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The anti-cancer, anti-inflammatory and tuberculostatic activities of a series of 6,7-substituted-5,8-quinolinequinones

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ABSTRACT

A variety of 6,7-substituted-5,8-quinolinequinones were synthesised and assessed for their anti-tumour and anti-inflammatory activities, and their ability to inhibit the growth of *Mycobacterium bovis* BCG. In particular, the introduction of a sulfur group at the 7-position of the quinolinequinone led to the discovery of two compounds, 6-methylamino-7-methylsulfanyl-5,8-quinolinequinone (**10a**) and 6-amino-7-methylsulfonyl-5,8-quinolinequinone (**12**), that exhibited selectivity for leukemic cells over T-cells, a highly desirable property for an anti-cancer drug. A number of anti-inflammatory (AI) compounds were also identified, with 6,7-bis-methylsulfanyl-5,8-quinolinequinone (**18a**) exhibiting the highest AI activity (0.11 μM), while 6,7-dichloro-5,8-quinolinequinone (**7a**), 6,7-dichloro-2-methyl-5,8-quinolinequinone (**7b**), and 6,7-bis-phenylsulfanyl-quinoline-5,8-diol (**19**) also exhibited good AI activity and specificity. Several quinolinequinone TB-drug candidates were identified. Of these, 6-amino-7-chloro-5,8-quinolinequinone (**11**) and 6-amino-7-methanesulfinyl-5,8-quinolinequinone (**14**), exhibited low MICs (1.56–3.13 μg/mL) for the 100% growth inhibition of *M. Bovis* BCG. Some general trends pertaining to the functional group substitution of the quinolinequinone core and biological activity were also identified.

1. Introduction

Derivatives of 5,8-quinolinequinones have a wide spectrum of biological properties that include anti-tumour, anti-inflammatory and anti-bacterial activities and accordingly, much effort is spent in developing new and more effective quinolinequinone-based therapeutics.[1–6] Notable 5,8-quinolinequinones include the anti-tumour agent streptonigrin (**1**) (Fig. 1), isolated from the bacterium *Streptomyces flocculus* in 1959.[7,8] Streptonigrin was one of the first compounds to be systematically modified in an attempt to correlate specific structural features with anti-cancer properties.[9] Earlier structure–activity studies concluded that the 7-amino-quinolinequinone-moiety of streptonigrin was crucial for anti-tumour activity,[10] however later work revealed that the addition of electron-withdrawing groups at the 6- and/or 7-positions of the quinolinequinone resulted in enhanced rates of DNA degradation,[11,12] a measure of anti-tumour activity. Unfortunately, the high toxicity of streptonigrin has limited its therapeutic value.[13,14] Other biologically active 5,8-quinolinequinones include the potent anti-inflammatory agents Ascidiathiazones A (**2**) and B (**3**), recently isolated from the New Zealand ascidian *Aplidium* sp.,[15] the anti-tuberculostatic thiazole-containing quinolinequinones, such as **4**,[16,17] and 7-heptadecylsulfanyl-6-hydroxy-5,8-quinolinequinone (**5**), which exhibited promising anti-malarial activity.[18,19]

Given the potential of quinolinequinones in the treatment of a variety of diseases and, in particular, the potential of less complex quinolinequinones (e.g., **5**) to have interesting biological activities, we synthesised and tested a number of 6,7-substituted-5,8-quinolinequinones for their anti-proliferative, anti-inflammatory, and tuberculostatic activities. Few 6,7-substituted-5,8-quinolinequinones have been screened in multiple assays and given the broad biological profiles of quinolinequinones, this makes it difficult to determine the drug's specificity. Moreover, relatively few bicyclic thiol-substituted quinolinequinones have been synthesised[12,16–18,20–28] and the subsequent biological studies performed on these compounds have primarily focussed on the role of mono- or di-thio-quinolinequinones as anti-malarial[18,19,29] and anti-fungal[24–26] agents. A comparison of the activities of a variety of 6,7-substituted-5,8-quinolinequinones in a number of different assays provides valuable information on how the substitution pattern affects biological activity and will highlight key factors important for the development of better and more

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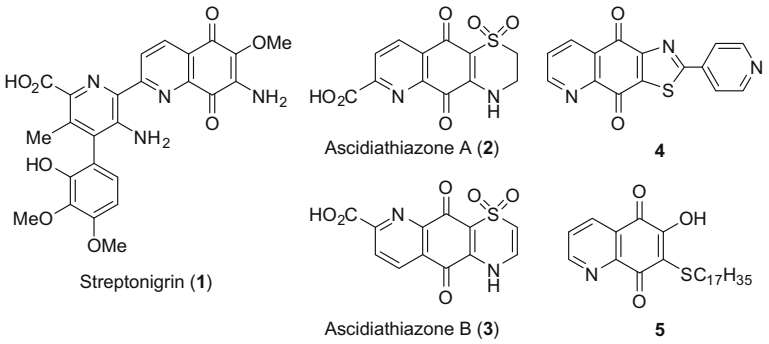


Figure 1. Representative 5,8-quinolinequinones.

specific quinolinequinone-based therapeutics. In this study, we focussed on the biological effects of chloro, amino, and thio-substituted quinolinequinones.

2. Chemistry

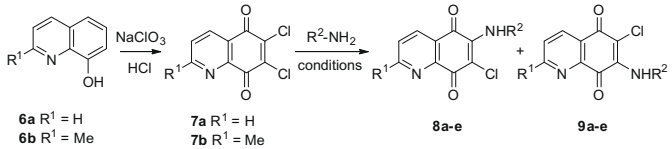
The synthesis of the 6,7-substituted-5,8-quinolinediones commenced with the formation of known dichloro-quinones **7a** and **7b** via the sodium chlorate oxidation[12] of 8-hydroxyquinoline **6a** or 8-hydroxy-2-methyl-quinoline **6b**, respectively (Table 1). The dichloro-quinones were then treated with a variety of amine nucleophiles to give the 6-substituted-products **8a–e** and/or the 7-substituted products **9a**, **9c–e** in good (65–96%) yield. Here, the presence or absence of a Lewis acid and the choice of solvent affected the regioselectivity of the reaction. The addition of $NiCl_2{\cdot}6H_2O$ favoured formation of the 6-isomer (Table 1, entries 1, 3, 4). This is thought to occur via the chelation of the Lewis acid to the nitrogen atom and C8 carbonyl of the quinolinequinone, thus increasing the electron-withdrawing power of the carbonyl and the reactivity at the conjugated 6-position.[30–33] The use of a more polar solvent also increased the yield of the 6-isomer, though the rationale for this is not well understood. In the absence of the Lewis acid, a mixture of the 6- and 7-substituted isomers was obtained (entries 2, 5–7). This ratio was improved in favour of the 7-isomer when a less polar solvent was used[31,32] (e.g., dioxane, entry 2). In all instances, the regio-isomers were separable by flash column chromatography.

To confirm the regioselectivity of the substitution reaction, it was initially hoped that the long range HMBC between the protons α to the aliphatic amine and the nearest carbonyl could be used. Unfortunately, such HMBC was only observed for the 6-substituted methylamine isomer **8a** (R^2 = Me). The tendency of quinolinequinones to tautomerise nevertheless allowed for the assignment of each regioisomer. As a consequence of tautomerisation (Scheme 1), C6, C7 and C8 are broader and less visible in the ^{13}C NMR spectra for the 6-substituted isomer, whilst C5, C6 and C7 are less visible for the 7-subsituted isomer. The HMBC between H4 to C5 can thus be used to determine the regioselectivity of addition.

It is also worthwhile to note that the ^1H NMR chemical shifts and polarity of the amino-5,8-quinolinequinone regioisomers can be used to determine the site of addition.[31] The chemical shift of H2 for the 7-isomer has been shown to be slightly upfield when compared to the 6-isomer, while that of H4 is slightly downfield, thus making the value of Δ(H2–H4) larger for the 6-isomer than the 7-isomer. The 6-isomer has also been reported to be more polar than the 7-isomer, as determined by R_f values (silica gel TLC). These correlations held true for the assignment of all of our compounds [e.g., for the 6-isomer **8a**: Δ(H2–H4) = 0.67 ppm, R_f = 0.23 (EtOAc) cf. 7-isomer **9a**: Δ(H2–H4) = 0.45 ppm, R_f = 0.32 (EtOAc)].

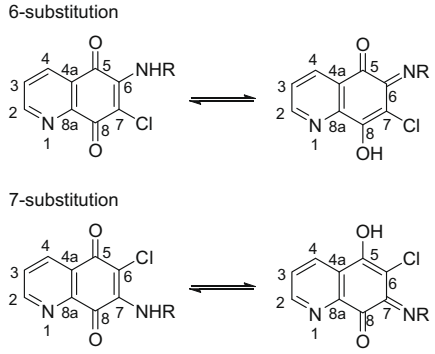
With the required amino-quinones in hand, the sulfur functionality was then introduced. Treatment of 7-chloro-quinolinequinone **8a** with sodium thiomethylate (NaSMe) in EtOH at rt gave the desired thio-quinone **10a** in good (88%) yield (Scheme 2), whilst the phenyl-substituted thiol **10b** was prepared via the reaction of dichloro-quinolinequinones **8a** in pyridine with thiophenol (PhSH). The sulfides **10a** and **10b** were then oxidised to the sulfoxides **13a** and **13b**, respectively, using one equivalent of $KMnO_4$ or *m*-CPBA, with the *m*-CPBA oxidation giving improved yields. Inter-

Table 1
Formation of 6- and 7-substituted quinolinequinones



Entry	Starting material	Conditions[a]	R^1	R^2	Yield[b] 6-isomer	Yield[b] 7-isomer
1	**7a**	A	H	Me	**8a**, 95%	**9a**, —
2	**7a**	B	H	Me	**8a**, 20%	**9a**, 67%
3	**7a**	A	H	$CHPh_2$	**8b**, 96%	**9b**, —
4	**7a**	C	H	CH_2CH_2Cl	**8c**, 82%	**9c**, —
5	**7a**	D	H	CH_2CH_2Cl	**8c**, 46%	**9c**, 48%
6	**7a**	D	H	CH_2CH_2Br	**8d**, 53%	**9d**, 25%
7	**7b**	D	Me	CH_2CH_2Br	**8e**, 44%	**9e**, 21%

[a] Conditions: (A) $NiCl_2{\cdot}6H_2O$ (1.1 equiv), EtOH; (B) 1,4-dioxane, pyr. (2.1 equiv); (C) EtOH/H_2O (2:1), $NiCl_2{\cdot}6H_2O$ (1.1 equiv), 2 M NaOH (2.1 equiv); (D) EtOH/H_2O (2:1), 2 M NaOH (2.1 equiv).
[b] Isolated yield.

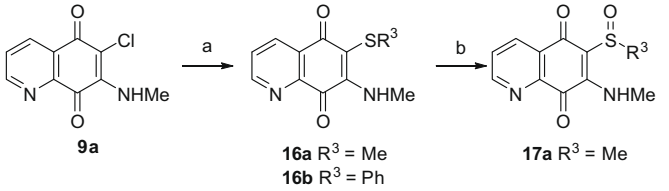


Scheme 1. Tautomerisation of amino-substituted quinolinequinones.

estingly, during column chromatography of the amine-substituted quinolinequinones using ammonia in the eluent, aminolysis products were observed. This reaction was subsequently used to our advantage to produce an additional series of compounds. Thus, by subjecting quinolinequinone **8b** to a solution of ammonia in water/methanol and subsequent workup, amine **11** was isolated in 84% yield. In an analogous manner, methylthio substituted quinone **15** was obtained from **10a** in good yield, while oxidation of sulfide **10a** to the corresponding sulfone, using two equivalents of *m*-CPBA, followed by aminolysis resulted in the formation of the free amine **12** in 93% yield. Aminolysis of sulfoxide **13a** also proceeded uneventfully to give amine **14** in 88% yield.

To explain the tendency of the N-alkylated amino-quinolinequinones to undergo aminolysis, it needs to be recognised that Michael addition to the 6-position of the quinolinequinone is favoured (Scheme 4). Here, enamine formation and delocalisation of the negative charge through both the quinone-ring and sulfoxide/sulfone substituent favours nucleophilic attack at the imine. Subsequent protonation of the alkylamino-substituent, followed by β-elimination results in reestablishment of the quinone system and net nucleophilic substitution at the 6-position. It is also conceivable that this type of substitution may occur in vitro/in vivo, rendering these compounds efficient electrophilic traps for irreversible/covalent inhibition of enzymatic targets.

The specificity of the aminolysis reaction, in that Michael addition takes place when ammonia is used as the nucleophile but not the thiolate, follows literature precedent[34] whereby hard nucleophiles, such as amines and alkoxides, predominantly result in displacement of the amine substituent. The large excess of ammonia also ensures complete aminolysis of the alkylamine. Similarly, displacement of the 7-chloride in chloro-quinolinequinone **8b** is not observed. Softer nucleophiles (e.g., thiols) favour substitution of chlorides,[35] and though amine substitution of chlorides is known

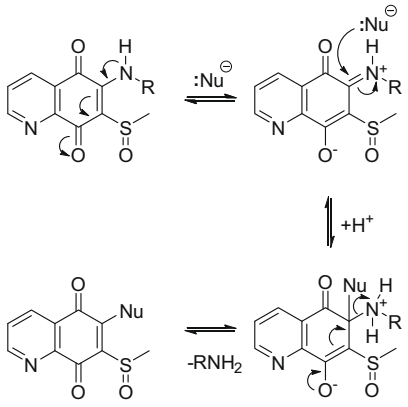


Scheme 3. Formation of 6-sulfur-substituted quinones. Regents and conditions: (a) NaSMe, EtOH, **16a**: 94% or HSPh, pyr., **16b**: 90%; (b) *m*-CPBA (1.0 equiv), CH_2Cl_2, 0 °C, 10 min, 79%.

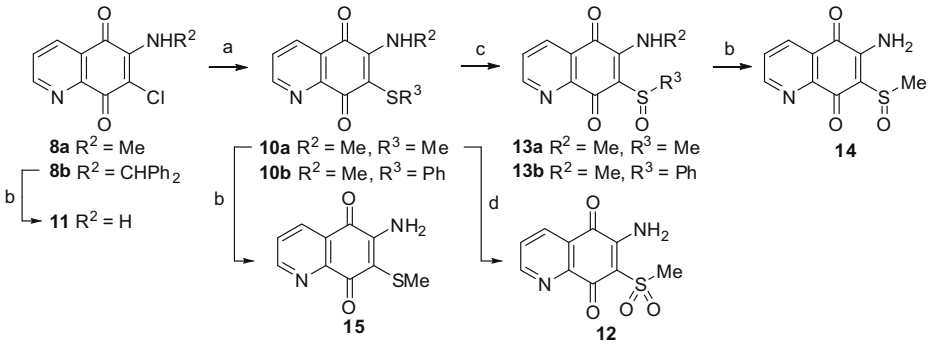
(e.g., **7**→**8**), this is disfavoured for quinolinequinones containing a 6- or 7-substituted amine (e.g., **8**)—a consequence of enamine formation (Scheme 4) and electron delocalisation over the quinoline quinone-ring leading to unfavourable orbital interactions with hard amine nucleophiles.

In the 7-amino-substituted series (Scheme 3), 7-methylamine quinolinequinone **9a** was treated with either NaSMe or PhSH to give sulfides **16a** and **16b** in 94% and 90% yield, respectively. Oxidation of sulfide **16a** with one equivalent of *m*-CPBA then gave sulfoxide **17a** in good yield.

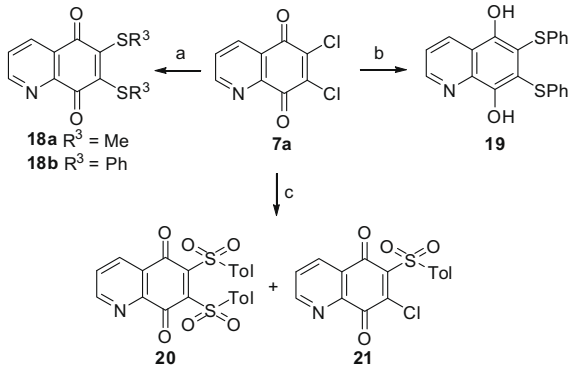
To prepare additional sulfur-substituted quinolinequinones for biological evaluation, dichloro-quinolinequinone **7a** was treated with 2.1 equiv of sodium thiomethoxide in MeCN to give the corresponding disulfide **18a** in excellent (90%) yield (Scheme 5). Alternatively, treatment of **7a** with 2.1 equiv of HSPh in pyridine gave the bis(phenylsulfanyl) analogue **18b**, also in excellent yield. When excess HSPh (3 equiv) was added to a solution of **7a** in pyridine, diol **19** was isolated in 42% yield—a consequence of the reduction of the quinone moiety by the excess HSPh, which itself was



Scheme 4. Nucleophilic substitution via a Michael/Retro Michael mechanism.



Scheme 2. Formation of 7-sulfur-substituted quinones. Reagents and conditions: (a) NaSMe, EtOH, **10a**: 88%; or HSPh, pyr., **10b**: 95%; (b) aq NH_3, **11**: 84%, **15**: 76%, **14**: 88%; (c) *m*-CPBA (1.0 equiv), CH_2Cl_2, 0 °C, 35 min, **13a**: 74%; or $KMnO_4$, AcOH, **13b**: 30%; (d) (i) *m*-CPBA (2.0 equiv), CH_2Cl_2, 0 °C, 30 min; (ii) aq NH_3, 93%.



Scheme 5. Formation of disulfides and sulfones. Reagents and conditions: (a) NaSMe (2.1 equiv), MeCN, **18a**: 90%; or HSPh (2.1 equiv), THF, pyr., **18b**: 92%; (b) HSPh (3 equiv), THF, pyr., 42%; (c) *p*-toluenesulfinic acid (2.1 equiv), H_2O, MeCN, **20**: 44%, **21**: 18%.

oxidised to the disulfide (PhSSPh). Somewhat unsurprisingly, subjection of dichloro-quinolinequinone **7a** to 1 equiv of HSPh under a variety of conditions led to an inseparable mixture of the 6- and 7-sulfur substituted products in a 1:1 ratio, even when $NiCl_2{\cdot}6H_2O$ was added as a Lewis acid. This lack of regioselectivity occurred as a consequence of the favourable reactivity of the soft sulfur nucleophile towards the α,β-unsaturated ketone—such nucleophilic addition is so facile, that the slight enhancement of electrophilicity at the C6 position brought about via the addition of the Lewis acid has no effect on chemoselectivity. To prepare further sulfone derivatives, dichloro-quinolinequinone **7a** was then treated with 2.1 equiv of *p*-toluenesulfinic acid. This resulted in the formation of the disulfone **20** in 44% yield, and the 6-isomer **21** (18% yield) following recrystallisation.

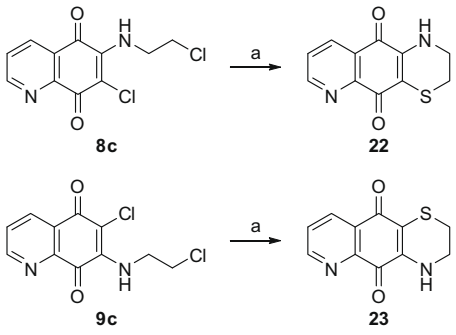
Finally, to follow-up from our previous studies whereby we synthesised and tested a variety of Ascidathiazone analogues,[5] the more simple tricyclic quinolinequinones **22** and **23** were prepared (Scheme 6). Here, ethanolic solutions of the β-chloro-ethylamine precursors **8c** and **9c** were subjected to a slight excess of sodium sulfide in H_2O,[16] which, following substitution of the primary chloride by the thiol and subsequent intra molecular cyclisation, then gave the simple ascidiathiazone quinolinequinone analogues **22** and **23** in 59% and 76% yield, respectively.

3. Biological results and discussion

3.1. Overview

Having synthesised a variety of quinolinequinones, we then tested them for anti-proliferative, anti-inflammatory and tuberculostatic activities. To investigate the potential of the compounds to act as anti-cancer drugs, their anti-proliferative activity was measured against HL60 cells (IC_{50}) and human T-cells (IC_{50}) from healthy volunteers using the MTT assay.[36,37] To determine the anti-inflammatory activity (AI) of the quinolinequinones, their ability to block the production of superoxide by activated human neutrophils,[37,38] a key readout linked to a number of inflammatory diseases including gout and cardiovascular disease,[39–41] was measured. The potential of the quinolinequinones in treating tuberculosis was determined via the Alamar Blue tuberculostatic assay[42,43] using *Mycobacterium bovis* BCG as the bacterial model. Here, the MIC was determined as the minimal amount of compound leading to 100% growth inhibition.

A summary of these assay results is presented (Table 2). The compounds have been broadly grouped into those containing two halogen substituents (entries 1 and 2), an amine and halogen functionality (entries 3–12), those with a mono-sulfur functionality (entries 13–24), and the di-sulfur containing derivatives (entries 25–28).



Scheme 6. Formation of tricyclic structures. Regents and conditions: (a) $Na_2S{\cdot}9H_2O$, EtOH, **22**: 59%, **23**: 76%.

3.2. Anti-proliferative activity

Since the early work on streptonigrin, it is well known that quinolinequinones show promise as anti-cancer agents; however, in general, their toxicity has limited therapeutic application. A similar trend was observed for our 6,7-amino and/or halogen functionalised quinolinequinones with most exhibiting potent anti-proliferative activity against HL60 cells [IC_{50} values of ca. 1–4 μM (entries 3–12)] and T-cells [IC_{50} values of ca. 1.5–5 μM (entries 3–12)]. There was little distinction between the anti-proliferative activities for the regioisomers (e.g., compare entries 5 and 6, 7 and 8, 9 and 10, 11 and 12), or with the type of amino substituent (cf. 3, 4, and 5). The dichloro-quinolinequinones (entries 1 and 2), exhibited notably lower anti-proliferative activity against T-cells and HL60 cells. The introduction of a sulfur atom, at either the 6- or 7-position however, provided more promising results. Most notable were the activities of thiol **10a** (entry 14), and the sulfoxide **12** (entry 23). With an HL60 IC_{50} of 3.4 μM and a T-cell IC_{50} of 19.5 μM, thiol **10a** showed modest selectivity towards tumour cells and is a worthy candidate for further evaluation. This somewhat selective activity appears to be specific to this particular thiol, as its amine-analogue **15** (entry 13), its regio-isomer **16a** (entry 15), and its thiophenyl analogue **10b** (entry 16) exhibited comparable anti-proliferative activity across the two assays. The sulfoxides (entries 20–22) did not exhibit very promising anti-proliferative activity; however the sulfone **12** (entry 23) exhibited good selectivity with the HL60 anti-proliferative activity (IC_{50} = 4.53 μM) being almost fivefold higher than the corresponding T-cell anti-proliferative activity (IC_{50} = 19.3 μM). The di-thiols **18a**, **18b**, and **19** (entries 25–27) exhibited little anti-proliferative specificity, though it is interesting to note that the reduction of the diphenyl quinolinequinone **18b** to the diol **19** led to a slight reduction in the anti-proliferative activities (cf. entry 26 and entry 27). Finally, of the quinolinequinones tested, the chloro-sulfone analogue **21** (entry 24) and the *bis*-tolyl-analogue **20** (entry 28) showed comparatively modest anti-proliferative activity.

Taken as a whole, these results indicate that 6,7-substituted-5,8-quinolinequinones containing an amine and a halogen at the 6- or 7-position or two sulfur-containing groups show little specificity towards HL60 cells compared to human T-cells, thus making them less desirable anti-cancer drugs. Of these two classes of compounds, the amine-functionalised quinolinequinones show greater anti-proliferative activity. Quinolinequinones with an amine- and thiol-functionality at the 6- or 7-position however, hold promise as selective anti-cancer drugs with two lead compounds, thiol **10a** and sulfone **12**, having been identified.

Table 2
Quinolinequinones and their biological activities

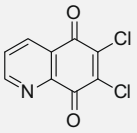
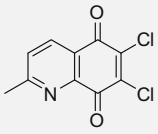
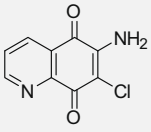
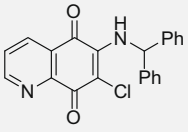
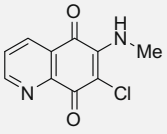
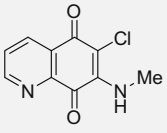
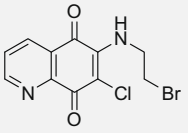
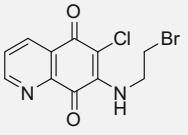
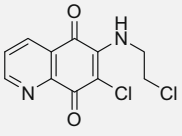
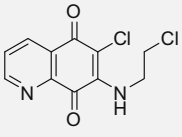
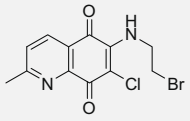
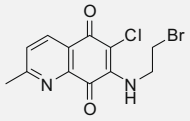
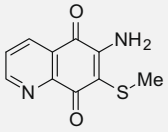
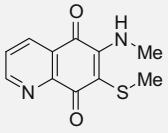
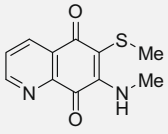
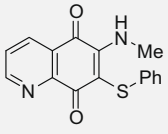
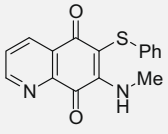
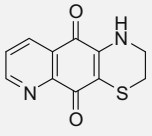
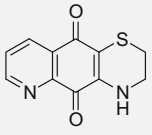
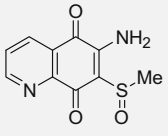
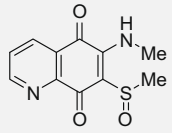
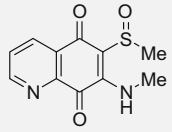
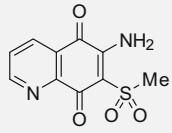
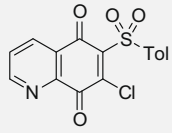
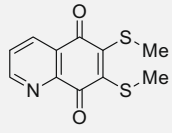
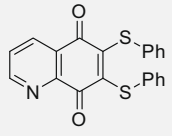
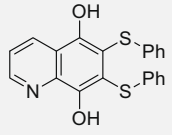
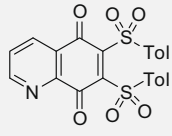
Entry	Compound	HL60 IC_{50} (μM)	T-cell IC_{50} (μM)	AI_{50} (μM)	Tuberculostatic activity; MIC (μg/mL)
1	 **7a**	31.8 ± 3.8	32.9 ± 15.3	1.3 ± 0.9	6.3–12.5
2	 **7b**	18.7 ± 3.4	44.9 ± 4.5	3.4 ± 1.9	6.3–12.5
3	 **11**	3.26 ± 0.12	2.04 ± 0.06	>200	1.56–3.13
4	 **8b**	2.45 ± 0.11	2.59 ± 0.01	33.9 ± 0.61	6.3–12.5
5	 **8a**	2.45 ± 0.46	3.56 ± 0.08	78.9 ± 0.36	12.5–25
6	 **9a**	4.30 ± 0.09	5.21 ± 0.34	41.1 ± 2.1	12.5–25
7	 **8d**	1.65 ± 0.25	2.56 ± 0.56	20.0 ± 4.2	3.13–6.3
8	 **9d**	1.20 ± 0.03	2.50 ± 0.69	20.7 ± 6.4	6.3–12.5
9	 **8c**	1.62 ± 0.17	2.29 ± 0.05	37.0 ± 2.3	6.3–12.5
10	 **9c**	1.94 ± 0.34	2.27 ± 0.09	31.0 ± 4.7	12.5–25

Table 2 (*continued*)

Entry	Compound	HL60 IC_{50} (μM)	T-cell IC_{50} (μM)	AI_{50} (μM)	Tuberculostatic activity; MIC (μg/mL)
11	 **8e**	1.30 ± 0.07	2.23 ± 0.10	34.2 ± 0.7	12.5–25
12	 **9e**	1.21 ± 0.02	1.59 ± 0.08	73.2 ± 1.5	12.5–25
13	 **15**	7.64 ± 0.49	8.32 ± 0.56	>150	6.3–12.5
14	 **10a**	3.44 ± 0.07	19.5 ± 1.0	>200	12.5–25
15	 **16a**	7.92 ± 0.23	7.57 ± 0.23	>200	>50
16	 **10b**	8.03 ± 0.30	12.2 ± 1.4	>150	12.5–25
17	 **16b**	5.88 ± 0.04	6.91 ± 0.42	92.8 ± 1.1	12.5–25
18	 **22**	4.24 ± 0.15	5.88 ± 1.40	>200	6.3–12.5
19	 **23**	3.52 ± 0.13	6.02 ± 0.40	>200	6.3–12.5
20	 **14**	1.30 ± 0.01	1.93 ± 0.07	>200	1.56–3.13

(*continued on next page*)

Table 2 (*continued*)

Entry	Compound	HL60 IC_{50} (μM)	T-cell IC_{50} (μM)	AI_{50} (μM)	Tuberculostatic activity; MIC (μg/mL)
21	 **13a**	12.1 ± 0.2	11.8 ± 2.0	>200	25–50
22	 **17a**	33.2 ± 0.4	13.4 ± 1.5	>200	>50
23	 **12**	4.53 ± 0.25	19.3 ± 1.4	>200	25–50
24	 **21**	38.1 ± 2.7	35.5 ± 2.8	>200	>50
25	 **18a**	1.23 ± 0.02	1.69 ± 0.07	0.11 ± 0.01	6.3–12.5
26	 **18b**	3.68 ± 0.15	7.77 ± 1.5	24.8 ± 0.6	>50
27	 **19**	6.95 ± 1.36	10.0 ± 1.6	2.3 ± 0.1	25–50
28	 **20**	61.6 ± 3.2	40.5 ± 0.9	>200	6.3–12.5

3.3. Anti-inflammatory activity

When considering the anti-inflammatory activities of the 6,7-substituted-5,8-quinolinequinones, it is interesting to note that there was an inverse correlation between anti-inflammatory activity and anti-proliferative activity. Of the quinolinequinones tested, two of the more promising anti-inflammatory candidates were the dichloro-derivatives **7a** and **7b**, which, with AI activities of 1.3 μM and 3.4 μM, respectively, exhibited greater than 10-fold selectivity for inhibition of superoxide production when compared to their anti-proliferative T-cell activities (entries 1 and 2). With the exception of the poorly inhibitory chloro-amine quinolinequinone **11** (entry 3), the quinolinequinones containing an amine- and chloro-functionality typically showed modest anti-inflammatory activity (entries 4–12), while the amine- and sulfur-containing quinolinequinones showed poor activity (entries 13–23). Here, it should be noted that the tricyclic quinolinequinones **22** and **23** (entries 18 and 19), the stripped-back analogues of ascidiathiazones A and B, respectively, showed poor anti-inflammatory activity. Previous AI studies on other ascidiathiazone analogues revealed that the addition of a polar-group to the pyridine ring greatly increases AI activity, and it was also suggested that the oxidation state and regiochemistry of the thiazine ring played a critical role in AI activity.[5] Our results support this statement.

As a class of compounds, the di-thiol containing quinolinequinones showed better AI activity. The di-thiomethyl derivative **18a**

(AI = 0.11 μM), exhibited 10-fold greater activity in the AI assay compared to its anti-proliferative activity (entry 25). Interestingly, the di-thiophenyl derivative **18b** exhibited modest AI activity (ca. 25 μM, entry 26), however in its reduced form, diol **19**, showed remarkably enhanced AI activity (ca. 2.3 μM, entry 27). Finally, the disulfone **20** (entry 28) exhibited particularly poor AI activity. Taken as a whole, these results illustrate that quinolinequinones containing an amine-functionality at the 6- or 7-position show poor AI activity compared to di-chloro or di-thio quinolinequinones. Based on our results dichloro **7a**, methyl-dichloro **7b**, di-thiomethyl **18a** and diol **19** have been identified as lead AI drug candidates.

3.4. Tuberculostatic activity

On the whole, most of the quinolinequinones tested exhibited good to modest tuberculostatic activity with typical MIC's ranging from approximately 3 to 50 μg/mL. Ethambutol, a front-line drug used for the treatment of tuberculosis, served as a positive control and exhibited an MIC = 3.1–6.3 μg/mL in our assay.

When considering the tuberculostatic activity of the quinolinequinones, it was more difficult to correlate specific structural features with biological activity. Generally, the quinolinequinones containing a halogen at the 6- and/or 7-position exhibited slightly better tuberculostatic activity than derivatives containing a thiol group at the 6- or 7-position (compare entries 1–12 with 13–19); however the precise levels of activity depended on the substitution pattern. Previous studies have revealed that chloro-ethylamino-quinolinequinones, such as **8c** (entry 9), and the subsequent tricyclic compound **22** (entry 18) exhibited promise as TB-drugs.[44] Though our halo-ethylamines **8d–e** (entries 7, 11) and **9c–e** (entries 8, 10, 12) showed good activities, as did the tricyclic derivatives **22** and **23** (entries 18, 19), these were not the most promising compounds identified in our assay. Of particular note was the tuberculostatic activity of the primary amine **11** (entry 3). With an MIC = 1.56–3.13 μg/mL, amine **11** was one of the more promising TB drug candidates identified in our assay. The quinolinequinones containing two thiol functionalities also exhibited mixed tuberculostatic activity with the di-thiomethyl derivative **18a** showing better activity than the di-thiophenyl derivative **18b** (entry 27 cf. entry 28).

The tuberculostatic activities of the quinolinequinones containing a sulfoxide or sulfone functionality were again mixed. A quinolinequinone containing a free amine, sulfone **14** (entry 20) exhibited good tuberculostatic activity (MIC = 1.56–3.13 μg/mL), however its free amine sulfone counterpart **12** exhibited poor activity (MIC = 25–50 μM, entry 23), thus preventing the correlation between the presence of a free amine and tuberculostatic activity to be made. [This lack of correlation is further illustrated when the activity of free amine **11** (MIC = 1.56–3.13 μg/mL, entry 3) is compared with the activity of the free amine thiol analogue **15** (MIC = 6.3–12.5, entry 13)]. Of special note, however, is the tuberculostatic activity of the di-sulfone **20** (entry 28). Though the mono-sulfones tested exhibited only poor tuberculostatic activity (entries 23 and 24), di-sulfone **20** showed markedly better activity and, more importantly, had tuberculostatic activity that was approximately fivefold greater than its anti-proliferative activity.

In summary, three lead quinolinequinone TB-drug candidates, amine **11**, sulfoxide **14**, and di-sulfone **20** were identified. Though the di-sulfone **20** had a lower MIC when compared to the other two quinolinequinones (6.3–12 μg/mL vs 1.56–3.13 μg/mL), it exhibited only modest anti-proliferative activity and therefore may also be a suitable TB-drug.

4. Conclusion

A variety of 6,7-substituted 5,8-quinolinequinolines have been synthesised and several lead candidates identified as potential anti-cancer, anti-inflammatory or tuberculostatic agents. In particular, the introduction of a sulfur group at the 7-position of the quinolinequinone led to the discovery of a number of biologically active compounds that exhibit selectivity for leukemic cells over T-cells, a highly desirable property for an anti-cancer drug. Several lead anti-inflammatory and tuberculostatic quinolinequinones have also been identified. When determining the biological specificity of the quinolinequinones, some broad functional group patterns appear to be important. Typically, a thiol and amine-functionalised quinolinequinone leads to better anti-proliferative activity, while a di-chloro or di-thiol-substituted quinolinequinone gives better anti-inflammatory activity. In the case of tuberculostatic activity, the functional-group distinctions are less clear and the observed activities may better reflect the differences in the quinolinequinones bioavailability and the inherent difficulties of drugs penetrating the thick mycobacterial cell wall. We are currently further investigating the biological profiles and modes of action of our lead candidates.

5. Experimental

5.1. Chemistry

5.1.1. General methods

Unless otherwise stated all reactions were performed under atmospheric air. THF (Lab-Scan) was distilled from $LiAlH_4$. MeCN (Panreac) was distilled from calcium hydride, and EtOAc (Pure Science) was also distilled. EtOH (absolute, Pure Science), hexanes (Pure Science), MeOH (Pure Science), AcOH (Aiax Finechem), CH_2Cl_2 (LabServ), 30% aqueous NH_3 (J. T. Baker Chemical Co.), Et_2O (Merk), TFA (Aldrich), sodium chlorate (BDH), Sodium chlorate (BDH), 8-hydroxyquinoline (BDH), concd HCl (Univar), 2-methyl-8-quinolinol (Aldrich), $NiCl_2{\cdot}6H_2O$ (Riedel de Häen), methylamine (Riedel de Häen), $NaHCO_3$ (Pure Science), $MgSO_4$ (Pure Science), pyridine (Roth), β-chloro-ethylamino hydrochloride (Aldrich), β-bromo-ethylamino hydrochloride (BDH), sodium thiomethoxide (Acros), Toluene (Fischer Scientific), thiophenol (Janssen Chemicals), *m*-CPBA (Janssen Chemicals), $KMnO_4$ (AnalaR), *p*-toluenesulfinic acid sodium salt (Aldrich), sodium sulfide (Univar), diphenylmethylamine (Aldrich), AcCl (B&M), NaOH (Pure Science), and NH_4OAc (AnalaR) were used as received. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC-analysis on ALUGRAM SIL G/UV_{254} TLC plates with either visual detection, or detection by UV-absorption (254 nm). Column chromatography was performed using Pure Science silica gel (40–63 μm) as the stationary phase and the solvent systems indicated. High-resolution mass spectra were recorded on a Waters Q-TOF Premier™ Tandem Mass Spectrometer using positive electro-spray ionisation. Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) sampling accessory, and are reported in wave numbers (cm^{-1}). Melting points (Mp) were obtained on a Gallenkamp Melting Point Apparatus and UV data obtained on an Agilent 8453 spectrometer. Nuclear magnetic resonance (NMR) spectra were acquired at 20 °C in $CDCl_3$, D_2O or DMSO-d_6 as indicated using a Varian Unity-INOVA 500 MHz spectrometer, where ^1H and ^{13}C were measured at 500 and 125 MHz, respectively. ^1H NMR spectral data are presented as illustrated: chemical shift (δ) in ppm, multiplicity [s (singlet), d (doublet), t (triplet), m (multiplet over the range specified)], number of protons, (nH), coupling constants (J) in hertz, assignment of protons).

^{1}H NMR spectra are referenced to the $CHCl_3$ (δ 7.26 ppm), H_2O (δ 4.79 ppm) or DMSO (δ 2.50 ppm) residual solvent peaks. ^{13}C NMR spectra were proton decoupled and referenced to TMS (δ 0 ppm). NMR peak assignments were made using COSY, HSQC, and HMBC experiments.

5.1.2. Procedures for the synthesis of the quinolinequinones

5.1.2.1. 6,7-Dichloro-5,8-quinolinequinone (7a). Sodium chlorate (53 g, 0.50 mol, 5 equiv) was added over a period of 1 h to a solution of 8-hydroxyquinoline (14.5 g, 0.10 mol) in concd HCl (600 mL) at 40 °C and the reaction mixture stirred for 2 h before being diluted with water to a total volume of 2 L. The white precipitate that formed was removed by filtration and discarded. The filtrate was then extracted with CH_2Cl_2 (6 × 250 mL), the organic phases were combined, washed with water and concentrated in vacuo to give a yellow solid. The solid was then recrystallised in MeOH to yield **7a** as bright yellow crystals (6.67 g, 29 mmol, 29%). R_f = 0.52 (EtOAc); Mp 219.4–221.0 °C (Lit.[12] 221–223 °C); IR (thin film): 2989, 2915, 2831, 1691, 1675, 1572, 1557, 1273, 1194, 1136, 1100, 1020, 949, 895, 828, 724, 694 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 201 (4.23), 240 (4.33), 272 (4.24) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.11 (dd, 1H, $J_{2,3}$ = 4.1, $J_{2,4}$ = 0.9 Hz, H-2), 8.54 (dd, 1H, $J_{3,4}$ = 8.0, $J_{2,4}$ = 1.2 Hz, H-4), 7.77 (dd, 1H, $J_{3,4}$ = 8.1, $J_{2,3}$ = 4.9 Hz, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.6 (C-5), 174.3 (C-8), 155.4 (C-2), 146.8 (C-8a), 144.4 (C-6 or C-7), 143.1 (C-6 or C-7), 135.6 (C-4), 128.3 (C-4a), 128.3 (C-3); HRMS(ESI) *m/z* calcd for $[C_9H_3NO_2Cl_2+Na]^+$: 249.9439, obsd.: 249.9445.

5.1.2.2. 6,7-Dichloro-2-methyl-5,8-quinolinequinone (7b). Sodium chlorate (16.5 g, 155 mmol, 5 equiv) was added over a period of 1 h to a solution of 8-hydroxy-2-methyl-quinoline (4.93 g, 31.0 mmol) in concd HCl (200 mL) at 40 °C and the reaction mixture stirred for 2 h before being diluted to 1 L with water. The white precipitate that formed was removed by filtration and discarded and the filtrate extracted with CH_2Cl_2 (6 × 100 mL). The organic phases were combined and washed with water, then concentrated in vacuo to give a yellow solid that was set to crystallise in the minimum volume of boiling MeOH to yield dichloride **7b** as bright yellow crystals (2.05 g, 8.4 mmol, 27%). R_f = 0.20 (EtOAc); Mp 179.1–181.3 °C (Lit.[12] Mp 180–181 °C); IR (film): 1699, 1691, 1676, 1593, 1569, 1466, 1290, 1260, 1208, 1146, 1084, 930, 864, 828, 727, 640 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 280 (4.10), 245 (4.17), 195 (3.82) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-4), 7.59 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-3), 2.78 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): 175.6 (C5), 174.5 (C8), 166.1 (C2), 146.3 (C8a), 143.9 (C7), 142.8 (C6), 135.6 (C4), 128.3 (C3), 126.1 (C4a), 25.3 (CH_3); HRMS(ESI) *m/z* calcd for $[C_{10}H_5NO_2Cl_2+Na]^+$: 263.9595, obsd.: 263.9600.

5.1.2.3. 7-Chloro-6-methylamino-5,8-quinolinequinone (8a). A solution of 6,7-dichloro-5,8-quinolinequinone (**7a**) (1.37 g, 5.99 mmol) and $NiCl_2{\cdot}6H_2O$ (1.57 g, 6.59 mmol, 1.1 equiv) in EtOH (25 mL) was stirred for 45 min at rt. Methylamine (40%, 0.62 mL, 7.19 mmol, 1.2 equiv) was then added and after stirring at rt for 1 h, the EtOH was removed under reduced pressure, the residue was dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$, followed by brine, then dried ($MgSO_4$). Filtration, concentration in vacuo and purification by column chromatography (CH_2Cl_2/EtOAc, 3/1→1/1, v/v) yielded a red solid which was crystallised from a minimal amount of boiling CH_2Cl_2/hexanes (1/4, v/v) to give quinone **8a** as deep brick-red crystals (1.26 g, 5.7 mmol, 95%). R_f = 0.23 (EtOAc); Mp 219.0–219.2 °C (Lit.[45] Mp 194–195 °C); IR (thin film): 3347, 2926, 2856, 1678, 1596, 1561, 1507, 1424, 1308, 1217, 1131, 912, 737, 682 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 200 (4.08), 232 (4.16), 270 (4.01), 470 (3.42) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.02 (dd, 1H, $J_{2,3}$ = 4.6, $J_{2,4}$ = 1.7 Hz, H-2), 8.35 (dd, 1H, $J_{3,4}$ = 7.8, $J_{2,4}$ = 1.7 Hz, H-4), 7.58 (dd, 1H, $J_{3,4}$ = 7.8, $J_{2,3}$ = 4.6 Hz, H-3) 6.09 (s, 1H, N–H), 3.48 (d, 3H, $J_{NH,N\text{-}Me}$ = 5.6 Hz, N-Me); ^{13}C NMR (125 MHz, $CDCl_3$): δ 180.1 (C-5), 175.3 (C-8), 155.3 (C-2), 148.5 (C-8a), 144.4 (C-6), 134.6 (C4), 126.7 (C-4a), 126.5 (C-3), 112.2 (C-7), 32.7 (CH_3); HRMS(ESI) *m/z* calcd for $[C_{10}H_7N_2O_2Cl+H]^+$: 223.0274, obsd.: 223.0274.

5.1.2.4. 6-(Benzhydryl-amino)-7-chloro-5,8-quinolinequinone (8b). A solution of 6,7-dichloro-5,8-quinolinequinone (**7a**) (0.20 g, 0.87 mmol) and $NiCl_2{\cdot}6H_2O$ (0.21 g, 0.95 mmol, 1.1 equiv) in EtOH (10 mL) was stirred for 45 min at rt. Diphenylmethylamine (0.18 mL, 0.95 mmol, 1.1 equiv) was then added and after stirring at rt for 1 h, the EtOH was removed under reduced pressure, the reaction mixture was dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$, followed by brine, then dried ($MgSO_4$). Filtration, concentration in vacuo and purification by column chromatography (CH_2Cl_2/EtOAc, 1/0→5/1, v/v) yielded a bright orange solid that was crystallised from CH_2Cl_2/hexanes to give quinolinequinone **8b** as a bright orange micro-crystalline powder (0.31 g, 0.83 mmol, 96%). R_f = 0.48 (EtOAc); Mp 172.2–173.5 °C; IR (thin film): 3335, 3061, 1676, 1598, 1568, 1508, 1454, 1309, 1266, 1203, 1142, 1070, 701 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 201 (6.47), 231 (6.31), 271 (6.19) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.00 (dd, 1H, $J_{2,3}$ = 4.7, $J_{2,4}$ = 1.7 Hz, H-2), 8.32 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,4}$ = 1.7 Hz, H-4), 7.56 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,3}$ = 4.7 Hz, H-3) 7. 24–7.42 (m, 10H, CH arom.), 6.92 (d, 1H, $J_{NH,CHPh2}$ = 8.5 Hz, CH), 6.45 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.9 (C-5), 175.3 (C-8), 155.2 (C-2), 148.2 (C-8a), 143.0 (C-4a), 141.6 (C-6), 134.8 (C-4) 129.1 (CH-*o* arom.), 128.1 (CH-*p* arom.), 127.2 (CH-*m* arom.), 126.9 (C-*ipso* arom.), 126.7 (C-3), 114.3 (C-7), 61.8 ($CHPh_2$); HRMS(ESI) *m/z* calcd for $[C_{22}H_{15}N_2O_2Cl+Na]^+$: 397.0720, obsd.: 397.0644.

5.1.2.5. 7-Chloro-6-(2-chloro-ethylamino)-5,8-quinolinequinone (8c). To a solution of 6,7-dichloro-5,8-quinolinequinone (**7a**) (0.11 g, 0.48 mmol, 1 equiv) in EtOH/H_2O (7.5 mL, 2:1, v/v) was added $NiCl_2{\cdot}6H_2O$ (0.13 g, 0.55 mmol, 1.15 equiv) and the solution stirred for 45 min at rt. 2-Chloro-ethylamine hydrochloride (0.075 g, 0.65 mmol, 1.35 equiv) and 2 m NaOH (0.5 mL, 1.0 mmol, 2.1 equiv) were then added and the solution refluxed for 15 min. The solvents were removed under reduced pressure and the residue taken up in CH_2Cl_2, washed with satd aq $NaHCO_3$ and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Purification of the residue by column chromatography (CH_2Cl_2/EtOAc, 35/1→1/2, v/v) gave quinolinequinone **8c** (0.12 g, 0.39 mmol, 82%) as dark red crystals. R_f = 0.23 (EtOAc); Mp 165.7–166.6 °C (Lit. Mp 151 °C, Ref. 29); IR (film): 1687, 1657, 1595, 1570, 1514, 1452, 1440, 1360, 1329, 1304, 1257, 1215, 1191, 1111, 1075, 822, 679 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 470 (3.53), 271 (4.11), 232 (4.24) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.99 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.7 Hz, 1H, H-2), 8.35 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-4), 7.59 (dd, $J_{2,3}$ = 4.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-3), 6.34 (br s, 1H, N–H), 4.21 (m, 2H, H-2′), 3.78 (t, $J_{H\text{-}2',H\text{-}3'}$ = 5.8 Hz, 2H, H-3′); ^{13}C NMR (125 MHz, $CDCl_3$) 179.7 (C5), 175.5 (C8), 155.3 (C2), 148.1 (C8a), 143.4 (C6), 134.8 (C4), 126.8 (C4a), 126.8 (C3), 113.4 (C7), 46.0 (C2′), 44.1 (C3′); HRMS(ESI) *m/z* calcd for $[C_{11}H_8N_2O_2Cl_2+Na]^+$: 292.9861, obsd.: 292.9865.

5.1.2.6. 6-(2-Bromo-ethylamino)-7-chloro-5,8-quinolinequinone (8d) and 7-(2-bromo-ethylamino)-6-chloro-5,8-quinolinequinone (9d). 2-Bromo-ethylamine hydrochloride (0.33 g, 2.9 mmol, 1.5 equiv) and 2 m NaOH (1.7 mL, 3.5 mmol, 1.8 equiv) were added to a solution of the 6,7-dichloro-5,8-quinolinequinone (**7a**) (0.43 g, 1.9 mmol, 1 equiv) in EtOH (35 mL) and H_2O (15 mL) and the solution refluxed for 15 min. The solvents were then removed under reduced pressure and the residue dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$ and brine, dried ($MgSO_4$), filtered and concen-

trated. Purification of the crude product by column chromatography (CH_2Cl_2/EtOAc, 3/1→0/1, v/v) yielded the 6-substituted isomer **8d** as dark red crystals (0.36 g, 1.03 mmol, 53%) and the 7-substituted isomer **9d** as orange crystals (0.17 g, 0.48 mmol, 25%). Data for 6-substituted isomer **8d**: R_f = 0.24 (EtOAc); Mp 166.0–167.3 °C; IR (film): 1679, 1592, 1548, 1445, 1324, 1264, 1219, 1147, 1082, 854, 681, 645 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 464 (3.69), 271 (4.22), 232 (4.34) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.03 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.7 Hz, 1H, H-2), 8.38 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-4), 7.62 (dd, $J_{2,3}$ = 4.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-3), 6.35 (br s, 1H, N–H), 4.28 (m, 2H, H-2′), 3.64 (m, 2H, H-3′); ^{13}C NMR (125 MHz, $CDCl_3$) 179.7 (C5), 175.2 (C8), 155.4 (C2), 148.1 (C8a), 143.3 (C6), 134.8 (C4), 126.8 (C4a), 126.8 (C3), 113.5 (C7), 45.8 (C2′), 32.7 (C3′); HRMS(ESI) *m/z* calcd for $[C_{11}H_8N_2O_2ClBr+Na]^+$: 336.9355, obsd.: 336.9356. Data for 7-substituted isomer **9d**: R_f = 0.36 (EtOAc); Mp 143.0 °C (decomp); IR (film): 1687, 1649, 1604, 1565, 1516, 1437, 1355, 1301, 1245, 1197, 1138, 1096, 850, 724, 544 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 465 (3.15), 288 (3.70) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.7 Hz, 1H, H-2), 8.47 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-4), 7.67 (dd, $J_{2,3}$ = 4.7 Hz, $_{3,4}$ = 7.9 Hz, 1H, H-3), 6.46 (br s, 1H, N–H), 4.26 (m, 2H, H-2′), 3.63 (t, $J_{H\text{-}2',H\text{-}3'}$ = 5.8 Hz, 2H, H-3′); ^{13}C NMR (125 MHz, $CDCl_3$) 178.5 (C8), 175.7 (C5), 153.7 (C2), 146.0 (C8a), 144.2 (C7), 134.8 (C4), 129.6 (C4a), 128.5 (C3), 111.8 (C6), 45.9 (C2′), 32.0 (C3′); HRMS(ESI) *m/z* calcd for $[C_{11}H_8N_2O_2ClBr+Na]^+$: 336.9355, obsd.: 336.9359.

5.1.2.7. 6-(2-Bromo-ethylamino)-7-chloro-2-methyl-5,8-quinolinequinone (8e) and 7-(2-Bromo-ethylamino)-6-chloro-2-methyl-5,8-quinolinequinone (9e). 2-Bromo-ethylamine hydrochloride (3.6 g, 33 mmol, 1.5 equiv) and 2 m NaOH (20 mL, 40 mmol, 1.8 equiv) were added to a solution of the 6,7-dichloro-2-methyl-5,8-quinolinequinone (**7b**) (4.7 g, 22 mmol, 1 equiv) in EtOH (400 mL) and H_2O (200 mL) and the solution refluxed for 15 min. The solvents were then removed under reduced pressure and the residue dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$ and brine, dried ($MgSO_4$), filtered and concentrated. Purification of the crude product by column chromatography (CH_2Cl_2/EtOAc, 3/1→0/1, v/v) yielded the 6-substituted isomer **8e** as dark red crystals (3.6 g, 9.7 mmol, 44%) and the 7-substituted isomer **9e** as orange crystals (1.73 g, 4.71 mmol, 21%). Data for 6-substituted isomer **8e**: R_f = 0.31 (EtOAc); Mp 168.4–169.3 °C; IR (film): 1676, 1604, 1571, 1515, 1449, 1404, 1357, 1311, 1230, 1143, 1116, 930, 859, 731 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 486 (3.50), 280 (4.26), 238 (4.40) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-4), 7.42 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-3), 6.32 (br s, 1H, N–H), 4.24 (m, 2H, H-2′), 3.61 (m, 2H, H-3′), 2.73 (s, 3H, H-Me); ^{13}C NMR (125 MHz, $CDCl_3$) 179.7 (C5), 175.5 (C8), 166.0 (C2), 147.7 (C8a), 143.1 (C6), 134.9 (C4), 126.7 (C3), 124.6 (C4a), 112.9 (C7), 45.8 (C2'), 32.2 (C3′), 25.4 (Me); HRMS(ESI) *m/z* calcd for $[C_{12}H_{10}N_2O_2ClBr+Na]^+$: 350.9512, obsd.: 350.9510. Data for 7-substituted isomer **9e**: R_f = 0.44 (EtOAc); Mp 211.3–211.7 °C; IR (film): 3313, 1693, 1605, 1583, 1555, 1513, 1445, 1330, 1300, 1262, 1147, 729 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 485 (3.53), 301 (4.00), 274 (4.07), 236 (4.16) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-4), 7.51 (d, $J_{3,4}$ = 8.0 Hz, 1H, H-3), 6.39 (br s, 1H, N–H), 4.25 (m, 2H, H-2′), 3.62 (m, 2H, H-3′); ^{13}C NMR (125 MHz, $CDCl_3$) 178.8 (C8), 176.0 (C5), 163.9 (C2), 145.4 (C8a), 144.0 (C7), 134.9 (C4), 128.5 (C3), 127.4 (C4a), 111.6 (C6), 45.9 (C2′), 32.0 (C3′), 25.0 (Me); HRMS(ESI) *m/z* calcd for $[C_{12}H_{10}N_2O_2ClBr+Na]^+$: 350.9512, obsd.: 350.9516.

5.1.2.8. 6-Chloro-7-methylamino-5,8-quinolinequinone (9a)[46]. Methylamine (40% aq, 0.072 mL, 2.33 mmol, 1.2 equiv) and pyridine (0.33 mL, 4.0 mmol, 2.1 equiv) were added to a solution of dichloro-quinolinequinone **7a** (0.44 g, 1.9 mmol) in 1,4-dioxane (10 mL). The reaction mixture was left to stir for 4 h, until TLC-analysis showed the complete consumption of starting material and the presence of two products. The solvent was removed under reduced pressure and the residue taken up in CH_2Cl_2, washed with satd aq $NaHCO_3$ and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Purification of the crude product by column chromatography (CH_2Cl_2/EtOAc, 1/0→4/1, v/v) gave the title compound **9a** (0.30 g, 1.27 mmol, 67%) as a mauve solid, together with small amounts of its structural isomer **8a** (0.090 g, 0.38 mmol, 20%) as a red solid. Both were crystallised from a minimum amount of boiling solvent (CH_2Cl_2/hexanes, 1/4, v/v). Data for 6-substituted isomer **9a**: R_f = 0.32 (EtOAc); Mp 220.3–221.9 °C; IR (thin film): 3306, 1690, 1594, 1562, 1508, 1416, 1327, 1305, 1206, 1097, 907, 724 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 201 (3.78), 236 (3.84), 273 (3.78) 480 (3.17) nm; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.92 (dd, 1H, $J_{2,3}$ = 4.6, $J_{2,4}$ = 1.7 Hz, H-2), 8.47 (dd, 1H, $J_{3,4}$ = 8.0, $J_{2,4}$ = 0.8 Hz, H-4), 7.66 (dd, 1H, $J_{3,4}$ = 8.0, $J_{2,3}$ = 4.6 Hz, H-3), 6.27 (s, 1H, N–H), 3.40 (d, 3H, $J_{NH,N\text{-}Me}$ = 5.8, N–CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.9 (C-5), 175.7 (C-8), 153.4 (C-2), 145.9 (C-8a), 145.3 (C-7), 134.7 (C-4), 130.0 (C-4a), 128.5 (C-3), 107.6 (C-6), 32.7 (CH_3); HRMS(ESI) *m/z* calcd for $[C_{10}H_7ClN_2O_2+Na]^+$: 245.0094, obsd.: 245.0094.

5.1.2.9. 6-Chloro-7-(2-chloro-ethylamino)-5,8-quinolinequinone (9c). 2-Chloro-ethylamine hydrochloride (0.82 g, 7.1 mmol, 1.5 equiv) and 2 m NaOH (4.3 mL, 8.5 mmol, 1.8 equiv) were added to a solution of the 6,7-dichloro-5,8-quinolinequinone (**7a**) (1.06 g, 4.7 mmol, 1 equiv) in EtOH (45 mL) and H_2O (22.5 mL) and the solution refluxed for 15 min. The solvents were then removed under reduced pressure and the residue dissolved in CH_2Cl_2, washed with sat aq $NaHCO_3$ and brine, dried ($MgSO_4$), filtered and concentrated. Purification of the crude product by column chromatography (CH_2Cl_2/EtOAc, 3/1→0/1, v/v) yielded the 7-substituted isomer **9c** as orange crystals (0.68 g, 2.2 mmol, 48%) and the 6-substituted isomer **8c** as dark red crystals (0.65 g, 2.1 mmol, 46%). Data for 7-substituted isomer **9c**: R_f = 0.36 (EtOAc); Mp 148.6–150.0 °C; IR (film): 1687, 1649, 1605, 1566, 1518, 1438, 1302, 1251, 1205, 1145, 1097, 859, 813, 723, 547 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 462 (3.32), 273 (3.97), 235 (4.00) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.93 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.7 Hz, 1H, H-2), 8.46 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-4), 7.67 (dd, $J_{2,3}$ = 4.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-3), 6.44 (br s, 1H, N–H), 4.23 (m, 2H, H-2′), 3.79 (t, $J_{H\text{-}2',H\text{-}3'}$ = 5.8 Hz, 2H, H-3′); ^{13}C NMR (125 MHz, $CDCl_3$) 178.6 (C8), 175.7 (C5), 153.7 (C2), 146.0 (C8a), 144.3 (C7), 134.7 (C4), 129.6 (C4a), 128.5 (C3), 111.8 (C6), 46.1 (C2′), 44.0 (C3′); HRMS(ESI) *m/z* calcd for $[C_{11}H_8N_2O_2Cl_2+Na]^+$: 292.9861, obsd.: 292.9866.

5.1.2.10. 6-Methylamino-7-methylsulfanyl-quinolinequinone (10a). To a solution of the 7-chloro-6-methylamino-quinoline (**8a**) (0.353 g, 1.59 mmol, 1 equiv) in EtOH (15 mL) was added sodium thiomethoxide (0.177 g, 2.54 mmol, 1.6 equiv) and the reaction stirred for 90 min at rt. The solvent was then removed under reduced pressure and the mixture dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$ followed by brine, then dried over $MgSO_4$ before being filtered and concentrated in vacuo. Purification by flash chromatography (CH_2Cl_2/ EtOAc, 10/1→0/1, v/v) gave the desired quinone **10a** (0.33 g, 2.24 mmol, 88%) as a red-brown solid, which yielded reddish-purple crystals after recrystallisation from toluene. R_f = 0.14 (EtOAc); IR (film): 3688, 3167, 2921, 2852, 1684, 1563, 1512, 1413, 1299, 1211, 1116 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.00 (dd, $J_{2,4}$ = 1.8 Hz, $J_{2,3}$ = 4.6 Hz, 1H, H-2), 8.33 (dd, $J_{2,4}$ = 1.8 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-4), 7.56 (dd, $J_{2,3}$ = 4.6 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-3), 6.52 (br s, 1H, N–H), 3.49 (d, $J_{9,N\text{–}H}$ = 5.9 Hz, 3H, H-9), 2.37 (s, 3H, H-10); ^{13}C NMR (125 MHz, $CDCl_3$) 181.5 (C5), 178.5 (C8), 155.4 (C2), 150.2 (C6), 149.6 (C8a), 134.7 (C4), 127.6 (C4a), 126.6 (C3), 112.4 (C7), 34.0 (NHMe), 19.0 (SMe); HRMS(ESI) *m/z* calcd for $[C_{11}H_{10}N_2O_2S+Na]^+$: 257.0361, obsd.: 257.0364.

5.1.2.11. 6-Methylamino-7-phenylsulfanyl-5,8-quinolinequinone (10b). Methylamino-quinolinequinone (**8a**) (0.030 g, 0.13 mmol) was dissolved in pyridine (12 mL) and thiophenol (0.016 mL, 0.16 mmol, 1.2 equiv) was added. After the reaction mixture was stirred for 16 h, the pyridine was removed under reduced pressure and the residue dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$ and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by column chromatography (hexanes/CH_2Cl_2, 10/1→0/1, v/v followed by CH_2Cl_2/EtOAc, 10/1→0/1) yielded a purple-red solid which was crystallised in the minimum volume of boiling solvent (CH_2Cl_2/hexanes, 1/3, v/v) to give **10b** as purple-red crystals (0.037 g, 0.12 mmol, 95%). R_f = 0.19 (EtOAc); Mp 176.4–177.5 °C; IR (thin film): 3263, 1740, 1682, 1558, 1306, 1273, 1212, 733, 688 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 202 (4.40), 237 (4.18), 253 (4.19) 454 (3.16) nm; ^1H NMR (500 MHz, $CDCl_3$): δ 9.05 (dd, 1H, $J_{2,3}$ = 4.6, $J_{2,4}$ = 1.7 Hz, H-2), 8.40 (dd, 1H, $J_{3,4}$ = 7.8, $J_{2,4}$ = 1.7 Hz, H-4), 7.60 (dd, 1H, $J_{3,4}$ = 7.8, $J_{2,3}$ = 4.6 Hz, H-3) 7.23 (m, 4H, CH-*o* and CH-*m* arom.), 7.11 (m, 1H, CH-*p* arom.), 6.69 (s, 1H, NH), 3.44 (d, 3H, $J_{NH,CH3}$ = 4.4 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 181.4 (C-8), 178.4 (C-5), 155.5 (C-2), 150.5 (C-6), 149.1 (C-8a), 134.7 (C-4), 129.1 (CH-*o* or CH-*m* arom.), 127.0 (CH-*o* or CH-*m* arom.), 126.4 (C-3), 125.7 (CH-*p* arom.), 33.4 (CH_3); HRMS(ESI) *m/z* calcd for $[C_{16}H_{12}N_2O_2S_2+Na]^+$: 319.0517, obsd.: 319.0517.

5.1.2.12. 7-Methanesulfinyl-6-methylamino-5,8-quinolinequinone (13a). To a solution of 6-methylamino-7-methylsulfanyl-5,8-quinolinedione (**10a**) (12 mg, 0.054 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added *m*-CPBA (13 mg, 0.052 mmol, 1.0 equiv). After stirring at rt for 30 min, the mixture was concentrated in vacuo and purified by flash chromatography (CH_2Cl_2/EtOH/MeOH/30% aq NH_3, 300/2/2/1→35/2/2/1, v/v) to give the desired sulfoxide **13a** as orange crystals (9 mg, 0.040 mmol, 74%). ^1H NMR (500 MHz, $CDCl_3$) δ 10.16 (br s, 1H, N–H), 9.01 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.8 Hz, 1H, H-2), 8.35 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-4), 7.61 (dd, $J_{2,3}$ = 4.8 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-3), 3.39 (d, $J_{Me,N-H}$ = 5.9 Hz, 3H, NMe), 3.02 (s, 3H, SMe); ^{13}C NMR (125 MHz, $CDCl_3$) 181.0 (C8), 176.4 (C5), 155.6 (C2), 152.2 (C7), 148.0 (C8a), 135.2 (C4), 129.4 (C4a), 126.2 (C3), 109.5 (C6), 39.8 (SOMe), 33.6 (NMe); HRMS(ESI) *m/z* calcd for $[C_{11}H_{10}N_2O_3S+H]^+$: 251.0485, obsd.: 251.0488.

5.1.2.13. 7-Benzenesulfinyl-6-methylamino-5,8-quinolinequinone (13b). To a solution of 6-methylamino-7-phenylsulfanyl-5,8-quinolinequinone (**10b**) (0.45 g, 1.66 mmol) in AcOH (10 mL) was added $KMnO_4$ (0.29 g, 1.83 mmol, 1.1 equiv) and the reaction stirred at rt for 1.5 h. The reaction was then neutralised with satd aq $NaHCO_3$, washed with brine, dried with $MgSO_4$ and filtered before being concentrated in vacuo. Purification by column chromatography (CH_2Cl_2/EtOAc, 1/0→0/1, v/v) lead to the isolation of sulfoxide **13b** as a bright orange film (0.16 g, 30%). R_f = 0.25 (EtOAc); IR (thin film): 2923, 1688, 1600, 1567, 1443, 1416, 1393, 1331, 1306, 1274, 1106, 1075, 994, 748, 686 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.96 (s, 1H, NH), 9.01 (s, 1H, H-2), 8.30 (s, 1H, H-3), 7.86 (s, 1H, CH-*p*), 7.55 (m, 5H, H3, CH-*o* and CH-*m*), 3.38, (d, $J_{NH,CH3}$ = 5.0 Hz, CH_3); HRMS(ESI) *m/z* calcd for $[C_{16}H_{12}N_2O_3S+Na]^+$: 335.0466, obsd.: 335.0463.

5.1.2.14. 6-Amino-7-chloro-5,8-quinolinequinone (11)[32]. To a solution of 6-(benzhydryl-amino)-7-chloro-5,8-quinolinequinone (**8b**) (15 mg, 0.040 mmol) in MeOH (1 mL) was added 30% aq NH_3 (0.2 mL). The resulting solution was stirred for 1 h, concentrated and purified via silica gel column chromatography (CH_2Cl_2→1% MeOH in CH_2Cl_2) to give amine **11** as a bright orange solid (7 mg, 0.033 mmol, 84%). R_f = 0.33 (EtOAc); IR (thin film): 3522, 3005, 1707, 1584, 1539, 1421, 1360, 1222, 1093, 1050, 905, 713 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 8.94 (dd, 1H, $J_{2,3}$ = 4.7, $J_{2,4}$ = 1.5 Hz, H-2), 8.32 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,4}$ = 1.5 Hz, H-4), 7.73 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,3}$ = 4.7 Hz, H-3) 7.65 (m, 2H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 179.6 (C-5), 174.3 (C-8), 154.8 (C-2), 148.7 (C-8a), 147.1 (C-6), 134.6 (C-4), 127.6 (C-4a), 127.3 (C-3), 110.8 (C-7); HRMS(ESI) *m/z* calcd for $[C_9H_5N2O_2Cl+Na]^+$: 230.9932, obsd.: 230.9937.

5.1.2.15. 6-Amino-7-methylsulfonyl-5,8-quinolinequinone (12). To a solution of 6-methylamino-7-methylsulfanyl-5,8-quinolinequinone (**10a**) (18 mg, 0.077 mmol) in CH_2Cl_2 (1 mL) was slowly added a solution of *m*-CPBA (40 mg, 0.16 mmol, 2.1 equiv) in CH_2Cl_2 (1 mL). After 30 min, the reaction mixture was quenched with satd aq $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in MeOH (1 mL), and 30% aq NH_3 (0.2 mL) was added. The resulting solution was stirred for 1 h, concentrated and purified via silica gel column chromatography (CH_2Cl_2→1% MeOH in CH_2Cl_2) to give amine **12** as an orange solid (0.018 g, 0.071 mmol, 93%). R_f = 0.28 (CH_2Cl_2/MeOH, 9/1, v/v), IR (film): 3301, 1697, 1602, 1575, 1419, 1330, 1288, 1219, 1170, 1064, 1008, 843 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 9.03 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.6 Hz, 1H, H-2), 8.54 (br s, 2H, NH_2), 8.39 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-4), 7.79 (dd, $J_{2,3}$ = 4.6 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-3), 3.31 (s, 3H, SO_2Me); ^{13}C NMR (125 MHz, DMSO-d_6) 180.1 (C5), 176.7 (C8), 155.8 (C2), 150.2 (C6), 148.2 (C8a), 134.9 (C4), 127.9 (C4a), 127.7 (C3), 109.8 (C7), 45.0 (SO_2Me); HRMS(ESI) *m/z* calcd for $[C_{10}H_9N_2O_4S]^+$: 253.0278, obsd.: 253.0283.

5.1.2.16. 6-Amino-7-methanesulfinyl-5,8-quinolinequinone (14). To a solution of 7-methanesulfinyl-6-methylamino-5,8-quinolinequinone (**13a**) (12 mg, 0.048 mmol) in MeOH (1 mL) was added 30% aq NH_3 (0.2 mL). The resulting solution was stirred for 1 h, concentrated and purified via silica gel column chromatography (CH_2Cl_2→1% MeOH in CH_2Cl_2) to give amine **14** as orange crystals (10 mg, 0.042 mmol, 88%). R_f = 0.44 (CH_2Cl_2/EtOH 13/1, v/v); Mp 239.1 °C (decomp.); IR (film): 3301, 1697, 1602, 1575, 1419, 1330, 1288, 1219, 1170, 1064, 1008, 843 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 441 (3.15), 267 (3.76), 231 (3.95) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 9.13 (br s, 1H, N–H), 9.05 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.7 Hz, 1H, H-2), 8.41 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-4), 7.64 (dd, $J_{2,3}$ = 4.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-3), 6.17 (br s, 1H, N–H), 3.03 (s, 3H, SMe); HRMS(ESI) *m/z* calcd for $[C_{10}H_8N_2O_3S+Na]^+$: 259.0153, obsd.: 259.0146.

5.1.2.17. 6-Amino-7-methylsulfanyl-5,8-quinolinequinone (15). To a solution of 6-methylamino-7-methylsulfanyl-5,8-quinolinequinone (**10a**) (14 mg, 0.060 mmol) in MeOH (1 mL) was added 30% aq NH_3 (0.2 mL). The resulting solution was stirred for 1 h, concentrated and purified via silica gel column chromatography (CH_2Cl_2→1% MeOH in CH_2Cl_2) to give amine **15** as brown crystals (10 mg, 0.045 mmol, 76%). R_f = 0.32 (CH_2Cl_2/MeOH, 8/1, v/v); Mp 222.6–223.6 °C (decomp.); Lit.[16] Mp 222 °C); IR (film): 3235, 1686, 1591, 1572, 1531, 1396, 1279, 1263, 1208, 1003, 750, 685 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 486 (3.31), 257 (3.89), 230 (3.99) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 9.01 (dd, $J_{2,4}$ = 1.6 Hz, $J_{2,3}$ = 4.6 Hz, 1H, H-2), 8.36 (dd, $J_{2,4}$ = 1.6 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-4), 7.58 (dd, $J_{2,3}$ = 4.6 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-3), 5.99 (br s, 2H, N–H), 2.44 (s, 3H, H-9); ^{13}C NMR (125 MHz, DMSO-d_6) 180.4 (C5), 177.6 (C8), 154.8 (C2), 151.4 (C6), 149.4 (C8a), 134.4 (C4), 127.8 (C4a), 127.1 (C3), 110.0 (C7), 17.0 (C9); HRMS(ESI) *m/z* calcd for $[C_{10}H_8N_2O_2S+H]^+$: 221.0385, obsd.: 221.0390.

5.1.2.18. 6-Methylsulfanyl-7-methylamino-5,8-quinolinequinone (16a). To a solution of the 7-chloro-6-methylamino-quinoline (**9a**) (1.1 g, 4.8 mmol, 1 equiv) in EtOH (50 mL) was added sodium thiomethoxide (0.53 g, 7.6 mmol, 1.6 equiv) and the reaction stir-

red for 90 min at rt. The solvent was then removed under reduced pressure and the mixture dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$ followed by brine, then dried over $MgSO_4$ before being filtered and concentrated in vacuo. Purification by flash chromatography (CH_2Cl_2/ EtOAc, 10/1→0/1, v/v) gave the desired sulfide **16a** (1.05 g, 4.5 mmol, 94%) as a mauve solid, which yielded reddish-purple crystals after recrystallisation from toluene. R_f = 0.17 (EtOAc); Mp 170.1–173.8 °C; IR (film): 3674, 3302, 3087, 2923, 2851, 1688, 1591, 1552, 1509, 1413, 1303, 1257, 1096 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 488 (3.64), 260 (4.31), 232 (4.34), 197 (4.25) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 8.90 (dd, $J_{2,4}$ = 1.1 Hz, $J_{2,3}$ = 3.4 Hz, 1H, H-2), 8.46 (dd, $J_{2,4}$ = 1.1 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-4), 7.64 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-3), 6.68 (br s, 1H, N–H), 3.51 (d, $J_{10,N-H}$ = 5.8 Hz, 3H, H-10), 2.34 (s, 3H, H-9); ^{13}C NMR (125 MHz, $CDCl_3$) 180.3 (C8), 179.2 (C5), 153.5 (C2), 151.2 (C7), 146.8 (C8a), 135.0 (C4), 131.3 (C4a), 128.6 (C3), 110.1 (C6), 34.0 (NHMe), 19.2 (SMe); HRMS(ESI) *m/z* calcd for $[C_{11}H_{10}N_2O_2S+Na]^+$: 257.0361, obsd.: 257.0368.

5.1.2.19. 7-Methylamino-6-phenylsulfanyl-5,8-quinolinequinone (16b). 7-Chloro,6-methylamino-5,8-quinolinequinone (**9a**) (0.17 g, 0.77 mmol) was dissolved in pyridine (25 mL) and thiophenol (0.094 mL, 0.91 mmol, 1.2 equiv) was added. The reaction mixture was refluxed for 24 h by which ^1H NMR analysis showed complete consumption of the starting material. The pyridine was removed under reduced pressure and the residue was dissolved in CH_2Cl_2, washed with satd aq $NaHCO_3$ and brine, dried ($MgSO_4$) and filtered before being concentrated in vacuo. Purification by column chromatography (hexanes/CH_2Cl_2, 10/1→0/1, v/v followed by CH_2Cl_2/EtOAc, 10/1→1/3) yielded a purple-red solid which was crystallised in a minimum amount of boiling solvent (CH_2Cl_2/hexanes, 1/3, v/v) to give thioether **16b** as purple-red crystals (0.203 g, 0.69 mmol, 90%). R_f = 0.52 (EtOAc); Mp 198.0–199.8 °C; IR (thin film): 3290, 1700, 1591, 1555, 1518, 1413, 1307, 1268, 1202, 1140, 1098, 731 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 201 (3.01), 248 (2.82) nm; ^1H NMR (500 MHz, $CDCl_3$): δ 8.95 (dd, 1H, $J_{2,3}$ = 4.4, $J_{2,4}$ = 1.6 Hz, H-2), 8.50 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,4}$ = 1.6 Hz, H-4), 7.67 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,3}$ = 4.4 Hz, H-3), 7.23 (m, 4H, CH-*o* and CH-*m* arom.), 7.13 (m, 1H, CH-*p* arom.), 6.88 (s, 1H, NH), 3.48 (d, 3H, $J_{NH,CH3}$ = 5.9 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 180.0 (C-8), 179.1 (C-5), 153.4 (C-2), 151.4 (C-7), 146.3 (C-8a), 138.2 (C_i arom), 135.2 (C-4), 131.0 (C-4a) 129.2 (CH-*o* or CH-*m* arom.), 128.6 (C-3), 126.4 (CH-*o* or CH-*m* arom.), 125.6 (CH-*p* arom.), 104.0 (C-6), 33.4 (CH_3); HRMS(ESI) *m/z* calcd for $[C_{16}H_{12}N_2O_2S_2+Na]^+$: 319.0517, obsd.: 319.0517.

5.1.2.20. 6-Methanesulfinyl-7-methylamino-5,8-quinolinequinone (17a). To a solution of 7-methylamino-6-methylsulfane-5,8-quinolinedione (**16a**) (0.025 g, 0.107 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added *m*-CPBA (0.027 g, 0.107 mmol, 1.0 equiv). After stirring at rt for 30 min, the mixture was concentrated in vacuo and purified by flash chromatography (CH_2Cl_2/EtOH/MeOH/30% aq NH_3, 300/2/2/1→35/2/2/1, v/v) to give the desired sulfoxide **17a** as orange crystals (0.021 g, 0.084 mmol, 79%). R_f = 0.48 (CH_2Cl_2/EtOH 13/1, v/v); Mp 151.4–152.7 °C; IR (film): 3076, 1703, 1596, 1560, 1470, 1415, 1393, 1341, 1306, 1283, 1142, 1096, 1081, 1003, 962, 726 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 430 (3.06), 289 (3.58), 258 (3.69), 232 (3.79), 210 (3.61) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 10.13 (br s, 1H, N–H), 8.93 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.7 Hz, 1H, H-2), 8.35 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-4), 7.66 (dd, $J_{2,3}$ = 4.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-3), 3.42 (d, $J_{Me,N-H}$ = 5.6 Hz, 3H, NMe), 2.99 (s, 3H, SMe); ^{13}C NMR (125 MHz, $CDCl_3$) 180.6 (C8), 176.8 (C5), 153.4 (C2), 152.7 (C7), 147.0 (C8a), 133.7 (C4), 129.3 (C4a), 128.4 (C3), 109.5 (C6), 39.8 (SMe), 33.6 (NMe); HRMS(ESI) *m/z* calcd for $[C_{11}H_{10}N_2O_3S+Na]^+$: 273.0310, obsd.: 273.0303.

5.1.2.21. 6,7-Bis-methylsulfanyl-5,8-quinolinequinone (18a). 6,7-Dichloro-5,8-quinolinequinone (**7a**) (0.326 g, 1.42 mmol) was dissolved in MeCN (5 mL) at 40 °C, and a saturated solution of sodium thiomethoxide (0.209 g, 2.98 mmol, 2.1 equiv) in water was added drop-wise. The solution was then refluxed overnight, cooled to rt, then extracted twice with CH_2Cl_2, washed with satd aq $NaHCO_3$ then brine and dried over $MgSO_4$ before and being filtered and concentrated in vacuo. Purification by column chromatography (CH_2Cl_2/EtOAc, 1/0→0/1, v/v) yielded a brown-red solid that was recrystallised (CH_2Cl_2/hexanes, 1/3, v/v) to give disulfide **18a** as dark red crystals (0.32 g, 1.28 mmol, 90%). R_f = 0.40 (EtOAc); Mp (decomp.) 93.0–95 °C; IR (thin film): 2929, 1659, 1582, 1476, 1429, 1303, 1284, 1203, 1140, 809, 692 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 202 (4.53), 231 (4.53), 285 (4.32) nm; ^1H NMR (500 MHz, $CDCl_3$): δ 8.97 (dd, 1H, $J_{2,3}$ = 4.6, $J_{2,4}$ = 1.5 Hz, H-2), 8.36 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,4}$ = 1.5 Hz, H-4), 7.63 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,3}$ = 4.6 Hz, H-3) 2.78 (s, 1H, S-CH_3) 2.73 (s, 1H, S-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.0 (C-5), 177.2 (C-8), 154.2 (C-2), 148.8 (C-6 or C-7), 148.5 (C-4a), 145.8 (C-6 or C-7), 134.7 (C-4), 129.8 (C-8a), 127.4 (C-3), 18.5 (CH_3), 18.4 (CH_3); HRMS(ESI) *m/z* calcd for $[C_{11}H_9NO_2S_2+Na]^+$: 273.9972, obsd.: 273.9972.

5.1.2.22. 6,7-Bis-phenylsulfanyl-5,8-quinolinequinone (18b). To a solution of 6,7-dichloro-5,8-quinolinequinone (**7a**) (0.307 g, 1.35 mmol) in THF (5 mL) were added thiophenol (0.291 mL, 2.84 mmol, 2.1 equiv) and pyridine (0.326 mL, 4.05 mmol, 3 equiv) and the reaction mixture stirred for 30 min at rt. The reaction mixture was then extracted with CH_2Cl_2, washed with satd aq $NaHCO_3$ then brine, dried over $MgSO_4$ and filtered before being concentrated in vacuo. Purification by column chromatography (CH_2Cl_2/EtOAc, 3/1→1/1, v/v) yielded a red solid that was recrystallised (CH_2Cl_2/hexanes) to give disulfide **18b** as dark red crystals (0.465 g, 1.24 mmol, 92%). R_f = 0.62 (EtOAc); Mp 180.7–182.0 °C; Lit. 177–178 °C;[24] IR (thin film): 3075, 2241, 1668, 1581, 1494, 1476, 1440, 1268, 1199, 1138, 744, 726, 689 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 462 (2.67), 240 (3.86), 201 (4.29) nm; ^1H NMR (500 MHz, $CDCl_3$): δ 8.91 (dd, 1H, $J_{2,3}$ = 4.7, $J_{2,4}$ = 1.5 Hz, H-2), 8.25 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,4}$ = 1.5 Hz, H-4), 7.57 (dd, 1H, $J_{3,4}$ = 7.9, $J_{2,3}$ = 4.7 Hz, H-3), 7.31 (m, 10H, CH arom.); ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.1 (C-5 or C-8), 177.2 (C-5 or C-8), 154.6 (C-2), 149.7 (C-6 or C-7), 148.3 (C-8a), 146.6 (C-6 or C-7), 135.1 (C-4), 133.2 (CH arom.), 132.7 (CH arom), 132.0 (CH arom.), 131.4 (CH arom.) 129.7 (C-4a), 129.2 (CH arom.), 128.3 (CH arom.), 128.1 (CH arom.) 127.6 (C-3.); HRMS(ESI) *m/z* calcd for $[C_{21}H_{13}NO_2S_2+Na]^+$: 398.0285, obsd.: 398.0289.

5.1.2.23. 6,7-Bis-methylsulfanyl-quinoline-5,8-diol (19). To a solution of 6,7-dichloro-5,8-quinolinequinone (**7a**) (0.272 g, 1.19 mmol) in THF (5 mL) was added thiophenol (0.550 mL, 5.00 mmol, 4.2 equiv) and pyridine (0.331 mL, 4.19 mmol, 3 equiv) and the solution stirred for 3 h at rt. The reaction mixture was then extracted with CH_2Cl_2, washed with satd aq $NaHCO_3$ then brine, dried over $MgSO_4$ and filtered before being concentrated in vacuo. Purification by column chromatography (CH_2Cl_2/EtOAc, 1/0→5/1, v/v) yielded a pale yellow solid that was recrystallised (CH_2Cl_2) to give quinolinediol **19** as long yellow needles (0.188 g, 0.50 mmol, 42%). R_f = 0.88 (EtOAc); Mp 160.4–161.6 °C, Lit.[24] 160.5–161 °C; IR (thin film): 1578, 1476, 1398, 1372, 1228, 1146, 1082, 740, 688 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 205 (4.56), 260 (4.31), 353 (3.56) nm; ^1H NMR (500 MHz, $CDCl_3$): δ 8.92 (dd, 1H, $J_{2,3}$ = 4.4, $J_{2,4}$ = 1.7 Hz, H-2), 8.63 (dd, 1H, $J_{3,4}$ = 8.5, $J_{2,4}$ = 1.7 Hz, H-4), 7.57 (dd, 1H, $J_{3,4}$ = 8.5, $J_{2,3}$ = 4.4 Hz, H-3) 7.15 (m, 10H, CH arom.); ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.9 (C-2), 149.1 (C-8), 148.1 (C-5), 138.8 (C-8a), 136.5 (C_i), 135.1 (C_i), 133.3 (C-4), 129.2 (CH arom.), 128.8 (CH arom.), 127.7 (CH arom.), 127.2 (CH arom.), 126.4 (CH arom.), 125.8 (CH arom.), 122.4 (C-3), 119.9 (C-4a),

117.5, (C-6 or C-7) 115.3 (C-6 or C-7); HRMS(ESI) *m/z* calcd for $[C_{21}H_{15}NO_2S_2+H]^+$: 378.0623, obsd.: 378.0622.

5.1.2.24. 6,7-Bis-(toluene-4-sulfonyl)-5,8-quinolinequinone (20) and 7-Chloro-6-(toluene-4-sulfonyl)-5,8-quinolinequinone (21). A solution of *p*-toluenesulfinic acid, sodium salt (0.198 g, 1.11 mmol, 2.1 equiv) in H_2O (3 mL) was added to a warm solution of 6,7-dichloro-5,8-quinolinequinone (**7a**) (0.121 g, 0.53 mmol) in MeCN (10 mL). The reaction was refluxed for 5 h, cooled, and neutralised with NaOH. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$), filtered and concentrated. Purification of the residue by column chromatography (CH_2Cl_2/EtOAc/MeOH, 1/1/0→0/1/0.01, v/v) yielded sulfone **21** (0.070 g, 0.10 mmol, 18%) as a yellow solid, which was crystallised in the minimum volume of boiling solvent (H_2O, and EtOH) to give yellow powder-like crystals, and bis-sulfone **20** (0.229 g, 0.23 mmol, 44%) as an orange film. Data for **21**: R_f = 0.58 (CH_2Cl_2/MeOH, 9:1); Mp (decomp.) 240.5–243.5 °C; IR (thin film): 3374, 2361, 2136, 1698, 1641, 1597, 1556, 1527, 1368, 1277, 1238, 1084 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 200 (3.56), 226 (3.42) nm; ^1H NMR (500 MHz, D_2O): δ 8.79 (s, 1H, H-2), 8.28 (s, 1H, H-4), 7.84 (s, 2H, CH-*o* or CH-*m*), 7.62 (s, 1H, H-3) 7.36 (s, 2H, CH-*o* or CH-*m*), 2.35 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 183.3.1 (C-5), 177.5 (C-8), 171.2 (C-8a), 154.4 (C-2), 149.1 (C-8a), 144.5 (C-6 or C-7), 139.4 (C_i arom.), 135.3 (C-4), 129.4 (CH-*m* arom.), 127.5 (C-4a), 127.2 (C-3) 126.1 (CH-*o* arom.), 114.6 (C6-or C-7), 20.6 (CH_3); Data for **20**: R_f = 0.40 (EtOAc); IR (thin film): 1708, 1644, 1595, 1360, 1273, 1166, 1116, 1066, 1021, 813, 738, 704, 667 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.77 (s, 1H, H-2), 8.60 (s, 1H, H-4), 7.91 (s, 4H, CH-*o* or CH-*m*), 7.34 (s, 1H, H-3) 7.27 (s, 4H, CH-*o* or CH-*m*), 2.43 (s, 6H, CH_3); HRMS(ESI) *m/z* calcd for $[C_{23}H_{19}NO_7S_2+Na]^+$: 508.0495, obsd.: 508.0503.

5.1.2.25. 2,3-Dihydro-1*H*-4-thia-1,5-diaza-anthraquinone (22). A solution of sodium sulfide (0.28 g, 1.2 mmol, 1.5 equiv) in H_2O (25 mL) was added to quinolinequinone (**8c**) (0.24 g, 0.72 mmol) in EtOH (50 mL) and the solution was stirred at rt for 50 min and refluxed for an additional 10 min. The solvents were removed under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/EtOAc, 50/1→1/2, v/v) to yield the title compound **22** as dark purple crystals (0.11 g, 0.46 mmol, 59%). R_f = 0.07 (EtOAc), R_f = 0.27 (CH_2Cl_2/MeOH, 11:1); Mp 262.5–263.3 °C (Lit.[16] Mp 263 °C); IR (film): 1672, 1593, 1558, 1510, 1399, 1336, 1320, 1270, 1150, 1119, 1086, 910, 734, 685 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 335 (3.26), 268 (3.51), 230 (3.71), 209 (3.72) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 8.93 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.8 Hz, 1H, H-2), 8.30 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-4), 7.53 (dd, $J_{2,3}$ = 4.8 Hz, $J_{3,4}$ = 7.8 Hz, 1H, H-3), 6.01 (br s, 1H, N–H), 3.80 (m, 2H, H-2′), 3.05 (m, 2H, H-3′); HRMS(ESI) *m/z* calcd for $[C_{11}H_8N_2O_2S+Na]^+$: 255.0204, obsd.: 255.0206.

5.1.2.26. 3,4-Dihydro-2*H*-1-thia-4,5-diaza-anthraquinone (23). A solution of sodium sulfide (0.26 g, 1.1 mmol, 1.5 equiv) in H_2O (25 mL) was added to quinolinequinone (**9c**) (0.22 g, 0.72 mmol) in EtOH (50 mL) and the solution was stirred at rt for 50 min and refluxed for an additional 10 min. The solvents were removed under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/EtOAc, 50/1→1/2, v/v) to yield the title compound **23** as dark purple crystals (0.13 g, 0.54 mmol, 76%). R_f = 0.17 (EtOAc), R_f = 0.33 (CH_2Cl_2/MeOH, 11:1); Mp 264.2 °C (decomp); IR (film): 1666, 1629, 1590, 1556, 1497, 1337, 1314, 1200, 1153, 1113, 910, 720 cm^{-1}; UV–vis (MeOH) λ_{max} (log ε): 579 (2.27), 330 (3.43), 269.0 (3.66), 230.0 (3.73), 201 (3.67) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 8.87 (dd, $J_{2,4}$ = 1.7 Hz, $J_{2,3}$ = 4.7 Hz, 1H, H-2), 8.37 (dd, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-4), 7.58 (dd, $J_{2,3}$ = 4.7 Hz, $J_{3,4}$ = 7.9 Hz, 1H, H-3), 6.20 (br s, 1H, N–H), 3.82 (m, 2H, H-2′), 3.04 (t, $J_{H\text{-}2',H\text{-}3'}$ = 4.7 Hz, 2H, H-3'); ^{13}C NMR (125 MHz, $CDCl_3$) 178.2 (C5), 176.2 (C8), 153.0 (C2), 146.7 (C8a), 141.4 (C7), 133.9 (C4), 130.5 (C4a), 127.8 (C3), 111.6 (C6), 41.8 (C2′), 23.8 (C3′); HRMS(ESI) *m/z* calcd for $[C_{11}H_8N_2O_2S+H]^+$: 233.0385, obsd.: 233.0391.

5.2. Biological assays

5.2.1. Reagents and chemicals

Unless otherwise stated, cell culture reagents were obtained from Invitrogen (New Zealand) and all other reagents were purchased from Sigma–Aldrich (New Zealand). HL60 cells, originally from ATCC, were obtained from Dr. Graeme Findlay (University of Auckland, NZ). RPMI-1640 media was obtained from GIBCO-BRL, Grand Island, NY, USA. The Vacutainer cell preparation tubes were obtained from BD, Franklin Lakes, NJ, USA. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Sigma–Aldrich and 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt (WST-1) from Dojindo (Kumamoto, Japan). Polymorphprep density gradient (Axis-Shield, Norway) was obtained through Media Pacifica Ltd (New Zealand). MACS T cell activation/expansion kit was obtained from Miltenyi Biotec, Gladbach, Germany. *M. bovis* BCG Pasteur strain 1173P was gifted by AgResearch Wallaceville Animal Research Centre, Upper Hutt, New Zealand. Dubos broth base and OADC (Oleic acid-albumin-dextrose-catalase) were supplied by Fort Richard, Auckland, New Zealand. Alamar Blue solution (BUF012B) was obtained from AbD serotec, UK.

5.2.2. HL60 cell culture

HL60 cells were maintained in RPMI-1640 supplemented with 5% (v/v) fetal calf serum (FCS), 2 mM glutamate, 100 U/mL penicillin, and 100 μg/mL streptomycin, and were kept at 37 °C in a humidified incubator maintained at 5% CO_2.

5.2.3. In vitro activation of T cells

Blood samples were obtained from healthy volunteers, and peripheral blood mononuclear cells (PBMC) were immediately isolated by the FICOLL™-hypaque™ method using BD Vacutainer cell preparation tubes containing sodium heparin. PBMC were washed in PBS and resuspended in RPMI-1640 medium with 10% FCS, 100 U/mL penicillin, and 100 μg/mL streptomycin. T cells were activated using a MACS T cell activation/expansion kit following the manufacturer's protocol. Briefly, PBMC (5×10^6) were incubated for two days at 37 °C with 2.5×10^6 anti-biotin MACS iBead particles that had been pre-loaded with CD2-biotin, CD3-biotin and CD28-biotin. Cells were then split into two equal parts with culture medium supplemented with 20 U/mL IL-2 and incubated for another two–four days. All assays were performed in triplicate and the mean result recorded.

5.2.4. Anti-proliferative MTT assay

Anti-proliferative activity was measured using the 2-day MTT assay.[36,37] Phenoxodiol was used as a positive control (IC_{50} = 3.2 μM).[47] HL60 cells and T cells were plated into 96-well plates in the presence of different concentrations of quinolinequinones (final cell concentration = 0.2×10^6 cells/mL in complete RPMI) and incubated for 48 h (37 °C, 5% CO_2). 10 μL of MTT (5 mg/mL in HBSS) was then added to each well and the cells incubated for 2 h before adding 100 μL of SDS lysing buffer (10% w/v SDS, 45% v/v dimethylformamide/H_2O, pH 4.7) and further overnight incubation. The absorbance at 570 nm was measured in a FLUOstar OPTIMA plate reader (BMG Labtechnologies Pty. Germany). All assays were performed in triplicate and the mean result recorded.

5.2.5. Superoxide assay

Measurement of superoxide production was performed using the tetrazolium salt, WST-1 as previously described and reviewed.[37,38] Blood was obtained from healthy volunteers by venous puncture and purified using Polymorphprep density gradient according to manufacturer's instructions. Purified neutrophils were cultured in 96-well plates in HBSS (1×10^5 per well, 100 μl) and the test compound or DMSO control added. After 30 min, 250 μg/ml WST-1 was added and neutrophils stimulated with 0.2 μg/ml phorbol 12-myristate 13-acetate (PMA). Immediately following PMA addition, the plate was loaded into a Versamax spectrophotometer and the absorbance measured at 450 nm over 20 min at 37 °C. The V_{max} was then calculated for each sample and normalised against the positive PMA-stimulated, DMSO cell control. All assays were performed in triplicate and the mean result recorded. Superoxide dismutase served as a positive control (0.8 ± 0.05 μM).

5.2.6. Alamar blue tuberculostatic assay

Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well optical bottom microplates using the Alamar Blue assay.[41] Briefly, stock suspensions of frozen *M. bovis* BCG were thawed, sonicated lightly to remove clumps, diluted in tween albumin broth [Dubos broth base/OADC] and 100 μL aliquots added to each well (final bacterial concentration 4×10^4 bacteria/well). Different concentrations of compound were added (PBS/2.5–5% DMSO). Wells containing compound only were prepared to test for autofluorescence. Control wells of bacteria only, medium only, or ethambutol (as a positive control) were used. The plates were then incubated at 37 °C for 7 days. 20 μL of Alamar Blue solution was then added and the bacteria incubated for 24 h. The fluorescence (excitation 544 nm, emission 590 nm) was then measured and a background autofluorescence (media only and compounds alone) subtracted from all wells. The MIC was calculated as the lowest drug concentration exhibiting 100% inhibition. All assays were performed in triplicate and the mean result recorded. Ethambutol served as a positive control (MIC = 3.1–6.3 μg/mL).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.03.021.

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