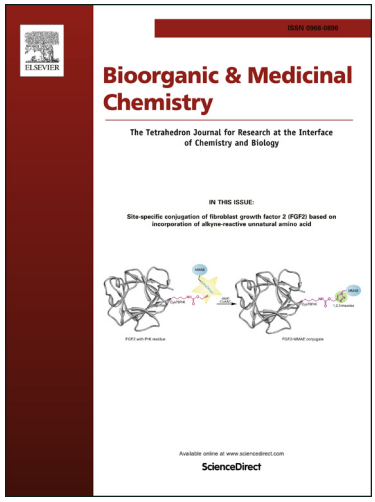

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Structure-activity relationship studies on thiaplidiaquinones A and B as novel inhibitors of *Plasmodium falciparum* and farnesyltransferase

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ABSTRACT

Marine meroterpenoids, thiaplidiaquinones A and B and their respective non-natural dioxothiazine regioisomers have been shown to inhibit mammalian and protozoal farnesyltransferase (FTase) with the regioisomers exhibiting activity in the nanomolar range. In order to explore the structure-activity relationship (SAR) of this class of marine natural products, analogues of thiaplidiaquinones A and B and their regioisomers were synthesised, with variation in the number of isoprene units present in their side chains to afford prenyl and farnesyl analogues. The previously reported geranyl series of compounds were found to be the most potent FTase inhibitors closely followed by the novel farnesyl series. The prenyl series exhibited the most potent anti-plasmodial activity but the series was also the most cytotoxic. Overall, the farnesyl series exhibited moderate anti-plasmodial activity with one analogue, **14** also exhibiting low cytotoxicity, identifying it as a scaffold worthy of further exploration.

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1. Introduction

Prenylating enzymes, farnesyltransferase (FTase), geranylgeranyltransferase type I (GGTase I) and Rab geranylgeranyltranferase (RabGGTase), are involved in posttranslational modification by farnesylation or geranylgeranylation of proteins which ensures membrane association.[1,2] Inhibitors of these enzymes are well known in the field of cancer drug discovery and have been recently investigated as potential treatments for several neglected diseases.[1,2] For example, *Plasmodium falciparum*, one of the causative agents of malaria which claims the lives of around 1–3 million people every year[1], expresses prenylating enzymes such as FTase whose inhibition has been shown to impair the growth of the parasite.[2] Given mammalian cells also utilise prenylating FTases, it is essential that FTase-inhibiting anti-malarials exhibit selectivity towards the parasitic enzyme over the mammalian variant.[1,2]

Benzo[*c*]chromene-7,10-dione natural products, reported from both terrestrial and marine sources, exhibit a variety of biological activities including the inhibition of FTases.[3–6] For example, thiaplidiaquinones A (**1**) and B (**2**) (**Figure 1**), isolated from the ascidian *Aplidium conicum*, have been shown to induce apoptosis in Jurkat cells by a mechanism involving the rapid production of reactive oxygen species.[7] A recent study by Harper *et al*. reported a more extensive biological evaluation of synthetically prepared thiaplidiaquinones A (**1**) and B (**2**) and their respective non-natural dioxothiazine regioisomers **3** and **4** from which **1**, **3** and **4** were identified to be potent inhibitors of human and *Trypanosoma brucei* FTase, with **4** exhibiting activity in the nanomolar range (**Table 1**).[8] In addition, both **3** and **4** exhibited moderate anti-plasmodial activity against *P. falciparum* (IC_{50} 4.56±0.76 µM and 4.39±0.77 µM, respectively) with **3** also exhibiting moderate anti-proliferative activity against melanoma cell lines leading to the conclusion that the dioxothiazine regioisomers were more bioactive than their natural product counterparts.[8]

Modifications of the prenylated side chains of natural products have been shown to modulate biological activity.[6,9] For example, analogue studies conducted on mallotojaponin B showed that an increase in the number of prenyl units on the side chain met with an increase in anti-plasmodial and FTase inhibitory activity while studies on tecomaquinone I have shown that such an increase leads to a decrease in FTase activity.[6,9] To expand on the previously reported SAR of the thiaplidiaquinones (**1**–**4**), a novel series of analogues bearing a prenyl (**7**–**10**) or farnesyl (**11**–**14**) side chain were prepared and evaluated for their anti-plasmodial, anti-bacterial and FTase inhibitory activity as well as their cytotoxicity against a rat skeletal muscle cell line. Herein we report the synthesis of a small set of analogues to explore the influence of the geranyl side chain of **1**–**4** on their observed biological activities.

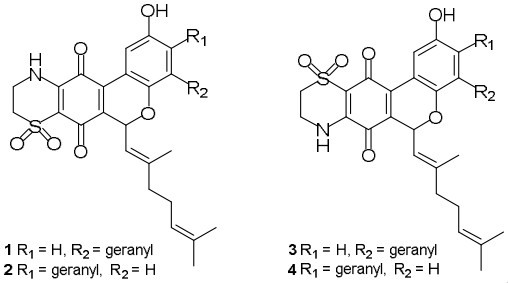


Figure 1 Structures of thiaplidiaquinones A (**1**) and B (**2**) and regioisomers **3** and **4**.

2. Results and discussion

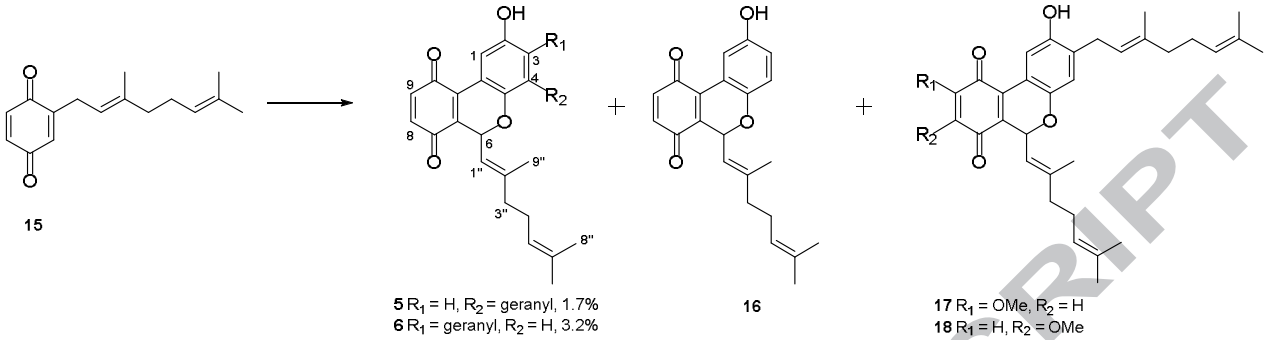
2.1. Synthesis

A biomimetic synthesis of thiaplidiaquinone A (**1**) and B (**2**) was reported by Khalil *et al*. using a strategy which also yielded their respective regioisomers **3** and **4**.[10] This synthetic approach was based upon ring closure of tautomerised benzochromenediones **5** and/or **6** by a facile oxa-6π-electrocyclisation process to construct the benzo[*c*]chromene-7,10-dione scaffold, followed by addition of hypotaurine to form the dioxothiazine ring.[10] To confirm the reproducibility of this route, thiaplidiaquinones A (**1**) and B (**2**) and regioisomers **3** and **4** were synthesised according to the procedure reported by Khalil *et al*..[10] Reaction of geranyl benzoquinone (**15**) with triethylamine for 2 minutes in CH_2Cl_2 followed by exposure to silica gel overnight afforded benzochromenediones **5** and **6** after purification in lower yields than Khalil *et al*. (**Scheme 1**). Further investigation of minor products from this reaction that eluted with increments of methanol identified trace amounts of the known benzochromenedione **16**[11] along with methoxy substituted benzochromenediones **17** and **18**, characterised as a 5:2 mixture. Benzochromenedione **16** was speculated to be a result of the reaction of starting material **15** with benzoquinone while methoxy benzochromenediones **17** and **18** were speculated to arise from the solvolytic incorporation of methanol onto benzochromenedione **6**. It is not possible however to discern whether **16** is a result of the reaction with benzoquinone, which was not detectable in samples of **15**, or reaction with a further equivalent of geranyl benzoquinone (**15**) followed by cleavage of the pendant geranyl group.

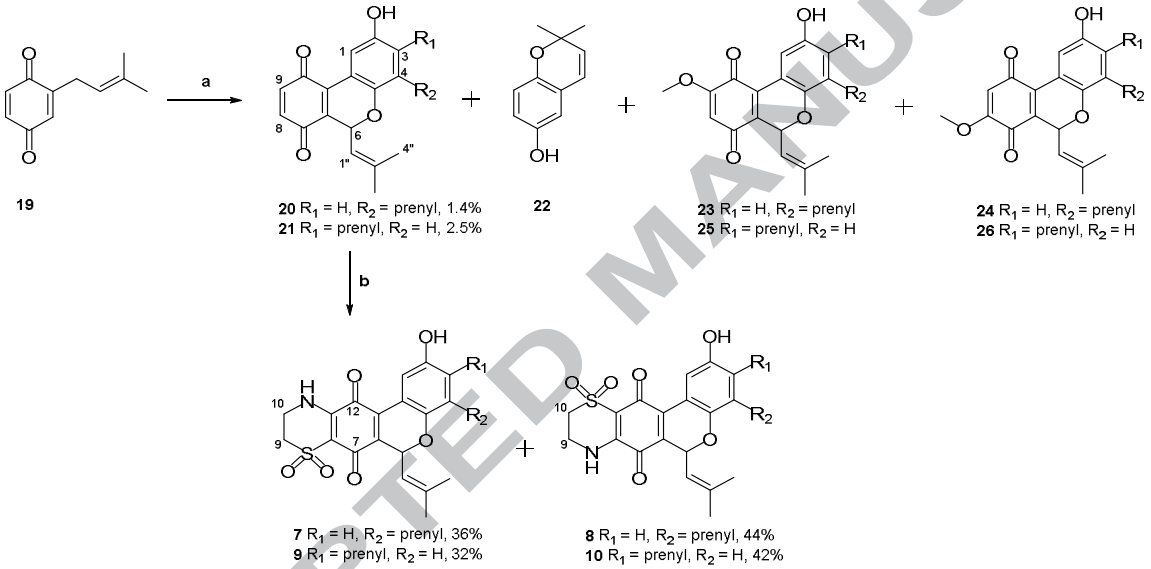
Reaction of benzochromenedione **5** with hypotaurine in CH_3CN/EtOH for two days followed by purification by silica gel column chromatography afforded thiaplidiaquinone A (**1**), as a red oil, and regioisomer **3**, as a green oil, in yields of 25% and 38%, respectively. Similarly, reaction of **6** with hypotaurine afforded thiaplidiaquinone B (**2**), as a purple oil, and regioisomer **4**, as a blue oil, in yields of 28% and 31%, respectively. Compounds **1** and **3** were synthesised in yields analogous to those reported by Khalil *et al*. while **2** and **4** were obtained in higher yields than those reported and was speculated to be a result of the increase in the scale of the reaction.

The prenyl series of analogues were prepared by reaction of prenyl benzoquinone (**19**) with triethylamine followed by exposure to silica for 2 days to afford prenyl benzochromenediones **20** and **21** as purple solids in 1.4% and 2.5% yield, respectively (**Scheme 2**). In addition, chromenol **22**, a known sea squirt natural product,[12] was also purified in trace amounts from the same reaction. As observed

for the geranyl series, minor reaction products, eluting with increments of methanol, comprising of inseparable mixtures of methoxy substituted benzochromenediones **23** and **24** (5:2) and **25** and **26** (5:2) were also purified from the crude reaction product.
Prenyl benzochromenedione **20** was reacted with hypotaurine followed by purification to afford prenyl thiaplidiaquinone **7**, as a pale red oil, and the dioxothiazine regioisomer **8**, as a grey oil, in yields of 36% and 44%, respectively. Similarly, reaction of prenyl regioisomer **21** with hypotaurine afforded prenyl thiaplidiaquinone **9**, as a purple oil, and regioisomer **10**, as a blue oil, in yields of 32% and 42%, respectively.



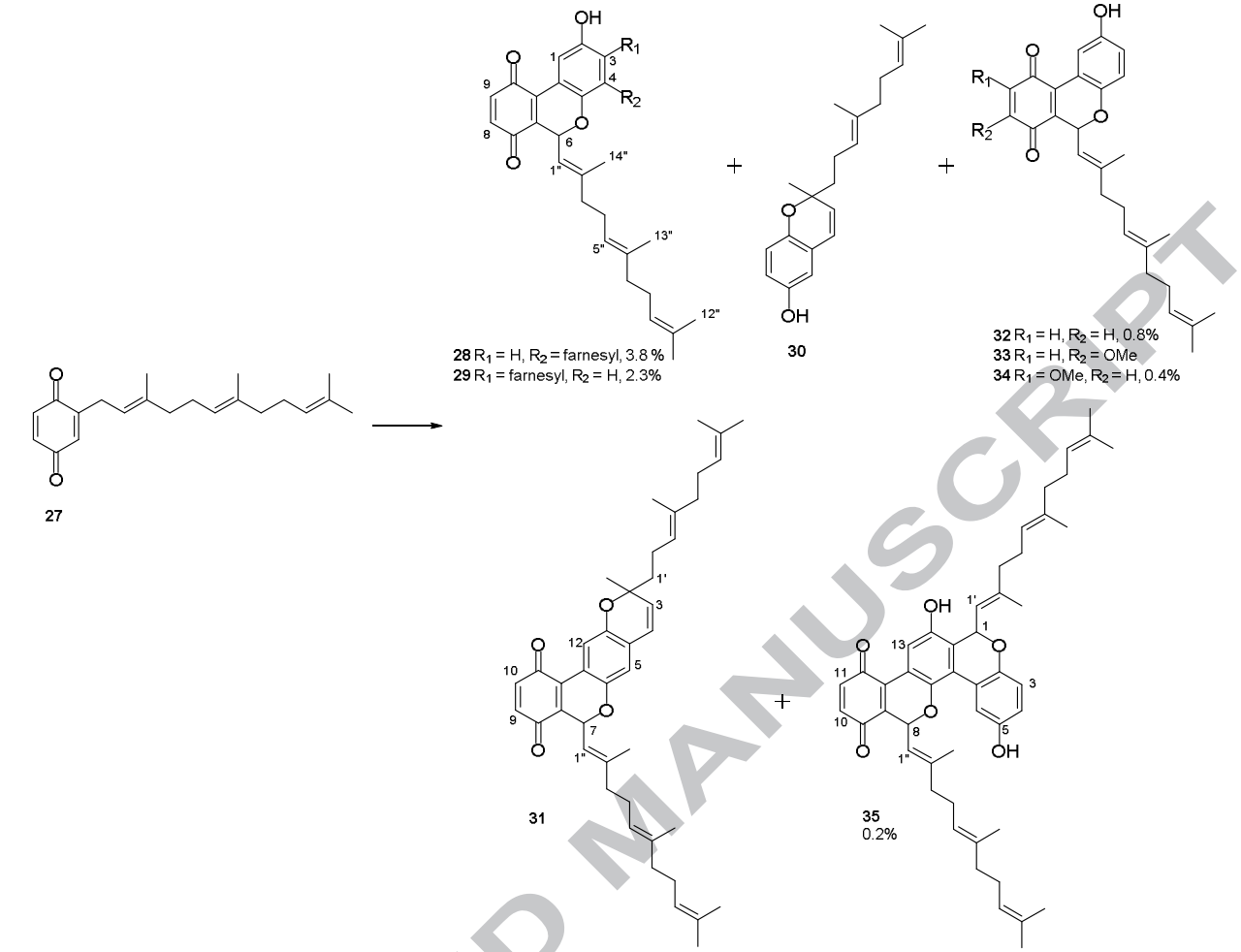
Scheme 1 Synthesis of geranyl benzochromenediones **5** and **6** from geranyl benzoquinone (**15**).
Reagents and conditions: (i) Et_3N (5 eq.), CH_2Cl_2, 2 min, (ii) SiO_2.



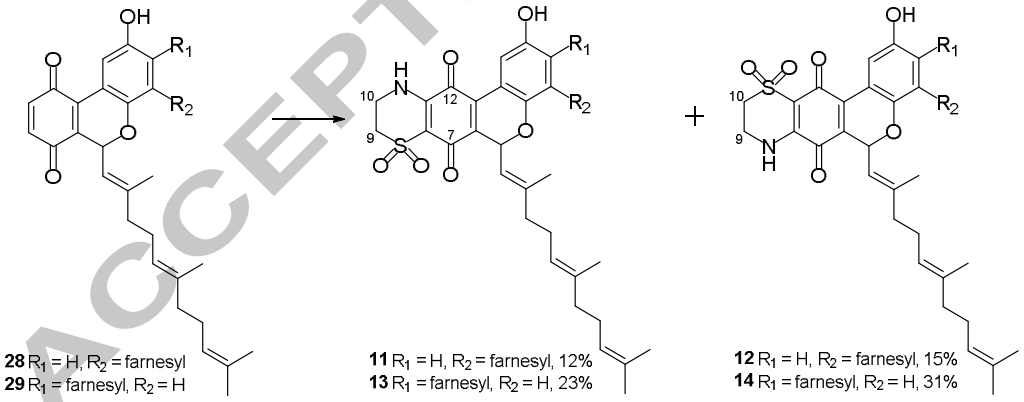
Scheme 2 Synthesis of prenyl thiaplidiaquinone analogues and regioisomers **7**–**10** from prenyl benzoquinone (**19**).
Reagents and conditions: (a) (i) Et_3N (5 eq.), CH_2Cl_2, 2 min, (ii) SiO_2; (b) hypotaurine (1 eq.), CH_3CN:EtOH (1:1), H_2O, 48 h.

Preparation of the farnesyl series of analogues proceeded in a similar manner. Reaction of farnesyl benzoquinone (**27**) with triethylamine and subsequent exposure to silica overnight afforded the desired benzochromenedione analogues **28** and **29** as purple oils in 3.8% and 2.3% yield, respectively. In addition, minor products including trace amounts of chromenol **30**, a known natural product isolated from *Dictyopteris undulata*,[13] and a mixture of diastereomeric bischromenes **31A** and **31B** were also purified (**Scheme 3**). Increasing the percentage of MeOH in the column chromatography eluent afforded farnesyl benzochromenedione **32** (0.8%), methoxy benzochromenedione **33** (as a 1:1 mixture with **34**), methoxy benzochromenedione **34** (0.4%) and benzochromene dimer **35** (0.2%). The structures of chromenols **30** and **31** represent products arising from the cyclisation of starting material **27** and benzochromenedione **29**, respectively, while products **32**–**35** appear to be a result of the reaction of starting material **27** with benzoquinone (**32**–**34**), as observed in the geranyl series, or of **29** with benzoquinone (**35**).

Farnesyl benzochromenedione **28** was reacted with hypotaurine for 2 days (**Scheme 4**) to afford farnesyl thiaplidiaquinone **11**, as a red oil, and regioisomer **12**, as a green oil, in yields of 12% and 15%, respectively. Similarly, reaction of benzochromenedione **29** with hypotaurine afforded farnesyl thiaplidiaquinone **13**, as a purple oil, and regioisomer **14**, as a blue oil, in yields of 23% and 31%, respectively.



Scheme 3 Synthesis of farnesyl benzochromenediones **28** and **29** from farnesyl benzoquinone (**27**).
Reagents and conditions: (i) Et_3N, CH_2Cl_2, 2 min, (ii) SiO_2.



Scheme 4 Synthesis of farnesyl thiaplidiaquinone analogues and regioisomers **11**–**14**.
Reagents and conditions: Hypotaurine (1 eq.), CH_3CN:EtOH (1:1), H_2O, 48 h.

2.2. Bioactivity of compounds

Biological testing was undertaken on prenyl and farnesyl thiaplidiaquinone analogues; **7**–**10** and **11**–**14** respectively, as well as methoxy benzochromenediones **23**–**26**, **33** and **34**, benzochromenedione **32** and dimer **35**. Methoxy analogues **23**–**26** were tested as their respective mixtures. These compounds were tested for their ability to inhibit both human and parasitic FTase enzymes (**Table 1**)

and for whole cell/organism growth inhibitory properties against *P. falciparum* (FcM29 or NF54 strains), rat skeletal myoblast cells (L6) and bacteria (*S. aureus* and *S. intermedius*) (**Table 2**).

The parasitic FTase used for the inhibition assays was from *Trypanosoma brucei* as it shows high sequence homology to *P. falciparum* FTase[1] and is more readily available while the human variant serves as a control to observe selectivity for parasitic FTase over human FTase. FTase assays revealed farnesyl thiaplidiaquinone A **11** as the most active of all the new analogues against both FTases with activity in the low micromolar range (**Table 1**). In addition, farnesyl thiaplidiaquinone **13** also exhibited potent inhibitory activity against the human variant of the enzyme. In general, the farnesyl series (**11**–**14**) was more active than the prenyl series (**7**–**10**), however, none of the analogues was as active as the geranyl series (**1**–**4**). None of the compounds in all three series exhibited good selectivity for parasitic FTase over human FTase.

Anti-plasmodial assays against NF54 strain of chloroquinone-sensitive *P. falciparum* revealed both the prenyl and farnesyl series of analogues (**7**–**14**) were more active than the geranyl series (**1**–**4**). The prenyl and farnesyl analogues exhibited moderate activity against *P. falciparum*, with the exception of the most potent prenyl regioisomer **10** which exhibited activity in the low micromolar range (IC_{50} 0.29 µM). Cytotoxic assays against an L6 rat skeletal myoblast cell line revealed the prenyl series to be more cytotoxic than the farnesyl series with prenyl regioisomer **10** (IC_{50} 0.4 µM) identified as the most toxic compound among all the analogues tested. Overall, the farnesyl series exhibited greater selectivity towards malaria activity over cytotoxicity (SI = IC_{50} L6/IC_{50} *P.f*) with analogue **14** exhibiting 17-fold selectivity while the prenyl series exhibited no such selectivity. Previously reported cytotoxicity evaluation of **1**–**4** at the NCI identified regioisomer **3** as the most active compound in the anti-cancer screening data, exhibiting moderate activity against melanoma cell lines.[8]

The compounds were evaluated for anti-bacterial activity against a panel of Gram-negative (*Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922)) and Gram-positive (*Staphylococcus aureus* (ATCC 25923) and *Staphylococcus intermedius* (1051997)) organisms. Benzochromenedione **32** inhibited Gram-positive bacteria potently while methoxy benzochromenediones **23**–**26** and **34** inhibited the bacteria modestly and all other compounds were inactive against Gram-positive bacteria. Furthermore, none of the compounds showed activity towards Gram-negative bacteria.

Table 1 Inhibitory activities of selected compounds against target enzymes, human and *T. brucei* FTase.

Analogues	IC_{50} (µM)	
	Human FTase[a]	*T. brucei* FTase[b]
1	0.78 ± 0.17[c]	0.74 ± 0.20[c]
2	1.22 ± 0.068[c]	3.04 ± 0.30[c]
3	0.14 ± 0.0017[c]	0.22 ± 0.034[c]
4	0.054 ± 0.005[c]	0.098 ± 0.008[c]
7	17.3 ± 1.2	>22
8	14.7 ± 0.4	19.6 ± 1.4
9	>22	>22
10	3.1 ± 0.5	2.3 ± 0.4
11	0.17 ± 0.008	0.35 ± 0.009
12	1.5 ± 0.2	2.9 ± 0.5
13	0.45 ± 0.03	1.0 ± 0.05
14	4.7 ± 0.8	5.2 ± 1.3
23 and **24**	7.3 ± 0.6	8.5 ± 1.1
25 and **26**	5.8 ± 0.3	5.4 ± 0.6
32	1.7 ± 0.1	3.3 ± 0.6
33 and **34**	>22.3	>22.3
34	>22.3	>22.3
35	1.0 ± 0.06	1.6 ± 0.2

[a] Human farnesyltransferase with FTI 276 as the positive control (IC_{50} 0.015 ± 0.004 µM) and values presented as the mean ± SEM (n = 3).
[b] *T. brucei* farnesyltransferase with FTI 276 as the positive control (IC_{50} 0.010 ± 0.002 µM) and values presented as the mean ± SEM (n = 3).
[c] Data taken from Harper *et al.*.[8]

Table 2 Anti-plasmodial (*P. falciparum*), cytotoxicity (L6) and anti-bacterial (*S. aureus* and *S. intermedius*) activities of selected compounds.

Analogues	IC_{50} (µM)		MIC (µM)	
	P. falc	Cytotox L6[a]	*S. aureus* (ATCC 25923)[b]	*S. intermedius* (1051997)[c]
1	>17[d,e]	n.t.	>200	>200
2	>17[d,e]	n.t.	>200	>200
3	4.56 ± 0.76[d,e]	n.t.	>200	>200
4	4.39 ± 0.77[d,e]	n.t.	>200	>200
7	8.0 ± 0.5[f]	12.8 ± 0.9	>200	>200
8	2.0 ± 0.2[f]	4.2 ± 1.0	>200	>200
9	4.8 ± 0.4[f]	5.2 ± 1.4	>200	>200
10	0.29 ± 0.03[f]	0.4 ± 0.07	>200	>200
11	4.0 ± 0.2[f]	30.6 ± 6.3	>200	>200
12	2.9 ± 0.2[f]	16.7 ± 1.7	>200	>200

13	3.4 ± 0.2[f]	25.8 ± 6.9	>200	>200
14	7.4 ± 1.0[f]	126.4 ± 5.5	>200	>200
23 and **24**	n.t.[g]	n.t.	100	100
25 and **26**	n.t.	n.t.	50	50
32	7.2 ± 0.2[f]	23.5 ± 2.5	6.25	3.12
33 and **34**	n.t.	n.t.	12.5	25
34	20.4 ± 0.8[f]	34.3 ± 11.8	25	50
35	33.9 ± 0.1[f]	53.2 ± 2.1	>200	>200

[a] L6 rat skeletal myoblast cell line with podophyllotoxin as the positive control (IC_{50} 0.012 μM) and values presented as the mean ± SEM (n = 2).
[b] *S. aureus* (ATCC 25923) with streptomycin (MIC 21.5 μM) and chloramphenicol (MIC 1.5–3 μM) used as positive controls and values presented as the mean (n = 3).
[c] *S. intermedius* (1051997) with streptomycin (MIC 10.7 μM) and chloramphenicol (MIC 3–6 μM) used as positive controls and values presented as the mean (n = 3).
[d] Data taken from Harper *et al.*.[8]
[e] *P. falciparum* (FcM29-Cameroon strain) with chloroquine as the positive control (IC_{50} 0.45 μM) and values presented as the mean ± SEM (n = 2).
[f] *P. falciparum* (NF54 strain, IEF stage) with chloroquine as the positive control (IC_{50} 0.006 μM) and values presented as the mean ± SEM (n = 2).
[g] Not tested.

3. Conclusion

In conclusion, the synthesis of thiaplidiaquinones A (**1**) and B (**2**) and regioisomers **3** and **4** were successfully undertaken along with their prenyl (**7**–**10**) and farnesyl (**11**–**14**) analogues utilising the reported biomimetic approach. Unexpectedly, during the synthesis of the precursors, the formation of several side products was observed across all three series (prenyl, geranyl and farnesyl).

Evaluation of the FTase activity, identified the farnesyl series as better inhibitors than the prenyl series though none were as active as the geranyl series. Both the prenyl and farnesyl series were more active in the anti-plasmodial assays than the geranyl series with prenyl regioisomer **10** identified as the most potent compound. Prenyl analogue **10** however was also the most cytotoxic compound, with the prenyl series observed to be more toxic than the farnesyl series. Of note was regioisomer **14**, which exhibited nearly 20-fold selectivity for anti-plasmodial activity over cytotoxicity identifying it as a suitable candidate for *in vivo* testing.

4. Experimental

4.1. General remarks

Infrared spectra were recorded on a Perkin-Elmer spectrometer. Mass spectra were acquired on a Bruker micrOTOF Q II spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 298 K on Bruker AC300, AVANCE 400 or 500 spectrometer using standard pulse sequences with TMS as an internal standard. Silica gel column chromatography was carried out using Davisil silica gel (40–60 μm) or Merck silica gel (15–40 μm). Thin layer chromatography was conducted on Merck DC-plastikfolien Kieselgel 60 F254 plates. Quinones **15**, **19** and **27** were prepared according to literature procedure.[10,14,15]

4.2. General procedure A: benzochromenedione synthesis

To a solution of quinone (1 eq.) in CH_2Cl_2 (20 mL) was added triethylamine (5 eq.) dropwise under an atmosphere of nitrogen. Reaction was stirred for 2 mins and loaded onto a silica gel column (CH_2Cl_2). After elution with copious amounts of CH_2Cl_2, the column was left overnight. Elution with CH_2Cl_2 afforded benzochromenediones.

4.3. General procedure B: hypotaurine addition

To a solution of benzochromenedione (1 eq.) in EtOH/MeCN (1:1) (4 mL) was added hypotaurine (1 eq.) in water (0.4 mL) at 0°C and the reaction was stirred at room temperature for 48 h. Water (2 mL) was added, the aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL) and the combined organic layers dried over anhydrous $MgSO_4$. Solvent was removed under reduced pressure and the crude products purified by silica gel column chromatography eluting with EtOAc/*n*-hexane (1:1) to afford thiaplidiaquinones.

4.4. Synthesis of compounds

4.4.1. Thiaplidiaquinone A (1)

Following general procedure B, benzochromenedione **5** (0.013 g, 0.03 mmol) was reacted with hypotaurine (0.003 g, 0.03 mmol) and the crude product purified to afford the title compound as a red oil (0.004 g, 25%) as well as regioisomer **3** as a green oil (0.006 g, 38%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.57 (1H, d, *J* = 2.8 Hz, H-1), 6.72 (1H, d, *J* = 2.8 Hz, H-3), 6.65 (1H, br s, H-11), 6.13 (1H, d, *J* = 9.4 Hz, H-6), 5.28–5.21 (2H, m, H-2', H-1''), 5.12–5.06 (1H, m, H-6'), 4.96–4.92 (1H, m, H-5''), 4.11–4.08 (2H, m, H_2-10), 3.32–3.30 (3H, m, H_2-9, H_2-1'$_A$), 3.26–3.22 (1H, m, H_2-1'$_B$), 2.09–2.06 (2H, m, H_2-5'), 2.03–1.99 (2H, m, H_2-4'), 1.99–1.96 (2H, m, H_2-4''), 1.96–1.92 (2H, m, H_2-3''), 1.94 (3H, br s, H_3-9''), 1.68 (3H, s, H_3-8'), 1.66 (3H, s, H_3-10'), 1.60 (6H, s, H_3-9', H_3-7''), 1.51 (3H, s, H_3-8''); (–)-HRESIMS $[M–H]^-$ *m/z* 590.2564 (calcd for $C_{34}H_{40}NO_6S$, 590.2582). ^{1}H NMR data were in agreement with literature.[10]

4.4.2. Regioisomer 3

Green oil; yield: 38%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (1H, d, *J* = 2.8 Hz, H-1), 6.79 (1H, d, *J* = 2.8 Hz, H-3), 6.57 (1H, br s, H-8), 6.00 (1H, d, *J* = 9.3 Hz, H-6), 5.32–5.29 (1H, m, H-1''), 5.23–5.20 (1H, m, H-2'), 5.10–5.07 (1H, m, H-6'), 4.94–4.91 (1H, m, H-5''), 4.09–4.08 (2H, m, H_2-9), 3.36–3.33 (3H, m, H_2-10, H_2-1'$_A$), 3.23–3.19 (1H, m, H_2-1'$_B$), 2.09–2.06 (2H, m, H_2-5'), 2.03–1.96 (4H, m, H_2-

4', H_2-4"), 1.95–1.93 (2H, m, H_2-3"), 1.91 (3H, br s, H_3-9"), 1.68 (3H, s, H_3-8'), 1.65 (3H, s, H_3-10'), 1.59 (6H, s, H_3-9', H_3-7"), 1.51 (3H, s, H_3-8"); (–)-HRESIMS $[M–H]^-$ *m/z* 590.2570 (calcd for $C_{34}H_{40}NO_6S$, 590.2582). ^{1}H NMR data were in agreement with literature.[10]

4.4.3. Thiaplidiaquinone B (2)

Following general procedure B, benzochromenedione **6** (0.024 g, 0.05 mmol) was reacted with hypotaurine (0.005 g, 0.05 mmol) and the crude product purified to afford the title compound as a purple oil (0.008 g, 28%) as well as regioisomer **4** as a blue oil (0.009 g, 31%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.72 (1H, s, H-1), 6.70 (1H, s, H-4), 6.63 (1H, br s, H-11), 6.08 (1H, d, *J* = 9.7 Hz, H-6), 5.30–5.29 (2H, m, H-2', H-1"), 5.09–5.05 (1H, m, H-6'), 4.96–4.94 (1H, m, H-5"), 4.11–4.08 (2H, m, H_2-10), 3.33–3.31 (4H, m, H_2-9, H_2-1'), 2.13–2.10 (2H, m, H_2-5'), 2.09–2.07 (2H, m, H_2-4'), 2.01–1.98 (2H, m, H_2-4"), 1.95–1.92 (2H, m, H_2-3"), 1.93 (3H, d, *J* = 1.0 Hz, H_3-9"), 1.74 (3H, s, H_3-10'), 1.69 (3H, s, H_3-8'), 1.60 (6H, s, H_3-9', H_3-7"), 1.52 (3H, s, H_3-8"); (+)-HRESIMS $[M+Na]^+$ *m/z* 614.2539 (calcd for $C_{34}H_{41}NNaO_6S$, 614.2547). ^{1}H NMR data were in agreement with literature.[10]

4.4.4. Regioisomer 4

Blue oil; yield: 31%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.99 (1H, s, H-1), 6.66 (1H, s, H-4), 6.55 (1H, br s, H-8), 5.96 (1H, d, *J* = 9.5 Hz, H-6), 5.35–5.34 (1H, m, H-1"), 5.30–5.26 (1H, m, H-2'), 5.09–5.06 (1H, m, H-6'), 4.95–4.93 (1H, m, H-5"), 4.08–4.07 (2H, m, H_2-9), 3.32–3.31 (4H, m, H_2-10, H_2-1'), 2.12–2.10 (2H, m, H_2-5'), 2.09–2.07 (2H, m, H_2-4'), 2.01–1.98 (2H, m, H_2-4"), 1.96–1.93 (2H, m, H_2-3"), 1.88 (3H, br s, H_3-9"), 1.70 (3H, s, H_3-10'), 1.68 (3H, s, H_3-8'), 1.60 (6H, s, H_3-9', H_3-7"), 1.52 (3H, s, H_3-8"); (+)-HRESIMS $[M+Na]^+$ *m/z* 614.2535 (calcd for $C_{34}H_{41}NNaO_6S$, 614.2547). ^{1}H NMR data were in agreement with literature.[10]

4.4.5. 6-((*E*)-2,6-Dimethylhepta-1,5-dien-1-yl)-4-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-6*H*-benzo[*c*]chromene-7,10-dione (5)

Following general procedure A, quinone **15** (0.76 g, 3 mmol) was reacted with triethylamine (1.57 g, 15 mmol), loaded onto a silica gel column (CH_2Cl_2) and left overnight. Elution with CH_2Cl_2 afforded the title compound as a red oil (0.013 g, 1.7%) as well as benzochromenedione **6** as a purple oil (0.024 g, 3.2%). Elution with CH_2Cl_2/MeOH (9:1) afforded benzochromenedione **16** in trace amounts and methoxy benzochromenediones **17** and **18** as a 5:2 mixture (0.002 g). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66 (1H, d, *J* = 3.0 Hz, H-1), 6.74 (1H, d, *J* = 3.0 Hz, H-3), 6.73 (2H, s, H-8, H-9), 6.04 (1H, d, *J* = 9.4 Hz, H-6), 5.33–5.30 (1H, m, H-1"), 5.26–5.22 (1H, m, H-2'), 5.11–5.07 (1H, m, H-6'), 4.95–4.90 (1H, m, H-5"), 4.53 (1H, br s, OH), 3.26–3.23 (2H, m, H_2-1'), 2.10–2.06 (2H, m, H_2-5'), 2.03–2.00 (2H, m, H_2-4'), 2.00–1.97 (2H, m, H_2-4"), 1.95–1.92 (2H, m, H_2-3"), 1.92 (3H, s, H_3-9"), 1.68 (3H, s, H_3-8'), 1.66 (3H, s, H_3-10'), 1.59 (6H, br s, H_3-9', H_3-7"), 1.51 (3H, s, H_3-8"); (+)-HRESIMS $[M+Na]^+$ *m/z* 509.2659 (calcd for $C_{32}H_{38}NaO_4$, 509.2662). ^{1}H NMR data were in agreement with literature.[10]

4.4.6. 6-((*E*)-2,6-Dimethylhepta-1,5-dien-1-yl)-3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-6*H*-benzo[*c*]chromene-7,10-dione (6)

Purple oil; yield: 3.2%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (1H, s, H-1), 6.72 (2H, s, H-8, H-9), 6.71 (1H, s, H-4), 5.99 (1H, d, *J* = 9.5 Hz, H-6), 5.36–5.34 (1H, m, H-1"), 5.31–5.28 (1H, m, H-2'), 5.10–5.06 (1H, m, H-6'), 4.95–4.91 (1H, m, H-5"), 4.81 (1H, br s, OH), 3.34–3.31 (2H, m, H_2-1'), 2.13–2.11 (2H, m, H_2-5'), 2.11–2.07 (2H, m, H_2-4'), 2.02–1.97 (2H, m, H_2-4"), 1.96–1.92 (2H, m, H_2-3"), 1.92 (3H, d, *J* = 1.2 Hz, H_3-9"), 1.73 (3H, s, H_3-10'), 1.69 (3H, s, H_3-8'), 1.60 (3H, s, H_3-7"), 1.59 (3H, s, H_3-9'), 1.51 (3H, s, H_3-8"); (+)-HRESIMS $[M+Na]^+$ *m/z* 509.2671 (calcd for $C_{32}H_{38}NaO_4$, 509.2662). ^{1}H NMR data were in agreement with literature.[10]

4.4.7. (*E*)-6-(2,6-Dimethylhepta-1,5-dien-1-yl)-2-hydroxy-6*H*-benzo[*c*]chromene-7,10-dione (16)

Red oil; trace; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.85 (1H, d, *J* = 2.8 Hz, H-1), 6.85 (1H, dd, *J* = 9.0, 2.8 Hz, H-3), 6.81 (1H, d, *J* = 9.0 Hz, H-4), 6.74 (2H, s, H-8, H-9), 6.00 (1H, d, *J* = 9.4 Hz, H-6), 5.33 (1H, d, *J* = 9.4 Hz, H-1'), 4.95–4.90 (1H, m, H-5'), 4.65 (1H, br s, OH), 2.01–1.98 (2H, m, H_2-4'), 1.96–1.94 (2H, m, H_2-3'), 1.92 (3H, br s, H_3-9'), 1.59 (3H, s, H_3-7'), 1.51 (3H, s, H_3-8'); (–)-HRESIMS $[M–H]^-$ *m/z* 349.1449 (calcd for $C_{22}H_{21}O_4$, 349.1445). ^{1}H NMR data were in agreement with literature.[11]

4.4.8. 6-((*E*)-2,6-Dimethylhepta-1,5-dien-1-yl)-3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-9-methoxy-6*H*-benzo[*c*]chromene-7,10-dione (17)

Purple oil; trace; R_f (CH_2Cl_2) 0.17; IR (ATR) ν_{max} 3415, 2928, 2853, 1639, 1598, 1424, 1224, 1005, 849 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (1H, s, H-1), 6.69 (1H, s, H-4), 6.01 (1H, d, *J* = 9.5 Hz, H-6), 5.89 (1H, s, H-8), 5.36–5.34 (1H, m, H-1"), 5.32–5.28 (1H, m, H-2'), 5.10–5.06 (1H, m, H-6'), 4.95–4.92 (1H, m, H-5"), 4.91 (1H, s, OH), 3.84 (3H, s, OMe), 3.34–3.31 (2H, m, H_2-1'), 2.13–2.11 (2H, m, H_2-5'), 2.11–2.07 (2H, m, H_2-4'), 2.02–1.97 (2H, m, H_2-4"), 1.96–1.92 (2H, m, H_2-3"), 1.91 (3H, br s, H_3-9"), 1.73 (3H, s, H_3-10'), 1.69 (3H, s, H_3-8'), 1.59 (6H, br s, H_3-9', H_3-7"), 1.51 (3H, s, H_3-8"); ^{13}C NMR ($CDCl_3$, 125 MHz, deduced from HSQC and HMBC) δ 184.6 (C-7), 181.4 (C-10), 158.1 (C-9), 148.7 (C-4a), 148.4 (C-2), 143.8 (C-2"), 138.7 (C-3'), 134.6 (C-6a), 132.9 (C-3), 131.9 (C-7'), 131.6 (C-6"), 128.6 (C-10a), 124.2 (C-6'), 123.7 (C-5"), 120.7 (C-2'), 118.5 (C-4), 118.1 (C-1"), 115.5 (C-10b), 114.8 (C-1), 106.9 (C-8), 67.5 (C-6), 56.5 (OMe), 39.8 (C-4', C-3"), 29.7 (C-1'), 26.6 (C-5'), 26.3 (C-4"), 25.8 (C-8'), 25.7 (C-7"), 17.8 (C-9', C-8"), 17.4 (C-9"), 16.3 (C-10'); (+)-HRESIMS $[M+Na]^+$ *m/z* 539.2764 (calcd for $C_{33}H_{40}NaO_5$, 539.2768).

4.4.9. 6-((*E*)-2,6-Dimethylhepta-1,5-dien-1-yl)-3-((*E*)-3,7-dimethylocta-2,6-dien-1-yl)-2-hydroxy-8-methoxy-6*H*-benzo[*c*]chromene-7,10-dione (18)

Purple oil; trace; R_f (CH_2Cl_2) 0.27; IR (ATR) ν_{max} 3415, 2928, 2853, 1639, 1598, 1424, 1224, 1005, 849 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.85 (1H, s, H-1), 6.69 (1H, s, H-4), 6.03 (1H, d, *J* = 9.3 Hz, H-6), 5.89 (1H, s, H-9), 5.36–5.34 (1H, m, H-1"), 5.32–5.28 (1H, m, H-2'), 5.10–5.06 (1H, m, H-6'), 4.95–4.92 (1H, m, H-5"), 4.88 (1H, s, OH), 3.83 (3H, s, OMe), 3.34–3.31 (2H, m, H_2-1'), 2.13–2.11 (2H, m, H_2-5'), 2.11–2.07 (2H, m, H_2-4'), 2.02–1.97 (2H, m, H_2-4"), 1.96–1.92 (2H, m, H_2-3"), 1.91 (3H, br s, H_3-9"), 1.73 (3H, s, H_3-10'), 1.69 (3H, s, H_3-8'), 1.59 (6H, br s, H_3-9', H_3-7"), 1.51 (3H, s, H_3-8"); ^{13}C NMR ($CDCl_3$, 125 MHz, deduced from HSQC and HMBC) δ 186.8 (C-10), 178.9 (C-7), 158.1 (C-8), 149.6 (C-4a), 149.2 (C-2), 144.6 (C-2"), 138.7 (C-3'), 133.5 (C-3, C-6a), 131.9 (C-7'), 131.6 (C-6"), 131.3 (C-10a), 124.2 (C-6'), 123.7 (C-5"), 120.7 (C-2'), 118.5 (C-4), 118.1 (C-1"), 115.5 (C-10b), 115.4 (C-1), 107.9

(C-9), 67.7 (C-6), 56.4 (OMe), 39.8 (C-4', C-3"), 29.8 (C-1'), 26.6 (C-5'), 26.3 (C-4"), 25.8 (C-8'), 25.7 (C-7"), 17.8 (C-9', C-8"), 17.4 (C-9"), 16.3 (C-10'); (+)-HRESIMS $[M+Na]^+$ *m/z* 539.2764 (calcd for $C_{33}H_{40}NaO_5$, 539.2768).

4.4.10. 2-Hydroxy-4-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (20)

Following general procedure A, quinone **19** (1.06 g, 6 mmol) was reacted with triethylamine (3.05 g, 30 mmol) and loaded onto a silica gel column. After two days, elution with CH_2Cl_2 afforded the title compound as a purple solid (0.015 g, 1.4%) as well as benzochromenedione **21** as a purple solid (0.026 g, 2.5%). Elution with CH_2Cl_2/MeOH (9:1) afforded chromenol **22** in trace amounts and methoxy benzochromenediones **23** and **24** as a 5:2 mixture as well as **25** and **26** as a 5:2 mixture. m.p. 169–171°C; R_f (CH_2Cl_2) 0.13; IR (ATR) ν_{max} 3376, 1717, 1469, 1363, 1223, 1132, 956 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.66 (1H, d, *J* = 3.0 Hz, H-1), 6.74 (1H, d, *J* = 3.0 Hz, H-3), 6.73 (2H, s, H-8, H-9), 6.03 (1H, d, *J* = 9.5 Hz, H-6), 5.34–5.31 (1H, m, H-1"), 5.24–5.20 (1H, m, H-2'), 4.52 (1H, s, OH), 3.30–3.25 (1H, m, H_2-1'$_A$), 3.21–3.15 (1H, m, H_2-1'$_B$), 1.94 (3H, d, *J* = 1.0 Hz, H_3-3"), 1.71 (3H, s, H_3-4'), 1.68 (3H, s, H_3-5'), 1.66 (3H, d, *J* = 1.0 Hz, H_3-4"); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 187.0 (C-10), 185.2 (C-7), 149.8 (C-2), 147.0 (C-4a), 140.9 (C-2"), 137.2 (C-8/C-9), 135.8 (C-8/C-9), 134.9 (C-6a), 133.2 (C-3'), 132.3 (C-4), 130.8 (C-10a), 121.8 (C-2'), 120.2 (C-3), 118.6 (C-1"), 117.7 (C-10b), 112.5 (C-1), 67.4 (C-6), 28.6 (C-1'), 26.1 (C-4"), 25.9 (C-4'), 19.0 (C-3"), 17.9 (C-5'); (–)-HRESIMS $[M–H]^-$ *m/z* 349.1457 (calcd for $C_{22}H_{21}O_4$, 349.1445).

4.4.11. 2-Hydroxy-3-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (21)

Purple solid; yield: 2.5%; m.p. 170–172°C; R_f (CH_2Cl_2) 0.31; IR (ATR) ν_{max} 3007, 1710, 1422, 1359, 1220, 1093, 905 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.81 (1H, s, H-1), 6.72 (2H, s, H-8, H-9), 6.70 (1H, s, H-4), 5.98 (1H, d, *J* = 9.6 Hz, H-6), 5.38 (1H, dq, *J* = 9.6, 1.0 Hz, H-1"), 5.31–5.28 (1H, m, H-2'), 4.89 (1H, s, OH), 3.32 (2H, t, *J* = 6.0 Hz, H_2-1'), 1.92 (3H, d, *J* = 1.0 Hz, H_3-3"), 1.77 (3H, s, H_3-4'), 1.74 (3H, s, H_3-5'), 1.67 (3H, d, *J* = 1.0 Hz, H_3-4"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 187.1 (C-10), 185.1 (C-7), 149.2 (C-4a), 149.0 (C-2), 140.7 (C-2"), 137.0 (C-8/C-9), 135.9 (C-8/C-9), 135.3 (C-3'), 133.7 (C-6a), 133.4 (C-3), 130.2 (C-10a), 120.8 (C-2'), 118.6 (C-4), 118.4 (C-1"), 115.6 (C-10b), 115.0 (C-1), 67.7 (C-6), 29.7 (C-1'), 26.2 (C-4"), 26.0 (C-4'), 19.0 (C-3"), 18.0 (C-5'); (–)-HRESIMS $[M–H]^-$ *m/z* 349.1432 (calcd for $C_{22}H_{21}O_4$, 349.1445).

4.4.12. 2,2-Dimethyl-2*H*-chromen-6-ol (22)

Red oil; trace; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.65 (1H, d, *J* = 8.6 Hz, H-8), 6.58 (1H, dd, *J* = 8.6, 3.0 Hz, H-7), 6.49 (1H, d, *J* = 3.0 Hz, H-5), 6.25 (1H, d, *J* = 9.8 Hz, H-4), 5.63 (1H, d, *J* = 9.8 Hz, H-3), 1.40 (6H, s, H_3-1'); (–)-HRESIMS $[M–H]^-$ *m/z* 175.0763 (calcd for $C_{11}H_{11}O_2$, 175.0765). ^1H NMR data were in agreement with literature.[16]

4.4.13. 2-Hydroxy-9-methoxy-4-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (23)

Purple oil; R_f (CH_2Cl_2) 0.04; IR (ATR) ν_{max} 3278, 2927, 1717, 1640, 1366, 1325, 1225, 1206 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.67 (1H, d, *J* = 3.0 Hz, H-1), 6.73 (1H, d, *J* = 3.0 Hz, H-3), 6.05 (1H, d, *J* = 9.2 Hz, H-6), 5.90 (1H, s, H-8), 5.32–5.30 (1H, m, H-1"), 5.24–5.21 (1H, m, H-2'), 4.66 (1H, s, OH), 3.84 (3H, s, OMe), 3.30–3.25 (1H, m, H_2-1'$_A$), 3.21–3.16 (1H, m, H_2-1'$_B$), 1.94 (3H, d, *J* = 0.9 Hz, H_3-3"), 1.71 (3H, s, H_3-4'), 1.68 (3H, s, H_3-5'), 1.66 (3H, d, *J* = 0.9 Hz, H_3-4"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.0 (C-7), 181.3 (C-10), 158.6 (C-9), 149.8 (C-2), 146.6 (C-4a), 140.9 (C-2"), 135.7 (C-6a), 133.2 (C-3'), 132.3 (C-4), 129.2 (C-10a), 121.9 (C-2'), 119.8 (C-3), 118.6 (C-1"), 117.6 (C-10b), 112.3 (C-1), 106.9 (C-8), 67.5 (C-6), 56.6 (OMe), 28.6 (C-1'), 26.1 (C-4"), 25.9 (C-4'), 19.0 (C-3"), 17.9 (C-5'); (+)-HRESIMS $[M+Na]^+$ *m/z* 403.1526 (calcd for $C_{23}H_{24}NaO_5$, 403.1516).

4.4.14. 2-Hydroxy-8-methoxy-4-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (24)

Purple oil; R_f (CH_2Cl_2) 0.04; IR (ATR) ν_{max} 3278, 2927, 1717, 1640, 1366, 1325, 1225, 1206 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.71 (1H, d, *J* = 3.0 Hz, H-1), 6.75 (1H, d, *J* = 3.0 Hz, H-3), 6.07 (1H, d, *J* = 8.8 Hz, H-6), 5.90 (1H, s, H-9), 5.32–5.30 (1H, m, H-1"), 5.24–5.21 (1H, m, H-2'), 4.66 (1H, s, OH), 3.83 (3H, s, OMe), 3.30–3.25 (1H, m, H_2-1'$_A$), 3.21–3.16 (1H, m, H_2-1'$_B$), 1.94 (3H, d, *J* = 0.9 Hz, H_3-3"), 1.71 (3H, s, H_3-4'), 1.68 (3H, s, H_3-5'), 1.65 (3H, d, *J* = 0.9 Hz, H_3-4"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 187.1 (C-10), 178.5 (C-7), 158.6 (C-8), 149.9 (C-2), 147.7 (C-4a), 140.9 (C-2"), 133.3 (C-3'), 133.21 (C-4/C-6a), 133.15 (C-4/C-6a), 130.8 (C-10a), 121.8 (C-2'), 120.4 (C-3), 118.4 (C-1"), 117.8 (C-10b), 113.0 (C-1), 108.0 (C-9), 67.6 (C-6), 56.4 (OMe), 28.6 (C-1'), 26.1 (C-4"), 25.9 (C-4'), 19.0 (C-3"), 17.9 (C-5'); (+)-HRESIMS $[M+Na]^+$ *m/z* 403.1526 (calcd for $C_{23}H_{24}NaO_5$, 403.1516).

4.4.15. 2-Hydroxy-9-methoxy-3-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (25)

Purple oil; R_f (CH_2Cl_2) 0.11; IR (ATR) ν_{max} 3430, 2974, 1717, 1648, 1364, 1223, 1132, 953, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.81 (1H, s, H-1), 6.70 (1H, s, H-4), 6.00 (1H, d, *J* = 9.5 Hz, H-6), 5.89 (1H, s, H-8), 5.38–5.35 (1H, m, H-1"), 5.32–5.29 (1H, m, H-2'), 4.94 (1H, s, OH), 3.84 (3H, s, OMe), 3.33–3.31 (2H, m, H_2-1'), 1.92 (3H, d, *J* = 1.0 Hz, H_3-3"), 1.77 (3H, s, H_3-4'), 1.74 (3H, s, H_3-5'), 1.67 (3H, d, *J* = 1.0 Hz, H_3-4"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.0 (C-7), 181.4 (C-10), 158.5 (C-9), 148.8 (C-4a), 148.7 (C-2), 140.7 (C-2"), 135.2 (C-3'), 134.4 (C-6a), 132.9 (C-3), 128.8 (C-10a), 120.9 (C-2'), 118.6 (C-4), 118.3 (C-1"), 115.5 (C-10b), 114.8 (C-1), 106.9 (C-8), 67.8 (C-6), 56.5 (OMe), 29.7 (C-1'), 26.2 (C-4"), 26.0 (C-4'), 19.0 (C-3"), 18.0 (C-5'); (+)-HRESIMS $[M+Na]^+$ *m/z* 403.1520 (calcd for $C_{23}H_{24}NaO_5$, 403.1516).

4.4.16. 2-Hydroxy-8-methoxy-3-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (26)

Purple oil; R_f (CH_2Cl_2) 0.11; IR (ATR) ν_{max} 3430, 2974, 1717, 1648, 1364, 1223, 1132, 953, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.86 (1H, s, H-1), 6.70 (1H, s, H-4), 6.02 (1H, d, *J* = 9.7 Hz, H-6), 5.89 (1H, s, H-9), 5.38–5.35 (1H, m, H-1"), 5.32–5.29 (1H, m, H-2'), 4.92 (1H, s, OH), 3.83 (3H, s, OMe), 3.33–3.31 (2H, m, H_2-1'), 1.92 (3H, d, *J* = 1.0 Hz, H_3-3"), 1.77 (3H, s, H_3-4'), 1.74 (3H, s, H_3-5'), 1.66 (3H, d, *J* = 1.0 Hz, H_3-4"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 187.2 (C-10), 178.4 (C-7), 158.5 (C-8), 149.8 (C-4a), 149.2 (C-2), 140.7 (C-2"), 135.2 (C-3'), 133.7 (C-3, C-6a), 131.0 (C-10a), 120.8 (C-2'), 118.6 (C-4), 118.3 (C-1"), 115.5 (C-10b), 115.4 (C-1), 107.9 (C-9), 67.9 (C-6), 56.4 (OMe), 29.6 (C-1'), 26.2 (C-4"), 26.0 (C-4'), 19.0 (C-3"), 18.0 (C-5'); (+)-HRESIMS $[M+Na]^+$ *m/z* 403.1520 (calcd for $C_{23}H_{24}NaO_5$, 403.1516).

4.4.17. 2-Hydroxy-4-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-10,11-dihydrobenzo[3,4]isochromeno[7,6-*b*][1,4]thiazine-7,12(6*H*,9*H*)-dione 8,8-dioxide (7)

Following general procedure B, benzochromenedione **20** (0.009 g, 0.027 mmol) was reacted with hypotaurine (0.003 g, 0.027 mmol) and the crude product purified to afford the title compound as a pale red oil (0.004 g, 36%) as well as regioisomer **8** as a grey oil (0.006 g, 44%). R_f (EtOAc) 0.64; IR (ATR) ν_{max} 3420, 1717, 1654, 1364, 1225, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.55 (1H, d, *J* = 3.0 Hz, H-1), 6.72 (1H, d, *J* = 3.0 Hz, H-3), 6.67 (1H, br s, H-11), 6.10 (1H, d, *J* = 9.5 Hz, H-6), 5.27–5.24 (1H, m, H-1"), 5.22–5.19 (1H, m, H-2'), 4.64 (1H, br s, OH), 4.11–4.10 (2H, m, H_2-10), 3.35–3.30 (2H, m, H_2-9), 3.30–3.26 (1H, m, H_2-1'$_A$), 3.18–3.13 (1H, m, H_2-1'$_B$), 1.93 (3H, d, *J* = 1.0 Hz, H_3-3"), 1.70 (3H, s, H_3-4'), 1.67 (3H, s, H_3-5'), 1.64 (3H, d, *J* = 1.0 Hz, H_3-4"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.3 (C-12), 175.3 (C-7), 149.7 (C-2), 146.2 (C-4a), 143.8 (C-11a), 142.1 (C-2"), 138.9 (C-6a), 133.3 (C-3'), 132.6 (C-4), 128.2 (C-12a), 121.7 (C-2'), 120.1 (C-3), 117.3 (C-1"), 116.8 (C-12b), 111.6 (C-1), 110.7 (C-7a), 67.6 (C-6), 48.8 (C-9), 40.1 (C-10), 28.5 (C-1'), 26.2 (C-4"), 25.9 (C-4'), 19.1 (C-3"), 17.9 (C-5'); (+)-HRESIMS $[M+Na]^+$ *m/z* 478.1284 (calcd for $C_{24}H_{25}NNaO_6S$, 478.1295).

4.4.18. 2-Hydroxy-4-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-9,10-dihydrobenzo[3,4]isochromeno[6,7-*b*][1,4]thiazine-7,12(6*H*,8*H*)-dione 11,11-dioxide (8)

Grey oil; yield: 44%; R_f (EtOAc) 0.74; IR (ATR) ν_{max} 3388, 2974, 1717, 1470, 1364, 1225, 1032, 953 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 7.69 (1H, d, *J* = 2.8 Hz, H-1), 6.70 (1H, d, *J* = 2.8 Hz, H-3), 5.99 (1H, d, *J* = 9.5 Hz, H-6), 5.34–5.32 (1H, m, H-1"), 5.22–5.18 (1H, m, H-2'), 3.99–3.95 (2H, m, H_2-9), 3.40–3.34 (2H, m, H_2-10), 3.27–3.23 (1H, m, H_2-1'$_A$), 3.13–3.09 (1H, m, H_2-1'$_B$), 1.93 (3H, d, *J* = 1.0 Hz, H_3-3"), 1.70 (3H, s, H_3-4'), 1.68 (3H, s, H_3-5'), 1.67 (3H, d, *J* = 1.0 Hz, H_3-4"); ^{13}C NMR (CD_3OD, 125 MHz) δ 178.8 (C-12), 177.8 (C-7), 152.7 (C-2), 148.3 (C-4a), 146.6 (C-7a), 141.7 (C-2"), 134.5 (C-12a), 133.5 (C-6a/C-3'), 133.4 (C-6a/C-3'), 132.9 (C-4), 123.3 (C-2'), 122.0 (C-3), 120.1 (C-1"), 119.4 (C-12b), 114.7 (C-11a), 114.6 (C-1), 68.4 (C-6), 49.9 (C-10), 40.7 (C-9), 29.4 (C-1'), 26.0 (C-4"), 25.9 (C-4'), 18.8 (C-3"), 17.8 (C-5'); (+)-HRESIMS $[M+Na]^+$ *m/z* 478.1288 (calcd for $C_{24}H_{25}NNaO_6S$, 478.1295).

4.4.19. 2-Hydroxy-3-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-10,11-dihydrobenzo[3,4]isochromeno[7,6-*b*][1,4]thiazine-7,12(6*H*,9*H*)-dione 8,8-dioxide (9)

Following general procedure B, benzochromenedione **21** (0.014 g, 0.042 mmol) was reacted with hypotaurine (0.005 g, 0.042 mmol) and the crude product purified to afford the title compound as a purple oil (0.006 g, 32%) as well as regioisomer **10** as a blue oil (0.008 g, 42%). R_f (EtOAc) 0.69; IR (ATR) ν_{max} 3388, 2979, 1715, 1653, 1364, 1225, 1033, 953 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.70 (1H, s, H-1), 6.71 (1H, s, H-4), 6.67 (1H, br s, H-11), 6.06 (1H, d, *J* = 9.8 Hz, H-6), 5.32–5.27 (2H, m, H-2', H-1"), 4.95 (1H, br s, OH), 4.11–4.08 (2H, m, H_2-10), 3.33–3.31 (4H, m, H_2-9, H_2-1'), 1.92 (3H, d, *J* = 0.9 Hz, H_3-3"), 1.77 (3H, s, H_3-4'), 1.75 (3H, s, H_3-5'), 1.66 (3H, d, *J* = 0.9 Hz, H_3-4"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.4 (C-12), 175.3 (C-7), 148.9 (C-2), 148.3 (C-4a), 143.7 (C-11a), 142.2 (C-2"), 137.6 (C-6a), 135.7 (C-3'), 133.1 (C-3), 127.9 (C-12a), 120.6 (C-2'), 118.9 (C-4), 117.1 (C-1"), 114.8 (C-12b), 114.3 (C-1), 110.5 (C-7a), 67.9 (C-6), 48.9 (C-9), 40.1 (C-10), 29.9 (C-1'), 26.3 (C-4"), 26.0 (C-4'), 19.2 (C-3"), 18.1 (C-5'); (+)-HRESIMS $[M+Na]^+$ *m/z* 478.1282 (calcd for $C_{24}H_{25}NNaO_6S$, 478.1295).

4.4.20. 2-Hydroxy-3-(3-methylbut-2-en-1-yl)-6-(2-methylprop-1-en-1-yl)-9,10-dihydrobenzo[3,4]isochromeno[6,7-*b*][1,4]thiazine-7,12(6*H*,8*H*)-dione 11,11-dioxide (10)

Blue oil; yield: 42%; R_f (EtOAc) 0.71; IR (ATR) ν_{max} 3375, 2974, 1715, 1470, 1364, 1225, 1032, 953 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 7.86 (1H, s, H-1), 6.57 (1H, s, H-4), 5.93 (1H, d, *J* = 9.6 Hz, H-6), 5.36–5.34 (1H, m, H-1"), 5.31–5.28 (1H, m, H-2'), 3.98–3.95 (2H, m, H_2-9), 3.40–3.35 (2H, m, H_2-10), 3.28–3.27 (2H, m, H_2-1'), 1.90 (3H, d, *J* = 1.1 Hz, H_3-3"), 1.75 (3H, s, H_3-4'), 1.70 (3H, s, H_3-5'), 1.67 (3H, d, *J* = 1.1 Hz, H_3-4"); ^{13}C NMR (CD_3OD, 125 MHz) δ 179.0 (C-12), 178.5 (C-7), 151.2 (C-2), 150.8 (C-4a), 146.7 (C-7a), 141.5 (C-2"), 136.8 (C-3), 134.3 (C-12a/C-3'), 134.2 (C-12a/C-3'), 131.9 (C-6a), 122.6 (C-2'), 120.0 (C-1"), 118.9 (C-4), 117.0 (C-12b), 116.0 (C-1), 114.1 (C-11a), 68.5 (C-6), 49.8 (C-10), 40.7 (C-9), 29.5 (C-1'), 26.0 (C-4"), 25.9 (C-4'), 18.8 (C-3"), 17.8 (C-5'); (+)-HRESIMS $[M+Na]^+$ *m/z* 478.1279 (calcd for $C_{24}H_{25}NNaO_6S$, 478.1295).

4.4.21. 2-Hydroxy-4-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-6-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (28)

Following general procedure A, quinone **27** (1.42 g, 4.5 mmol) was reacted with triethylamine (2.30 g, 22.7 mmol), loaded onto a silica gel column and left overnight. Elution with CH_2Cl_2 afforded the title compound as a purple oil (0.054 g, 3.8%) as well as benzochromenedione **29** as a purple oil (0.032 g, 2.3%). Elution with CH_2Cl_2/MeOH (9:1) afforded benzochromenedione **32** (0.008 g, 0.8%), methoxybenzochromene **33** (as a 1:1 mixture with **34**) (0.006 g), **34** (0.004 g, 0.4%) and dimer **35** (0.003 g, 0.2%). R_f (CH_2Cl_2) 0.42; IR (ATR) ν_{max} 3056, 1712, 1363, 1221, 737, 729, 702 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.66 (1H, d, *J* = 3.0 Hz, H-1), 6.74 (1H, d, *J* = 3.0 Hz, H-3), 6.73 (2H, s, H-8, H-9), 6.04 (1H, d, *J* = 9.5 Hz, H-6), 5.33 (1H, dq, *J* = 9.5, 1.1 Hz, H-1"), 5.25 (1H, t, *J* = 7.4 Hz, H-2'), 5.12–5.08 (2H, m, H-6', H-10'), 5.05–5.02 (1H, m, H-9"), 4.95–4.92 (1H, m, H-5"), 4.54 (1H, s, OH), 3.24 (2H, d, *J* = 7.4 Hz, H_2-1'), 2.10–2.07 (2H, m, H_2-5'), 2.04–2.00 (4H, m, H_2-4', H_2-9'), 2.00–1.96 (6H, m, H_2-4", H_2-8', H_2-8"), 1.96–1.92 (2H, m, H_2-3"), 1.94 (3H, s, H_3-14"), 1.89–1.86 (2H, m, H_2-7"), 1.67 (6H, s, H_3-11", H_3-12'), 1.66 (3H, s, H_3-15'), 1.59 (6H, s, H_3-13', H_3-14'), 1.57 (3H, s, H_3-12"), 1.50 (3H, s, H_3-13"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 187.0 (C-10), 185.2 (C-7), 149.8 (C-2), 147.0 (C-4a), 144.4 (C-2"), 137.1 (C-8/C-9), 137.0 (C-3'), 135.8 (C-8/C-9), 135.6 (C-6"), 135.2 (C-7'), 134.9 (C-6a), 132.3 (C-4), 131.5 (C-11', C-10"), 130.5 (C-10a), 124.5 (C-10'), 124.4 (C-6'), 124.2 (C-9"), 123.4 (C-5"), 121.6 (C-2'), 120.2 (C-3), 118.3 (C-1"), 117.6 (C-10b), 112.5 (C-1), 67.3 (C-6), 39.91 (C-4'/C-3"), 39.86 (C-4'/C-3"), 39.82 (C-8'/C-7"), 39.78 (C-8'/C-7"), 28.3 (C-1'), 26.9 (C-5'/C-9'), 26.8 (C-5'/C-9'), 26.7 (C-8"), 26.3 (C-4"), 25.8 (C-12', C-11"), 17.8 (C-13', C-12"), 17.4 (C-14"), 16.3 (C-15'), 16.2 (C-13"), 16.1 (C-14'); (–)-HRESIMS $[M–H]^-$ *m/z* 621.3947 (calcd for $C_{42}H_{53}O_4$, 621.3949).

4.4.22. 2-Hydroxy-3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-6-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (29)

Purple oil; yield: 2.3%; R_f (CH_2Cl_2) 0.56; IR (ATR) ν_{max} 3493, 1710, 1420, 1361, 1222, 1090, 735 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.80 (1H, s, H-1), 6.71 (2H, s, H-8, H-9), 6.69 (1H, s, H-4), 5.99 (1H, d, *J* = 9.6 Hz, H-6), 5.36 (1H, d, *J* = 9.6 Hz, H-1''), 5.32–5.28 (1H, m, H-2'), 5.11–5.07 (2H, m, H-6', H-10'), 5.07–5.01 (1H, m, H-9''), 4.96–4.93 (1H, m, H-5''), 4.86 (1H, s, OH), 3.33 (2H, t, *J* = 7.8 Hz, H_2-1'), 2.13–2.10 (2H, m, H_2-5'), 2.10–2.05 (4H, m, H_2-4', H_2-9'), 2.04–2.01 (2H, m, H_2-4''), 2.01–1.97 (4H, m, H_2-8', H_2-8''), 1.96–1.93 (2H, m, H_2-3''), 1.92 (3H, d, *J* = 1.3 Hz, H_3-14''), 1.91–1.86 (2H, m, H_2-7''), 1.74 (3H, s, H_3-15'), 1.67 (3H, s, H_3-11''), 1.66 (3H, s, H_3-12'), 1.60 (6H, s, H_3-13', H-14'), 1.57 (3H, s, H_3-12''), 1.51 (3H, s, H_3-13''); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 187.1 (C-10), 185.1 (C-7), 149.2 (C-4a), 149.1 (C-2), 144.0 (C-2''), 139.0 (C-3'), 137.0 (C-8/C-9), 135.9 (C-8/C-9), 135.65 (C-7'/C-6''), 135.58 (C-7'/C-6''), 133.7 (C-3), 133.4 (C-6a), 131.5 (C-11'/C-10''), 131.4 (C-11'/C-10''), 130.2 (C-10a), 124.6 (C-10'), 124.4 (C-6'), 123.9 (C-9''), 123.5 (C-5''), 120.7 (C-2'), 118.6 (C-4), 118.1 (C-1''), 115.7 (C-10b), 115.1 (C-1), 67.6 (C-6), 39.9 (C-4', C-3''), 39.81 (C-8'/C-7''), 39.76 (C-8'/C-7''), 29.7 (C-1'), 26.9 (C-5', C-9'), 26.6 (C-8''), 26.2 (C-4''), 25.8 (C-12', C-11''), 17.8 (C-13', C-12''), 17.4 (C-14''), 16.4 (C-15'), 16.19 (C-14'/C-13''), 16.15 (C-14'/C-13''); (–)-HRESIMS $[M–H]^-$ *m/z* 621.3951 (calcd for $C_{42}H_{53}O_4$, 621.3949).

4.4.23. (*E*)-2-(4,8-Dimethylnona-3,7-dien-1-yl)-2-methyl-2*H*-chromen-6-ol (30)

Red oil; trace; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.64 (1H, d, *J* = 8.6 Hz, H-8), 6.57 (1H, dd, *J* = 8.6, 2.9 Hz, H-7), 6.48 (1H, d, *J* = 2.9 Hz, H-5), 6.27 (1H, d, *J* = 9.9 Hz, H-4), 5.60 (1H, d, *J* = 9.9 Hz, H-3), 5.12–5.06 (2H, m, H-3', H-7'), 4.40 (1H, s, OH), 2.11–2.07 (2H, m, H_2-2'), 2.06–1.99 (2H, m, H_2-6'), 1.97–1.93 (2H, m, H_2-5'), 1.89–1.85 (2H, m, H_2-1'), 1.67 (3H, s, H_3-9'), 1.59 (6H, s, H_3-10', H_3-11'), 1.37 (3H, s, H_3-9); HRESIMS $[M+Na]^+$ *m/z* 335.1992 (calcd for $C_{21}H_{28}NaO_2$, 335.1982). ^1H NMR data were in agreement with literature.[17]

4.4.24. 10-((*E*)-4,8-Dimethylnona-3,7-dien-1-yl)-10-methyl-5-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)benzo[*c*]pyrano[2,3-*g*]chromene-1,4(5*H*,10*H*)-dione (31A and 31B)

Purple oil; trace; R_f (CH_2Cl_2) 0.89; IR (ATR) ν_{max} 2976, 1738, 1378, 1264, 730, 704 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.79 (1H, s, H-12), 6.71 (2H, s, H-9, H-10), 6.53 (1H, s, H-5), 6.29 (1H, d, *J* = 10.0 Hz, H-4), 5.98 (1H, d, *J* = 9.6 Hz, H-7), 5.73 (1H, d, *J* = 10.0 Hz, H-3), 5.36 (1H, dq, *J* = 9.6, 1.0 Hz, H-1''), 5.12–5.06 (2H, m, H-3', H-7'), 5.06–5.04 (1H, m, H-9''), 4.96–4.94 (1H, m, H-5''), 2.17–2.03 (4H, m, H_2-2', H_2-6'), 2.03–1.99 (2H, m, H_2-4''), 1.99–1.94 (4H, m, H_2-5', H_2-8''), 1.94–1.89 (5H, m, H_2-3'', H_3-14''), 1.89–1.86 (2H, m, H_2-7''), 1.70–1.62 (11H, m, H_2-1', H_3-9', H_3-11', H_3-11''), 1.59 (3H, s, H_3-10'), 1.57 (3H, s, H_3-12''), 1.50 (3H, s, H_3-13''), 1.40 (3H, br s, H_3-13); ^{13}C NMR ($CDCl_3$, 125 MHz, deduced from HSQC and HMBC) δ 186.9 (C-11), 186.2 (C-8), 148.8 (C-5a), 147.9 (C-12a), 144.1 (C-2''), 137.1 (C-9/C-10), 135.9 (C-9/C-10), 135.8 (C-4', C-6''), 133.7 (C-3, C-7a), 131.5 (C-8', C-10''), 128.4 (C-11a), 124.5 (C-7'), 124.4 (C-3', C-9''), 123.6 (C-5''), 122.5 (C-4, C-4a), 118.3 (C-1''), 117.0 (C-11b), 115.9 (C-12), 114.8 (C-5), 78.5 (C-2), 67.6 (C-7), 41.2 (C-1'), 39.8 (C-5', C-3'', C-7''), 26.9 (C-6', C-8''), 26.8 (C-13), 26.2 (C-4''), 25.8 (C-9', C-11''), 22.8 (C-2'), 17.8 (C-10', C-12''), 17.3 (C-14''), 16.1 (C-11', C-13''); HRESIMS $[M+Na]^+$ *m/z* 643.3740 (calcd for $C_{42}H_{52}NaO_4$, 643.3758).

Purple oil; trace; R_f (CH_2Cl_2) 0.83; IR (ATR) ν_{max} 2976, 1738, 1378, 1264, 730, 704 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.78 (1H, s, H-12), 6.72 (2H, s, H-9, H-10), 6.53 (1H, s, H-5), 6.29 (1H, d, *J* = 9.8 Hz, H-4), 5.98 (1H, d, *J* = 9.4 Hz, H-7), 5.72 (1H, d, *J* = 9.8 Hz, H-3), 5.36 (1H, dq, *J* = 9.4, 1.0 Hz, H-1''), 5.12–5.06 (2H, m, H-3', H-7'), 5.06–5.04 (1H, m, H-9''), 4.96–4.94 (1H, m, H-5''), 2.17–2.03 (4H, m, H_2-2', H_2-6'), 2.03–1.99 (2H, m, H_2-4''), 1.99–1.94 (4H, m, H_2-5', H_2-8''), 1.94–1.89 (7H, m, H_2-1', H_2-3'', H_3-14''), 1.89–1.86 (2H, m, H_2-7''), 1.67 (3H, br s, H_3-9'), 1.66 (3H, br s, H_3-11''), 1.59 (6H, s, H_3-10', H_3-11'), 1.57 (3H, s, H_3-12''), 1.51 (3H, s, H_3-13''), 1.41 (3H, br s, H_3-13); ^{13}C NMR ($CDCl_3$, 125 MHz, deduced from HSQC and HMBC) δ 186.9 (C-11), 186.2 (C-8), 148.8 (C-5a), 147.9 (C-12a), 144.2 (C-2''), 137.1 (C-9/C-10), 135.9 (C-9/C-10), 135.8 (C-4', C-6''), 133.7 (C-3, C-7a), 131.6 (C-8', C-10''), 128.4 (C-11a), 124.5 (C-7'), 124.4 (C-3', C-9''), 123.6 (C-5''), 122.5 (C-4, C-4a), 119.7 (C-1''), 117.0 (C-11b), 115.9 (C-12), 114.8 (C-5), 78.5 (C-2), 67.6 (C-7), 39.8 (C-5', C-3'', C-7''), 38.5 (C-1'), 26.9 (C-8''), 26.8 (C-6'), 26.7 (C-13), 26.2 (C-4''), 25.8 (C-9', C-11''), 22.6 (C-2'), 17.8 (C-10', C-12''), 17.4 (C-14''), 16.1 (C-11', C-13''); HRESIMS $[M+Na]^+$ *m/z* 643.3740 (calcd for $C_{42}H_{52}NaO_4$, 643.3758).

4.4.25. 2-Hydroxy-6-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (32)

Red oil; yield: 0.8%; R_f (CH_2Cl_2) 0.21; IR (ATR) ν_{max} 3233, 2916, 1638, 1599, 1335, 1315, 1276, 1229, 1200, 1000, 842, 827, 766 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.86 (1H, d, *J* = 2.9 Hz, H-1), 6.85 (1H, dd, *J* = 8.6, 2.9 Hz, H-3), 6.81 (1H, d, *J* = 8.6 Hz, H-4), 6.74 (2H, s, H-8, H-9), 6.01 (1H, d, *J* = 9.5 Hz, H-6), 5.34 (1H, dq, *J* = 9.5, 1.1 Hz, H-1'), 5.05–5.02 (1H, m, H-9'), 5.01 (1H, br s, OH), 4.95–4.93 (1H, m, H-5'), 2.04–1.98 (2H, m, H_2-4'), 1.98–1.94 (4H, m, H_2-3', H_2-8'), 1.93 (3H, d, *J* = 1.1 Hz, H_3-14'), 1.89–1.86 (2H, m, H_2-7'), 1.66 (3H, s, H_3-11'), 1.57 (3H, s, H_3-12'), 1.50 (3H, s, H_3-13'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 187.0 (C-10), 185.1 (C-7), 150.4 (C-2), 149.0 (C-4a), 144.5 (C-2'), 137.1 (C-8/C-9), 135.8 (C-8/C-9), 135.6 (C-6'), 134.9 (C-6a), 131.5 (C-10'), 130.0 (C-10a), 124.4 (C-9'), 123.4 (C-5'), 119.9 (C-3), 118.7 (C-4), 117.8 (C-10b, C-1'), 115.0 (C-1), 67.6 (C-6), 39.8 (C-3'), 39.7 (C-7'), 26.8 (C-8'), 26.1 (C-4'), 25.8 (C-11'), 17.8 (C-12'), 17.4 (C-14'), 16.2 (C-13'); HRESIMS $[M+Na]^+$ *m/z* 441.2043 (calcd for $C_{27}H_{30}NaO_4$, 441.2036).

4.4.26. 2-Hydroxy-8-methoxy-6-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (33)

Purple oil; R_f (CH_2Cl_2) 0.11; IR (ATR) ν_{max} 3412, 2966, 2915, 2852, 1636, 1602, 1569, 1441, 1382, 1313, 1225, 1192, 1131, 1002, 824, 808 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.93 (1H, d, *J* = 2.9 Hz, H-1), 6.86 (1H, dd, *J* = 8.9, 2.9 Hz, H-3), 6.80 (1H, d, *J* = 8.9 Hz, H-4), 6.04 (1H, d, *J* = 9.5 Hz, H-6), 5.91 (1H, s, H-9), 5.34 (1H, dq, *J* = 9.5, 1.1 Hz, H-1'), 5.06–5.02 (2H, m, H-9'), 4.96–4.93 (1H, m, H-5'), 3.84 (3H, s, OMe), 2.02–2.00 (2H, m, H_2-4'), 1.99–1.94 (4H, m, H_2-3', H_2-8'), 1.93 (3H, d, *J* = 1.1 Hz, H_3-14'), 1.89–1.86 (2H, m, H_2-7'), 1.66 (3H, s, H_3-11'), 1.57 (3H, s, H_3-12'), 1.50 (3H, s, H_3-13'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 187.3 (C-10), 179.9 (C-7), 158.2 (C-8), 150.5 (C-2), 149.3 (C-4a), 144.3 (C-2'), 135.6 (C-6'), 133.3 (C-6a), 131.5 (C-10'), 130.4 (C-10a), 124.5 (C-9'), 123.4 (C-5'), 120.2 (C-3), 118.7 (C-4), 117.9 (C-10b), 117.7 (C-1'), 115.5 (C-1), 108.0 (C-9), 67.8 (C-6), 56.5 (OMe), 39.9 (C-3'), 39.7 (C-7'), 26.9 (C-8'), 26.1 (C-4'), 25.8 (C-11'), 17.8 (C-12'), 17.4 (C-14'), 16.2 (C-13'); (–)-HRESIMS $[M–H]^-$ *m/z* 447.2185 (calcd for $C_{28}H_{31}O_5$, 447.2177).

4.4.27. 2-Hydroxy-9-methoxy-6-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)-6*H*-benzo[*c*]chromene-7,10-dione (34)

Purple oil; yield: 0.4%; R_f (CH_2Cl_2) 0.08; IR (ATR) ν_{max} 3412, 2966, 2915, 2852, 1636, 1602, 1569, 1441, 1382, 1313, 1225, 1192, 1131, 1002, 824, 808 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.86 (1H, d, *J* = 2.8 Hz, H-1), 6.83 (1H, dd, *J* = 8.8, 2.8 Hz, H-3), 6.80 (1H, d, *J* = 8.8 Hz, H-4), 6.03 (1H, d, *J* = 9.5 Hz, H-6), 5.90 (1H, s, H-8), 5.32 (1H, dq, *J* = 9.5, 1.0 Hz, H-1'), 5.06–5.02 (2H, m, H-9'), 4.95–4.93 (1H, m, H-5'), 4.83 (1H, br s, OH), 3.84 (3H, s, OMe), 2.04–2.00 (2H, m, H_2-4'), 1.99–1.95 (4H, m, H_2-3', H_2-8'), 1.92 (3H, d, *J* = 1.0 Hz, H_3-14'), 1.89–1.86 (2H, m, H_2-7'), 1.66 (3H, s, H_3-11'), 1.57 (3H, s, H_3-12'), 1.50 (3H, s, H_3-13'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 184.8 (C-7), 181.2 (C-10), 158.5 (C-9), 150.2 (C-2), 148.7 (C-4a), 144.5 (C-2'), 135.7 (C-6a), 135.6 (C-6'), 131.5 (C-10'), 128.7 (C-10a), 124.4 (C-9'), 123.4 (C-5'), 119.5 (C-3), 118.7 (C-4), 117.9 (C-1'), 117.8 (C-10b), 114.9 (C-1), 106.9 (C-8), 67.7 (C-6), 56.6 (OMe), 39.9 (C-3'), 39.8 (C-7'), 26.9 (C-8'), 26.1 (C-4'), 25.8 (C-11'), 17.8 (C-12'), 17.4 (C-14'), 16.2 (C-13'); (–)-HRESIMS $[M–H]^-$ *m/z* 447.2189 (calcd for $C_{28}H_{31}O_5$, 447.2177).

4.4.28. 5,14-Dihydroxy-1,8-bis((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)benzo[*c*]chromeno[3,4-*h*]chromene-9,12(1*H*,8*H*)-dione (35)

Purple oil; yield: 0.2%; R_f (CH_2Cl_2) 0.34; IR (ATR) ν_{max} 3351, 2916, 2853, 1647, 1567, 1420, 1383, 1304, 1274, 1234, 1195, 1092, 1000, 839, 816, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.84 (1H, d, *J* = 2.9 Hz, H-6), 7.73 (1H, s, H-13), 6.81 (1H, d, *J* = 8.6 Hz, H-3), 6.76 (1H, s, H-10/H-11), 6.75 (1H, s, H-10/H-11), 6.70 (1H, dd, *J* = 8.6, 2.9 Hz, H-4), 6.14 (2H, d, *J* = 9.3 Hz, H-1, H-8), 5.32 (1H, d, *J* = 9.3 Hz, H-1'), 5.24 (1H, d, *J* = 9.3 Hz, H-1"), 5.04–4.99 (2H, m, H-9', H-9"), 4.96–4.93 (1H, m, H-5'), 4.85–4.82 (1H, m, H-5"), 4.80 (1H, br s, 14-OH), 4.48 (1H, br s, 5-OH), 2.02–1.99 (2H, m, H_2-4'), 1.97–1.91 (6H, m, H_2-3', H_2-8', H_2-8"), 1.94 (3H, d, *J* = 0.9 Hz, H_3-14'), 1.91–1.88 (2H, m, H_2-4"), 1.88–1.84 (4H, m, H_2-3", H_2-7'), 1.87 (3H, d, *J* = 1.0 Hz, H_3-14"), 1.81–1.78 (2H, m, H_2-7"), 1.65 (3H, s, H_3-11'/ H_3-11"), 1.64 (3H, s, H_3-11'/ H_3-11"), 1.56 (6H, s, H_3-12', H_3-12"), 1.50 (3H, s, H_3-13'), 1.43 (3H, s, H_3-13"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.8 (C-12), 184.9 (C-9), 149.6 (C-5), 147.2 (C-2a), 146.6 (C-6c), 145.3 (C-14/C-2"), 145.2 (C-14/C-2"), 142.2 (C-2'), 137.2 (C-10/C-11), 135.9 (C-10/C-11), 135.6 (C-6'), 135.5 (C-6"), 134.8 (C-8a, C-14a), 131.3 (C-10', C-10"), 130.1 (C-12a), 128.4 (C-6b), 124.59 (C-9'/C-9"), 124.56 (C-9'/C-9"), 123.6 (C-5'), 123.3 (C-5"), 121.6 (C-6a), 120.6 (C-1'), 118.4 (C-3), 117.9 (C-1"), 117.3 (C-12b), 116.1 (C-4), 115.6 (C-6), 115.1 (C-13), 69.0 (C-8), 67.4 (C-1), 39.82 (C-3'/C-3"), 39.79 (C-3'/C-3"), 39.7 (C-7"), 39.6 (C-7'), 26.8 (C-8', C-8"), 26.2 (C-4'), 26.1 (C-4"), 25.8 (C-11', C-11"), 17.8 (C-12', C-12"), 17.5 (C-14"), 17.1 (C-14'), 16.2 (C-13'), 16.0 (C-13"); HRESIMS $[M+Na]^+$ *m/z* 751.3969 (calcd for $C_{48}H_{56}NaO_6$, 751.3969).

4.4.29. 2-Hydroxy-4-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-6-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)-10,11-dihydrobenzo[3,4]isochromeno[7,6-*b*][1,4]thiazine-7,12(6*H*,9*H*)-dione 8,8-dioxide (11)

Following general procedure B, benzochromenedione **28** (0.045 g, 0.072 mmol) was reacted with hypotaurine (0.008 g, 0.072 mmol) and the crude product purified to afford the title compound as a red oil (0.006 g, 12%) as well as regioisomer **12** as a green oil (0.008 g, 15%). R_f (EtOAc) 0.79; IR (ATR) ν_{max} 3423, 2927, 1708, 1677, 1537, 1462, 1394, 1378, 1349, 1326, 1115, 957, 822, 786, 746, 727, 713 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.56 (1H, d, *J* = 2.6 Hz, H-1), 6.72 (1H, br s, H-11), 6.71 (1H, d, *J* = 2.6 Hz, H-3), 6.11 (1H, d, *J* = 9.6 Hz, H-6), 5.27–5.21 (2H, m, H-2', H-1"), 5.12–5.08 (2H, m, H-6', H-10'), 5.06–5.03 (1H, m, H-9"), 4.96–4.94 (1H, m, H-5"), 4.10–4.07 (2H, m, H_2-10), 3.34–3.28 (2H, m, H_2-9), 3.28–3.24 (1H, m, H_2-1'$_A$), 3.21–3.17 (1H, m, H_2-1'$_B$), 2.11–2.08 (2H, m, H_2-5'), 2.07–2.03 (2H, m, H_2-9'), 2.03–2.00 (2H, m, H_2-4'), 2.00–1.96 (4H, m, H_2-8', H_2-8"), 1.96–1.90 (4H, m, H_2-3", H_2-4"), 1.94 (3H, d, *J* = 0.9 Hz, H_3-14"), 1.90–1.86 (2H, m, H_2-7"), 1.67 (3H, s, H_3-11"), 1.66 (3H, s, H_3-12'), 1.65 (3H, s, H_3-15'), 1.59 (6H, br s, H_3-13', H_3-14'), 1.57 (3H, s, H_3-12"), 1.50 (3H, s, H_3-13"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.2 (C-12), 175.3 (C-7), 149.7 (C-2), 146.2 (C-4a), 145.6 (C-2"), 143.9 (C-11a), 138.9 (C-6a), 137.2 (C-3'), 135.6 (C-6"), 135.2 (C-7'), 132.5 (C-4), 131.5 (C-10"), 131.4 (C-11'), 128.2 (C-12a), 124.5 (C-6', C-10'), 124.2 (C-9"), 123.6 (C-5"), 121.4 (C-2'), 120.0 (C-3), 116.9 (C-1"), 116.8 (C-12b), 111.7 (C-1), 110.6 (C-7a), 67.6 (C-6), 48.8 (C-9), 40.1 (C-10), 39.92 (C-4', C-3"), 39.86 (C-8'), 39.8 (C-7"), 28.2 (C-1'), 26.9 (C-5', C-9'), 26.7 (C-8"), 26.3 (C-4"), 25.8 (C-12', C-11"), 17.8 (C-13', C-12"), 17.6 (C-14"), 16.3 (C-14'), 16.2 (C-15'), 16.1 (C-13"); (+)-HRESIMS $[M+Na]^+$ *m/z* 750.3808 (calcd for $C_{44}H_{57}NNaO_6S$, 750.3799).

4.4.30. 2-Hydroxy-4-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-6-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)-9,10-dihydrobenzo[3,4]isochromeno[6,7-*b*][1,4]thiazine-7,12(6*H*,8*H*)-dione 11,11-dioxide (12)

Green oil; yield: 15%; R_f (EtOAc) 0.86; IR (ATR) ν_{max} 3427, 2927, 1708, 1677, 1535, 1462, 1390, 1377, 1329, 1113, 823, 784, 743, 727, 713 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.74 (1H, d, *J* = 3.0 Hz, H-1), 6.71 (1H, d, *J* = 3.0 Hz, H-3), 6.62 (1H, br s, H-8), 5.91 (1H, d, *J* = 9.5 Hz, H-6), 5.23 (1H, dq, *J* = 9.5, 0.9 Hz, H-1"), 5.15–5.13 (1H, m, H-2'), 5.04–5.00 (2H, m, H-6', H-10'), 4.98–4.96 (1H, m, H-9"), 4.89–4.86 (1H, m, H-5"), 4.05–4.01 (2H, m, H_2-9), 3.31–3.28 (2H, m, H_2-10), 3.18–3.13 (1H, m, H_2-1'$_A$), 3.11–3.06 (1H, m, H_2-1'$_B$), 2.04–1.97 (4H, m, H_2-5', H_2-9'), 1.96–1.93 (2H, m, H_2-4'), 1.91–1.86 (8H, m, H_2-8', H_2-3", H_2-4", H_2-8"), 1.83 (3H, d, *J* = 0.9 Hz, H_3-14"), 1.82–1.80 (2H, m, H_2-7"), 1.60 (3H, s, H_3-11"), 1.59 (3H, s, H_3-12'), 1.58 (3H, s, H_3-15'), 1.52 (6H, br s, H_3-13', H_3-12"), 1.50 (3H, s, H_3-14'), 1.44 (3H, s, H_3-13"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.7 (C-12), 177.5 (C-7), 150.4 (C-2), 148.4 (C-4a), 144.8 (C-2'), 143.7 (C-7a), 137.1 (C-3'), 135.7 (C-6"), 135.3 (C-7'), 133.6 (C-12a), 132.2 (C-4), 131.6 (C-10"), 131.4 (C-11'), 130.8 (C-6a), 124.5 (C-6'/C-10'), 124.4 (C-6'/C-10'), 124.2 (C-9"), 123.4 (C-5"), 122.1 (C-3), 121.4 (C-2'), 118.2 (C-1"), 117.5 (C-12b), 113.8 (C-1), 111.3 (C-11a), 67.1 (C-6), 49.1 (C-10), 40.2 (C-9), 39.9 (C-4'), 39.85 (C-3"), 39.79 (C-8'/C-7"), 39.75 (C-8'/C-7"), 28.2 (C-1'), 26.9 (C-5'/C-9'), 26.8 (C-5'/C-9'), 26.7 (C-8"), 26.3 (C-4"), 25.8 (C-12', C-11"), 17.8 (C-13', C-12"), 17.4 (C-14"), 16.3 (C-14'), 16.2 (C-15', C-13"); (+)-HRESIMS $[M+Na]^+$ *m/z* 750.3820 (calcd for $C_{44}H_{57}NNaO_6S$, 750.3799).

4.4.31. 2-Hydroxy-3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-6-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)-10,11-dihydrobenzo[3,4]isochromeno[7,6-*b*][1,4]thiazine-7,12(6*H*,9*H*)-dione 8,8-dioxide (13)

Following general procedure B, benzochromenedione **29** (0.022 g, 0.035 mmol) was reacted with hypotaurine (0.004 g, 0.035 mmol) and the crude product purified to afford the title compound as a purple oil (0.006 g, 23%) as well as regioisomer **14** as a blue oil (0.008 g, 31%). R_f (EtOAc) 0.74; IR (ATR) ν_{max} 3427, 2927, 1708, 1677, 1534, 1462, 1392, 1378, 1350, 1116, 822, 783, 746, 729, 711 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.70 (1H, s, H-1), 6.69 (2H, br s, H-4, H-11), 6.07 (1H, d, *J* = 9.6 Hz, H-6), 5.31–5.28 (2H, m, H-2', H-1"), 5.10–5.08 (2H, m, H-6', H-10'), 5.07–5.03 (1H, m, H-9"), 4.98–4.95 (1H, m, H-5"), 4.11–4.07 (2H, m, H_2-10), 3.34–3.29 (4H, m, H_2-9, H_2-1'), 2.14–2.10 (2H, m, H_2-5'), 2.10–2.06 (2H, m, H_2-4'), 2.06–2.03 (2H, m, H_2-9'), 2.01–1.97 (4H, m, H_2-8', H_2-8"), 1.97–1.90

(4H, m, H_2-3", H_2-4"), 1.93 (3H, d, *J* = 0.9 Hz, H_3-14"), 1.90–1.86 (2H, m, H_2-7"), 1.75 (3H, s, H_3-15'), 1.67 (3H, s, H_3-11"), 1.66 (3H, s, H_3-12'), 1.59 (6H, br s, H_3-13', H_3-14'), 1.58 (3H, s, H_3-12"), 1.51 (3H, s, H_3-13"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.3 (C-12), 175.3 (C-7), 149.1 (C-4a), 148.3 (C-2), 145.5 (C-2"), 143.7 (C-11a), 139.5 (C-3'), 137.6 (C-6a), 135.8 (C-6"), 135.6 (C-7'), 133.0 (C-3, C-11'), 131.4 (C-10"), 127.9 (C-12a), 124.5 (C-6', C-10'), 123.8 (C-9"), 123.6 (C-5"), 120.5 (C-2'), 118.9 (C-4), 116.8 (C-1"), 114.9 (C-12b), 114.4 (C-1), 110.7 (C-7a), 67.8 (C-6), 48.8 (C-9), 40.1 (C-10), 40.0 (C-3"), 39.9 (C-4'), 39.80 (C-8'/C-7"), 39.76 (C-8'/C-7"), 29.8 (C-1'), 26.9 (C-5'/C-9'), 26.8 (C-5'/C-9'), 26.6 (C-8"), 26.2 (C-4"), 25.8 (C-12', C-11"), 17.8 (C-13', C-12"), 17.6 (C-14"), 16.4 (C-14'), 16.2 (C-15'), 16.1 (C-13"); (+)-HRESIMS $[M+Na]^+$ *m/z* 750.3814 (calcd for $C_{44}H_{57}NNaO_6S$, 750.3799).

4.4.32. 2-Hydroxy-3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-6-((1*E*,5*E*)-2,6,10-trimethylundeca-1,5,9-trien-1-yl)-9,10-dihydrobenzo[3,4]isochromeno[6,7-*b*][1,4]thiazine-7,12(6*H*,8*H*)-dione 11,11-dioxide (14)

Blue oil; yield: 31%; R_f (EtOAc) 0.88; IR (ATR) ν_{max} 3431, 2923, 1710, 1675, 1539, 1466, 1390, 1375, 1120, 819, 783, 746, 730, 716 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.98 (1H, s, H-1), 6.65 (1H, s, H-4), 6.56 (1H, br s, H-8), 5.96 (1H, d, *J* = 9.7 Hz, H-6), 5.36 (1H, dq, *J* = 9.7, 0.9 Hz, H-1"), 5.30–5.27 (1H, m, H-2'), 5.11–5.09 (1H, m, H-6'), 5.09–5.06 (1H, m, H-10'), 5.06–5.04 (1H, m, H-9"), 4.97–4.95 (1H, m, H-5"), 4.09–4.05 (2H, m, H_2-9), 3.33–3.30 (4H, m, H_2-10, H_2-1'), 2.13–2.08 (2H, m, H_2-5'), 2.08–2.04 (4H, m, H_2-4', H_2-9'), 2.04–2.00 (2H, m, H_2-4"), 2.00–1.93 (6H, m, H_2-8', H_2-3", H_2-8"), 1.93–1.89 (2H, m, H_2-7"), 1.89 (3H, d, *J* = 0.9 Hz, H_3-14"), 1.71 (3H, s, H_3-15'), 1.67 (3H, s, H_3-11"), 1.66 (3H s, H_3-12'), 1.59 (6H, br s, H_3-13', H_3-14'), 1.58 (3H, s, H_3-12"), 1.52 (3H, s, H_3-13"); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.8 (C-12), 177.2 (C-7), 150.7 (C-4a), 149.4 (C-2), 144.5 (C-2"), 143.6 (C-7a), 138.9 (C-3'), 136.1 (C-3), 135.7 (C-6"), 135.6 (C-7'), 133.3 (C-12a), 131.6 (C-10"), 131.4 (C-11'), 129.5 (C-6a), 124.5 (C-6'/C-10'), 124.4 (C-6'/C-10'), 124.0 (C-9"), 123.4 (C-5"), 120.3 (C-2'), 118.5 (C-4), 117.9 (C-1"), 116.0 (C-1), 115.6 (C-12b), 111.4 (C-11a), 67.3 (C-6), 49.1 (C-10), 40.1 (C-9), 39.9 (C-4'), 39.80 (C-3"), 39.76 (C-8', C-7"), 29.5 (C-1'), 26.83 (C-5'/C-9'), 26.81 (C-5'/C-9'), 26.6 (C-8"), 26.2 (C-4"), 25.9 (C-12', C-11"), 17.8 (C-13', C-12"), 17.3 (C-14"), 16.4 (C-14'), 16.18 (C-15'/C-13"), 16.17 (C-15'/C-13"); (+)-HRESIMS $[M+Na]^+$ *m/z* 750.3812 (calcd for $C_{44}H_{57}NNaO_6S$, 750.3799).

4.5 *In vitro* anti-plasmodial assays

In vitro activity against the erythrocytic stage of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay,[18] using a strain susceptible to known antimalarial drugs (*P. falciparum* NF54) and all the test compounds were compared for activity with the standard drug chloroquine. Compounds were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL), AlbumaxR (5 g/L) and washed human red cells Aþ at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 mg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 ºC; 4% CO_2, 3% O_2, 93% N_2. After 48 h 50 mL of ^{3}H-hypoxanthine (¼0.5 mCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fibre filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate liquid scintillation counter (Wallac, Zürich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression using Microsoft Excel.[19]

4.6 *In vitro* cytotoxicity against L6

Assays were performed in 96-well microtiter plates, each well containing 100 µL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4×10^4 L6 cells (a primary cell line derived from rat skeletal myoblasts). Serial drug dilutions of seven 3-fold dilution steps covering a range from 90 to 0.123 µg/mL were prepared. After 72 h of incubation, the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. Alamar Blue solution (10 µL) was then added to each well and the plates incubated for another 2 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analysed using the microplate reader software Softmax Pro. Podophyllotoxin was the reference drug used.[20]

4.7 *In vitro* FTase inhibition assays

4.7.1 Inhibition of human FTase

Assays were conducted on 96-well plates and read on a Wallac Victor fluorimeter from Perkin-Elmer. To each well, 20µL of farnesyl pyrophosphate (10 µM) was added to 180 µL of a solution containing 2 µL of varied concentrations of potential inhibitors (dissolved in DMSO) and 178 µL of a solution composed by 5 µL of partially purified human recombinant FTase (1.5 mg/mL) and 1.0 mL of dansyl-GCVLS peptide in the following buffer: 5.8 mM DTT, 6 mM $MgCl_2$, 12 µM $ZnCl_2$ and 0.18% (w/v) octyl-D-glucopyranoside, 53 mM Tris/HCl, pH 7.5.[21] Fluorescence development was recorded for 15 min (0.7 seconds per well, 15 repeats) at 30 °C with an excitation filter at 340 nm and an emission filter at 486 nm. Each measurement was performed in triplicate.

4.7.2 Inhibition of *T. brucei* FTase

T. brucei assays were conducted on 96-well plates, as described for human FTase with the dansylated peptide dansyl-GCAIM and the solution contains 15 µL of partially purified recombinant *Tb*FTase (1.0 mg/mL) in 1 mL peptide solution.[22]

4.8 *In vitro* anti-bacterial assays

4.8.1 Strains

Reference strains used were *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 and were purchased from Pasteur Institute (France). *S. Intermedius* 1051997 was provided by VIRBAC Company (Carros, France).

4.8.2 Anti-bacterial evaluation

Anti-bacterial activity of the compounds was studied by determination of minimal inhibitory concentrations (MIC) according to the NCCLS guidelines M7-A3 using the microbroth dilution methods. The bacterial strains were grown on trypticase soy agar (Becton Dickinson) at 37 °C for 24 h. Inocula were prepared in Mueller-Hinton broth by measuring the turbidity at 623 nm to obtain 2-6 10^5 CFU/mL.

Anti-bacterial activities of the compounds were determined by using a broth microdilution method performed in sterile 96-well microplates. All compounds were solubilized in DMSO at a concentration of 5 mg/mL and were transferred to each microplate well (in all cases concentrations of the desired molecules in DMSO do not exceed 2% of the total proportion), to obtain a two-fold serial dilution in 100 µL of broth and 100 µL of inoculum containing 2-6 10^5 CFU of each bacteria and yeast were added to each well. Several wells were reserved for positive controls, inoculum viability and solvent effect. After 24 h incubation, MIC was defined for each agent from duplicate observations as the lowest concentration of compound allowing no visible growth.[23]

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Supplementary Material

Supplementary data (comprised of copies of ^{1}H and ^{13}C NMR spectra) associated with this article can be found, in the online version, at http://

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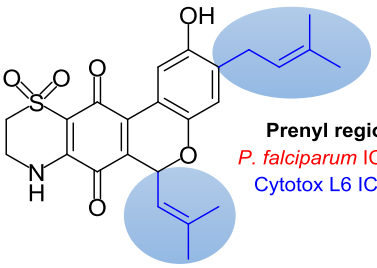
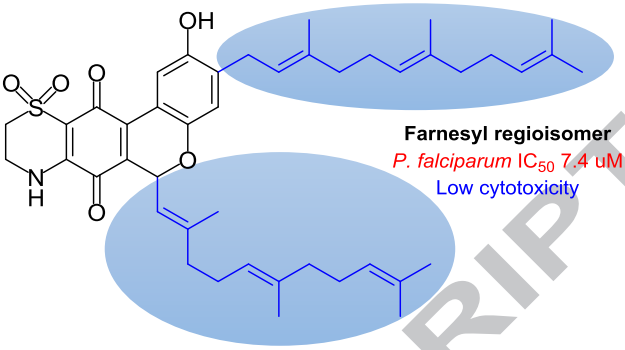


Prenyl regioisomer
P. falciparum IC_{50} 0.29 uM
Cytotox L6 IC_{50} 0.4 uM



Farnesyl regioisomer
P. falciparum IC_{50} 7.4 uM
Low cytotoxicity