7.22 (t, *J* = 7.2 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 8.05 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.4, 29.3, 29.4, 29.5, 29.6, 31.9, 36.9, 39.6, 111.1, 113.2, 118.8, 119.5, 121.9, 122.3, 127.4, 136.4, 173.0. IR ν cm^{-1}: 3387, 3264, 3089, 2955, 2918, 2851, 1650, 1629, 1554, 1470, 1456, 1424, 1377, 1340, 1294, 1271, 1246, 1222, 1130, 1094, 1068, 1009, 913, 847, 801, 739, 719, 671, 608. MS(ESI) *m/z*: 379 ([M+Na]$^+$, 100). ESI-HRMS Calcd for [M+Na]$^+$ $C_{23}H_{36}N_2ONa$: 379.2725. Found: 379.2714.

3.3.2.2. *N*-(2-(1*H*-Indol-3-yl)ethyl)pentadecanamide (1b). Compound **1b** (270 mg, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.88 (t, *J* = 6.8 Hz, 3H), 1.25 (m, 22H), 1.61 (m, 4H), 2.10 (t, *J* = 7.0 Hz, 2H), 2.98 (t, *J* = 6.8 Hz, 2H), 3.58 (t, *J* = 6.4 Hz, 1H), 3.65 (t, *J* = 6.4 Hz, 1H), 5.49 (s, 1H), 7.04 (s, 1H), 7.12 (t, *J* = 7.0 Hz, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 8.04 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 14.1, 22.6, 22.9, 23.7, 25.3, 28.9, 29.2, 29.4, 29.6, 31.8, 36.8, 39.6, 111.2, 112.9, 118.6, 119.3, 122.0, 127.3, 136.4, 173.1. IR ν cm^{-1}: 3387, 3267, 2955, 2918, 2850, 1650, 1630, 1555, 1470, 1456, 1425, 1377, 1340, 1297, 1225, 1130, 1094, 1069, 1010, 931, 801, 739, 718, 607. MS(ESI) *m/z*: 407 ([M+Na]$^+$, 100). ESI-HRMS Calcd for [M+Na]$^+$ $C_{25}H_{40}N_2O$-Na: 407.3038. Found: 407.3030.

3.3.2.3. *N*-(2-(1*H*-Indol-3-yl)ethyl)hexadecanamide (1c). Compound **1c** (254 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.88 (t, *J* = 6.9 Hz, 3H), 1.25 (m, 30H), 1.57 (m, 4H), 2.09 (t, *J* = 7.2 Hz, 2H), 2.98 (t, *J* = 6.9 Hz, 2H), 3.60 (t, *J* = 6.6 Hz, 1H), 3.62 (t, *J* = 6.6 Hz, 1H), 5.56 (s, 1H), 7.04 (d, *J* = 2.1 Hz, 1H), 7.13 (t, *J* = .8 Hz, 1H), 7.22 (t, *J* = 6.9 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.61 (d, *J* = 7.2 Hz, 1H), 8.06 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.2, 25.3, 25.7, 26.9, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 33.4, 36.9, 39.7, 111.2, 113.0, 118.7, 119.4, 122.0, 122.1, 127.4, 136.4, 173.1. IR ν cm^{-1}: 3387, 3267, 2918, 2850, 1630, 1553, 1456, 1377, 1340, 1293, 1225, 1094, 1011, 801, 739, 718, 608. MS(ESI) *m/z*: 421 ([M+Na]$^+$, 100). ESI-HRMS Calcd for [M+Na]$^+$ $C_{26}H_{42}N_2ONa$: 421.3195. Found: 421.3183.

3.3.2.4. *N*-(2-(1*H*-Indol-3-yl)ethyl)heptadecanamide (1d). Compound **1d** (256 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.88 (t, *J* = 6.6 Hz, 3H), 1.25 (m, 26H), 1.57 (m, 2H), 2.09 (t, *J* = 7.6 Hz, 2H), 2.98 (t, *J* = 6.8 Hz, 2H), 3.60 (t, *J* = 6.6 Hz, 1H), 3.62 (t, *J* = 6.6 Hz, 1H), 5.49 (s, 1H), 7.04 (d, *J* = 2.1 Hz, 1H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 8 Hz, 1H), 8.09 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.4, 25.7, 29.3, 29.4, 29.6, 29.7, 31.9, 36.9, 39.6, 111.2, 113.1, 118.7, 119.5, 121.9, 122.2, 127.3, 136.4, 173.1. IR ν cm^{-1}: 3393, 3267, 2918, 2850, 1649, 1629, 1552, 1471, 1456, 1423, 1377, 1339, 1300, 1225, 1094, 1067, 1010, 800, 739, 717. MS(ESI) *m/z*: 435 ([M+Na]$^+$, 100). Anal. Calcd for $C_{27}H_{44}N_2O{\cdot}1.16H_20$: C, 75.74, H, 10.76, N, 6.54. Found: C, 75.75, H, 10.75, N, 6.75.

3.3.2.5. *N*-(2-(1*H*-Indol-3-yl)ethyl)octadecanamide (1e). Compound **1e** (245 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.88 (t, *J* = 6.9 Hz, 3H), 1.25 (m, 27H), 1.57 (m, 2H), 2.09 (t, *J* = 7.2 Hz, 2H), 2.98 (t, *J* = 6.6 Hz, 2H), 3.60 (t, *J* = 6.3 Hz, 1H), 3.62 (t, *J* = 6.3 Hz, 1H), 5.45 (s, 1H), 7.03 (d, *J* = 2.1 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.61 (d, *J* = 7.2 Hz, 1H), 8.07 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.4, 25.7, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 36.9, 39.6, 111.2, 113.2, 118.8, 119.5, 121.9, 122.2, 127.4, 136.4, 173.1. IR ν cm^{-1}: 3392, 3267, 2918, 2850, 1649, 1629, 1553, 1456, 1424, 1377, 1339, 1298, 1224, 1094, 1013, 800, 739, 718, 609. MS(ESI) *m/z*: 449 ([M+Na]$^+$,

100). ESI-HRMS Calcd for $[M+Na]^+$ $C_{28}H_{46}N_2ONa$: 449.3508. Found: 449.3491.

3.3.2.6. *N*-(2-(1*H*-Indol-3-yl)ethyl)icosadecanamide (1f). Compound **1f** (563 mg, 50%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 0.88 (t, J = 6.8 Hz, 3H), 1.25 (m, 30H), 1.57 (m, 2H), 2.09 (t, J = 7.6 Hz, 2H), 2.98 (t, J = 6.8 Hz, 2H), 3.60 (t, J = 6.4 Hz, 1H), 3.62 (t, J = 6.4 Hz, 1H), 5.05 (s, 1H), 7.04 (s, 1H), 7.13 (t, J = 8.0 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 7.6 Hz, 1H), 8.10 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.4, 25.7, 29.3, 29.4, 29.5, 29.7, 31.9, 33.7, 36.9, 39.6, 111.2, 113.1, 118.7, 119.5, 121.9, 122.2, 136.6, 173.1. IR ν cm^{-1}: 3394, 3269, 2917, 2850, 1649, 1629, 1552, 1472, 1456, 1424, 1377, 1339, 1299, 1222, 1095, 1012, 913, 799, 739, 718, 669. MS(ESI) m/z: 477 ($[M+Na]^+$, 100). Anal. Calcd for $C_{30}H_{50}N_2O{\cdot}2.2H_20$: C, 77.84, H, 11.08, N, 6.05. Found: C, 77.84, H, 11.27, N, 6.31.

3.3.2.7. *N*-(2-(1*H*-Indol-3-yl)ethyl)tricosadecanamide (1g). Compound **1g** (96 mg, 88%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 0.88 (t, J = 6.6 Hz, 3H), 1.25 (m, 38H), 1.57 (m, 2H), 2.09 (t, J = 7.2 Hz, 2H), 2.98 (t, J = 6.4 Hz, 2H), 3.60 (t, J = 6.4 Hz, 1H), 3.62 (t, J = 6.4 Hz, 1H), 5.47 (s, 1H), 7.04 (d, J = 2.0 Hz, 1H), 7.13 (t, J = 7.6 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H), 8.02 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.4, 25.7, 29.3, 29.4, 29.5, 29.7, 31.9, 33.7, 36.9, 39.6, 111.2, 113.1, 118.7, 119.5, 121.9, 122.2, 136.6, 173.1. IR ν cm^{-1}: 3396, 3258, 2959, 2917, 2848, 1628, 1564, 1472, 1462, 1377, 1342, 1225, 1093, 983, 800, 738, 719, 612, 580, 560. MS(ESI) m/z: 497 ($[M+H]^+$, 100).

3.3.2.8. *N*-(2-(1*H*-Indol-3-yl)ethyl)-octadeca-9*Z*,12*Z*-dienamide (2). Compound **2** (372 mg, 81%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 0.90 (t, J = 6.8 Hz, 3H), 1.25 (m, 14H), 1.59 (t, J = 6.8 Hz, 2H), 2.06 (m, 4H), 2.10 (t, J = 7.2 Hz, 2H), 2.79 (t, J = 6.4 Hz, 2H), 2.98 (t, J = 6.4 Hz, 2H), 3.59 (t, J = 6.4 Hz, 1H), 3.62 (t, J = 6.4 Hz, 1H), 5.37 (m, 4H), 5.63 (s, 1H), 7.00 (d, J = 1.6 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 8.47 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 14.0, 22.5, 25.3, 25.6, 25.7, 27.1, 29.1, 29.2, 29.3, 29.4, 29.6, 31.5, 36.8, 39.7, 111.3, 112.8, 118.6, 119.3, 122.0, 127.3, 127.8, 128.0, 130.0, 130.2, 136.4, 173.2. IR ν cm^{-1}: 3386, 3264, 3010, 2920, 2851, 1650, 1629, 1555, 1456, 1425, 1377, 1340, 1299, 1269, 1225, 1126, 1094, 1069, 1011, 931, 801, 739, 719, 608, 580, 560. MS(ESI) m/z: 445 ($[M+Na]^+$, 100). Anal. Calcd for $C_{28}H_{42}N_2O{\cdot}1.0H_20$: C, 79.20, H, 10.44, N, 6.60. Found: C, 79.33, H, 10.37, N, 6.46.

3.3.2.9. *N*-(2-(1*H*-Indol-3-yl)ethyl)-15-hydroxypentadecanamide (3). To a solution of tryptamine (320 mg, 2 mmol, 1 equiv) and hydroxypentadecanoic acid (518 mg, 2 mmol, 1 equiv) in dichloromethane (40 mL) under nitrogen at room temperature was added triethylamine (6 mmol, 3 equiv), EDC (768 mg, 4 mmol, 2 equiv) and HOBt (405 mg, 3 mmol, 1.5 equiv). The mixture was stirred overnight at room temperature then quenched by adding water (20 mL) and extracted three times with dichloromethane (3 × 60 mL). The combined organic layers were washed three times dichloromethane (3 × 30 mL). Combined organic layers were washed with a saturated NH_4Cl aqueous solution then dried over $MgSO_4$, filtered and concentrated in vacuo. A flash chromatography on silica gel with a mixture of cyclohexane and ethyl acetate (95:5) led to a clear beige powder (182 mg, 60%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.25 (m, 22H), 1.57 (m, 4H), 2.09 (t, J = 7.2 Hz, 2H), 2.98 (t, J = 6.8 Hz, 2H), 3.59-3.65 (m, 4H), 5.48 (s, 1H), 7.04 (d, J = 1.6 Hz, 1H), 7.13 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H), 8.06 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 24.8, 25.4, 25.7, 26.3, 29.3, 29.4, 29.5, 30.4, 39.6, 63.1, 105.1, 111.2, 118.5, 118.8, 119.5, 121.9, 122.3, 127.4, 183.4. IR ν cm^{-1}: 3394, 3275, 2917, 2849, 2037, 1982, 1669, 1629, 1556, 1457, 1375, 1357, 1267, 1224, 1166, 1109, 1057, 1026, 1012, 974, 758, 741, 727, 704, 606. MS(ESI) m/z: 423 ($[M+Na]^+$, 100). ESI-HRMS Calcd for $[M+Na]^+$ $C_{25}H_{40}N_2O_2Na$: 423.2987. Found: 423.2978.

3.3.3. General procedure for syntheses of (4a–g, 5, 6)

To a solution of amide **1a** (356 mg, 1 mmol, 1 equiv) in tetrahydrofuran (10 mL) under nitrogen at 0 °C was added $LiAlH_4$ (8 mmol, 8 equiv) in small pieces. The mixture was refluxed and stirred for 2 h, then quenched by adding water (1 mL) and an aqueous solution of NaOH (1 M, 1 mL) was added until obtaining of a white precipitate. A filtration on celite® using ethyl acetate was used to remove the precipitate. The organic layer was then dried over $MgSO_4$, filtered and concentrated in vacuo. A flash chromatography on silica with a mixture of cyclohexane and ethyl acetate (60:40) led to the expected product.

3.3.3.1. *N*-(2-(1*H*-Indol-3-yl)ethyl)tridecan-1-amine (4a). Compound **4a** (287 mg, 84%). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 0.88 (t, J = 6.9 Hz, 3H), 1.25 (m, 21H), 1.46 (m, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.97 (m, 4H), 7.04 (d, J = 2.1 Hz, 1H), 7.12 (td, J = 7.8 Hz, J = 0.9 Hz, 1H), 7.20 (td, J = 7.2 Hz, J = 1.2 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 8.10 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.8, 27.4, 29.3, 29.6, 29.7, 30.1, 32.0, 50.0, 111.1, 114.2, 118.9, 119.2, 121.8, 122.0, 127.5, 136.4. IR ν cm^{-1}: 3387, 3275, 3139, 3063, 2916, 2849, 1622, 1553, 1504, 1467, 1453, 1376, 1358, 1341, 1309, 1221, 1104, 1078, 1010, 914, 886, 842, 804, 739, 722, 611, 592, 565. MS(ESI) m/z: 343 ($[M+H]^+$, 100). ESI-HRMS Calcd for $[M+H]^+$ $C_{23}H_{38}N_2$: 343.3113. Found: 343.3120. Anal. Calcd for $C_{23}H_{38}N_2{\cdot}1.4H_20$: C, 77.72, H, 11.18, N, 7.88. Found: C, 77.86, H, 11.11, N, 7.82.

3.3.3.2. *N*-(2-(1*H*-Indol-3-yl)ethyl)pentadecan-1-amine (4b). Compound **4b** (118 mg, 83%). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 0.89 (t, J = 6.9 Hz, 3H), 1.26 (m, 24H), 1.46 (m, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.97 (m, 4H), 7.04 (d, J = 1.8 Hz, 1H), 7.12 (td, J = 7.8 Hz, J = 1.2 Hz, 1H), 7.20 (td, J = 8.1 Hz, J = 1.2 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.64 (d, J = 7.5 Hz, 1H), 8.18 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.8, 27.4, 27.5, 29.4, 29.6, 29.7, 30.1, 31.9, 50.0, 111.1, 114.1, 118.9, 119.2, 121.8, 122.0, 128.4, 133.4. IR ν cm^{-1}: 3387, 2916, 2649, 1630, 1454, 1341, 1222, 1104, 1011, 914, 874, 805, 739, 721, 611, 592. MS(ESI) m/z: 371 ($[M+H]^+$, 100). ESI-HRMS Calcd for $[M+H]^+$ $C_{25}H_{42}N_2$: 371.3426. Found: 371.3419.

3.3.3.3. *N*-(2-(1*H*-Indol-3-yl)ethyl)hexadecan-1-amine (4c). Compound **4c** (121 mg, 70%). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 0.88 (t, J = 6.9 Hz, 3H), 1.25 (m, 26H), 1.47 (m, 2H), 2.61 (t, J = 7.2 Hz, 2H), 2.97 (m, 4H), 7.04 (d, J = 1.8 Hz, 1H), 7.11 (td, J = 8.1 Hz, J = 1.2 Hz, 1H), 7.19 (td, J = 8.1 Hz, J = 1.2 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 8.21 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.6, 27.3, 29.3, 29.5, 29.6, 29.7, 29.8, 30.0, 31.9, 50.0, 111.1, 113.8, 118.8, 119.2, 122.0, 127.4, 136.4. IR ν cm^{-1}: 3139, 3053, 2916, 2849, 1621, 1454, 1341, 1222, 1105, 1011, 910, 886, 803, 739, 721, 611, 592. MS(ESI) m/z: 385 ($[M+H]^+$, 100). ESI-HRMS Calcd for $[M+H]^+$ $C_{26}H_{44}N_2$: 385.3583. Found: 385.3569.

3.3.3.4. *N*-(2-(1*H*-Indol-3-yl)ethyl)heptadecan-1-amine (4d). Compound **4d** (122 mg, 90%). ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 0.88 (t, J = 6.9 Hz, 3H), 1.25 (m, 28H), 1.46 (m, 2H), 2.61 (t, J = 7.2 Hz, 2H), 2.97 (m, 4H), 7.05 (d, J = 2.1 Hz, 1H), 7.12 (t, J = 7.8 Hz, 1H), 7.20 (t, J = 7.5 Hz, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 8.05 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.9, 27.4, 29.3, 29.6, 29.7, 30.2, 31.9, 50.0, 111.1, 114.2, 118.9, 119.2, 121.8, 122.0, 127.5, 136.4. IR ν cm^{-1}: 3141, 3064, 2916, 2849, 1622, 1505, 1454, 1358, 1342, 1223, 1105,

1011, 891, 871, 841, 805, 739, 721, 612, 592. MS(ESI) *m/z*: 399 ([M+H]$^+$, 100). Anal. Calcd for $C_{27}H_{46}N_2 \cdot 0.98H_20$: C, 77.76, H, 11.61, N, 6.72. Found: C, 77.76, H, 11.36, N, 6.56.

3.3.3.5. *N*-(2-(1*H*-Indol-3-yl)ethyl)octadecan-1-amine (4e). Compound **4e** (156 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 0.88 (t, J = 6.9 Hz, 3H), 1.25 (m, 30H), 1.46 (m, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.98 (m, 4H), 7.04 (d, J = 1.8 Hz, 1H), 7.12 (t, J = 7.8 Hz, 1H), 7.20 (t, J = 7.8 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 8.11 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.4, 27.4, 29.4, 29.7, 30.0, 32.0, 49.9, 111.1, 114.0, 118.9, 119.2, 121.8, 121.9, 122.0, 127.5, 136.4. IR ν cm^{-1}: 3417, 2916, 2848, 1619, 1455, 1339, 1223, 1125, 1010, 805, 735, 600, 577. MS(ESI) *m/z*: 413 ([M+H]$^+$, 100). ESI-HRMS Calcd for [M+H]$^+$ $C_{28}H_{48}N_2$: 413.3896. Found: 413.3897.

3.3.3.6. *N*-(2-(1*H*-Indol-3-yl)ethyl)icosadecan-1-amine (4f). Compound **4f** (216 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 0.88 (t, J = 6.9 Hz, 3H), 1.25 (m, 34H), 1.54 (m, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.97 (m, 4H), 7.04 (d, J = 2.1 Hz, 1H), 7.11 (t, J = 7.8 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.98 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.8, 27.4, 29.4, 29.7, 30.1, 31.9, 49.9, 111.1, 114.1, 119.1, 119.6, 121.8, 122.0, 127.4, 136.4. IR ν cm^{-1}: 3244, 2916, 2849, 1626, 1455, 1341, 1221, 1104, 1011, 868, 806, 740, 609, 592. MS(ESI) *m/z*: 441 ([M+H]$^+$, 100). ESI-HRMS Calcd for [M+H]$^+$ $C_{30}H_{52}N_2$: 441.4209. Found: 441.4192.

3.3.3.7. *N*-(2-(1*H*-Indol-3-yl)ethyl)tricosadecan-1-amine (4g). Compound **4g** (110 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 0.88 (t, J = 6.8 Hz, 3H), 1.25 (m, 40H), 1.47 (m, 2H), 2.63 (t, J = 7.6 Hz, 2H), 2.99 (m, 4H), 7.06 (s, 1H), 7.12 (t, J = 7.6 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 8.03 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 14.1, 22.7, 25.6, 27.3, 29.2, 29.3, 29.4, 29.5, 29.7, 31.9, 49.8, 111.1, 113.9, 118.9, 119.3, 122.0, 127.4, 136.4. IR ν cm^{-1}: 3418, 2916, 2849, 1619, 1463, 1215, 1091, 805, 746, 735, 720, 668, 577. MS(ESI) *m/z*: 483 ([M+H]$^+$, 100).

3.3.3.8. *N*-(2-(1*H*-Indol-3-yl)ethyl)-octadeca-9*Z*,12*Z*-dien-1-amine (5). Compound **5** (307 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 0.89 (t, J = 6.8 Hz, 3H), 1.25 (m,14H), 1.49 (m, 2H), 2.04 (m, 4H), 2.64 (t, J = 7.2 Hz, 2H), 2.77 (t, J = 6.4 Hz, 2H), 3.00 (m, 4H), 5.34 (m, 4H), 7.04 (s, 1H), 7.11 (t, J = 7.6 Hz, 1H), 7.19 (t, J = 7.2 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 8.10 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 14.1, 22.6, 25.5, 25.6, 27.2, 27.3, 29.2, 29.3, 29.5, 29.6, 29.7, 31.5, 49.8, 111.1, 113.8, 118.9, 119.3, 121.9, 122.0, 127.4, 127.9, 128.0, 130.1, 130.2, 136.4. IR ν cm^{-1}: 3415, 3009, 2923, 2853, 1620, 1456, 1354, 1231, 1108, 1010, 803, 738, 625. MS(ESI) *m/z*: 409 ([M+H]$^+$, 100).

3.3.3.9. *N*-(2-(1*H*-Indol-3-yl)ethyl)-15-hydroxypentadecan-1-amine (6). Compound **6** (80 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.24 (m, 24H), 1.47 (m, 4H), 2.62 (t, J = 7.6 Hz, 2H), 2.97 (m, 4H), 3.64 (t, J = 6.8 Hz, 2H), 7.04 (d, J = 1.6 Hz, 1H), 7.11 (t, J = 7.6 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 8.03 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 25.7, 25.8, 27.4, 29.4, 29.5, 30.1, 32.8, 50.0, 63.0, 111.1, 114.2, 118.9, 119.3, 121.8, 122.0, 124.5, 136.4. IR ν cm^{-1}: 3425, 3299, 2923, 2848, 1668, 1466, 1457, 1374, 1355, 1216, 1149, 1108, 1044, 1024, 1010, 974, 760, 740, 727, 669, 587. MS(ESI) *m/z*: 387 ([M+H]$^+$, 100). Anal. Calcd for $C_{23}H_{42}N_2O \cdot 1.0H_20$: C, 74.21, H, 10.96, N, 6.92. Found: C, 74.30, H, 10.85, N, 6.63.

4. Conclusions

This study describes for the first time the biological properties of a rare group of tryptamine-derived natural alkaloids, highlighting promising neurotrophic properties.[41] Structure–activity relationships could be drawn, and from synthesis of their analogs with an improvement of activity by reducing the amide function into a secondary amine. This nitrogen seems to play a major role increasing dramatically the free-radical scavenging activity, in this series. The antioxidative capacity seems to correlate with the neuroprotective ability which could explain the dual activity of *N*-alkyltryptamines and also why *N*-acyltryptamines failed to protect DA neurons from death. Considering that neuroprotective effect is mainly due to an antioxidant mechanism, complementary experiments are required to elucidate the signaling pathways inherent to the neuritogenesis promotion by these compounds. Furthermore, a mass spectrometry peak area based quantification approach has been developed for the measurement of compound **4c** in brain tissues. The ability of compound **4c** to cross biological barriers, specially the BBB, is encouraging for further in vivo experiments in PD animal models. Despite the fact that these compounds could find applications in neurodegenerative disorders, this work should provide encouragement to study Annonaceae extracts for original biological activities such as their neurotrophic potential.

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