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Article

Discovery and Evaluation of Thiazinoquinones as Anti-Protozoal Agents

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Abstract: Pure compound screening has identified the dioxothiazino-quinoline-quinone ascidian metabolite ascidiathiazone A (**2**) to be a moderate growth inhibitor of *Trypanosoma brucei rhodesiense* (IC_{50} 3.1 μM) and *Plasmodium falciparum* (K1 dual drug resistant strain) (IC_{50} 3.3 μM) while exhibiting low levels of cytotoxicity (L6, IC_{50} 167 μM). A series of C-7 amide and $\Delta^{2(3)}$ analogues were prepared that explored the influence of lipophilicity and oxidation state on observed anti-protozoal activity and selectivity. Little variation in anti-malarial potency was observed (IC_{50} 0.62–6.5 μM), and no correlation was apparent between anti-malarial and anti-*T. brucei* activity. Phenethylamide **7e** and $\Delta^{2(3)}$-glycine analogue **8k** exhibited similar anti-*Pf* activity to **2** but with slightly enhanced selectivity (SI 72 and 93, respectively), while $\Delta^{2(3)}$-phenethylamide **8e** (IC_{50} 0.67 μM, SI 78) exhibited improved potency and selectivity towards *T. brucei rhodesiense* compared to the natural product hit. A second series of analogues were prepared that replaced the quinoline ring of **2** with benzofuran or benzothiophene moieties. While esters **10a**/**10b** and **15** were once again found to exhibit cytotoxicity, carboxylic acid analogues exhibited potent anti-*Pf* activity (IC_{50} 0.34–0.035 μM) combined with excellent selectivity (SI 560–4000). *In vivo*

evaluation of a furan carboxylic acid analogue against *P. berghei* was undertaken, demonstrating 85.7% and 47% reductions in parasitaemia with ip or oral dosing respectively.

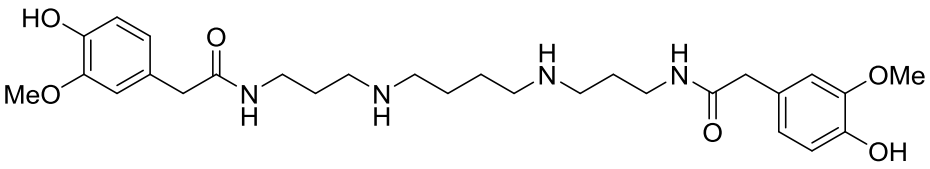
Keywords: marine natural products; protozoa; malaria; *Plasmodium falciparum*; *Trypanosoma brucei rhodesiense*; quinone; dioxothiazine; alkaloid

1. Introduction

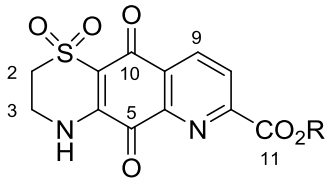
Natural products have historically played an important role in the discovery of new treatments for malaria [1]. From quinine and artemisinin [2,3] starting points, a diverse range of anti-malarials have been developed and have been the mainstay of frontline treatment for decades. Unfortunately with time has come loss of therapeutic efficacy due to the growing prevalence of drug resistant strains [4]. In the hunt for novel scaffolds from which to develop the next generation of anti-malarials, a structurally-diverse array of natural products, including those obtained from marine organisms, have been reported to exhibit activity towards *Plasmodium falciparum* [5–7].

As part of our own continuing search for new leads for the development of treatments for neglected human diseases [8–12] we have screened a library of synthesized and isolated marine natural products against a panel of four parasitic protozoa: *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum* K1 dual drug-resistant strain, with concurrent counter-screening for toxicity towards the non-malignant L6 rat myoblast cell line. We recently disclosed details of the first hit from this screen, the previously reported anti-inflammatory polyamine diamide ascidian metabolite orthidine F (**1**) [13–15] (Figure 1).

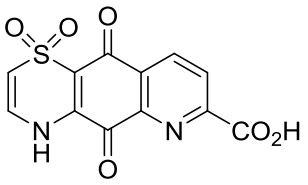
Figure 1. Structures of orthidine F (**1**), ascidiathiazone A (**2**) and analogues **3** and **4**.



1 orthidine F



2 R = H ascidiathiazone A
3 R = Me



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A second series of hits identified in this screening program were ascidiathiazone A (**2**), also previously reported by us as an anti-inflammatory alkaloid from a New Zealand ascidian, and synthetic analogues **3** and **4** [16]. The anti-protozoal evaluation of **2** (Table 1, entry 1) established the natural product to be a moderately potent *in vitro* growth inhibitor of *P. falciparum* K1 strain (IC_{50} 3.3 μM) and *Trypanosoma brucei rhodesiense* (IC_{50} 3.1 μM) while being effectively inactive towards *T. cruzi*

and *Leishmania donovani* and exhibiting low levels of cytotoxicity against a mammalian cell-line (L6, IC_{50} 170 μM). Similar levels of potency and selectivity were observed for ester **3** (Table 1, entry 2), while $\Delta^{2(3)}$ analogue **4** (Table 1, entry 3) exhibited more potent anti-malarial activity (IC_{50} 0.6 μM) with enhanced selectivity (SI *Pf* 300). Herein we report the results of a preliminary structure-activity relationship study investigating the influence of C-2 amide functionalization and thiazine-$\Delta^{2(3)}$ oxidation on the biological activity of **2**. In addition, we report that novel furan and thiophene analogues of **2** exhibit potent *in vitro* anti-malarial activity and that one analogue exhibits *in vivo* activity towards *P. berghei*.

Table 1. Anti-protozoal, cytotoxic activities and clogP values of **2**–**4**, **7a**–**h**, **j**, **8a**–**k**.

Entry	Compound	IC_{50} (μM) [a]					SI *Pf* [g]	clogP [h]
		T. b. rhod. [b]	*T. cruzi* [c]	*L. don.* [d]	*P. falc.* K1 [e]	L6 [f]		
1	**2**	3.1	>290	270	3.3	170	50	−1.1 ± 1.1
2	**3**	6.6	180	31	2.5	140	56	−0.5 ± 0.9
3	**4**	4.0	>290	190	0.60	180	300	−1.1 ± 1.0
4	**7a**	5.5	63	>280	0.94	24	26	0.3 ± 0.5
5	**7b**	1.8	15	29	0.62	23	37	0.8 ± 0.6
6	**7c**	3.9	15	48	1.1	12	10	2.3 ± 0.6
7	**7d**	1.9	43	21	1.1	15	14	0.6 ± 0.5
8	**7e**	2.4	140	160	1.5	110	72	0.9 ± 0.5
9	**7f**	2.4	27	47	1.4	13	10	1.4 ± 0.4
10	**7g**	3.4	41	83	1.6	24	15	2.1 ± 0.6
11	**7h**	>150	53	170	2.4	41	17	−0.6 ± 0.7
12	**7j**	120	250	>260	3.4	110	31	−0.9 ± 0.5
13	**8a**	3.7	63	>280	0.70	23	33	0.3 ± 0.8
14	**8b**	3.6	48	>270	1.5	4.8	3	0.8 ± 0.8
15	**8c**	2.4	42	53	0.98	6.5	6	2.2 ± 0.8
16	**8d**	4.2	160	>250	4.7	34	7	0.8 ± 0.6
17	**8e**	0.67	140	160	6.5	52	8	0.9 ± 0.7
18	**8f**	5.9	59	>240	1.2	6.5	5	1.3 ± 0.7
19	**8g**	2.5	42	150	1.1	4.9	4	2.1 ± 0.7
20	**8h**	10	150	230	1.7	50	29	−0.6 ± 0.8
21	**8i**	13	140	150	1.5	99	67	−1.0 ± 0.9
22	**8j**	35	160	220	1.8	100	57	−1.1 ± 1.0
23	**8k**	42	160	>280	1.2	110	93	−1.2 ± 0.7
	Melarsoprol [i]	0.005						
	Benznidazole [i]		1.8					
	Miltefosine [i]			0.53				
	Chloroquine [i]				0.28			
	Podophyllotoxin [i]					0.019		

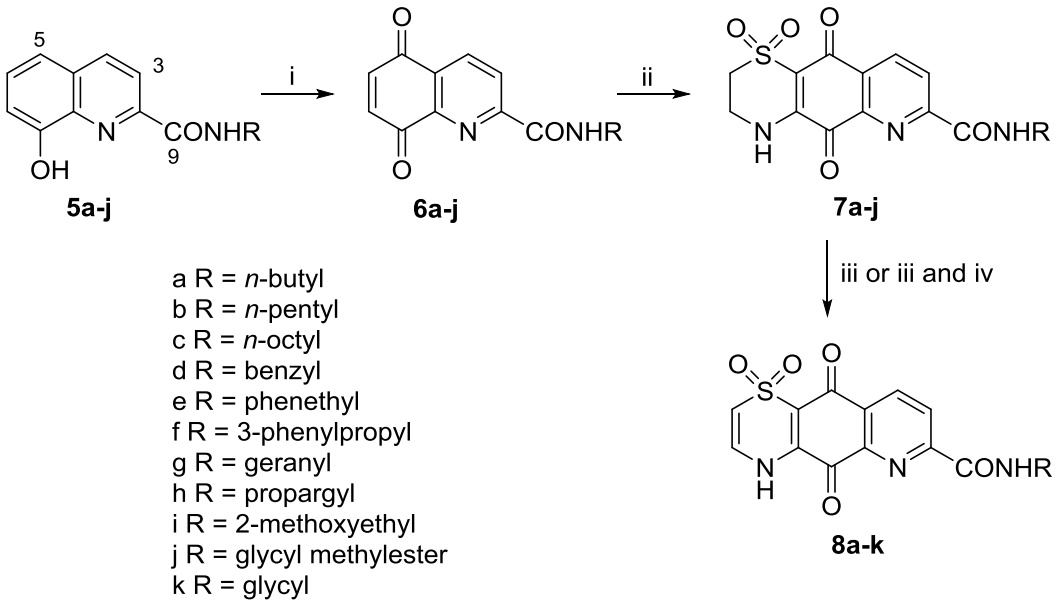
[a] IC_{50} values reported are the average of two independent assays. Assay protocols are described in [5]; [b] *Trypanosoma brucei rhodesiense*, STIB 900 strain, trypomastigotes stage; [c] *Trypanosoma cruzi*, Tulahuen C4 strain, amastigotes stage; [d] *Leishmania donovani*, MHOM-ET-67/L82 strain, amastigote/axenic stage; [e] *Plasmodium falciparum*, K1 strain, IEF stage; [f] L6 rat skeletal myoblast cell line; [g] Selectivity index for *P. falciparum* = IC_{50} L6/IC_{50} *Pf*; [h] cLogP calculated using ALOGPS 2.1, as described in [17,18]; [i] Melarsoprol, benznidazole, miltefosine, chloroquine and podophyllotoxin were used as positive controls.

2. Results and Discussion

2.1. Chemistry

We undertook a preliminary structure-activity relationship study to explore the effect of carboxylic acid functionalization and thiazine ring oxidation state towards the observed anti-protozoal activity of **2**. Efforts to directly prepare amide derivatives of **2** by reaction of the synthesized natural product [16] with various amines in the presence of peptide coupling reagents, led to the formation of complex product mixtures and low yields (data not shown). Instead we made use of a longer four step reaction sequence (Scheme 1). Commercially available 8-hydroxyquinoline-2-carboxylic acid was converted to amides **5a**–**5j** by reaction with the appropriate amine using PyBOP as the coupling agent in DMF. Subsequent oxidation using PIFA (phenyliodine bis(trifluoroacetate)) in MeCN/H_2O yielded unstable quinones **6a**–**6j**.

Scheme 1. General reaction sequence for the preparation of analogues **7a**–**j** and **8a**–**k**. *Reagents and conditions*: (i) PIFA (2–3 equiv.), MeCN/H_2O, 0 °C, 20 min; (ii) Hypotaurine (0.8 equiv.), $CeCl_3 \cdot 7H_2O$, MeCN/EtOH, rt, 2 days; (iii) 2 N NaOH, DMF, rt, 2 h; (iv) $SOCl_2$, MeOH, 0 °C then rt, then 65 °C, 2 h, 93% yield.

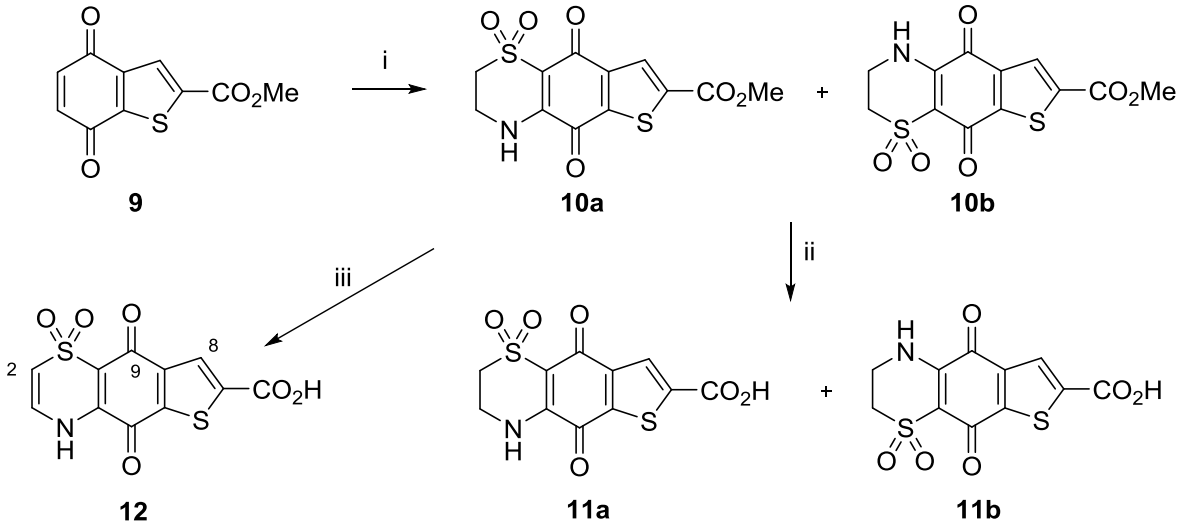


Previous studies by ourselves [16,19] and others [20,21] have found that hypotaurine addition to quinones typically yields a mixture of regio-isomeric thiazine adducts. In the present study, we found that slow addition of a dilute solution of hypotaurine in MeCN/EtOH at room temperature afforded, after filtration and washing, analogues **7a**–**7j** in yields of 14%, 27%, 57%, 17%, 49%, 57%, 29%, 29%, 26% and 20%, respectively. The regio-isomeric identity of the product in each case was established by 2D-NMR data, particularly HMBC experiments optimized for 8.3 and 2.0 Hz, which showed correlations from H-9 to quinone C-10 (8.3 Hz) and from NH-4 to quinone C-5 (2.0 Hz) [16]. Reaction of each of **7a**–**7j** with 2 N NaOH in DMF for 2 h [16] afforded the desired hydrolysed and autoxidised $\Delta^{2(3)}$-thiazine analogues **8a**–**8i** and **8k** in variable yield (30%–83%). In the specific case of the glycine

methylester **7j** the product of this reaction was the $\Delta^{2(3)}$-thiazino carboxylic acid **8k**, methylation of which ($SOCl_2$, MeOH, 93% yield) afforded ester **8j**.

Thiophene analogues of ascidiathiazone A were prepared (Scheme 2) starting from the literature quinone **9** [22]. Reaction with hypotaurine yielded two isomeric products **10a** and **10b** in a ratio of 1:0.3, as determined by NMR. Despite extensive attempts using chromatography, the isomers could not be separated and so were used as a mixture in the following steps. The regio-isomeric identity of **10a** and **10b** could not be established, as no relevant long range ^{1}H-^{13}C correlations were observed in HMBC data. Acid-mediated ester hydrolysis afforded carboxylic acids **11a** and **11b**, again characterized as an inseparable 1:0.3 mixture. HMBC data obtained for this isomeric mixture however was able to establish that the major regio-isomer was **11a** as shown. Thus correlations observed between the major isomer H-8 resonance (δ_H 7.84) to quinonoid resonance δ_C 171.7 (C-9) and from the thiazine NH (δ_H 9.31) to a second quinonoid resonance δ_C 173.1 (C-5) confirmed the identity of **11a**. In the case of base hydrolysis/autoxidation, reaction of the isomeric mixture **10a**/**10b** with 1N NaOH in a biphasic reaction in EtOAc, yielded the expected $\Delta^{2(3)}$ product **12**.

Scheme 2. Preparation of thiophene analogues **10a**/**10b**, **11a**/**11b** and **12**. *Reagents and conditions*: (i) Hypotaurine (1 equiv.), $CeCl_3 \cdot 7H_2O$ (1 equiv.), MeCN/EtOH, rt, 2 days, 18% yield (**10a** + **10b**); (ii) conc. HCl, rt, 5 h, 57% yield (**11a** + **11b**); (iii) 1 N NaOH, EtOAc, rt, 1 h, 78% yield.

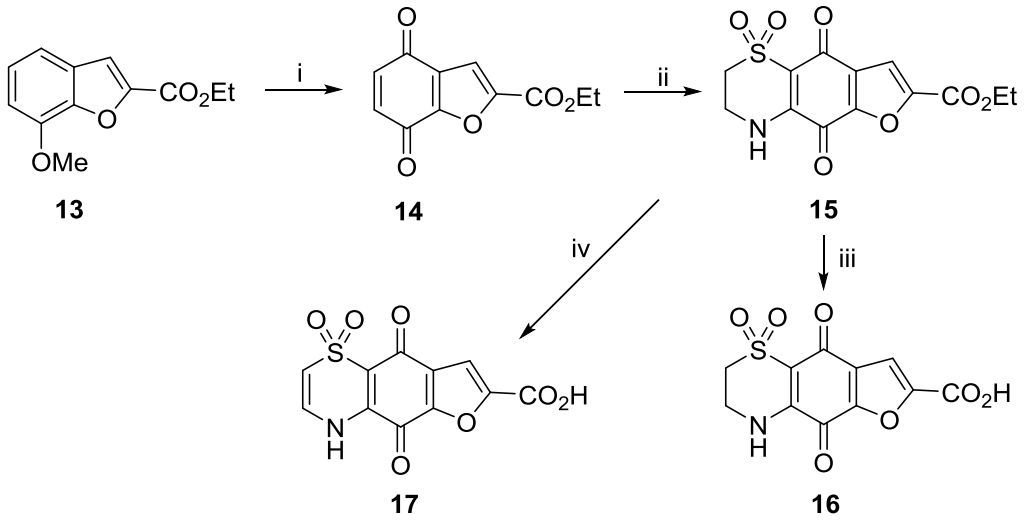


Column chromatography in this case was successful in affording the major regio-isomeric product in pure form. HMBC data analysis, in particular the observation of correlations from H-2 (δ_H 6.57) and H-8 (δ_H 7.82) to the same quinonoid carbon resonance at δ_C 175.2 (C-9) established the dioxothiazine ring regiochemistry of **12** as shown.

A series of furan analogues were prepared in analogous fashion, this time starting from commercially available 7-methoxy-benzofuran-2-carboxylic acid ethyl ester **13** (Scheme 3). Oxidation using acidified ceric ammonium sulfate afforded quinone **14** in 85% yield. Slow addition of hypotaurine to the quinone afforded a single regio-isomer **15** of the expected dioxothiazine product (43% yield). As demonstrated earlier, acidic hydrolysis of ester **15** yielded the carboxylic acid **16**

(63% yield), while biphasic 1N NaOH/EtOAc hydrolysis and autoxidation afforded the $\Delta^{2(3)}$ carboxylic acid **17** in 47% yield.

Scheme 3. Preparation of furan analogues **15**–**17**. *Reagents and conditions*: (i) $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$, MeCN/$H_2SO4$, 60 °C, 90 min, 85% yield; (ii) Hypotaurine (1 equiv.), $CeCl_3 \cdot 7H_2O$ (0.5 equiv.), MeCN/EtOH, rt, 1 days, 43% yield; (iii) conc. HCl, 100 °C, 2 h, 63% yield; (iv) 1 N NaOH, EtOAc, rt, 2 h, 47% yield.



2.2. Biological Activities

2.2.1. *In Vitro* Biological Evaluation

The library of target analogues were screened against a set of four protozoa and for cytotoxicity towards the rat skeletal myoblast cell line L6 and the results are summarized in Table 1 (amide and oxidized analogues of **2**) and Table 2 (thiophene and ester analogues). All of the amide analogues **7a**–**h**,**j** evaluated were either equipotent or slightly more active against *P. falciparum* than the natural product **2**. A similar observation was made for activities towards *T. brucei rhodesiense*, except for propargyl **7h** (Table 1, entry 11) and glycyl ester **7j** (Table 1, entry 12) both of which were significantly less active than **2**. Notable in this series, unfortunately, was the lack of selectivity with most analogues exhibiting selectivity indices (SI) of 40 or less. Of this sub-set, only phenethyl amide **7e** (Table 1, entry 8) exhibited anti-protozoal activity and cytotoxic selectivity similar to those observed for **2**. The corresponding $\Delta^{2(3)}$ analogues **8a**–**k** while being typically equipotent or slightly more active against *P. falciparum*, were on the whole more cytotoxic with low selectivity. Significant amongst the series were the $\Delta^{2(3)}$ phenethyl amide **8e** (Table 1, entry 17), which was the most active anti-*T. brucei rhodesiense* analogue, and ether **8i**, ester **8j** and carboxylic acid **8k** (Table 1, entries 21–23) which maintained the anti-*Pf* activity of **2** but with modestly enhanced selectivity. There was no apparent correlation between calculated logP and observed biological activity (Table 1).

Table 2. Anti-protozoal and cytotoxic activities of **10a**/**10b**, **11a**/**11b**, **12**, **15–17**.

Entry	Compound	IC_{50} (μM) [a]					SI *Pf* [g]	clogP [h]
		T. b. rhod. [b]	*T. cruzi* [c]	*L. don.* [d]	*P. falc.* K1 [e]	L6 [f]		
1	**10a/10b**	0.39	0.51	6.3	0.028	0.52	18	0.1 ± 0.8
2	**11a/11b**	5.4	>290	260	0.086	230	2700	−0.2 ± 0.7
3	**12**	2.2	210	>290	0.035	140	4000	−0.2 ± 0.7
4	**15**	1.1	4.7	40	0.11	5.1	46	−0.1 ± 0.8
5	**16**	7.5	>300	>300	0.12	290	2400	−0.8 ± 0.7
6	**17**	2.7	>300	120	0.34	190	560	−0.8 ± 0.9
	Melarsoprol [i]	0.01						
	Benznidazole [i]		1.4					
	Miltefosine [i]			0.53				
	Chloroquine [i]				0.28			
	Podophyllotoxin [i]					0.019		

[a] IC_{50} values reported are the average of two independent assays. Assay protocols are described in [5]; [b] *Trypanosoma brucei rhodesiense*, STIB 900 strain, trypomastigotes stage; [c] *Trypanosoma cruzi*, Tulahuen C4 strain, amastigotes stage; [d] *Leishmania donovani*, MHOM-ET-67/L82 strain, amastigote/axenic stage; [e] *Plasmodium falciparum*, K1 strain, IEF stage; [f] L6 rat skeletal myoblast cell line; [g] Selectivity index for *P. falciparum* respectively = IC_{50} L6/IC_{50} *Pf*; [h] cLogP calculated using ALOGPS 2.1, as described in [17,18]; [i] Melarsoprol, benznidazole, miltefosine, chloroquine and podophyllotoxin were used as positive controls.

Thiophene and furan analogues **10a**/**10b**, **11a**/**11b**, **12**, and **15–17** were evaluated against the same selection of protozoa and for cytotoxicity (Table 2). Potent anti-*Pf* activity was observed for the thiophene examples, with the isomerically pure carboxylic acid **12** (Table 2, entry 3) showing a desirable combination of nanomolar potency (*Pf* IC_{50} 35 nM) and excellent selectivity (SI *Pf* 4000). The furan analogues **15–17** (Table 2, entries 4–6) were slightly less active towards *P. falciparum*, exhibiting IC_{50}′s in the 110–340 nm range, with carboxylic acid **16** (Table 2, entry 5) exhibiting the best selectivity (SI *Pf* 2400). It is interesting to note the broad-range activities of esters **10a**/**10b** (Table 2, entry 1) and **15** (Table 2, entry 4): such pan-panel activity suggests the presence of an underlying general cytotoxic mechanism for these analogues. Once again, there was no apparent correlation between biological activity and calculated logP values.

2.2.2. *In Vivo* Anti-Malarial Evaluation

Furan carboxylic acid analogue **16** was selected for preliminary proof-of-principle *in vivo* evaluation in *Plasmodium berghei* infected mice. Preliminary ip acute toxicity of **16** showed no toxicity up to the highest test dose of 150 mg/kg. Using a standard test protocol [23], a repeated ip dose of 50 (mg/kg)/day for four days led to an 85.7% reduction in parasitaemia, and an increase in mean survival time from 4–6 days (untreated control) to 9.6 days. Switching to an oral dosing experiment (100 mg/kg once per day for 4 days) yielded a 47% reduction of parasitaemia. Although not considered significant, these levels of activity for both ip and po dosing clearly identifies heterocyclic dioxothiazinoquinone carboxylic acids to be a novel anti-malarial drug scaffold warranting further structure-activity relationship studies.

3. Experimental Section

3.1. General

HRMS data were acquired on a Bruker micrOTOF-QII mass spectrometer. Infrared spectra were recorded on a Perkin-Elmer Spectrum 100 Fourier-transform IR spectrometer equipped with a universal ATR accessory. Melting points were obtained on an Electrothermal melting point apparatus and are uncorrected. NMR spectra were recorded using either a Bruker Avance DRX 300 or 400 spectrometer operating at 300 MHz or 400 MHz for ^{1}H nuclei and 75 MHz or 100 MHz for ^{13}C nuclei. Resonance assignments were made by interpretation of 2D data. NMR assignments marked by an asterisk are interchangeable. Proto-deutero solvent signals were used as internal references (DMSO-d_6: δ_H 2.50, δ_C 39.52; $CDCl_3$: δ_H 7.25, δ_C 77.0; CD_3OD: δ_H 3.30, δ_C 49.05). Flash column chromatography was performed using reversed-phase Merck Lichroprep RP-18, or Kieselgel 60 PF silica gel. Thin layer chromatography used 0.2 mm thick plates of Kiesegel F_{254} (Merck, Manakau, New Zealand). The syntheses of **2**–**4** [16] and **9** [22] have been reported previously.

3.2. Synthetic Procedures

3.2.1. General Procedure for the Preparation of 8-Hydroxyquinoline-2-carboxamides **5a**–**5j**

To a solution of 8-hydroxyquinoline-2-carboxylic acid and PyBOP (1.25 equiv.) in dry DMF (3–6 mL), amine (1–2 equiv.) and triethylamine (1.25 equiv.) were added under N_2. The reaction mixture was then stirred under N_2 at rt for 12 h, after which time the mixture was dried in vacuo. The residue was purified by reversed-phase C_{18} flash column chromatography (0%–80% MeOH in H_2O (0.05% TFA)) and silica gel column chromatography (0%–1% MeOH in CH_2Cl_2).

3.2.1.1. *N*-*n*-Butyl-8-hydroxyquinoline-2-carboxamide (**5a**)

From 8-hydroxyquinoline-2-carboxylic acid (100 mg, 0.529 mmol), PyBOP (330 mg, 0.64 mmol), *n*-butylamine (104 μL, 1.05 mmol) and triethylamine (88 μL, 0.632 mmol) in DMF (6 mL) to give **5a** as a yellow oil (108 mg, 84% yield).

R_f = 0.68 (1% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3291, 1650, 1539, 1502, 1465, 1159 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.34 (1H, d, *J* = 8.4 Hz, H-3), 8.29 (1H, d, *J* = 8.4 Hz, H-4), 8.01 (1H, br s, NH-2′), 7.86 (1H, br s, OH), 7.53 (1H, t, *J* = 7.8 Hz, H-6), 7.40 (1H, d, *J* = 8.4 Hz, H-5), 7.23 (1H, d, *J* = 8.0 Hz, H-7), 3.53 (2H, dt, *J* = 7.2, 7.2 Hz, H_2-3′), 1.63 (2H, p, *J* = 7.3 Hz, H_2-4′), 1.40 (2H, sex., *J* = 7.6 Hz, H_2-5′), 0.92 (3H, t, *J* = 7.6 Hz, H_3-6′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 164.1 (C-1′), 152.2 (C-8), 148.2 (C-2), 137.8 (C-4), 136.1 (C-8a), 129.7 (C-4a), 129.2 (C-6), 119.9 (C-3), 118.3 (C-5), 111.2 (C-7), 39.5 (C-3′), 31.8 (C-4′), 20.2 (C-5′), 13.8 (C-6′); (+)-ESIMS *m/z* 245 $[M + H]^+$; (+)-HRESIMS *m/z* 245.1287 $[M + H]^+$ (calcd. for $C_{14}H_{17}N_2O_2$, 245.1285).

3.2.1.2. *N*-*n*-Pentyl-8-hydroxyquinoline-2-carboxamide (**5b**)

From 8-hydroxyquinoline-2-carboxylic acid (50 mg, 0.26 mmol), PyBOP (165 mg, 0.32 mmol), *n*-pentylamine (61 μL, 0.53 mmol) and triethylamine (44 μL, 0.32 mmol) in DMF (3 mL) to give **5b** as a colorless oil (65 mg, 97% yield).

R_f = 0.65 (5% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3266, 2929, 1647, 1500 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 8.46 (1H, t, *J* = 5.8 Hz, NH-2′), 8.33 (1H, d, *J* = 8.6 Hz, H-3), 8.24 (1H, d, *J* = 8.6 Hz, H-4), 7.49 (1H, t, *J* = 8.2 Hz, H-6), 7.34 (1H, d, *J* = 8.2 Hz, H-5), 7.20 (1H, d, *J* = 8.2 Hz, H-7), 3.46 (2H, dt, *J* = 7.4, 5.8 Hz, H_2-3′), 1.58 (2H, p, *J* = 7.4 Hz, H_2-4′), 1.30–1.18 (4H, m, H_2-5′/H_2-6′), 0.81 (3H, t, *J* = 7.4 Hz, H_3-7′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 164.4 (C-1′), 152.4 (C-8), 148.0 (C-2), 137.6 (C-4), 136.6 (C-8a), 129.7 (C-4a), 129.2 (C-6), 119.7 (C-3), 118.1 (C-5), 111.2 (C-7), 39.8 (C-3′), 29.4 (C-4′), 29.1 (C-5′), 22.3 (C-6′), 13.8 (C-7′); (+)-ESIMS *m/z* 281 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 281.1259 (calcd. for $C_{15}H_{18}N_2NaO_2$, 281.1260).

3.2.1.3. *N*-*n*-Octyl-8-hydroxyquinoline-2-carboxamide (**5c**)

From 8-hydroxyquinoline-2-carboxylic acid (50 mg, 0.26 mmol), PyBOP (165 mg, 0.32 mmol), *n*-octylamine (87 μL, 0.527 mmol) and triethylamine (44 μL, 0.32 mmol) in DMF (3 mL) to give **5c** as a colorless oil (73 mg, 94% yield).

R_f = 0.80 (5% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3297, 2924, 1648, 1501 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 8.69 (1H, t, *J* = 5.7 Hz, NH-2′), 8.33 (1H, d, *J* = 8.6 Hz, H-3), 8.22 (1H, d, *J* = 8.6 Hz, H-4), 7.48 (1H, t, *J* = 8.0 Hz, H-6), 7.32 (1H, d, *J* = 8.0 Hz, H-5), 7.18 (1H, d, *J* = 8.0 Hz, H-7), 3.45 (2H, dt, *J* = 7.2, 5.7 Hz, H_2-3′), 1.55 (2H, p, *J* = 7.2 Hz, H_2-4′), 1.27–1.09 (10H, m, H_2-5′/H_2-6′/H_2-7′/H_2-8′/H_2-9′), 0.80 (3H, t, *J* = 7.2 Hz, H_3-10′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 164.6 (C-1′), 152.5 (C-8), 147.8 (C-2), 137.6 (C-4), 136.6 (C-8a), 129.6 (C-4a), 129.2 (C-6), 119.6 (C-3), 118.1 (C-5), 111.2 (C-7), 39.9 (C-3′), 31.7 (C-6′*), 29.6 (C-4′), 29.2 (C-7′*), 29.1 (C-8′*), 27.0 (C-5′), 22.5 (C-9′*), 14.0 (C-10′); (+)-ESIMS *m/z* 323 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 323.1740 (calcd. for $C_{18}H_{24}N_2NaO_2$, 323.1730).

3.2.1.4. *N*-Benzyl-8-hydroxyquinoline-2-carboxamide (**5d**)

From 8-hydroxyquinoline-2-carboxylic acid (50 mg, 0.26 mmol), PyBOP (165 mg, 0.32 mmol), benzylamine (58 μL, 0.53 mmol) and triethylamine (44 μL, 0.32 mmol) in DMF (3 mL) to give **5d** as a colorless oil (57 mg, 79% yield).

R_f = 0.72 (5% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3251, 3062, 1642, 1501 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 8.90 (1H, br s, NH-2′), 8.29 (1H, d, *J* = 8.4 Hz, H-3), 8.20 (1H, d, *J* = 8.4 Hz, H-4), 7.48 (1H, t, *J* = 7.9 Hz, H-6), 7.33 (1H, d, *J* = 7.9 Hz, H-5), 7.25–7.13 (6H, m, H-7/2H-5′/2H-6′/H-7′), 4.61 (2H, br s, H_2-3′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 164.6 (C-1′), 152.4 (C-8), 147.5 (C-2), 137.9 (C-4′), 137.6 (C-4), 136.5 (C-8a), 129.7 (C-4a), 129.3 (C-6), 128.5 (C-5′), 127.7 (C-6′), 127.3 (C-7′), 119.7 (C-3), 118.1 (C-5), 111.3 (C-7), 43.6 (C-3′); (+)-ESIMS *m/z* 301 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 301.0949 (calcd. for $C_{17}H_{14}N_2NaO_2$, 301.0947).

3.2.1.5. *N*-Phenethyl-8-hydroxyquinoline-2-carboxamide (**5e**)

From 8-hydroxyquinoline-2-carboxylic acid (50 mg, 0.26 mmol), PyBOP (165 mg, 0.32 mmol), phenethylamine (66 μL, 0.53 mmol) and triethylamine (44 μL, 0.32 mmol) in DMF (3 mL) to give **5e** as a colorless oil (65 mg, 86% yield).

R_f = 0.65 (5% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3288, 3073, 1643, 1501 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 8.33 (1H, d, *J* = 8.5 Hz, H-3), 8.28 (1H, d, *J* = 8.5 Hz, H-4), 7.53 (1H, t, *J* = 7.8 Hz, H-6), 7.40–7.20 (7H, m, H-5/H-7/2H-6′/2H-7′/H-8′), 3.77 (2H, dt, *J* = 7.0, 6.8 Hz, H_2-3′), 2.96 (2H, t, *J* = 7.0 Hz, H_2-4′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 164.1 (C-1′), 152.3 (C-8), 147.9 (C-2), 138.8 (C-5′), 137.8 (C-4), 136.5 (C-8a), 129.7 (C-4a), 129.3 (C-6), 128.8 (C-6′), 128.7 (C-7′), 126.7 (C-8′), 119.7 (C-3), 118.1 (C-5), 111.2 (C-7), 40.7 (C-3′), 35.8 (C-4′); (+)-ESIMS *m/z* 293 $[M + H]^+$; (+)-HRESIMS *m/z* $[M + H]^+$ 293.1292 (calcd. for $C_{18}H_{17}N_2O_2$, 293.1285).

3.2.1.6. *N*-(3-Phenylpropyl)-8-hydroxyquinoline-2-carboxamide (**5f**)

From 8-hydroxyquinoline-2-carboxylic acid (50 mg, 0.26 mmol), PyBOP (165 mg, 0.32 mmol), 3-phenylpropylamine (66 μL, 0.46 mmol) and triethylamine (44 μL, 0.32 mmol) in DMF (3 mL) to give **5f** as a colorless oil (67 mg, 84% yield).

R_f = 0.65 (5% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3257, 2929, 1647, 1500 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 8.64 (1H, br s, NH-2′), 8.32 (1H, d, *J* = 8.5 Hz, H-3), 8.20 (1H, d, *J* = 8.5 Hz, H-4), 7.49 (1H, t, *J* = 7.9 Hz, H-6), 7.33 (1H, d, *J* = 7.9 Hz, H-5), 7.22–7.00 (6H, m, H-7/2H-7′/2H-8′/H-9′), 3.53–3.46 (2H, m, H_2-3′), 2.57 (2H, t, *J* = 7.2 Hz, H_2-5′), 1.89 (2H, p, *J* = 7.2 Hz, H_2-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 164.6 (C-1′), 152.5 (C-8), 147.7 (C-2), 141.2 (C-6′), 137.6 (C-4), 136.5 (C-8a), 129.6 (C-4a), 129.2 (C-6), 128.2 (C-7′), 128.1 (C-8′), 125.8 (C-9′), 119.5 (C-3), 118.1 (C-5), 111.2 (C-7), 39.4 (C-3′), 33.2 (C-5′), 31.0 (C-4′); (+)-ESIMS *m/z* 329 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 329.1267 (calcd. for $C_{19}H_{18}N_2NaO_2$, 329.1260).

3.2.1.7. *N*-Geranyl-8-hydroxyquinoline-2-carboxamide (**5g**)

From 8-hydroxyquinoline-2-carboxylic acid (50 mg, 0.26 mmol), PyBOP (165 mg, 0.32 mmol), geranylamine (98 μL, 0.53 mmol) and triethylamine (44 μL, 0.32 mmol) in DMF (3 mL) to give **5g** as a colorless oil (85 mg, 100% yield).

R_f = 0.66 (5% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3276, 2914, 1646, 1501 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 8.38 (1H, t, *J* = 5.6 Hz, NH-2′), 8.33 (1H, d, *J* = 8.5 Hz, H-3), 8.23 (1H, d, *J* = 8.5 Hz, H-4), 7.49 (1H, t, *J* = 6.1 Hz, H-6), 7.34 (1H, dd, *J* = 6.1, 1.1 Hz, H-5), 7.19 (1H, dd, *J* = 6.1, 1.1 Hz, H-7), 5.27 (1H, t, *J* = 6.9 Hz, H-4′), 4.99 (1H, t, *J* = 6.9 Hz, H-8′), 4.12 (2H, dd, *J* = 6.3, 6.3 Hz, H_2-3′), 2.04–1.95 (2H, m, H_2-7′), 1.93–1.86 (2H, m, H_2-6′), 1.62 (6H, s, H_3-11′/H_3-12′), 1.53 (3H, s, H_3-10′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 164.2 (C-1′), 152.4 (C-8), 148.0 (C-2), 139.7 (C-5′), 137.6 (C-4), 136.5 (C-8a), 131.6 (C-9′), 129.6 (C-4a), 129.2 (C-6), 123.7 (C-8′), 119.7 (C-4′), 119.6 (C-3), 118.1 (C-5), 111.2 (C-7), 39.4 (C-6′), 37.7 (C-3′), 26.3 (C-7′), 25.6 (C-12′), 17.6 (C-10′), 16.3 (C-11′); (+)-ESIMS *m/z* 325 $[M + H]^+$; (+)-HRESIMS *m/z* $[M + H]^+$ 325.1903 (calcd. for $C_{20}H_{25}N_2O_2$, 325.1911).

3.2.1.8. *N*-Propargyl-8-hydroxyquinoline-2-carboxamide (**5h**)

From 8-hydroxyquinoline-2-carboxylic acid (50 mg, 0.26 mmol), PyBOP (165 mg, 0.32 mmol), propargylamine (29 mg, 0.53 mmol) and triethylamine (44 μL, 0.32 mmol) in DMF (3mL) to give **5h** as a colorless oil (47 mg, 80% yield).

R_f = 0.56 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3303, 3273, 1646, 1504 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 10.17 (1H, br s, OH-9), 9.99 (1H, t, *J* = 5.8 Hz, NH-2′), 8.49 (1H, d, *J* = 8.4 Hz, H-4), 8.14 (1H, d, *J* = 8.4 Hz, H-3), 7.56 (1H, t, *J* = 7.8 Hz, H-6), 7.46 (1H, d, *J* = 7.8 Hz, H-5), 7.18 (1H, d, *J* = 7.8, Hz, H-7), 4.23 (2H, dd, *J* = 5.8, 2.4 Hz, H_2-3′), 3.22 (1H, t, *J* = 2.4 Hz, H_2-5′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 163.5 (C-1′), 153.7 (C-8), 147.0 (C-2), 137.8 (C-4), 136.4 (C-8a), 129.6 (C-4a and C-6), 118.8 (C-3), 117.5 (C-5), 111.6 (C-7), 81.1 (C-4′), 73.3 (C-5′), 28.1 (C-3′); (+)-ESIMS *m/z* 227 $[M + H]^+$; (+)-HRESIMS *m/z* $[M + H]^+$ 227.0814 (calcd. for $C_{13}H_{11}N_2O_2$, 227.0815).

3.2.1.9. *N*-(2-Methoxyethyl)-8-hydroxyquinoline-2-carboxamide (**5i**)

From 8-hydroxyquinoline-2-carboxylic acid (100 mg, 0.529 mmol), PyBOP (330 mg, 0.64 mmol), 2-methoxyethylamine (92.6 μL, 1.07 mmol) and triethylamine (88 μL, 0.632 mmol) in DMF (6 mL) to give **5i** as a colorless oil (106 mg, 82% yield).

R_f = 0.83 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3315, 1648, 1542, 1501, 1120 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 8.68 (1H, br s, NH-2′), 8.38 (1H, br s, OH), 8.32 (1H, d, *J* = 8.4 Hz, H-3), 8.26 (1H, d, *J* = 8.4 Hz, H-4), 7.50 (1H, t, *J* = 8.0 Hz, H-6), 7.36 (1H, d, *J* = 8.4 Hz, H-5), 7.21 (1H, d, *J* = 7.6 Hz, H-7), 3.73 (2H, dt, *J* = 5.6, 5.2 Hz, H_2-3′), 3.61 (2H, t, *J* = 5.2 Hz, H_2-4′), 3.36 (3H, s, H_3-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 164.6 (C-1′), 152.6 (C-8), 147.9 (C-2), 137.6 (C-4), 136.6 (C-8a), 129.7 (C-4a), 129.3 (C-6), 119.7 (C-3), 118.1 (C-5), 111.3 (C-7), 71.3 (C-4′), 58.8 (C-5′), 39.5 (C-3′); (+)-ESIMS *m/z* 247 $[M + H]^+$; (+)-HRESIMS *m/z* 247.1076 $[M + H]^+$ (calcd. for $C_{13}H_{15}N_2O_3$, 247.1077).

3.2.1.10. *N*-Glycine(methylester)-8-hydroxyquinoline-2-carboxamide (**5j**)

From 8-hydroxyquinoline-2-carboxylic acid (100 mg, 0.529 mmol), PyBOP (330 mg, 0.64 mmol), glycine methyl ester hydrochloride (94.4 mg, 0.76 mmol) and triethylamine (220 μL, 1.58 mmol) in DMF (6 mL) to give **5j** as a colorless oil (128 mg, 93% yield).

R_f = 0.86 (10% MeOH/CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ_H 9.08 (1H, t, *J* = 6.1 Hz, NH-2′), 8.38 (1H, br s, OH), 8.03 (2H, s, H-3 and H-4), 7.43 (1H, t, *J* = 8.0 Hz, H-6), 7.23 (1H, dd, *J* = 8.4, 1.0 Hz, H-5), 7.13 (1H, dd, *J* = 7.6, 1.0 Hz, H-7), 4.27 (2H, d, *J* = 6.1 Hz, H_2-3′), 3.72 (3H, s, H_3-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 171.2 (C-4′), 165.0 (C-1′), 152.7 (C-8), 146.8 (C-2), 137.2 (C-4), 136.4 (C-8a), 129.6 (C-4a), 129.5 (C-6), 119.3 (C-3), 117.9 (C-5), 111.3 (C-7), 52.5 (C-5′), 41.3 (C-3′); (+)-ESIMS *m/z* 261 $[M + H]^+$; (+)-HRESIMS *m/z* 261.0863 $[M + H]^+$ (calcd. for $C_{13}H_{13}N_2O_4$, 261.0870).

3.2.2. General Procedure for Preparation of Quinones **6a**–**6j**

A solution of PIFA (2–3 equiv.) in MeCN/H_2O (2:1 mL) was cooled to 0 °C, followed by the addition of the appropriate 8-hydroxyquinoline-2-carboxamide in CH_2Cl_2 (1 mL). The dark brown suspension was stirred for 20 min. at 0 °C before being poured into a mixture of CH_2Cl_2 (20 mL) and H_2O (30 mL). The organic phase was dried in vacuo and the crude product used in the subsequent reaction without further purification.

3.2.2.1. *N*-*n*-Butyl-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6a**)

From *N*-*n*-butyl-8-hydroxyquinoline-2-carboxamide (**5a**) (48 mg, 0.20 mmol), PIFA (254 mg, 0.59 mmol) to give **6a** (44 mg, 85% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.59 (1H, d, *J* = 8.0 Hz, H-3*), 8.56 (1H, d, *J* = 8.0 Hz, H-4*), 8.28 (1H, br s, NH-2′), 7.19 (1H, d, *J* = 10.4 Hz, H-7), 7.11 (1H, d, *J* = 10.4 Hz, H-6), 3.50 (2H, dt, *J* = 6.4, 6.4 Hz, H_2-3′), 1.64 (2H, p, *J* = 7.3 Hz, H_2-4′), 1.41 (2H, sex., *J* = 7.2 Hz, H_2-5′), 0.94 (3H, t, *J* = 7.6 Hz, H_3-6′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 183.8 (C-5), 182.6 (C-8), 162.4 (C-1′), 153.9 (C-2), 145.8 (C-8a), 139.3 (C-7*), 138.3 (C-6*), 136.5 (C-3*), 130.2 (C-4a), 126.2 (C-4*), 39.6 (C-3′), 31.6 (C-4′), 20.1 (C-5′), 13.8 (C-6′); (+)-ESIMS *m/z* 281 $[M + Na]^+$; (+)-HRESIMS *m/z* 281.0893 $[M + Na]^+$ (calcd. for $C_{14}H_{14}N_2NaO_3$, 281.0897).

3.2.2.2. *N*-*n*-Pentyl-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6b**)

From *N*-*n*-pentyl-8-hydroxyquinoline-2-carboxamide (**5b**) (38 mg, 0.15 mmol), PIFA (127 mg, 0.30 mmol) to give **6b** (31 mg, 76% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 8.57 (2H, s, H-3/H-4), 8.30 (1H, br s, NH-2′), 7.20 (1H, d, *J* = 10.3 Hz, H-7), 7.12 (1H, d, *J* = 10.3 Hz, H-6), 3.53–3.44 (2H, m, H_2-3′), 1.71–1.61 (2H, m, H_2-4′), 1.39–1.33 (4H, m, H_2-5′/H_2-6′), 0.90 (3H, t, *J* = 7.3 Hz, H_3-7′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 183.7 (C-5*), 182.4 (C-8*), 162.4 (C-1′), 154.0 (C-2), 145.7 (C-8a), 139.2 (C-7), 138.2 (C-6), 136.3 (C-3*), 130.2 (C-4a), 126.1 (C-4*), 39.7 (C-3′), 29.2 (C-4′), 29.0 (C-5′), 22.2 (C-6′), 13.9 (C-7′); (+)-ESIMS *m/z* 295 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 295.1061 (calcd. for $C_{15}H_{16}NaN_2O_3$, 295.1053).

3.2.2.3. *N*-*n*-Octyl-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6c**)

From *N*-*n*-octyl-8-hydroxyquinoline-2-carboxamide (**5c**) (73 mg, 0.24 mmol), PIFA (127 mg, 0.30 mmol) to give **6c** (64 mg, 85% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 8.56 (2H, s, H-3/H-4), 8.23 (1H, br s, NH-2′), 7.18 (1H, d, *J* = 10.5 Hz, H-7), 7.10 (1H, d, *J* = 10.5 Hz, H-6), 3.48 (2H, dt, *J* = 6.7, 6.0 Hz, H_2-3′), 1.65 (2H, p, *J* = 7.2 Hz, H_2-4′), 1.42–1.20 (10H, br s, H_2-5′/H_2-6′/H_2-7′/H_2-8′/H_2-9′), 0.86 (3H, t, *J* = 7.1 Hz, H_3-10′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 183.8 (C-5), 182.5 (C-8), 162.4 (C-1′), 154.1 (C-2), 145.0 (C-8a), 139.3 (C-7), 138.2 (C-6), 136.4 (C-3*), 130.3 (C-4a), 126.2 (C-4*), 39.9 (C-3′), 31.8 (C-6′*), 29.6 (C-4′), 29.2 (C-7′*), 29.1 (C-8′*), 27.0 (C-5′), 22.6 (C-9′*), 14.0 (C-10′); (+)-ESIMS *m/z* 337 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 337.1531 (calcd. for $C_{18}H_{22}N_2NaO_3$, 337.1523).

3.2.2.4. *N*-Benzyl-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6d**)

From *N*-benzyl-8-hydroxyquinoline-2-carboxamide (**5d**) (51 mg, 0.18 mmol), PIFA (127 mg, 0.30 mmol) to give **6d** (44 mg, 84% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.67 (1H, br s, NH-2′), 8.60 (1H, d, *J* = 8.1 Hz, H-3*), 8.55 (1H, d, *J* = 8.1 Hz, H-4*), 7.36–7.26 (5H, m, 2H-5′/2H-6′/H-7′), 7.14 (1H, d, *J* = 10.6 Hz, H-7), 7.09 (1H, d, *J* = 10.6 Hz, H-6), 4.69 (2H, d, *J* = 6.3 Hz, H_2-3′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 183.7 (C-5), 182.4 (C-8), 162.5 (C-1′), 153.8 (C-2), 145.8 (C-8a), 139.2 (C-7), 138.2 (C-6), 137.4 (C-4′), 136.4 (C-3*), 130.2 (C-4a), 128.7 (C-5′), 127.8 (C-6′), 127.5 (C-7′), 126.4 (C-4*), 43.6 (C-3′); (+)-ESIMS *m/z* 315 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 315.0748 (calcd. for $C_{17}H_{12}N_2NaO_3$, 315.0740).

3.2.2.5. *N*-Phenethyl-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6e**)

From *N*-phenethyl-8-hydroxyquinoline-2-carboxamide (**5e**) (58 mg, 0.20 mmol), PIFA (127 mg, 0.30 mmol) to give **6e** (42 mg, 69% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 8.57 (2H, s, H-3/H-4), 8.32 (1H, br s, NH-2′), 7.35–7.22 (5H, m, 2H-6′/2H-7′/H-8′), 7.17 (1H, d, *J* = 10.5 Hz, H-7), 7.10 (1H, d, *J* = 10.5 Hz, H-6), 3.75 (2H, dt, *J* = 7.7, 6.9 Hz, H_2-3′), 2.98 (2H, t, *J* = 7.7 Hz, H_2-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 183.7 (C-5), 182.3 (C-8), 162.4 (C-1′), 153.9 (C-2), 145.8 (C-8a), 139.3 (C-7), 138.2 (C-6), 137.4 (C-5′), 136.4 (C-3*), 130.2 (C-4a), 128.7 (C-6′*), 128.6 (C-7′*), 126.5 (C-8′), 126.1 (C-4*), 41.2 (C-3′), 35.8 (C-4′); (+)-ESIMS *m/z* 329 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 329.0904 (calcd. for $C_{18}H_{14}N_2NaO_3$, 329.0897).

3.2.2.6. *N*-(3-Phenpropyl)-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6f**)

From *N*-(3-phenylpropyl)-8-hydroxyquinoline-2-carboxamide (**5f**) (65 mg, 0.21 mmol), PIFA (127 mg, 0.30 mmol) to give **6f** (45 mg, 67% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.57 (2H, s, H-3/H-4), 8.34 (1H, br s, NH-2′), 7.33–7.19 (5H, m, 2H-7′/2H-8′/H-9′), 7.17 (1H, d, *J* = 10.4 Hz, H-7), 7.10 (1H, d, *J* = 10.4 Hz, H-6), 3.54 (2H, dt, *J* = 7.5, 6.8 Hz, H_2-3′), 2.72 (2H, t, *J* = 7.5 Hz, H_2-5′), 2.01 (2H, p, *J* = 7.5 Hz, H_2-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 183.7 (C-5), 182.4 (C-8), 162.4 (C-1′), 153.9 (C-2), 145.7 (C-8a), 139.2 (C-7), 138.2 (C-6), 137.4 (C-6′), 136.4 (C-3*), 130.2 (C-4a), 128.4 (C-7′), 128.3 (C-8′), 126.1 (C-9′), 125.9 (C-4*), 39.3 (C-3′), 33.2 (C-5′), 31.0 (C-4′); (+)-ESIMS *m/z* 343 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 343.1063 (calcd. for $C_{19}H_{16}N_2NaO_3$, 343.1053).

3.2.2.7. *N*-Geranyl-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6g**)

From *N*-geranyl-8-hydroxyquinoline-2-carboxamide (**5g**) (48 mg, 0.15 mmol), PIFA (127 mg, 0.30 mmol) to give **6g** (35 mg, 69% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.57 (1H, d, *J* = 8.1 Hz, H-3), 8.54 (1H, d, *J* = 8.1 Hz, H-4), 8.18 (1H, br s, NH-2′), 7.19 (1H, d, *J* = 10.5 Hz, H-7), 7.09 (1H, d, *J* = 10.5 Hz, H-6), 5.28 (1H, t, *J* = 5.6 Hz, H-4′), 5.06 (1H, t, *J* = 6.8 Hz, H-8′), 4.10 (2H, dd, *J* = 6.4, 5.6 Hz, H_2-3′), 2.11–2.05

(2H, m, H_2-7′), 2.04–1.99 (2H, m, H_2-6′), 1.72 (3H, s, H_3-11′), 1.65 (3H, s, H_3-12′), 1.58 (3H, s, H_3-10′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 183.8 (C-5), 182.5 (C-8), 162.2 (C-1′), 154.1 (C-2), 145.8 (C-8a), 140.0 (C-5′), 139.3 (C-7), 138.2 (C-6), 136.3 (C-3*), 131.7 (C-9′), 130.3 (C-4a), 126.2 (C-4*), 123.8 (C-8′), 119.4 (C-4′), 39.5 (C-6′), 37.7 (C-3′), 26.3 (C-7′), 25.6 (C-12′), 17.7 (C-10′), 16.4 (C-11′); (+)-ESIMS *m/z* 361 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 361.1526 (calcd. for $C_{20}H_{22}N_2NaO_3$, 361.1523).

3.2.2.8. *N*-Propargyl-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6h**)

From *N*-propargyl-8-hydroxyquinoline-2-carboxamide (**5h**) (45 mg, 0.20 mmol), PIFA (229 mg, 0.53 mmol) to give **6h** (40 mg, 83% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.57 (2H, br s, H-3/H-4), 7.19 (1H, d, *J* = 10.5 Hz, H-7), 7.12 (1H, d, *J* = 10.5 Hz, H-6), 4.30 (2H, dd, *J* = 5.6, 2.5 Hz, H_2-3′), 2.28 (1H, t, *J* = 2.5 Hz, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 183.6 (C-5), 182.4 (C-8), 162.3 (C-1′), 153.3 (C-2), 145.8 (C-8a), 139.3 (C-6*), 138.3 (C-7*), 136.5 (C-3*), 130.2 (C-4a), 126.4 (C-4*), 78.9 (C-4′), 71.8 (C-5′), 29.3 (C-3′); (+)-ESIMS *m/z* 263 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 263.0431 (calcd. for $C_{13}H_8N_2NaO_3$, 263.0427).

3.2.2.9. *N*-(2-Methoxyethyl)-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6i**)

From *N*-(2-methoxyethyl)-8-hydroxyquinoline-2-carboxamide (**5i**) (86 mg, 0.35 mmol), PIFA (300 mg, 0.70 mmol) to give **6i** (78 mg, 86% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.58 (2H, s, H-3/H-4), 8.49 (1H, br s, NH-2′), 7.19 (1H, d, *J* = 10.4 Hz, H-7), 7.10 (1H, d, *J* = 10.4 Hz, H-6), 3.72 (2H, dt, *J* = 5.6, 5.2 Hz, H_2-3′), 3.61 (2H, t, *J* = 5.2 Hz, H_2-4′), 3.40 (3H, s, H_3-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 183.8 (C-5), 182.3 (C-8), 162.7 (C-1′), 153.9 (C-2), 145.9 (C-8a), 139.3 (C-7*), 138.2 (C-6*), 136.4 (C-3*), 130.2 (C-4a), 126.2 (C-4*), 70.9 (C-4′), 58.9 (C-5′), 39.6 (C-3′); (+)-ESIMS *m/z* 283 $[M + Na]^+$; (+)-HRESIMS *m/z* 283.0696 $[M + Na]^+$ (calcd. for $C_{13}H_{12}N_2NaO_4$, 283.0689).

3.2.2.10. *N*-Glycine(methylester)-5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6j**)

From *N*-glycine(methylester)-8-hydroxyquinoline-2-carboxamide (**5j**) (50 mg, 0.19 mmol), PIFA (132 mg, 0.31 mmol) to give **6j** (40 mg, 77% yield) as a brown oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 8.77 (2H, br t, *J* = 5.2 Hz, NH-2′), 8.59 (1H, d, *J* = 8.3 Hz, H-3), 8.55 (1H, d, *J* = 8.3 Hz, H-4), 7.20 (1H, d, *J* = 10.5 Hz, H-7), 7.13 (1H, d, *J* = 10.5 Hz, H-6), 4.31 (2H, d, *J* = 6.1 Hz, H_2-3′), 3.79 (3H, s, H_3-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 183.7 (C-5), 182.4 (C-8), 169.7 (C-4′), 163.1 (C-1′), 153.2 (C-2), 145.9 (C-8a), 139.4 (C-7), 138.3 (C-6), 136.5 (C-4), 130.2 (C-4a), 126.4 (C-3), 52.5 (C-5′), 41.4 (C-3′); (+)-ESIMS *m/z* 297 $[M + Na]^+$; (+)-HRESIMS *m/z* 297.0477 $[M + Na]^+$ (calcd. for $C_{13}H_{10}N_2NaO_5$, 297.0482).

3.2.3. General Procedure for the Preparation of Carboxamide Analogues **7a**–**7j**

A solution of 5,8-dioxo-5,8-dihydroquinoline-2-carboxamide (**6a**–**6j**) was dissolved in MeCN/EtOH (1:1) before being cooled to 0 °C. In some cases, $CeCl_3 \cdot 7H_2O$ (1 equiv.) was also added to the reaction. Hypotaurine (0.8 equiv.) in H_2O was added dropwise over 3.5 h. The reaction mixture changed color from dark brown to dark orange, and was stirred at rt for 2 days. The product was purified either by filtration and washing with H_2O (3 × 20 mL) and MeOH (3 × 20 mL), or by reversed-phase C_{18} flash column chromatography (0%–80% MeOH in H_2O (0.05% TFA)).

3.2.3.1. *N*-*n*-Butyl-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**7a**)

From **6a** (54 mg, 0.21 mmol) in MeCN/EtOH (1:1, 20 mL) and hypotaurine (16.0 mg, 0.15 mmol) in H_2O (3 mL). Filtration gave **7a** as an orange powder (11.0 mg, 14% yield).

Mp 200 °C (decomp.); R_f = 0.49 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3300, 3237, 1682, 1594, 1580, 1508, 1336, 1280, 1170, 1107 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 9.35 (1H, br s, NH-4), 8.70 (1H, t, *J* = 6.4 Hz, NH-2′), 8.53 (1H, d, *J* = 8.2 Hz, H-9), 8.40 (1H, d, *J* = 8.2 Hz, H-8), 3.92–3.87 (2H, m, H_2-3), 3.43–3.36 (obscured by solvent, H_2-2 and H_2-3′), 1.55 (2H, p, *J* = 7.2 Hz, H_2-4′), 1.33 (2H, sex., *J* = 7.6 Hz, H_2-5′), 0.91 (3H, t, *J* = 7.6 Hz, H_3-6′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 176.2 (C-5), 173.4 (C-10), 162.5 (C-1′), 152.6 (C-7), 147.7 (C-4a), 145.3 (C-5a), 136.0 (C-9), 131.4 (C-9a), 126.5 (C-8), 110.7 (C-10a), 48.2 (C-2), 39.3 (obscured by solvent, C-3 and C-3′), 31.3 (C-4′), 19.6 (C-5′), 13.7 (C-6′); (+)-ESIMS *m/z* 386 $[M + Na]^+$; (+)-HRESIMS *m/z* 386.0791 $[M + Na]^+$ (calcd. for $C_{16}H_{17}N_3NaO_5S$, 386.0781).

3.2.3.2. *N*-*n*-Pentyl-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**7b**)

From **6b** (31 mg, 0.11 mmol), $CeCl_3.7H_2O$ (37 mg, 98 μmol) in MeCN/EtOH (1:1, 14 mL) and hypotaurine (8.2 mg, 0.075 mmol) in H_2O (2 mL). Filtration gave **7b** as a red-brown powder (11.0 mg, 27% yield).

Mp 200 °C (decomp.); R_f = 0.44 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3234, 2933, 1686, 1508 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 9.36 (1H, br s, NH-4), 8.72 (1H, t, *J* = 5.9 Hz, NH-2′), 8.53 (1H, d, *J* = 8.2 Hz, H-9), 8.40 (1H, d, *J* = 8.2 Hz, H-8), 3.90 (2H, br s, H_2-3), 3.43–3.33 (4H, obscured by H_2O, H_2-2, H_2-3′), 1.57 (2H, p, *J* = 6.8 Hz, H_2-4′), 1.34–1.27 (4H, m, H_2-5′/H_2-6′), 0.88 (3H, t, *J* = 6.8 Hz, H_3-7′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 176.2 (C-5), 173.5 (C-10), 162.5 (C-1′), 152.6 (C-7), 147.7 (C-4a), 145.4 (C-5a), 136.0 (C-9), 131.4 (C-9a), 126.5 (C-8), 110.7 (C-10a), 48.2 (C-2), 40.8 (C-3), 38.8 (C-3′), 28.8 (C-4′), 28.7 (C-5′*), 21.9 (C-6′*), 13.9 (C-7′); (+)-ESIMS *m/z* 378 $[M + H]^+$; (+)-HRESIMS *m/z* $[M + H]^+$ 378.1107 (calcd. for $C_{17}H_{20}N_3O_5S$, 378.1118).

3.2.3.3. *N*-*n*-Octyl-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**7c**)

From **6c** (32 mg, 0.10 mmol), $CeCl_3 \cdot 7H_2O$ (39 mg, 0.10 mmol) in MeCN/EtOH (1:1, 14 mL) and hypotaurine (9.7 mg, 0.089 mmol) in H_2O (2 mL). Filtration and solvent wash gave **7c** as a red-brown powder (24.0 mg, 57% yield).

Mp 200 °C (decomp.); R_f = 0.44 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3240, 2925, 1669, 1521 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 9.36 (1H, br s, NH-4), 8.71 (1H, t, *J* = 6.1 Hz, NH-2′), 8.53 (1H, d, *J* = 8.0 Hz, H-9), 8.39 (1H, d, *J* = 8.0 Hz, H-8), 3.90 (2H, br s, H_2-3), 3.41 (2H, br t, *J* = 6.1 Hz, H_2-2), 3.32 (2H, obscured by H_2O, H_2-3′), 1.61–1.53 (2H, m, H_2-4′), 1.37–1.20 (10H, m, H_2-5′/H_2-6′/H_2-7′/H_2-8′/H_2-9′), 0.85 (3H, t, *J* = 6.7 Hz, H_3-10′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 176.1 (C-5), 173.4 (C-10), 162.4 (C-1′), 152.5 (C-7), 147.6 (C-4a), 145.2 (C-5a), 135.9 (C-9), 131.3 (C-9a), 126.4 (C-8), 110.6 (C-10a), 48.1 (C-2), 39.5 (C-3/C-3′), 31.1 (C-8′), 29.0 (C-4′), 28.6 (C-6′), 26.4 (C-5′), 22.0 (C-9′), 13.9 (C-10′); (+)-ESIMS *m/z* 420 $[M + H]^+$; (+)-HRESIMS *m/z* $[M + H]^+$ 420.1581 (calcd. for $C_{20}H_{26}N_3O_5S$, 420.1588).

3.2.3.4. *N*-Benzyl-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**7d**)

From **6d** (44 mg, 0.15 mmol), $CeCl_3.7H_2O$ (55 mg, 0.15 mmol) in MeCN/EtOH (1:1, 14 mL) and hypotaurine (13.0 mg, 0.12 mmol) in H_2O (2 mL). Filtration and solvent wash gave **7d** as a red-brown powder (10.0 mg, 17% yield).

Mp 200 °C (decomp.); R_f = 0.44 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3267, 1676, 1595, 1513 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ_H 9.37 (1H, br s, NH-4), 9.28 (1H, t, *J* = 6.2 Hz, NH-2′), 8.55 (1H, d, *J* = 8.2 Hz, H-9), 8.43 (1H, d, *J* = 8.2 Hz, H-8), 7.37–7.23 (5H, m, 2H-5′/2H-6′/H-7′), 4.57 (2H, d, *J* = 6.4 Hz, H_2-3′), 3.90 (2H, br s, H_2-3), 3.41 (2H, t, *J* = 6.2 Hz, H_2-2); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 176.2 (C-5), 173.4 (C-10), 162.8 (C-1′), 152.5 (C-7), 147.7 (C-4a), 145.5 (C-5a), 139.3 (C-4′), 136.0 (C-9), 131.5 (C-9a), 128.4 (C-5′), 127.5 (C-6′), 126.9 (C-8), 126.7 (C-7′), 110.7 (C-10a), 48.2 (C-2), 42.7 (C-3′), 39.4 (C-3); (+)-ESIMS *m/z* 420 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 420.0618 (calcd. for $C_{19}H_{15}N_3NaO_5S$, 420.0625).

3.2.3.5. *N*-Phenethyl-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**7e**)

From **6e** (22.8 mg, 0.075 mmol), $CeCl_3.7H_2O$ (51 mg, 0.14 mmol) in MeCN/EtOH (1:1, 14 mL) and hypotaurine (12.0 mg, 0.11 mmol) in H_2O (2 mL). Filtration and solvent wash gave **7e** as a red-brown powder (15.0 mg, 49% yield).

Mp 240 °C (decomp.); R_f = 0.48 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3230, 1678, 1580, 1555 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ_H 9.37 (1H, br s, NH-4), 8.77 (1H, t, *J* = 5.9 Hz, NH-2′), 8.54 (1H, d, *J* = 8.1 Hz, H-9), 8.40 (1H, d, *J* = 8.1 Hz, H-8), 7.33–7.19 (5H, m, 2H-6′/2H-7′/H-8′), 3.93–3.87 (2H, m, H_2-3), 3.60 (2H, dt, *J* = 7.7, 5.9 Hz, H_2-3′), 3.40 (2H, br t, *J* = 5.4 Hz, H_2-2), 2.90 (2H, t, *J* = 7.7 Hz, H_2-4′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 176.2 (C-5), 173.4 (C-10), 162.5 (C-1′),

152.4 (C-7), 147.7 (C-4a), 145.3 (C-5a), 139.2 (C-5′), 136.0 (C-9), 131.4 (C-9a), 128.6 (C-6′), 128.4 (C-7′), 126.5 (C-8′), 126.2 (C-8), 110.7 (C-10a), 48.2 (C-2), 40.7 (C-3′), 38.6 (C-3), 35.1 (C-4′); (+)-ESIMS *m/z* 434 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 434.0768 (calcd. for $C_{20}H_{17}N_3NaO_5S$, 434.0781).

3.2.3.6. *N*-(3-Phenylpropyl)-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino[2,3-*g*] quinoline-7-carboxamide 1,1-Dioxide (**7f**)

From **6f** (39.0 mg, 0.12 mmol), $CeCl_3.7H_2O$ (51.0 mg, 0.14 mmol) in MeCN and EtOH (1:1, 14 mL) and hypotaurine (12.0 mg, 0.11 mmol) in H_2O (2 mL). Filtration and solvent wash gave **7f** as a red-brown powder (29.0 mg, 57% yield).

Mp 204 °C (decomp.); R_f = 0.52 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3247, 2922, 1670, 1528 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ_H 9.36 (1H, br s, NH-4), 8.77 (1H, t, *J* = 6.0 Hz, NH-2′), 8.54 (1H, d, *J* = 8.1 Hz, H-9), 8.40 (1H, d, *J* = 8.1 Hz, H-8), 7.31–7.15 (5H, m, H-7′/H-8′/H-9′), 3.90 (2H, t, *J* = 5.5 Hz, H_2-3), 3.44–3.35 (4H, m, H_2-2/H_2-3′), 2.64 (2H, t, *J* = 7.8 Hz, H_2-5′), 1.89 (2H, p, *J* = 7.8 Hz, H_2-4′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 176.2 (C-5), 173.5 (C-10), 162.6 (C-1′), 152.6 (C-7), 147.7 (C-4a), 145.4 (C-5a), 141.6 (C-6′), 136.0 (C-9), 131.4 (C-9a), 128.3 (C-7′/C-8′), 126.6 (C-9′), 125.8 (C-8), 110.7 (C-10a), 48.2 (C-2), 39.1 (C-3), 38.8 (C-3′), 32.6 (C-5′), 30.8 (C-4′); (+)-ESIMS *m/z* 426 $[M + H]^+$; (+)-HRESIMS *m/z* $[M + H]^+$ 426.1119 (calcd. for $C_{21}H_{20}N_3O_5S$, 426.1118).

3.2.3.7. (E)-*N*-(3,7-Dimethylocta-2,6-dien-1-yl)-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino [2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**7g**)

From **6g** (26.6 mg, 0.079 mmol), $CeCl_3.7H_2O$ (31 mg, 0.083 mmol) in MeCN/EtOH (1:1, 14 mL) and hypotaurine (7.2 mg, 0.066 mmol) in H_2O (2 mL). Filtration and solvent wash gave **7g** as a dark orange powder (10.0 mg, 29% yield).

Mp 200 °C (decomp.); R_f = 0.45 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3230, 3076, 1693, 1561 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ_H 9.35 (1H, br s, NH-4), 8.73 (1H, t, *J* = 5.7 Hz, NH-2′), 8.53 (1H, d, *J* = 8.1 Hz, H-9), 8.41 (1H, d, *J* = 8.1 Hz, H-8), 5.27 (1H, t, *J* = 6.2 Hz, H-4′), 5.07 (1H, t, *J* = 6.2 Hz, H-8′), 3.97 (2H, dd, *J* = 6.2, 5.7 Hz, H_2-3′), 3.90 (2H, br t, *J* = 5.5 Hz, H_2-3), 3.41 (2H, t, *J* = 5.5 Hz, H_2-2), 2.09–2.02 (2H, m, H_2-7′), 2.01–1.95 (2H, m, H_2-6′), 1.71 (3H, s, H_3-11′), 1.62 (3H, s, H_3-12′), 1.56 (3H, s, H_3-10′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 176.2 (C-5), 173.4 (C-10), 162.3 (C-1′), 152.6 (C-7), 147.7 (C-4a), 145.3 (C-5a), 137.5 (C-5′), 136.0 (C-9), 131.4 (C-9a), 130.9 (C-9′), 126.5 (C-8), 123.9 (C-8′), 121.0 (C-4′), 110.7 (C-10a), 48.2 (C-2), 40.3 (C-3), 38.6 (C-6′), 37.1 (C-3′), 25.9 (C-7′), 25.5 (C-12′), 17.5 (C-10′), 16.1 (C-11′); (+)-ESIMS *m/z* 466 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 466.1395 (calcd. for $C_{22}H_{25}N_3NaO_5S$, 466.1407).

3.2.3.8. *N*-(Prop-2-yn-1-yl)-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino[2,3-*g*] quinoline-7-carboxamide 1,1-Dioxide (**7h**)

From **6h** (35 mg, 0.15 mmol), $CeCl_3.7H_2O$ (46.5 mg, 0.12 mmol) in MeCN/EtOH (1:1, 14 mL) and hypotaurine (9.5 mg, 0.087 mmol) in H_2O (2 mL). The crude reaction mixture was purified by

reversed-phase C_{18} flash column chromatography to give **7h** as a bright yellow powder (15.0 mg, 29% yield).

Mp 280 °C (decomp.); R_f = 0.52 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3369, 3255, 2936, 1667, 1595 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ_H 9.38 (1H, t, J = 5.4 Hz, NH-4), 9.09 (1H, t, J = 6.0 Hz, NH-2′), 8.55 (1H, d, J = 8.1 Hz, H-9), 8.41 (1H, d, J = 8.1 Hz, H-8), 4.13 (2H, dd, J = 6.0, 2.4 Hz, H_2-3′), 3.93–3.87 (2H, m, H_2-3), 3.40 (2H, obscured by water, H_2-2), 3.12 (1H, t, J = 2.4 Hz, H-5′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 176.1 (C-5), 173.3 (C-10), 162.6 (C-1′), 152.0 (C-7), 147.7 (C-4a), 145.5 (C-5a), 136.0 (C-9), 131.5 (C-9a), 126.7 (C-8), 110.7 (C-10a), 80.9 (C-4′), 72.8 (C-5′), 48.2 (C-2), 39.4 (C-3), 28.7 (C-3′); (+)-ESIMS *m/z* 368 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 368.0294 (calcd. for $C_{15}H_{11}N_3NaO_5S$, 368.0312).

3.2.3.9. *N*-(2-Methoxyethyl)-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino[2,3-*g*] quinoline-7-carboxamide 1,1-Dioxide (**7i**)

From **6i** (31 mg, 0.12 mmol) in MeCN/EtOH (1:1, 20 mL) and hypotaurine (7.8 mg, 0.072 mmol) in H_2O (3 mL). The crude reaction mixture was purified by reversed-phase C_{18} flash column chromatography to give **7i** as an orange powder (11.2 mg, 26% yield).

Mp 200 °C (decomp.); R_f = 0.54 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3546, 3251, 1673, 1594, 1581, 1556, 1339, 1122 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 9.37 (1H, br s, NH-4), 8.64 (1H, t, J = 5.4 Hz, NH-2′), 8.54 (1H, d, J = 8.0 Hz, H-9), 8.41 (1H, d, J = 8.0 Hz, H-8), 3.92–3.88 (2H, m, H_2-3), 3.56–3.51 (4H, m, H_2-3′ and H_2-4′), 3.43–3.39 (2H, m, H_2-2), 3.29 (3H, s, H_3-5′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 176.2 (C-5), 173.4 (C-10), 162.5 (C-1′), 152.2 (C-7), 147.7 (C-4a), 145.4 (C-5a), 136.1 (C-9), 131.4 (C-9a), 126.5 (C-8), 110.7 (C-10a), 70.3 (C-4′), 57.9 (C-5′), 48.2 (C-2), 39.2 (C-3), 38.7 (C-3′); (+)-ESIMS *m/z* 388 $[M + Na]^+$; (+)-HRESIMS *m/z* 388.0566 $[M + Na]^+$ (calcd. for $C_{15}H_{15}N_3NaO_6S$, 388.0574).

3.2.3.10. Methyl 2-(1,1-Dioxido-5,10-dioxo-3,4,5,10-tetrahydro-2*H*-[1,4]thiazino[2,3-*g*] quinoline-7-carboxamido)acetate (**7j**)

From **6j** (50 mg, 0.18 mmol) in MeCN/EtOH (1:1, 20 mL) and hypotaurine (11.9 mg, 0.11 mmol) in H_2O (3 mL). Reversed-phase C_{18} flash column chromatography gave **7j** as a bright red powder (13.8 mg, 20% yield).

Mp 200 °C (decomp.); R_f = 0.46 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3576, 3335, 1748, 1666, 1594, 1581, 1557, 1346, 1271, 1212, 1164, 1115 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 9.40 (1H, br t, J = 3.4 Hz, NH-4), 9.08 (1H, t, J = 6.1 Hz, NH-2′), 8.56 (1H, d, J = 8.0 Hz, H-9), 8.42 (1H, d, J = 8.0 Hz, H-8), 4.15 (2H, d, J = 6.1 Hz, H-3′), 3.92–3.88 (2H, m, H_2-3), 3.67 (3H, s, H_3-5′), 3.43–3.40 (2H, m, H_2-2); ^{13}C (DMSO-d_6, 100 MHz) δ_C 176.2 (C-5), 173.4 (C-10), 170.0 (C-4′), 163.0 (C-1′), 151.7 (C-7), 147.7 (C-4a), 145.6 (C-5a), 136.1 (C-9), 131.6 (C-9a), 126.6 (C-8), 110.8 (C-10a), 51.9 (C-5′), 48.2 (C-2), 41.2 (C-3′), 39.2 (C-3); (+)-ESIMS *m/z* 380 $[M + H]^+$; (+)-HRESIMS *m/z* 380.0538 $[M + H]^+$ (calcd. for $C_{15}H_{14}N_3O_7S$, 380.0547).

3.2.4. General Procedure for Preparation of $\Delta^{2(3)}$ Analogues **8a**–**8i**, **8k**

Thiazine-quinoline-carboxamide (**7a**–**7j**) in DMF (1–3 mL) was stirred in 2 N NaOH (3 mL) at rt for 2 h. HCl (10% vol) was added dropwise until the reaction mixture was pH 5 and the mixture was then purified by reversed-phase C_{18} flash column chromatography (0%–10% MeOH (0.05% TFA)) to give the desired product.

3.2.4.1. *N*-*n*-Butyl-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**8a**)

From **7a** (34.0 mg, 0.094 mmol) using the general procedure to give **8a** as a yellow solid (13.0 mg, 38% yield).

Mp 200 °C (decomp.); R_f = 0.53 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3402, 3058, 1714, 1653, 1632, 1527, 1503, 1318, 1125 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 11.42 (1H, br s, NH-4), 8.76 (1H, t, *J* = 6.0 Hz, NH-2′), 8.58 (1H, d, *J* = 8.0 Hz, H-9), 8.43 (1H, d, *J* = 8.0 Hz, H-8), 7.17 (1H, d, *J* = 9.0 Hz, H-3), 6.62 (1H, d, *J* = 9.0 Hz, H-2), 3.38 (2H, dt, *J* = 6.9, 6.9 Hz, H_2-3′), 1.56 (2H, p, *J* = 7.0 Hz, H_2-4′), 1.33 (2H, sex., *J* = 7.5 Hz, H_2-5′), 0.91 (3H, t, *J* = 7.5 Hz, H_3-6′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.7 (C-10), 175.5 (C-5), 162.4 (C-1′), 153.3 (C-7), 145.5 (C-5a), 141.4 (C-4a), 136.1 (C-9), 130.6 (C-9a), 130.5 (C-3), 126.4 (C-8), 115.2 (C-10a), 112.0 (C-2), 38.6 (C-3′), 31.3 (C-4′), 19.6 (C-5′), 13.7 (C-6′); (+)-ESIMS *m/z* 384 $[M + Na]^+$; (+)-HRESIMS *m/z* 384.0632 $[M + Na]^+$ (calcd. for $C_{16}H_{15}N_3NaO_5S$, 384.0625).

3.2.4.2. *N*-*n*-Pentyl-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**8b**)

From **7b** (10.0 mg, 0.027 mmol) using the general procedure to give **8b** as a yellow solid (4.0 mg, 40% yield).

Mp 280 °C (decomp.); R_f = 0.44 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3319, 3057, 1710, 1635, 1528 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ_H 11.44 (1H, br s, NH-4), 8.78 (1H, t, *J* = 6.1 Hz, NH-2′), 8.58 (1H, d, *J* = 8.1 Hz, H-9), 8.43 (1H, d, *J* = 8.1 Hz, H-8), 7.17 (1H, d, *J* = 8.9 Hz, H-3), 6.62 (1H, d, *J* = 8.9 Hz, H-2), 3.40–3.34 (2H, m, H_2-3′), 1.58 (2H, p, *J* = 7.5 Hz, H_2-4′), 1.34–1.27 (4H, m, H_2-5′/H_2-6′), 0.88 (3H, t, *J* = 7.5 Hz, H_3-7′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 177.7 (C-10), 175.5 (C-5), 162.4 (C-1′), 153.3 (C-7), 145.6 (C-5a), 141.4 (C-4a), 136.1 (C-9), 130.6 (C-9a), 130.5 (C-3), 126.4 (C-8), 115.2 (C-10a), 112.0 (C-2), 38.8 (C-3′), 28.9 (C-4′), 28.7 (C-5′), 21.9 (C-6′), 14.0 (C-7′); (+)-ESIMS *m/z* 398 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 398.0776 (calcd. for $C_{17}H_{17}N_3NaO_5S$, 3798.0781).

3.2.4.3. *N*-*n*-Octyl-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**8c**)

From **7c** (12.0 mg, 0.029 mmol) using the general procedure to give **8c** as a yellow solid (6.0 mg, 50% yield).

Mp 280 °C (decomp.); R_f = 0.41 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3289, 2924, 1635, 1527 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ_H 11.44 (1H, d, *J* = 5.3 Hz, NH-4), 8.79 (1H, t, *J* = 5.8 Hz, NH-2′), 8.58 (1H, d, *J* = 8.0 Hz, H-9), 8.43 (1H, d, *J* = 8.0 Hz, H-8), 7.17 (1H, dd, *J* = 8.8, 5.3 Hz, H-3), 6.63 (1H, d, *J* = 8.8 Hz, H-2), 3.37 (obscured by solvent, H_2-3′), 1.60–1.52 (2H, m, H_2-4′), 1.31–1.23 (10H, m, H_2-5′/H_2-6′/H_2-7′/H_2-8′/H_2-9′), 0.85 (3H, t, *J* = 6.8 Hz, H_3-10′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 177.7 (C-10), 175.5 (C-5), 162.4 (C-1′), 153.3 (C-7), 145.6 (C-5a), 141.4 (C-4a), 136.1 (C-9), 130.6 (C-9a), 130.5 (C-3), 126.4 (C-8), 115.2 (C-10a), 112.0 (C-2), 38.9 (C-3′), 31.3 (C-8′), 29.2 (C-4′), 28.8 (C-6′), 28.7 (C-7′), 26.5 (C-5′), 22.1 (C-9′), 14.0 (C-10′); (+)-ESIMS *m/z* 440 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 440.1232 (calcd. for $C_{20}H_{23}N_3NaO_5S$, 440.1251).

3.2.4.4. *N*-Benzyl-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**8d**)

From **7d** (10.0 mg, 0.025 mmol) using the general procedure to give **8d** as a yellow solid (3.0 mg, 30% yield).

Mp 280 °C (decomp.); R_f = 0.47 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3213, 1706, 1634, 1513 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 11.41 (1H, d, *J* = 5.6 Hz, NH-4), 9.33 (1H, t, *J* = 6.3 Hz, NH-2′), 8.59 (1H, d, *J* = 8.2 Hz, H-9), 8.46 (1H, d, *J* = 8.2 Hz, H-8), 7.38–7.30 (4H, m, 2H-5′/2H-6′), 7.27–7.23 (1H, m, H-7′), 7.17 (1H, dd, *J* = 8.9, 5.6 Hz, H-3), 6.61 (1H, d, *J* = 8.9 Hz, H-2), 4.58 (2H, d, *J* = 6.3 Hz, H_2-3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.6 (C-10), 175.4 (C-5), 162.7 (C-1′), 153.1 (C-7), 145.6 (C-5a), 141.3 (C-4a), 139.2 (C-4′), 136.1 (C-9), 130.7 (C-9a), 130.5 (C-3), 128.3 (C-5′), 127.5 (C-6′), 126.9 (C-8), 126.6 (C-7′), 115.2 (C-10a), 112.0 (C-2), 42.7 (C-3′); (+)-ESIMS *m/z* 418 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 418.0470 (calcd. for $C_{19}H_{13}N_3NaO_5S$, 418.0468).

3.2.4.5. *N*-Phenethyl-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**8e**)

From **7e** (11.0 mg, 0.027 mmol) using the general procedure to give **8e** as a yellow solid (4.0 mg, 36% yield).

Mp 290 °C (decomp.); R_f = 0.47 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3103, 3067, 1714, 1678, 1512 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ_H 11.45 (1H, d, *J* = 5.6, NH-4), 8.85 (1H, t, *J* = 6.1 Hz, NH-2′), 8.58 (1H, d, *J* = 8.1 Hz, H-9), 8.44 (1H, d, *J* = 8.1 Hz, H-8), 7.34–7.22 (5H, m, 2H-6′/2H-7′/H-8′), 7.17 (1H, dd, *J* = 8.7, 5.6 Hz, H-3), 6.62 (1H, d, *J* = 8.7 Hz, H-2), 3.61 (2H, dt, *J* = 6.9, 6.1 Hz, H_2-3′), 2.90 (2H, t, *J* = 6.9 Hz, H_2-4′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.7 (C-10), 175.5 (C-5), 162.4 (C-1′), 153.0 (C-7), 145.6 (C-5a), 141.4 (C-4a), 139.3 (C-5′), 136.2 (C-9), 130.7 (C-9a), 130.5 (C-3), 128.7 (C-6′), 128.5 (C-7′), 126.4 (C-7′), 126.2 (C-8), 115.2 (C-10a), 112.0 (C-2), 40.8 (C-3′), 35.1 (C-4′); (+)-ESIMS *m/z* 432 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 432.0618 (calcd. for $C_{20}H_{15}N_3NaO_5S$, 432.0625).

3.2.4.6. *N*-(3-Phenylpropyl)-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**8f**)

From **7f** (20.0 mg, 0.047 mmol) using the general procedure to give **8f** as a yellow solid (6.0 mg, 30% yield).

Mp 230 °C (decomp.); R_f = 0.41 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3059, 2930, 1653, 1511 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 11.45 (1H, br s, NH-4), 8.86 (1H, t, *J* = 6.1 Hz, NH-2′), 8.58 (1H, d, *J* = 8.2 Hz, H-9), 8.44 (1H, d, *J* = 8.2 Hz, H-8), 7.31–7.15 (6H, m, H-3/2H-7′/2H-8′/H-9′), 6.63 (1H, d, *J* = 9.1 Hz, H-2), 3.40 (2H, dt, *J* = 7.5, 6.1 Hz, H_2-3′), 2.64 (2H, t, *J* = 7.5 Hz, H_2-5′), 1.89 (2H, p, *J* = 7.5 Hz, H_2-4′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.7 (C-10), 175.5 (C-5), 162.5 (C-1′), 153.3 (C-7), 145.6 (C-5a), 141.6 (C-6′), 141.4 (C-4a), 136.1 (C-9), 130.6 (C-9a), 130.5 (C-3), 128.3 (C-7′/C-8′), 126.5 (C-9′), 125.8 (C-8), 115.2 (C-10a), 112.0 (C-2), 38.9 (C-3′), 32.7 (C-5′), 30.8 (C-4′); (+)-ESIMS *m/z* 446 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 446.0790 (calcd. for $C_{21}H_{17}N_3NaO_5S$, 446.0781).

3.2.4.7. (*E*)-*N*-(3,7-Dimethylocta-2,6-dien-1-yl)-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*] quinoline-7-carboxamide 1,1-Dioxide (**8g**)

From **7g** (10.0 mg, 0.023 mmol) using the general procedure to give **8g** a yellow solid (5.0 mg, 50% yield).

Mp 280 °C (decomp.); R_f = 0.44 (10% MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 11.44 (1H, d, *J* = 5.7 Hz, NH-4), 8.81 (1H, t, *J* = 6.2 Hz, NH-2′), 8.58 (1H, d, *J* = 7.8 Hz, H-9), 8.44 (1H, d, *J* = 7.9 Hz, H-8), 7.17 (1H, dd, *J* = 8.6, 5.7 Hz, H-3), 6.62 (1H, d, *J* = 8.6 Hz, H-2), 5.27 (1H, t, *J* = 6.6 Hz, H-4′), 5.07 (1H, t, *J* = 7.0 Hz, H-8′), 3.98 (2H, dd, *J* = 6.2, 5.8 Hz, H_2-3′), 2.07–2.03 (2H, m, H_2-6′), 2.01–1.96 (2H, m, H_2-7′), 1.71 (3H, s, H_3-11′), 1.62 (3H, s, H_3-12′), 1.56 (3H, s, H_3-10′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.6 (C-10), 175.5 (C-5), 162.2 (C-1′), 153.2 (C-7), 145.6 (C-5a), 141.4 (C-4a), 137.6 (C-5′), 136.1 (C-9), 131.0 (C-9a), 130.6 (C-9′), 130.5 (C-3), 126.4 (C-8), 123.9 (C-8′), 121.1 (C-4′), 115.2 (C-10a), 112.0 (C-2), 38.9 (C-6′), 37.1 (C-3′), 26.0 (C-7′), 25.5 (C-12′), 17.6 (C-10′), 16.2 (C-11′); (+)-ESIMS *m/z* 464 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 464.1254 (calcd. for $C_{22}H_{23}N_3NaO_5S$, 464.1251).

3.2.4.8. *N*-(Prop-2-yn-1-yl)-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*]quinoline-7-carboxamide 1,1-Dioxide (**8h**)

From **7h** (8.0 mg, 0.023 mmol) using the general procedure to give **8h** as a yellow solid (6.0 mg, 76% yield).

Mp 230 °C (decomp.); R_f = 0.46 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3310, 3058, 1636, 1509 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 11.45 (1H, d, *J* = 5.5 Hz, NH-4), 9.16 (1H, t, *J* = 6.2 Hz, NH-2′), 8.60 (1H, d, *J* = 8.2 Hz, H-9), 8.45 (1H, d, *J* = 8.2 Hz, H-8), 7.17 (1H, dd, *J* = 8.8, 5.5 Hz, H-3), 6.62 (1H, d, *J* = 8.8 Hz, H-2), 4.15 (2H, dd, *J* = 5.9, 2.5 Hz, H_2-3′), 3.13 (1H, t, *J* = 2.5 Hz, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 177.6 (C-10), 175.4 (C-5), 162.5 (C-1′), 152.7 (C-7), 145.8 (C-5a), 141.4 (C-4a), 136.1 (C-9), 130.8 (C-9a), 130.5 (C-3), 126.6 (C-8), 115.3 (C-10a), 112.0 (C-2), 80.9

(C-4′), 72.9 (C-5′), 28.7 (C-3′); (+)-ESIMS *m/z* 366 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 366.0151 (calcd. for $C_{15}H_9N_3NaO_5S$, 366.0155).

3.2.4.9. *N*-(2-Methoxyethyl)-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*] quinoline-7-carboxamide 1,1-Dioxide (**8i**)

From **7i** (18.8 mg, 0.052 mmol) using the general procedure to give **8i** as a yellow solid (15.6 mg, 83% yield).

Mp 200 °C (decomp.); R_f = 0.54 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3250, 3057, 1633, 1508, 1278, 1097 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 11.44 (1H, s, NH-4), 8.70 (1H, t, *J* = 5.6 Hz, H-2′), 8.59 (1H, d, *J* = 8.0 Hz, H-9), 8.45 (1H, d, *J* = 8.0 Hz, H-8), 7.17 (1H, d, *J* = 9.0 Hz, H-3), 6.62 (1H, d, *J* = 9.0 Hz, H-2), 3.59–3.50 (4H, m, H_2-3′ and H_2-4′), 3.29 (3H, s, H_3-5′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.6 (C-10), 175.4 (C-5), 162.4 (C-1′), 152.8 (C-7), 145.5 (C-5a), 141.3 (C-4a), 136.2 (C-9), 130.7 (C-9a), 130.5 (C-3), 126.3 (C-8), 115.3 (C-10a), 112.0 (C-2), 70.2 (C-4′), 57.9 (C-5′), 38.4 (C-3′); (+)-ESIMS *m/z* 364 $[M + H]^+$; (+)-HRESIMS *m/z* 364.0606 $[M + H]^+$ (calcd. for $C_{15}H_{14}N_3O_6S$, 364.0598).

3.2.4.10. 2-(1,1-Dioxido-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*] quinoline-7-carboxamido)acetic Acid (**8k**)

From **7j** (13.8 mg, 0.036 mmol) using the general procedure to give carboxylic acid **8k** as a yellow oil (8.2 mg, 62% yield).

R_f = 0.20 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3582, 3250, 3057, 1748, 1634, 1508, 1279, 1127 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 11.95 (1H, br s, NH-4), 9.00 (1H, t, *J* = 6.0 Hz, NH-2′), 8.60 (1H, d, *J* = 8.0 Hz, H-9), 8.45 (1H, d, *J* = 8.0 Hz, H-8), 7.18 (1H, d, *J* = 8.8 Hz, H-3), 6.62 (1H, d, *J* = 8.8 Hz, H-2), 4.07 (2H, d, *J* = 6.0 Hz, H_2-3′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.6 (C-10), 175.5 (C-5), 170.9 (C-4′), 162.7 (C-1′), 152.4 (C-7), 145.7 (C-5a), 141.6 (C-4a), 136.2 (C-9), 130.8 (C-9a), 130.6 (C-3), 126.4 (C-8), 115.2 (C-10a), 112.0 (C-2), 41.3 (C-3′); (−)-ESIMS *m/z* 362 $[M - H]^-$; (−)-HRESIMS *m/z* 362.0083 $[M - H]^-$ (calcd. for $C_{14}H_8N_3O_7S$, 362.0088).

3.2.5. Methyl 2-(1,1-dioxido-5,10-dioxo-5,10-dihydro-4*H*-[1,4]thiazino[2,3-*g*] quinoline-7-carboxamido)acetate (**8j**)

Thionyl chloride (8.4 µL, 0.116 mmol) was added to a solution of **8k** (7.0 mg, 0.019 mmol) in dry MeOH (3 mL) at 0 °C. The reaction mixture was stirred at that temperature for 20 min, then heated to 65 °C and stirred for an additional 2 h. The solution was then cooled to rt and loaded directly onto a C_{18} reversed-phase chromatography column. The crude material was washed with two column volumes of H_2O and the product eluted with 100% MeOH (+0.05% TFA) to afford **8j** as a yellow oil (6.8 mg, 93% yield).

R_f = 0.49 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3247, 3056, 1753, 1635, 1508, 1273, 1128 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 11.46 (1H, br d, *J* = 5.2 Hz, NH-4), 9.14 (1H, t, *J* = 6.2 Hz, NH-2′), 8.61 (1H, d, *J* = 8.0 Hz, H-9), 8.45 (1H, d, *J* = 8.0 Hz, H-8), 7.17 (1H, dd, *J* = 8.8, 5.2 Hz, H-3), 6.63

(1H, d, J = 8.8 Hz, H-2), 4.16 (2H, d, J = 6.2 Hz, H_2-3′), 3.68 (3H, s, H_3-5′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 177.6 (C-10), 175.4 (C-5), 170.0 (C-4′), 162.9 (C-1′), 152.3 (C-7), 145.7 (C-5a), 141.4 (C-4a), 136.2 (C-9), 130.9 (C-9a), 130.4 (C-3), 126.4 (C-8), 115.3 (C-10a), 112.0 (C-2), 51.9 (C-5′), 41.3 (C-3′); (+)-ESIMS *m/z* 400 $[M + Na]^+$; (+)-ESIMS *m/z* 400.0222 $[M + Na]^+$ (calcd. for $C_{15}H_{11}N_3NaO_7S$, 400.0210).

3.2.6. Methyl 5,9-Dioxo-3,4,5,9-tetrahydro-2*H*-thieno[2′,3′:4,5]benzo[1,2-*b*][1,4]thiazine-7-carboxylate 1,1-Dioxide (**10a**) and Methyl 5,9-Dioxo-2,3,5,9-tetrahydro-1*H*-thieno[3′,2′:4,5]benzo [1,2-*b*][1,4]thiazine-7-carboxylate 4,4-Dioxide (**10b**)

A solution of methyl 4,7-dioxo-4,7-dihydrobenzo[*b*]thiophene-2-carboxylate (**9**) [22] (74 mg, 0.33 mmol) and $CeCl_3 \cdot 7H_2O$ (124 mg, 0.33 mmol) in MeCN (10 mL) and EtOH (10 mL) was cooled to 0 °C. Hypotaurine (36 mg, 0.33 mmol) in H_2O (2 mL) was added dropwise to the mixture leading to a color change from yellow to orange. The reaction was stirred at rt for 2 days. The residue was filtered and washed with H_2O (3 × 20 mL) and MeOH (3 × 20 mL), to give a mixture of regioisomers (**10a**/**10b**, 1:0.3 ratio determined by NMR)) as an orange solid (20 mg, 18% yield).

Mp 280 °C (decomp.); R_f = 0.36 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3222, 3003, 1726, 1683, 1579 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 9.36 (1H, br s, NH), 8.03 (1H, s, H-8 minor isomer), 7.93 (1H, s, H-8), 3.91 (3H, s, H_3-2′), 3.89 (3H, s, H_3-2′ minor isomer), 3.88–3.84 (2H, m, H_2-3), 3.37 (2H, obscured by water, H_2-2); ^{13}C NMR (DMSO-d_6, 75 MHz) **10a** δ_C 173.1 (C-5), 171.5 (C-9), 160.8 (C-1′), 148.0 (C-4a), 142.6 (C-5a*), 141.8 (C-8a*), 141.1 (C-7), 130.2 (C-8), 109.6 (C-9a), 53.3 (C-2′), 48.1 (C-2), 39.2 (C-3); (+)-FABMS *m/z* 328 $[M + H]^+$; (+)-HRFABMS *m/z* $[M + H]^+$ 327.9950 (calcd. for $C_{12}H_{10}NO_6S_2$, 327.9950).

3.2.7. 5,9-Dioxo-3,4,5,9-tetrahydro-2*H*-thieno[2′,3′:4,5]benzo[1,2-*b*][1,4]thiazine-7-carboxylic Acid 1,1-Dioxide (**11a**) and 5,9-Dioxo-2,3,5,9-tetrahydro-1*H*-thieno[3′,2′:4,5]benzo[1,2-*b*][1,4] thiazine-7-carboxylic Acid 4,4-Dioxide (**11b**)

Methyl ester (as a mixture of regioisomers) **10a**/**10b** (20.0 mg, 0.061 mmol) was dissolved in conc. HCl (3 mL), and stirred at rt for 5 h, after which time, the mixture was heated to 100 °C and stirred for a further 2 h. The crude reaction mixture was subjected to reversed-phase C_{18} column chromatography (0%–10% MeOH/H_2O (0.05% TFA)) to give **11a**/**11b** as a mixture of regioisomers (1:0.3, 11.0 mg, 57% yield) as a bright orange solid.

Mp 200 °C (decomp.); R_f = 0.25 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3357, 3230, 1674, 1577; ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 9.42 (br s, NH minor isomer), 9.31 (1H, br s, NH), 7.93 (s, H-8 minor isomer), (7.84 (1H, s, H-8), 3.86 (2H, br s, H_2-3), 3.40–3.34 (2H, br m, H_2-2); ^{13}C NMR (DMSO-d_6, 75 MHz) **11a** δ_C 173.1 (C-5), 171.7 (C-9), 161.8 (C-1′), 147.9 (C-4a), 144.2 (C-7), 142.8 (C-8a), 141.2 (C-5a), 129.6 (C-8), 109.6 (C-9a), 48.1 (C-2), 39.2 (C-3); (+)-ESIMS *m/z* 336 $[M + Na]^+$; (+)-HRESIMS *m/z* $[M + Na]^+$ 335.9605 (calcd. for $C_{11}H_7NNaO_6S_2$, 335.9607).

3.2.8. 5,9-Dioxo-5,9-dihydro-4*H*-thieno[2′,3′:4,5]benzo[1,2-*b*][1,4]thiazine-7-carboxylic Acid 1,1-Dioxide (**12**)

Thiophene methyl ester (**10a**/**10b**) (20.0 mg, 0.061 mmol) was dissolved in hot EtOAc (2 mL), followed by the addition of 1 N NaOH (1 mL). The biphasic mixture was stirred at rt for 1.5 h. HCl (10% vol) was added dropwise until the reaction mixture turned acidic. The crude mixture was subjected to reversed-phase C_{18} column chromatography (0%–10% MeOH/H_2O (0.05% TFA)) to give **12** (single regio-isomer) (15 mg, 78% yield) as a bright orange solid.

Mp 290 °C (decomp.); R_f = 0.27 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3227, 3068, 1689, 1637, 1510; ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 11.41 (1H, br s, NH), 7.82 (1H, s, H-8), 7.13 (1H, d, J = 8.9 Hz, H-3), 6.57 (1H, d, J = 8.9 Hz, H-2); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 175.2 (C-9), 172.5 (C-5), 161.7 (C-1′), 147.4 (C-7), 141.5 (C-5a*), 141.4 (C-4a*), 141.2 (C-8a*), 130.3 (C-3), 128.1 (C-8), 114.5 (C-9a), 112.1 (C-2); (+)-FABMS *m/z* 312 $[M + H]^+$; (+)-HRFABMS *m/z* $[M + H]^+$ 311.9642 (calcd. for $C_{11}H_6NO_6S_2$, 311.9637).

3.2.9. Methyl 4,7-Dihydroxybenzo[*b*]thiophene-2-carboxylate (**14**)

Commercially available 7-methoxy-benzofuran-2-carboxylic acid ethyl ester (**13**) (105 mg, 0.477 mmol) in MeCN/4 N H_2SO_4 (20 mL/5 mL) was stirred at rt, before addition of $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ (1.80 g, 3.02 mol) in 4 N H_2SO_4 (25 mL). The reaction mixture was heated to 60 °C for 90 min. changing the colour from orange to yellow as well as inducing the formation of a white precipitate. The reaction was cooled, filtered, and the filtrate was extracted repeatedly with CH_2Cl_2 (5 × 50 mL). The combined organic phases were then dried ($MgSO_4$) and the solvent removed *in vacuo* to give **14** as a yellow solid (89 mg, 85% yield). The product was used immediately in the next step without further purification.

IR ν_{max} (ATR) 3570, 2955, 1752, 1726, 1534, 1475, 1367, 1187, 1160, 1139 cm^{-1}; ^{1}H NMR (DMSO-d_6, 75 MHz) δ_H 7.49 (1H, s, H-3), 6.82 (2H, s, H-5/H-6), 4.45 (2H, q, J = 7.2 Hz, H_2-3′), 1.42 (3H, t, J = 7.2 Hz, H_3-4′); EIMS *m/z* 220 $[M]^+$; (+)-HREIMS *m/z* $[M]^+$ 220.0369 (calcd. for $C_{11}H_8O_5$, 220.0372).

3.2.10. Ethyl 5,9-Dioxo-3,4,5,9-tetrahydro-2*H*-benzofuro[5,6-*b*][1,4]thiazine-7-carboxylate 1,1-Dioxide (**15**)

A solution of quinone **14** (100 mg, 0.45 mmol) and $CeCl_3.7H_2O$ (78 mg, 0.21 mmol) in MeCN (10 mL) and EtOH (10 mL) was cooled to 0 °C. Hypotaurine (49 mg, 0.45 mmol) in H_2O (1 mL) was added dropwise to the reaction mixture, changing the colour from yellow to orange. The reaction was stirred at rt for 24 h. The residue was filtered and washed with H_2O (3 × 20 mL) and MeOH (3 × 20 mL), to give **15** (62 mg, 43% yield) as a red solid.

Mp 277 °C; R_f = 0.36 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3225, 1733, 1695, 1566 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ_H 9.38 (1H, br s, NH), 7.58 (1H, s, H-8), 4.37 (2H, q, J = 7.1 Hz, H_2-3′), 3.85 (2H, dt, J = 5.7, 5.7 Hz, H_2-3), 3.34 (2H, t, J = 5.7 Hz, H_2-2), 1.33 (3H, t, J = 7.1 Hz, H-4′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 172.2 (C-9), 167.8 (C-5), 157.1 (C-1′), 149.2 (C-5a), 148.7 (C-7), 147.4

(C-4a), 130.1 (C-8a), 114.2 (C-8), 108.9 (C-9a), 62.0 (C-3′), 48.1 (C-2), 39.2 (C-3), 14.0 (C-4′); (+)-FABMS *m/z* 326 $[M + H]^+$; (+)-HRFABMS *m/z* $[M + H]^+$ 326.0341 (calcd. for $C_{13}H_{12}NO_7S$, 326.0335).

3.2.11. 5,9-Dioxo-3,4,5,9-tetrahydro-2*H*-benzofuro[5,6-*b*][1,4]thiazine-7-carboxylic Acid 1,1-Dioxide (**16**)

Ethyl ester **15** (64 mg, 0.20 mmol) was dissolved in conc. HCl (3 mL), and the mixture was heated to 100 °C and stirred for 2 h. The crude reaction mixture was purified by reversed-phase C_{18} column chromatography (0%–10% MeOH/H_2O (0.05% TFA)), to give **16** (37 mg, 63% yield) as a bright red solid.

Mp 210 °C (decomp.); R_f = 0.23 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3234, 3093, 1635, 1694, 1561 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ_H 9.35 (1H, br s, NH-4), 7.47 (1H, s, H-8), 3.87–3.82 (2H, m, H_2-3), 3.37–3.31 (2H, m, H_2-2); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 172.5 (C-9), 167.8 (C-5), 158.5 (C-1′), 150.3 (C-5a*), 149.0 (C-7*), 147.4 (C-4a), 130.3 (C-8a), 113.5 (C-8), 108.9 (C-9a), 48.1 (C-2), 39.5 (C-3); (+)-ESIMS *m/z* 298 $[M + H]^+$; (+)-HRESIMS *m/z* $[M + H]^+$ 298.0009 (calcd. for $C_{11}H_8NO_7S$, 298.0016).

3.2.12. 5,9-Dioxo-5,9-dihydro-4*H*-benzofuro[5,6-*b*][1,4]thiazine-7-carboxylic Acid 1,1-Dioxide (**17**)

Ethyl ester **15** (15.0 mg, 0.046 mmol) was dissolved in hot EtOAc (2 mL), followed by the addition of 1 N NaOH (1 mL). The biphasic mixture was stirred at rt for 2 h. HCl (10% vol) was added dropwise until the reaction mixture turned acidic. The crude product was purified by reversed-phase C_{18} column chromatography (0%–10% MeOH/H_2O (0.05% TFA)), to give **17** (6.4 mg, 47% yield) as a red solid.

Mp 280 °C (decomp.); R_f = 0.36 (10% MeOH/CH_2Cl_2); IR ν_{max} (ATR) 3223, 3072, 1677, 1577, 1516 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 11.42 (1H, br s, NH-4), 7.47 (1H, s, H-8), 7.12 (1H, d, J = 8.8 Hz, H-3), 6.58 (1H, d, J = 8.8 Hz, H-2); ^{13}C NMR (DMSO-d_6, 75 MHz) δ_C 176.1 (C-9), 167.4 (C-5), 158.5 (C-1′), 152.0 (C-7), 149.2 (C-5a), 140.4 (C-4a), 130.2 (C-3), 129.1 (C-8a), 113.9 (C-9a), 112.3 (C-2 and C-8); (+)-FABMS *m/z* 296 $[M + H]^+$; (+)-HRFABMS *m/z* $[M + H]^+$ 295.9861 (calcd. for $C_{11}H_6NO_7S$, 295.9865).

3.3. Biological Assays

3.3.1. *In Vitro* Anti-Protozoal Activity

The *in vitro* activities against the protozoan parasites *T.b. rhodesiense*, *T. cruzi*, *L. donovani*, and *P. falciparum* and cytotoxicity assessment against L6 cells were determined as reported elsewhere [5]. The following strains, parasite forms and positive controls were used: *T.b. rhodesiense*, STIB900, trypomastigote forms, melarsoprol, IC_{50} of 0.01 μM (4 ng/mL); *T. cruzi*, Tulahuen C2C4, amastigote forms in L6 rat myoblasts, benznidazole, IC_{50} of 1.4 μM (0.352 μg/mL); *L. donovani*, MHOM/ET/67/L82, axenic amastigote forms, miltefosine, IC_{50} of 0.5 μM (0.213 μg/mL); *P. falciparum*, K1 (chloroquine and pyrimethamine resistant), erythrocytic stages, chloroquine, IC_{50} of

0.20 μM (0.065 μg/mL) and L6 cells, rat skeletal myoblasts, podophyllotoxin, IC_{50} of 0.01 μM (0.004 μg/mL).

3.3.2. *In Vivo* Anti-Malarial Efficacy Studies

In vivo anti-malarial activity was assessed as previously described [23]. Groups of three female NMRI mice (20–22 g) were intravenously infected with 2×10^7 parasitized erythrocytes on day 0 with GFP-transfected *P. berghei* strain ANKA [24]. Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water and administered intraperitoneally in a volume of 10 ml kg^{-1} on four consecutive days (4, 24, 48 and 72 h post infection). Control experiments used DMSO-H_2O vehicle alone. Parasitemia was determined on day 4 post infection (24 h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean per cent parasitaemia for the control (n = 5 mice) and treated groups expressed as a per cent relative to the control group. The survival of the animals was usually monitored up to 30 days: a compound was considered curative if the animal survived to day 30 after infection with no detectable parasites. *In vivo* efficacy studies in mice were conducted according to the rules and regulations for the protection of animal rights ("Tierschutzverordnung") of the Swiss "Bundesamt für Veterinärwesen". They were approved by the veterinary office of Canton Basel-Stadt, Switzerland.

4. Conclusions

The dioxothiazinoquinone marine natural product ascidiathiazone A (**2**) has been identified as a moderate *in vitro* growth inhibitor of *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum*. A series of C-7 amide and $\Delta^{2(3)}$ analogues were prepared that explored the influence of lipophilicity and oxidation state on observed anti-protozoal activity and selectivity. Little variation in anti-malarial potency was observed (IC_{50} 0.62–6.5 μM), and no correlation was apparent between anti-malarial and anti-*T. brucei* activity. Changing the quinoline-based structure of **2** to incorporate benzofuran or benzothiophene moieties yielded particularly potent anti-malarials. The finding of ip and oral dosing anti-malarial activity for benzofuran carboxylic acid **16** is highly encouraging, suggesting that future studies should be directed at exploring this novel antiprotozoal pharmacophore.

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Conflict of Interest

The authors declare no conflict of interest.

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