SUPPLEMENTARY MATERIAL

Isolation, *in vitro* evaluation and molecular docking of acetylcholinesterase inhibitors from South African Amaryllidaceae

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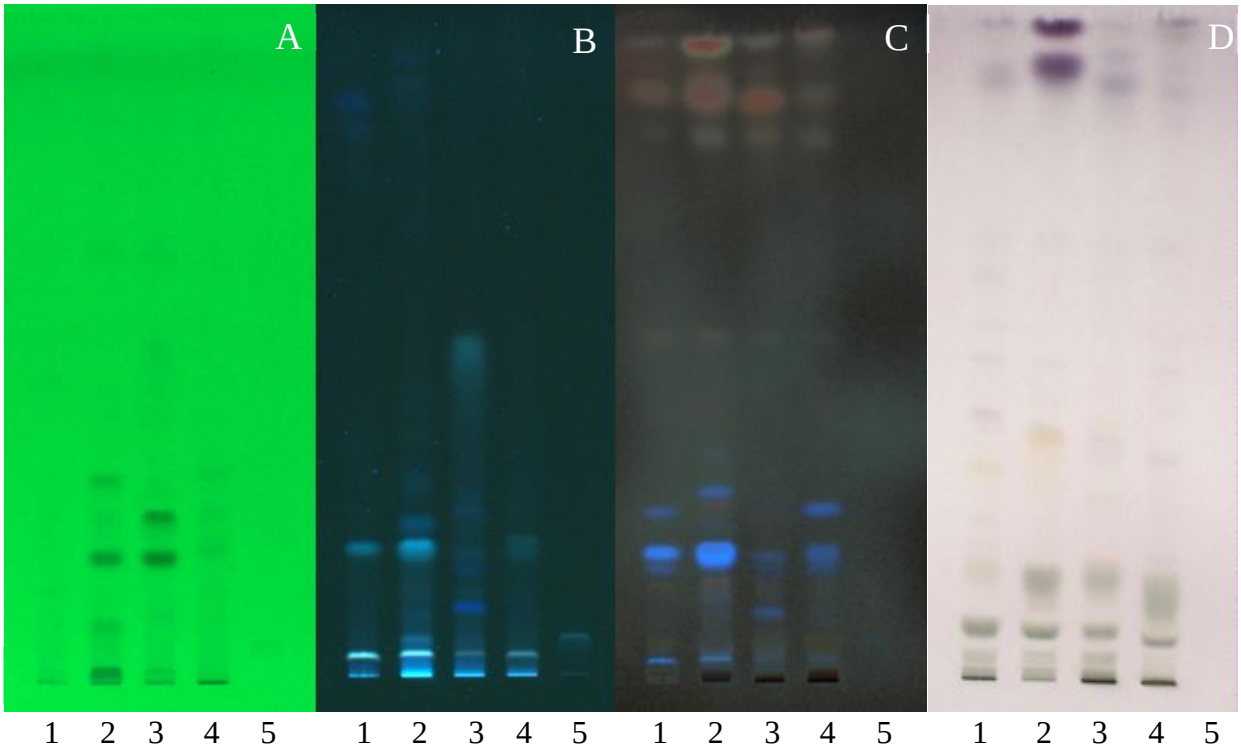

Fig. S1. HPTLC plates of the methanolic bulb extracts of some Amaryllidaceae species. Plates were viewed under A) UV 254 nm, B) UV 366 nm, C) UV 366 nm after derivatization with *p*-anisaldehyde-sulfuric acid (AS) and D) under white light. Solvent system: EtOAc: MeOH: H_2O (100:20:15), tracks (1) *Nerine undulata*, (2) *Amaryllis belladonna*, (3) *Haemanthus albiflos*, (4) *Nerine huttoniae* and (5) galanthamine.

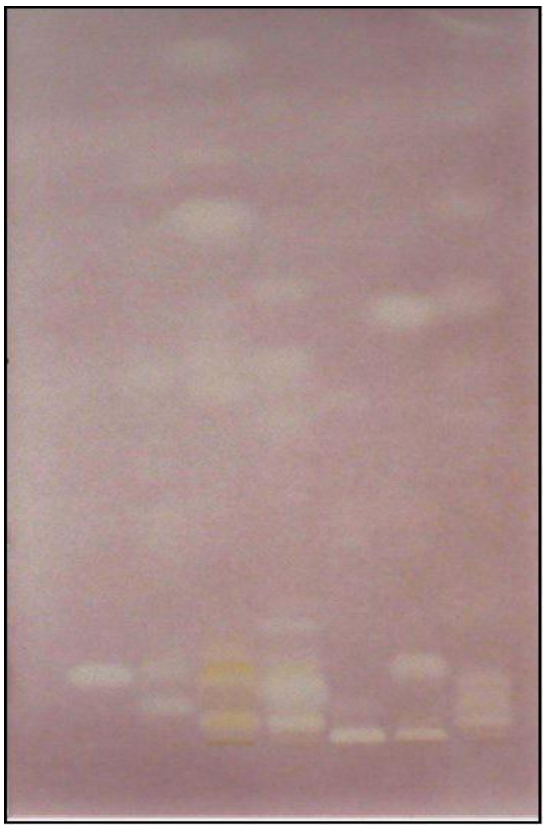

Fig. S2. Bio-autography plate of galanthamine and the methanolic extracts of (1) *Brunsvigia marginata*, (2) *Amaryllis belladonna*, (3) *Nerine huttoniae*, (4) *Haemanthus montanus*, (5) *Haemanthus albiflos*, (6) *Nerine undulata* (7), indicating the presence of several compounds with acetylcholinesterase inhibitory activity.

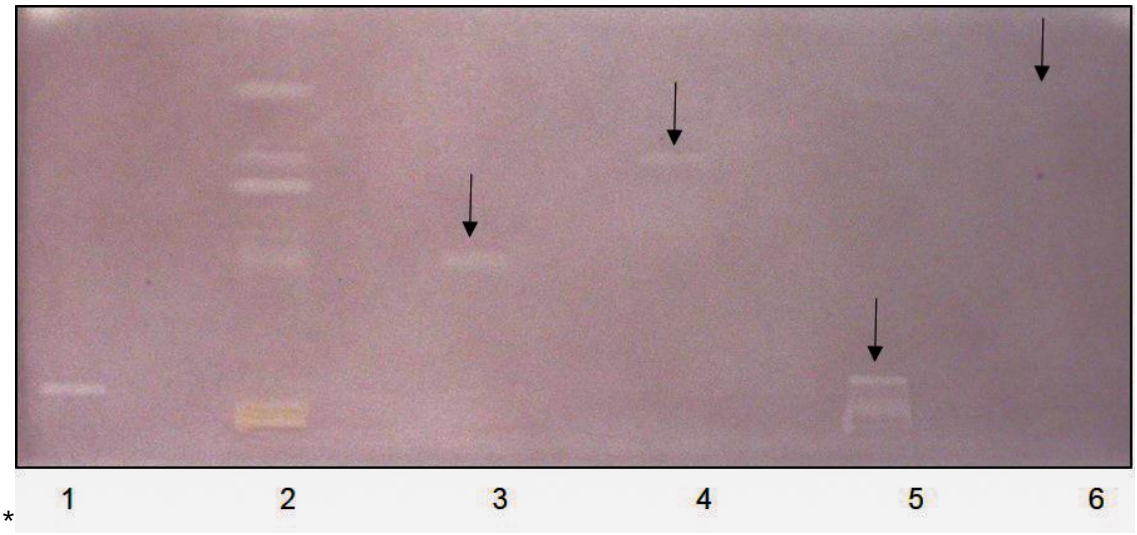

Fig. S3. TLC bio-autography plate indicating zones of acetylcholinesterase inhibition by pure compounds isolated from *Amaryllis belladonna*. Active zones of the isolated compounds are indicated with black arrows and observed as white bands against a purple background. Tracks (1) galanthamine, (2) *Amaryllis belladonna*, (3) compound [1], (4) compound [2], (5) compound [3] and (6) compound [4].

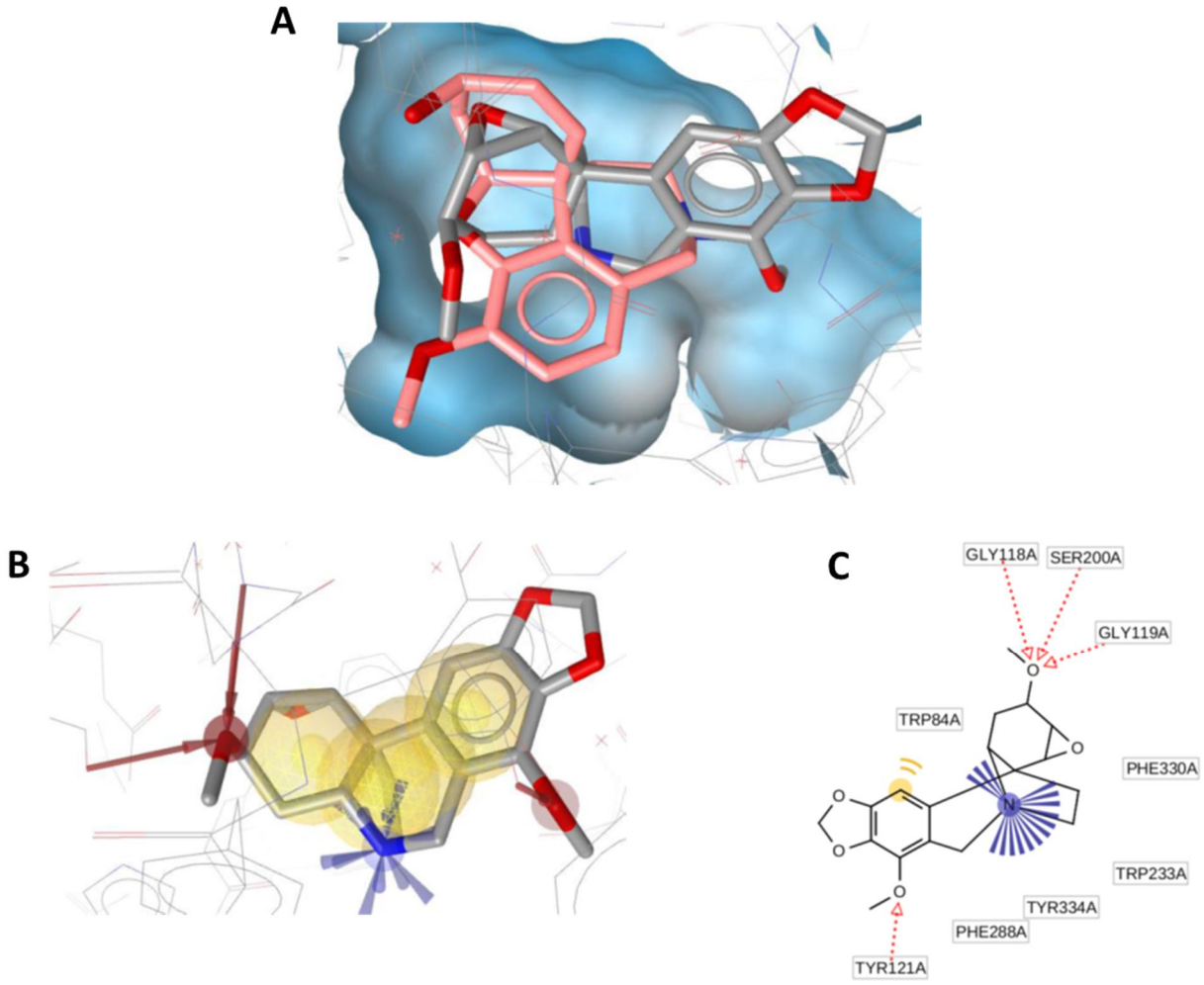

Fig. S4. Results of docking experiments using undalatine, (A) Predicted best-ranked binding pose for undulatine (grey), compared to the co-crystallized ligand galanthamine (rose red). (B) Protein contacts for AChE for the ligand undulatine (C) showing interactions with the aromatic cage amino acids.

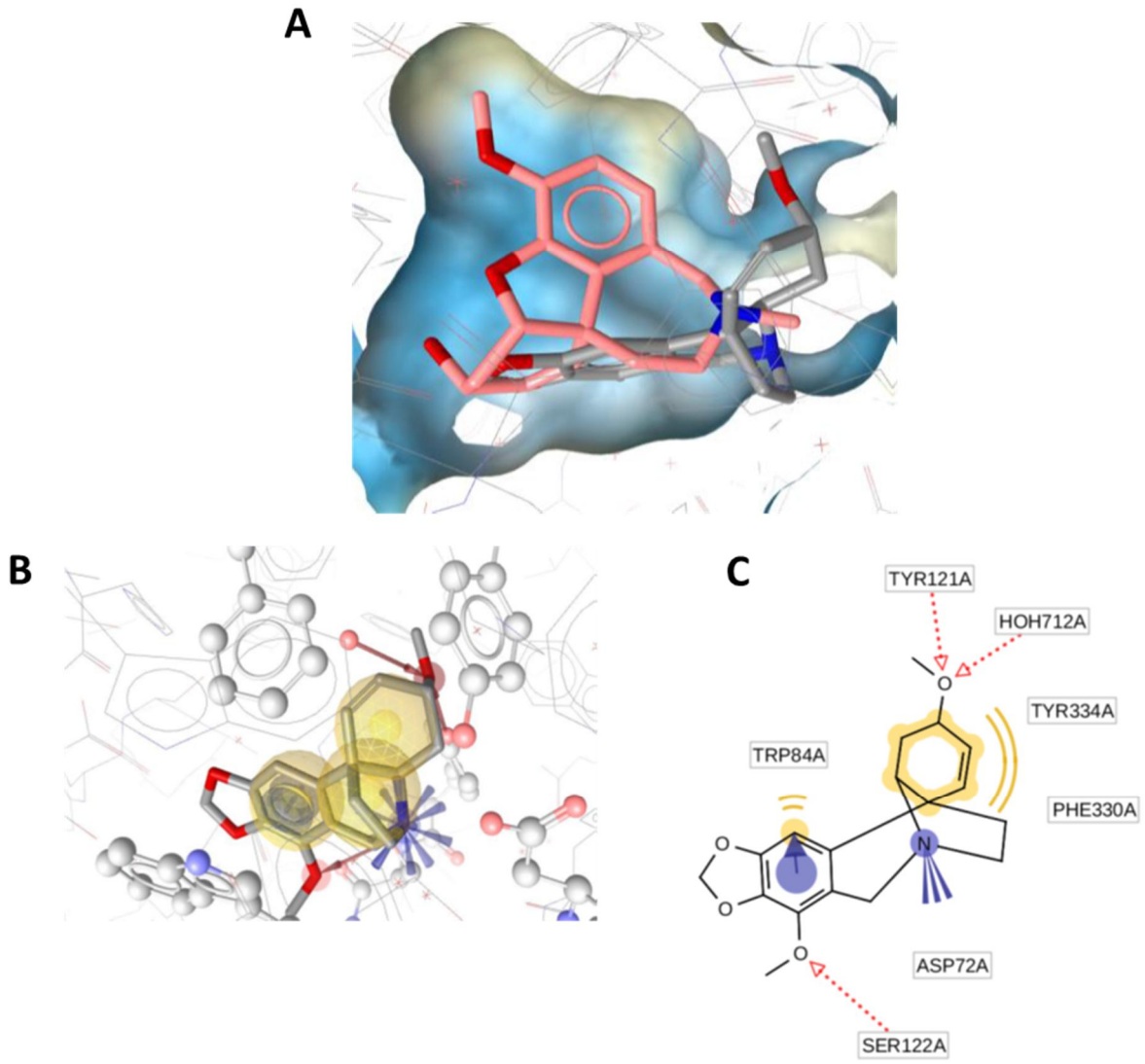

Fig. S5. Results of docking experiments using buphanidrine (A) Predicted best-ranked binding pose for buphanidrine (grey), compared to the co-crystallized ligand galanthamine (rose red). (B) Protein contacts for AChE for the ligand buphanidrine (C) showing interactions with the aromatic cage amino acids.

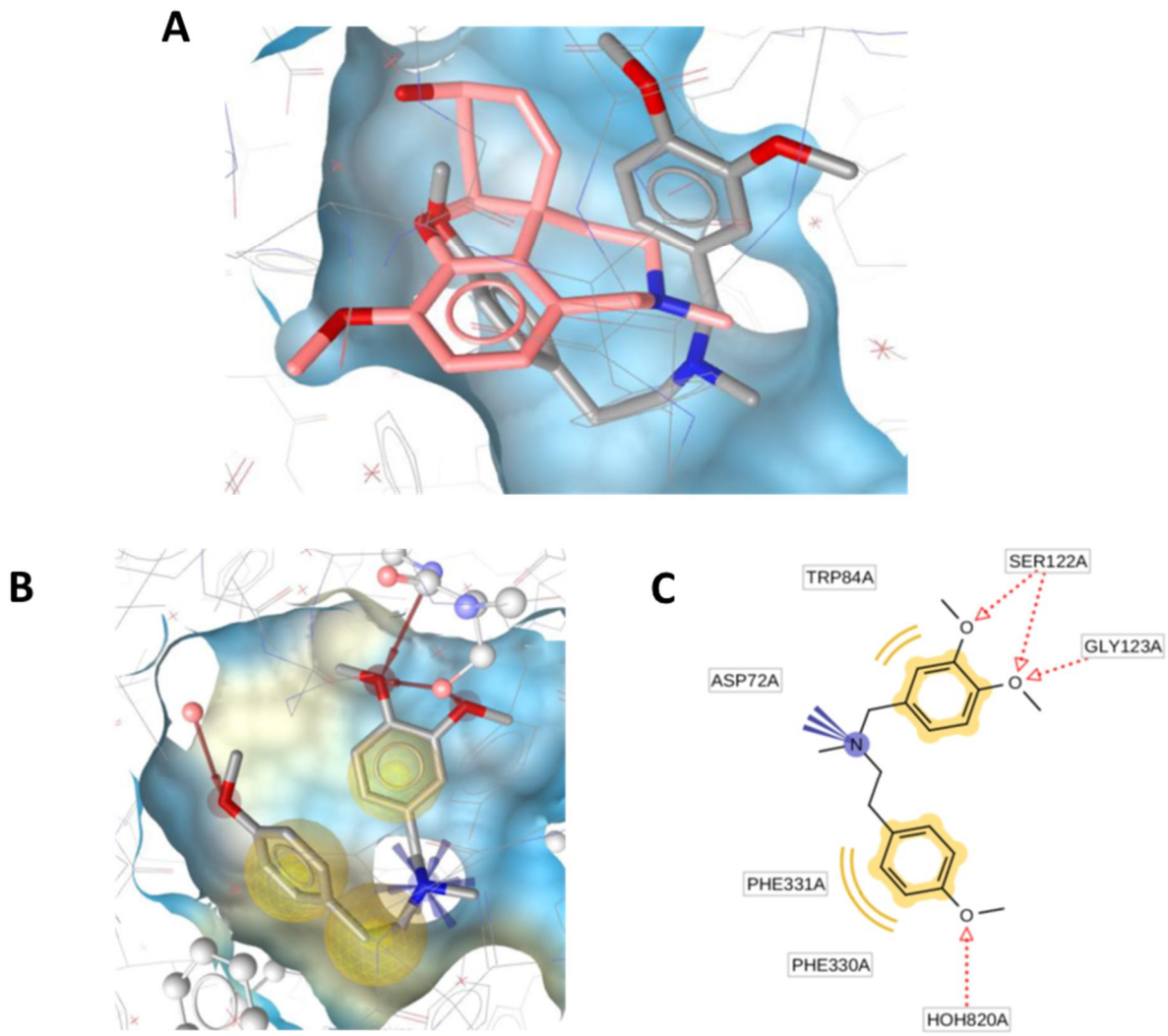

Fig. S6. Results of docking experiments using belladine (A) Predicted best-ranked binding pose for belladine (grey), compared to the co-crystallized ligand galanthamine (rose red). (B) Protein contacts for AChE for the ligand belladine (C) showing interactions with the aromatic cage amino acids.

Table S1
^{1}H NMR and ^{13}C NMR data for undulatine [1] (methanol-d4) compared to those reported by Viladomat et al. [24] ($CDCl_3$).

Position	δ^{1}H (observed) (ppm), *J** (Hz)	δ^{1}H (literature) (ppm), *J* (Hz)	δ^{13}C observed (ppm)	δ^{13}C (literature) (ppm)
1	3.85, 1H, d, *J* 3.5	3.71, 1H, d, *J* 3.5	54.30, d	53.9, d
2	3.33, 1H, m*	3.32, 1H, dd, *J* 2.5, 3.5	55.82, d	55.1, d
3	3.99, 1H, m	3.96, 1H, m	76.06, d	74.8, d
4α	2.0, 1H, ddd, *J* 3.7, 9.1, 12.9	2.11, 1H, br d	25.9, t	25.2, t
4β	1.44, 1H, ddd, *J* 3.0, 13.7, 13.7	1.42, 1H, ddd *J* 3.0, 13.5, 13.5		
4a	3.33, 1H, dd	3.25, 1H, dd, *J* 3.4, 13.5	62.52, d	61.2, d
6a	-	-	117.60, s	117.8, s
6α	4.17, 1H, d, *J* 17.1	4.35, 1H, d, *J* 17.0	59.27, t	58.6, t
6β	3.77, 1H, d, *J* 16.9	3.88, 1H, d, *J* 17.0		
7	-	-	142.31, s	140.9, s
8	-	-	134.87, s	133.3, s
9	-	-	150.05, s	147.9, s
10	6.78, 1H, s	6.60, 1H, s	97.60, d	96.3, d
10a	-	-	139.92, s	138.9, s
10b	-	-	43.01, s	41.4, s
11 endo	1.73, 1H, dd, *J* 13, 8.4, 13	2.10, 1H, ddd, *J* 5.0, 9.0, 12.5	39.84, t	39.2, t
11 exo	2.44, 1H, *J* 5.5, 11.5, 11.9	2.49, 1H, ddd, *J* 6.0, 11.0, 12.5		
12 endo	2.83, 1H, ddd, J 5.8, 9.4, 13.2	2.92, 1H, ddd, *J* 6.0, 9.0, 12.8	53.03, t	52.5, t
12 exo	3.33,1H, m*	3.42, 1H, m	-	-
3-OMe	3.44, 3H, s	3.42, 3H, s	57.90, q	57.5, q
7-OMe	3.99, 3H, s	3.97, 3H, s	59.67, q	59.0, q
O-CH_2-O	5.85, 2H, d	5.87-5.88, 2H, d, *J* 1.5	102.14, t	100.5, t

*Overlap of signals, m: multiplet, br: broad, s: singlet, d: doublet, ddd: doublet-doublet-doublet, t: triplet, q: quartet

Table S2
^{1}H NMR and ^{13}C NMR data for buphanidrine [2] (methanol-d4) compared to those reported by Viladomat et al. [24] ($CDCl_3$).

Position	$\delta^{1}H$ (observed) (ppm) / *J* (Hz)	$\delta^{1}H$ (literature) (ppm)/ *J* (Hz)	$\delta^{13}C$ (observed) (ppm)	$\delta^{13}C$ (literature (ppm)
1	6.64, 1H, d, *J* 8.6	6.58, d, *J* 9.2	133.4, d	132.1, d
2	6.02,1H, dd, *J* 10.0, 5.3	5.96, ddd, *J* 10.0, 5.2, 1.3	126.4, d	125.5, d
3	3.84, 1H, m[&]	3.82, ddd, *J* 1.9, 4.0, 5.5	73.7, t	72.2, d
4	-	-	29.3, t	28.0 , t
4α	2.05, 1H, m	2.11, m	-	-
4β	1.70,1H, dt, *J* 3.2, 12.9	1.60, dt, *J* 4.1, 13.5	-	-
4a	3.31, 1H, m^	3.31, dd, *J* 4.2, 13.5	64.1, d	62.8, d
6	4.21, 1H, d, *J* 16.9	4.25, d, *J* 17.4	59.1, t	58.1, t
6a	3.84, 1H, m[&]	3.81, d, *J* 17.4	116.7, s	115.8, s
7	-	-	142.2, s	140.8, s
8	-	-	134.9, s	133.4, s
9	-	-	150.2, s	148.2, s
10	6.64, 1H, s	6.56, s	98.0, d	96.9, d
10a	-	-	140.1, s	138.6, s
10b	-	-	45.7, s	44.3, s
11	-	-	44.5, t	43.4, t
11 endo	2.17, 1H, m	2.15, ddd, *J* 4.4, 9.3, 12.6	-	-
11 exo	1.95, 1H, m	1.92, ddd, *J* 6.0, 10.6, 12.2	-	-
12	-	-	54.1, t	53.3, t
12 endo	2.99, 1H, m	2.88, ddd, *J* 6.0, 9.2, 13.5	-	-
12 exo	3.37,1H, m^	3.37, m	-	-
3-OMe	3.36, 3H, s	3.36, s	56.7, q	56.5, q
7-OMe	3.99,3H, s	3.95, s	59.7, q	59.1, q
O-CH_2-O	5.87, 2H, s	5.84, d, - 5.85, d, *J* 1.5	102.1, t	100.6, t

^, [&] peaks overlap, m: multiplet, br: broad, s: singlet, d: doublet, dd: doublet-doublet

Table S3
^{1}H NMR and ^{13}C NMR data for belladine [3] (methanol-d4) compared to those reported by Nair et al. [25].

Position	δ ^{1}H observed (ppm), *J* (Hz)	δ^{1}H (literature) (ppm), *J* (Hz)	δ^{13}C observed (ppm)	δ^{13}C (literature) (ppm)
1	2.52, 2H, m	2.60 m	60.0, t	59.1, t
2	2.67, 2H, m	2.77 m	33.1, t	32.9, t
3	-	-	133.1, s	132.5, s
4	6.98, 1H, d, *J* 8.5	7.10, *J* 8.7	130.7, d	129.6, d
5	6.74-6.84, d*	6.82, d, *J* 8.7	114.9, d	113.7, d
6	-	-	159.6, s	157.8, s
7	6.74-6.84, d*	6.82, d, *J* 8.7	114.9, d	113.7, d
8	6.98, 1H, d, *J* 8.5	7.10, d, *J* 8.7	130.7, d	129.6, d
1’	3.46, 2H, br s	3.49, br s	62.5, t	62.0, t
2’	-	-	131.4, d	131.6, s
3’	6.74-6.84, d**	6.85, br s	112.6, d	110.6, d
4’	-	-	150.4, s	148.8, s
5’	-	-	150.0, s	147.9, s
6’	6.74-6.84, d**	6.79, br s	114.3, d	111.9, d
7’	6.74-6.84, d*	6.79, br s	123.3, d	121.0, d
6-OCH_3	3.65, 3H, s	3.78, s	56.4, q	55.2, q
4’-OCH_3	3.70, 3H, s,	3.86, s	56.4, q	55.8, q
5’-OCH_3	3.71, 3H, s	3.87, s	55.6, q	55.8, q
N-CH_3	2.21, 3H, s	2.28, s	42.2, q	42.2, q

*overlap of aromatic signals, m: multiplet, br: broad, s: singlet, d: doublet, dd: doublet-doublet

Table S4
^{1}H NMR and ^{13}C NMR data for acetylcaranine [4] (methanol-d4) compared to those reported by Pettit et al. [26] ($CDCl_3$)

Position	δ^{1}H (observed) (ppm) /*J* (Hz)	δ^{1}H (literature) (ppm)/ *J* (Hz)	δ^{13}C (observed) (ppm)	δ^{13}C (literature) (ppm)
1	5.80,$^{\$}$ 1H, br dd (*J* 4.1, 1.8)	5.84, 1H, br dd (*J* 4.5, 2.0)	67.94, d	66.60, d
2 α	2.35, 1H, m	2.39, 1H, m	34.17, t	33.55, t
2 β	2.61, 1H, m	2.64, 1H, m	34.17, t	33.55, t
3	5.34,1H, dd, *J* 4.7, 2.3	5.39, 1H, dd, *J* 4.9, 2.4	116.00, d	114.43, d
4	-	-	139.75, s	139.56, s
4a	2.79, 1H, d, *J* 10.7	2.82, 1H, d, *J* 10.18	29.25, d	61.51, d
6α,	3.44, d, *J* 14.3	3.54, 1H, d, *J* 14.3	57.67, t	57.09, t
6β	4.01, d, *J* 14.3	4.13, 1H, d, *J* 14.1	57.67, t	57.09, t
6a	-	-	130.29, s	129.54, s
7	6.53, 1H, s	6.57, 1H, br s	108.27, d	107.34, d
8	-	-	147.9, s	146.24, s
9	-	-	148.13, s	146.49, s
10	6.65,1H, s	6.72, 1H, d, *J* 1.0	105.98, d	105.25, d
10a	-	-	128.79, s	127.79, s
10b	2.61, 1H, m	2.67, 1H, m	44.22, d	43.56, d
11 α,	2.48-2.63, 1H, m	2.61, 1H, m	29.26, t	28.70, t
11 β	2.48-2.63, 1H, m	2.61, 1H, m	29.26, t	28.70, t
12 α	2.36, 1H, m	2.39, 1H, m	54.61, t	53.83, t
12 β	3.21, 1H, m$^{\&}$	3.33, 1H, ddd (*J* 9.4, 4.9, 4.4)	54.61, t	53.83, t
O-CH_2-O	5.80$^{\$}$, br s	5.91-5.92, 2H, d, *J* 1.5	102.44, t	101.16, t
$OCOCH_3$	1.78, 3H, s	1.93, 3H, s	20.95, q	21.53, q
$OCOCH_3$	-	-	172.42, s	171.03, s

$^{\&}$ overlap with the solvent peak, $^{\$}$overlap of signals, m: multiplet, s: singlet, d: doublet