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Synthesis and anti-inflammatory structure–activity relationships of thiazine–quinoline–quinones: Inhibitors of the neutrophil respiratory burst in a model of acute gouty arthritis

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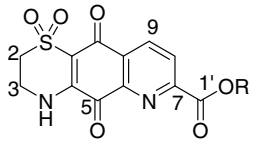
ABSTRACT

Sixteen new thiazine–quinoline–quinones have been synthesised, plus one bicyclic analogue. These compounds inhibited neutrophil superoxide production in vitro with IC_{50}s as low 60 nM. Compounds with high in vitro anti-inflammatory activity were also tested in a mouse model of acute inflammation. The most active compounds inhibited both neutrophil infiltration and superoxide production at doses 2.5 μmol/kg, highlighting their potential for development as novel NSAIDs.

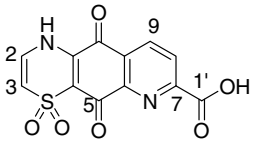
1. Introduction

Neutrophil infiltration and activation are linked with inflammation in a variety of diseases including gouty and rheumatoid arthritis, asthma, cardiovascular disease and intestinal disease.[1–5] One of the hallmark features of neutrophil activation is the 'respiratory burst' resulting in the production of destructive reactive oxygen species such as superoxide that, if left uncontrolled, can lead to tissue damage.[6–8] Therefore the ability to block overproduction or chronic production of superoxide has broad clinical potential for the improved management of inflammatory conditions.

In previous work involving screening for inhibitors of human neutrophil superoxide production, we isolated and characterized two thiazine-containing 2-quinolinequinone carboxylic acid derivatives ascidiathiazones A (**1**) and B (**23**) from a New Zealand ascidian *Aplidium* sp.[9] These natural products suppressed superoxide production with anti-inflammatory IC_{50} (AI_{50}) values of 1.55 for **1** and 0.44 μM for **23**. The anti-inflammatory effect of these compounds also translated into suppression of superoxide production by infiltrating neutrophils in vivo in a murine model of gouty inflammation highlighting the potential for further development of synthetic analogues of the natural products as novel anti-inflammatory agents. To this end, we now report a more comprehensive study into the structure–activity relationship of these thiazine–quinoline–quinones to determine the key structural features required for anti-inflammatory activity.



1 R = H
8 R = Me



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2. Chemistry

In general, the thiazine–quinoline–quinones were prepared by the addition reaction of hypotaurine to quinoline–quinones

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E-mail address: jharper@malaghan.org.nz (J.L. Harper).

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(Scheme 1). The required quinoline–quinones were prepared either from commercially available or synthesised 8-hydroxyquinolines by oxidation using potassium nitrosodisulfonate (Fremy's salt). The hypotaurine addition gave varying proportions of the two regioisomers of the dioxothiazine ring. Separation of the two regioisomers formed was accomplished by column chromatography. Proof of the regiochemistry of the various purified thiazine-quinoline-quinones was based on X-ray crystal structures, of the natural product ascidiathiazone A (**1**) previously reported[9] and of the synthetic methyl derivative **3** reported here (Fig. 1). The ^{13}C NMR chemical shift of the quinone carbonyl C-10 in **3**, assigned by an HMBC correlation with H-9, was 174.3 ppm, whereas the C-10 in regioisomer **22** resonated at 178.8 ppm (Table 1). This change in the ^{13}C NMR signals of C-10 in the different regioisomers was comparable to that previously reported for methyl ester **8** and its regiosomer[9] and was used as the basis for assignment of the regiochemistry of the other thiazines (Table 1).

Compounds that could not be formed by hypotaurine addition were prepared by modification of other thiazine–quinoline–quinones. Thus aldehyde **5** was prepared from a selenium dioxide oxidation of **3** and the unsaturated thiazine **25** was formed by a base catalysed oxidation, using aqueous potassium hydroxide, of **3** (previously reported during the preparation of **24** from **8**[9]). Amide **11** was prepared from acid **1** by a modified coupling reaction using *N*,*N*-dimethylethylenediamine and *N*,*N*-diisopropylethylamine (DIPEA) with benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP). The ester **12** was formed from the reaction of the alcohol **7** with DIPEA, dimethylaminopyridine (DMAP) and dimethylaminopropionyl chloride. The surprisingly rapid formation of stable hemiacetals when the aldehyde **5** was dissolved in alcohols was used to form the hemiacetal **13**. The opened thiazine ring analogue **15** was prepared by a sequential addition of sodium methanesulfinate followed by methylamine to the quinoline–quinone. Addition of methylamine first, or at the same time as the sodium methanesulfinate, resulted in a single addition of methylamine.

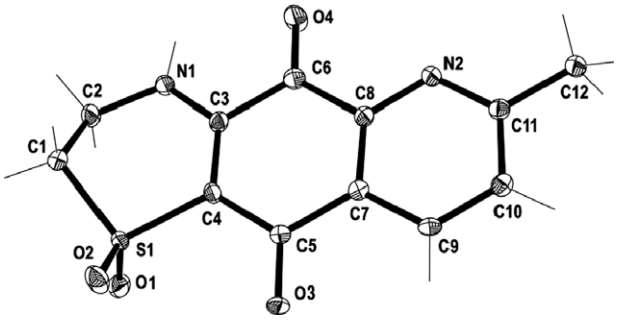


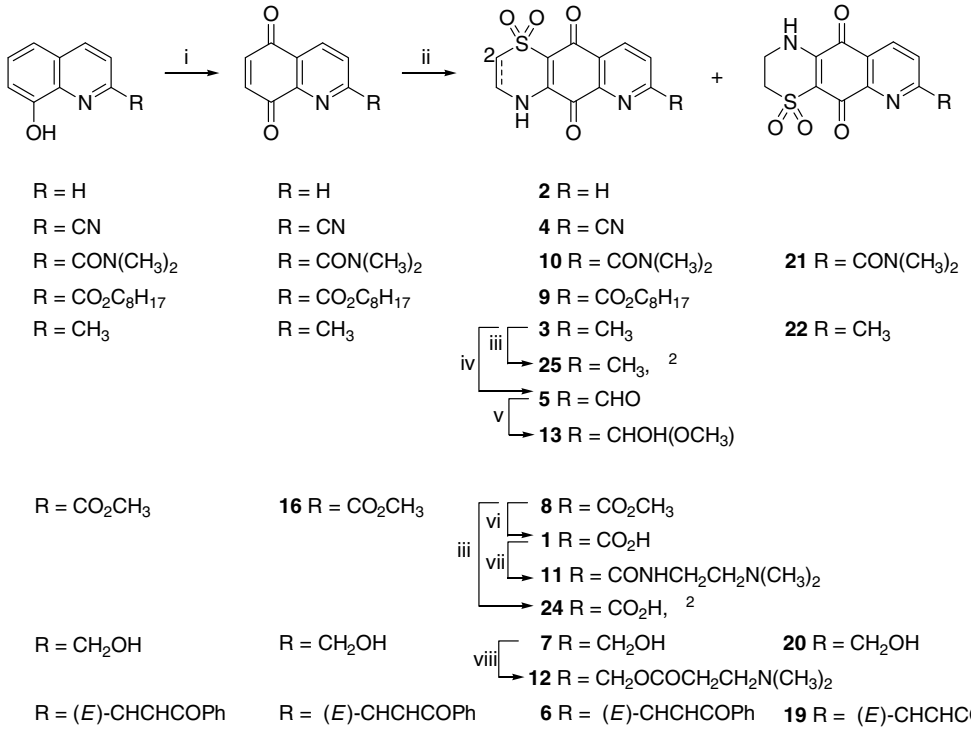
Figure 1. The crystal structure of compound **3** showing crystallographic numbering. Selected bond lengths (Å) and angles (°):C1–C2 1.519(3), C1–S1 1.7725(19), C2–N1 1.457(2), C3–N1 1.326(2), C3–C4 1.384(2), C3–C6 1.520(2), C4–C5 1.450(2), C4–S1 1.7646(17), C5–O3 1.230(2), C5–C7 1.500(2), C6–O4 1.208(2), C6–C8 1.479(2), C7–C8 1.385(2); C2 C1 S1 110.34(13), N1 C2 C1 109.81(15), N1 C3 C4 126.60(17), N1 C3 C6 113.27(15), C4 C3 C6 120.13(16), C3 C4 C5 122.35(16), C3 C4 S1 119.21(14), C5 C4 S1 118.44(13).

3. Biological assays

3.1. In vitro anti-inflammatory activity

The natural product ascidiathiazone A, **1**, was reported previously to act as an inhibitor of neutrophil superoxide production in vitro and in vivo.[9] To investigate the structural requirements for anti-inflammatory (AI) activity, analogues of the natural products were tested for their ability to suppress superoxide production by human neutrophils. Compounds were also tested for anti-proliferative (AP) activity to identify compounds exhibiting selectivity for high AI activity and low AP activity.

Initial structural modifications focussed on the carboxylic acid group at the 7-position, **2-7** (Table 2). The absence of the carboxylic acid functionality **2** resulted in a significant decrease in AI activity and an undesirable increase in AP activity and therefore



Scheme 1. Reagents and conditions: (i) Fremy's salt, aq acetone; (ii) hypotaurine, EtOH, CH_3CN, H_2O; (iii) 1 M KOH; (iv) SeO_2, dioxane; (v) MeOH; (vi) 3 M HCl; (vii) dimethylethylenediamine, DIPEA, PyBOP, DMF (viii) 3-dimethylaninopropionyl chloride, DMAP, DIPEA, DMF.

Table 1
^{13}C NMR data for thiazine-quinoline-quinones[a]

C	2	3	4[b]	5	6	7	9	10	11	12[c]	13	15[d]	19	22	25
2	48.3	48.3	50.3	48.3	48.3	48.3	48.1	48.3	49.3	48.4	48.9	45.6	40.0	39.7	111.8
3	39.9	39.6	41.5	39.5	39.5	39.1	39.0	39.6	40.6	39.8	39.6	34.4	48.4	48.4	131.5
4a	147.5	147.2	148.7	147.9	146.6	147.3	147.8	147.6	148.8	148.6	148.1	151.5	146.8	112.1	140.5
5	177.0	177.1	177.8	176.3	176.6	177.0	176.1	176.6	178.2	177.5	177.5	181.1	172.8	173.3	175.8
5a	146.4	145.7	148.5	146.9	147.6	145.5	146.4	145.4	146.2	145.8	146.0	145.9	148.0	146.5	145.8
7	153.3	162.7	137.8	154.0	156.2	166.6	150.1	157.3	153.4	160.5	164.1	164.3	158.1	165.4	163.5
8	134.0	128.8	134.8	125.9	128.6	125.3	129.1	127.6	128.2	128.5	126.0	129.1	129.1	126.7	128.5
9	129.1	134.2	137.9	136.0	135.2	134.7	135.8	135.3	137.5	136.8	135.6	134.7	135.6	134.6	134.2
9a	130.0	127.9	133.7	132.4	130.0	128.6	131.7	129.8	132.9	129.9	130.3	127.5	127.0	125.3	127.3
10	174.1	174.3	174.2	173.3	173.7	174.3	173.2	173.6	175.1	176.2	174.6	177.1	178.3	178.8	178.1
10a	110.6	110.3	114.0	111.0	110.8	110.4	110.7	110.6	112.3	109.4	111.1	109.1	112.5	147.3	114.8
1′		24.4	117.9	192.6	141.4	64.1	163.6	166.9	165.8	66.9	98.1	25.0	141.4	24.9	24.4
2′					128.0								127.5		
3′					189.5		65.8	38.2	35.8	172.0	54.6		189.5		
4′					137.1		31.2	34.9	58.2	29.5			137.1		
5′					128.4		28.6		43.8	53.4			128.7		
6′					129.1		28.6		43.8	43.5			129.1		
7′					133.7		28.0			43.5			133.8		
8′					129.1		25.3						129.1		
9′					128.4		22.0						128.7		
10′							13.9								

[a] In d_6-DMSO unless otherwise noted, chemical shifts in ppm, all assignments based on 2D NMR experiments.
[b] In d_6-acetone.
[c] In D_2O.
[d] In d_4-$CDCl_3$.

loss of selectivity. A methyl group at the 7-position (**3**) resulted in a further decrease in AI activity again with increased AP activity compared to **1**. The presence of electron-withdrawing groups at C-7 (**4**, **5**, **6**) resulted in AI activity comparable to that of **1**, but selectivity was lost. A methyl alcohol (**7**) at the 7-position also had diminished AI activity and selectivity. Methylation of the carboxylic acid group to give the methyl ester **8** (previously reported[9]) had little effect on AI activity whereas increasing the alkyl chain length to give the octyl ester **9** resulted in loss of AI activity and a significant increase in AP activity into the submicromolar range.

To determine whether increased aqueous solubility would enhance AI activity, a series of polar analogues **10-13** were prepared with dimethylaminoethylamide **11** exhibiting notable AI activity and greater selectivity compared to **1**. Reduction of the methyl ester to give **13** resulted in lowered AI potency. Together these results indicate that the functional group in the 7-position plays a key role in the efficacy and specificity of compounds with **11** exhibiting the lowest AI:AP ratio and therefore highest selectivity for AI activity.

Next we looked at the contribution of the different ring systems of **1** to AI activity (Table 2, compounds **14-18**). The dioxothiazinenaphthoquinone analogue **14** was essentially devoid of AI activity compared to **2** indicating that the presence of a ring nitrogen favours AI activity. Opening the thiazine ring (**15**) resulted in a loss of both AI and AP activity compared to **3**. Interestingly, without the thiazine ring, quinoline quinones **16** and **17** had similar AI activity compared to **8** and **5**, respectively but exhibited modest increases in AP activity. It appears that the presence of the thiazine ring lowers AP activity thereby improving the AI selectivity of the thiazine–quinoline–quinones. The structural fragment pyridine-2-carboxylic acid **18** was completely inactive identifying the quinoline-quinone as the minimum structure required for bioactivity.

To better understand the influence of the dioxothiazine ring on the pattern of bioactivity, compounds **19-22** were used to investigate the effect of ring regiochemistry on AI activity and selectivity. All four compounds exhibited less potent AI activity with a comparatively small loss of AP activity in our assays compared to their regioisomers, **6**, **7**, **10** and **3,** respectively (Table 2) indicating that [2,3-*g*] regiochemistry favours higher AI activity and low AP activity. Taken together, these data illustrate key contributions by the pyridine and dioxothiazine rings to the AI activity and selectivity of the ascidiathiazone structural class.

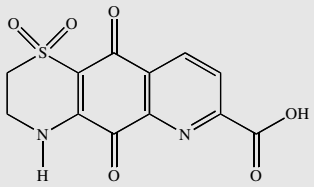
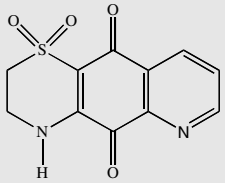
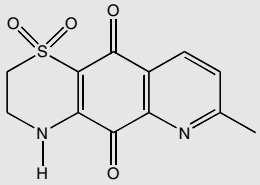
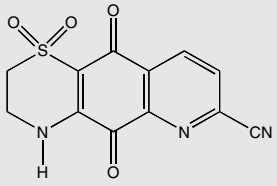
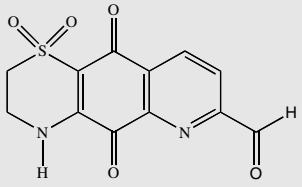
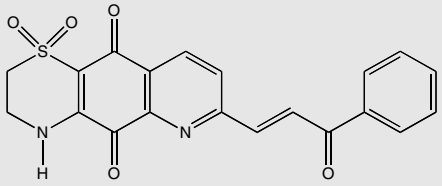
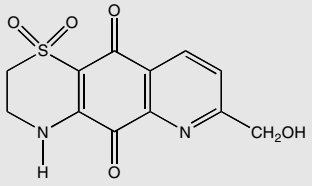
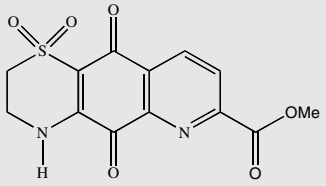
In addition to ascidiathiazone A **1**, our previous natural product isolation study also yielded the oxidized regioisomer ascidiathiazone B **23**.[9] This 4*H*-1,4-benzothiazine-1,1-dioxide exhibited submicromolar activity in the AI assay but with less selectivity than that observed for **1** (Table 2). Regioisomer **24** exhibited enhanced AI potency and selectivity compared to **23**. Compound **24** also exhibited enhanced AI activity and selectivity compared to **1**. These findings indicate that both the saturation state and the regiochemistry of the thiazine ring strongly influence AI activity and selectivity. Replacement of the carboxylic acid group **24** with a methyl **25** significantly lowered AI activity and increased anti-proliferative activity.

3.2. In vivo activity

Acute gouty arthritis is characterised by the recruitment and activation of neutrophils, and the production of superoxide, in response to the known inflammatory stimulus, MSU crystals. A peritoneal model of gouty arthritis was used to determine whether the in vitro AI activity of the compounds translated into an inhibitory effect in vivo. Compounds **1**, **8**, **11**, **23** and **24** were selected for in vivo testing on the basis of their high AI activity and low AP activity in vitro.

While modest suppression of MSU crystal-induced neutrophil superoxide production was observed for natural products **1** and **23** at the higher dose of 25 μmol/kg, the synthetic analogues **8** and **24** exhibited significantly greater in vivo activity at 2.5 μmol/kg (Table 3). Despite potent in vitro AI activity, **11** failed to exhibit any inhibitory effect in vivo. The lack of in vivo activity by **11** could result from a variety of different factors: differences in the uptake and bioavailability of the compounds, susceptibility to first pass clearance associated with delivery via the oral route and the half-life of the compounds in vivo. Interestingly, **8** and **24** also inhibited neutrophil infiltration indicating that these compounds have the potential to target neutrophilic inflammation by

Table 2
In vitro anti-inflammatory activity of ascidiathiazone analogues[a]

	Compound	AI_{50} (μM)[b]	AP_{50} (μM)[c]
1		1.55 ± 0.8	73 ± 16
2		29 ± 0.5	6.79 ± 0.6
3		115 ± 33	14 ± 8
4		1.86 ± 0.88	2.62 ± 2.00
5		5.34 ± 0.70	3.52 ± 3.0
6		8.01 ± 1.38	3.08 ± 1.22
7		35 ± 8	1.2 ± 0.2
8		2.26 ± 0.61	12 ± 3

(*continued on next page*)

Table 2 (*continued*)

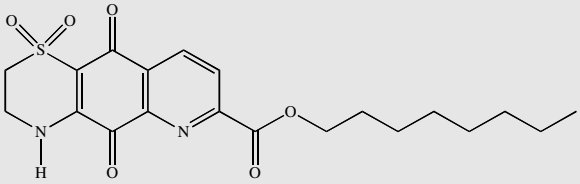
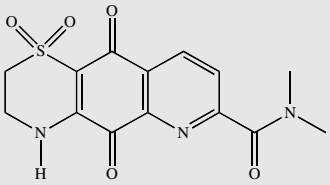
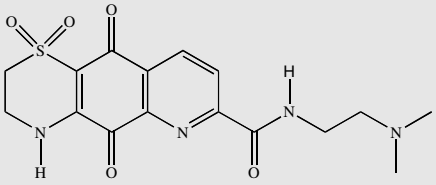
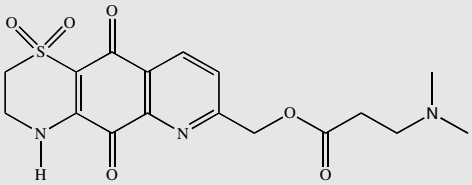
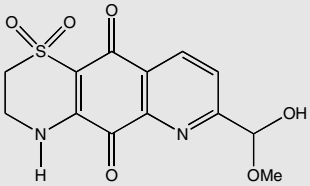
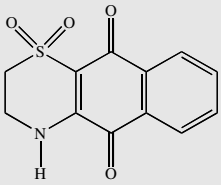
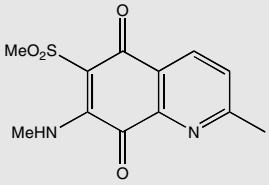
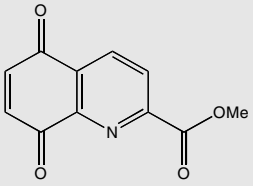
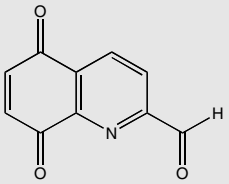
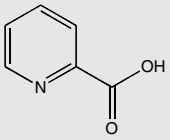
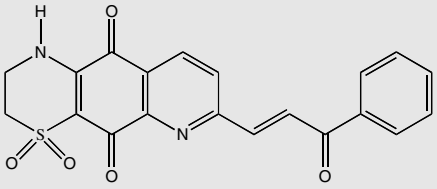
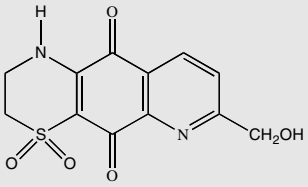
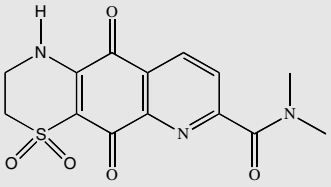
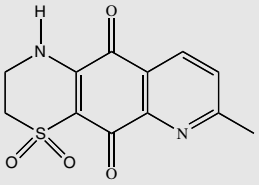
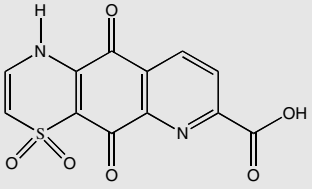
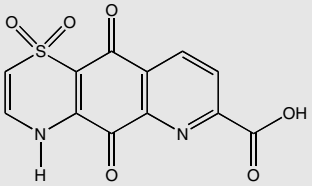
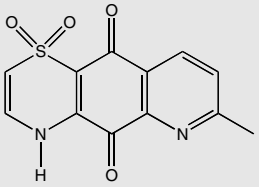
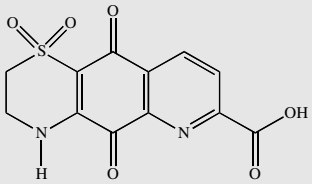
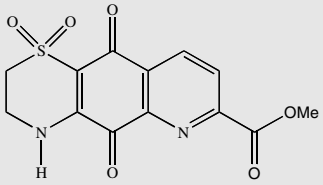
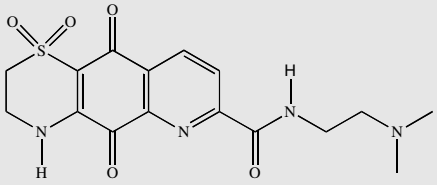
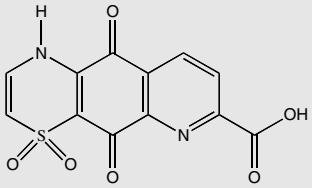
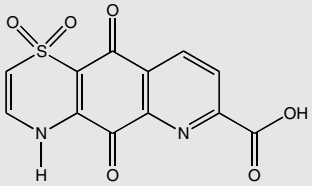
	Compound	AI_{50} (μM)[b]	AP_{50} (μM)[c]
9		15 ± 6	0.42 ± 0.03
10		26 ± 3	35 ± 5
11		1.57 ± 0.60	91 ± 20
12		62 ± 5	4.17 ± 1.21
13		13 ± 0.1	n.d
14		>167	2.55 ± 1.00
15		>333	97 ± 13
16		1.74 ± 1.01	4.00 ± 1.56

Table 2 (*continued*)

	Compound	AI_{50} (μM)[b]	AP_{50} (μM)[c]
17		7.29 ± 2.53	1.70 ± 0.59
18		>406	n.d.
19		>127	5.73 ± 1.42
20		64 ± 8	3.25 ± 0.86
21		>149	6.13 ± 1.44
22		>180	6.87 ± 3.35
23		0.44 ± 0.18	16 ± 3.26
24		0.06 ± 0.02	27 ± 6
25		2.56 ± 0.49	0.59 ± 0.20

[a] Values are means of two dose–response experiments ±SEM ($n = 3$).
[b] IC_{50} for inhibition of PMA-induced superoxide production by neutrophils.
[c] IC_{50} for inhibition of proliferation of HL60 cells.

Table 3
In vivo inhibition of neutrophil superoxide production and neutrophil infiltration by compounds **1**, **8**, **11**, **23** and **24**

#	Structure	AI (μM)	% Inhibition[a]	
			Superoxide Production	Neutrophil Infiltration
1		1.55	23[b] ($p < 0.05$)	25[b] ($p < 0.05$)
8		2.26	93 ($p < 0.05$)	49 ($p < 0.05$)
11		1.57	2 (*n.s.*)	5 (*n.s.*)
23		0.44	59[b] ($p < 0.05$)	0[b] (*n.s.*)
24		0.06	60 ($p < 0.05$)	65 ($p < 0.05$)

n.s., not significant
[a] All compounds were tested in vivo at 2.5 μmol/kg.
[b] Compounds with no effect at 2.5 μmol/kg were retested at 25 μmol/kg and results reported.

reducing both neutrophil recruitment and neutrophil superoxide production.

None of the compounds tested induced acute liver or renal toxicity measured as the levels of the liver enzymes and creatinine in the serum compared with untreated mice and mice treated with the anti-inflammatory agent colchicine. In all cases serum levels did not significantly exceed those observed in control mice (Supplementary Data Figures S1–4) although colchicine-treated mice showed significantly elevated enzyme and creatinine levels, consistent with colchicine's narrow therapeutic window and acute toxicity.

4. Conclusions

In conclusion, we have identified the 7-position substituent, the oxidation state of the thiazine ring and the regiochemistry of the thiazine ring as playing a key role in the anti-inflammatory activity and selectivity of the thiazine–quinoline–quinones. Selected thiazine–quinoline–quinones inhibited both neutrophil superoxide production and infiltration in an in vivo model of gouty arthritis thus identifying this structural class of inhibitors as promising candidates for development as anti-inflammatory drugs for the treatment neutrophil-driven disease.

5. Experimental

5.1. Chemistry: general methods

All chemicals were purchased from Sigma–Aldrich or Acros Organics. Octadecyl functionalised silica gel (C18), used for reversed-phase (RP) chromatography; silica gel 60, 200–400 mesh, 40–63 μm, for column chromatography and TLC plates silica gel 60 F_{254} were obtained from Merck. All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 35 °C. High resolution mass spectrometry was recorded using a VG70-250S double focussing magnetic sector mass spectrometer or a Bruker MicroTOFQ mass spectrometer. UV spectra were recorded in methanol using a Jasco V-550. NMR spectra were recorded at 500 or 300 MHz for ^{1}H and 125 MHz or 75 MHz for ^{13}C

on Varian INOVA-500 or VXR-300 spectrometers. HPLC analyses were carried out using an Agilent HP1100 on a C18 column (Phenomenex Luna ODS(3) 5 μm 100 A 150 × 3 mm) at 20 °C with a 2 × 4 mm C18 guard column. Peaks were detected at 210 and 254 nm and UV spectra recorded from 190 to 600 nm. Mobile phase A (water/0.1% formic acid) and B (acetonitrile/0.1% formic acid): t_0 = 10% B, $t_{12.5}$ = 100% B, t_{15} = 100% B, t_{16} = 10% B, t_{20} = 10% B. The flow rate was 0.5 mL/min, with an injection volume of 5 μL of 1 mg/mL solutions in methanol or DMSO. Preparative HPLC were carried out using a Gilson Preparative HPLC System with a C18 column (Synergi-Max RP, 4 μm, 21.20 × 250 mm), monitoring at 275 nm and 300 nm.

Potassium nitrosodisulfonate (Fremy's salt) was freshly prepared as described by Teuber.[10] Compounds **1**, **8**, **16**, **23** and **24** were prepared as described by Pearce et al.[9]; 8-hydroxyquinoline-2-carboxaldehyde as described by Hassani et al.[11]; and 8-hydroxyquinoline-2-carbonitrile as described by Schrader et al.[12] and 2-hydroxymethyl-8-hydroxyquinoline as described by Yoneda et al.[13] **14** was prepared as described by Schmitz and Bloor[14] and our data on **14** (see Registry No. 873536-72-2)[15] match those reported by Schmitz and Bloor, but the structure was drawn incorrectly in the 1988 paper and consequently recorded incorrectly in the Chemical Abstracts Registry file (see RN 113831-02-0).

5.1.1. General procedure for the preparation of 8-hydroxyquinolines

To a suspension of 8-hydroxyquinoline-2-carboxylic acid (190 mg, 1.0 mmol) in oxalyl chloride (3 mL) under nitrogen was added 2 drops of DMF. The solution was left at RT for 3 h then evaporated in vacuo to give the acid chloride. To a stirred solution of the acid chloride in dry CH_2Cl_2 (4 mL/mmol acid) under nitrogen was added excess nucleophile with triethylamine. The solution was refluxed for 30 min and then stirred at room temperature for 24 h. The reaction mixture was poured into CH_2Cl_2, washed with sat. $NaHCO_3$ then water and dried in vacuo. Column chromatography over silica gel gave the 8-hydroxyquinoline products.

5.1.1.1. 8-Hydroxyquinoline-*N*,*N*-dimethyl-2-carboxamide (RN 138878-37-2). From acid (250 mg, 1.32 mmol) and dimethyl amine (33% in ethanol, 10 mL, 222 mmol). Elution with CH_2Cl_2/EtOAc gave the product as a yellow gum (160 mg, 56%).[16] ^{1}H NMR ($CDCl_3$) 8.24 (1H, d, J = 8.4 Hz), 7.94 (1H, s), 7.71 (1H, d, J = 8.4 Hz), 7.50 (1H, td, J = 8, 1 Hz), 7.35 (1H, d, J = 8.1 Hz), 7.21 (1H, br t, J = 7.5 Hz), 3.20 (3H, s), 3.12 (3H, s).

5.1.1.2. Octyl 8-hydroxyquinoline-2-carboxylate (RN 880137-68-8). From acid (50 mg, 0.26 mmol), 1-octanol (200 μL, 1.30 mmol) and triethylamine (109 μL, 0.78 mmol). Purified by reversed-phase phenyl flash column chromatography eluting with MeOH, gave the product as a bright orange solid (71 mg, 91%). ^{1}H NMR ($CDCl_3$) 8.24 (1H, d, J = 8.5 Hz), 8.13 (1H, d, J = 8.5 Hz), 7.53 (1H, dd, J = 8.0, 7.7 Hz), 7.34 (1H, d, J = 8.0 Hz), 7.21 (1H, d, J = 7.7 Hz), 4.44 (2H, t, J = 6.8 Hz), 1.83 (2H, m), 1.49-1.28 (10H, m), 0.88 (3H, t, J = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) 165.0, 153.5, 145.4, 137.8, 137.1, 130.1, 129.7, 121.4, 117.4, 111.1, 66.2, 31.7, 29.1, 29.0, 28.6, 25.9, 22.5, 13.9. EIMS m/z 301.1677 (calcd for $C_{18}H_{23}NO_3$, 301.1678).

5.1.1.3. (*E*)-3-(8-Hydroxyquinolin-2-yl)-1-phenylprop-2-en-1-one (RN 880137-67-7). To a stirred solution of 8-hydroxyquinoline-2-carboxaldehyde (470 mg, 2.71 mmol) and acetophenone (326 mg, 2.71 mmol) in ethanol (95%, 20 mL) was added aq KOH (1M, 5 mL). The mixture was left at RT for 2 days, then aq HCl (3 M) added until neutral and the product extracted into CH_2Cl_2. Purification by column chromatography over silica gel eluting with ethyl acetate 0–20% in CH_2Cl_2 gave the product as a yellow oil (0.33 g, 44%). Found: C, 78.52; H, 4.79; N, 5.09. $C_{18}H_{13}NO_2$ requires C, 78.53; H, 4.76; N, 5.09; ^{1}H NMR (500 MHz, $CDCl_3$) 8.23 (1H, br s), 8.20 (1H, d, J = 8.5 Hz), 8.08 (2H, dd, J = 7.5, 1.5 Hz), 8.07 (1H, d, J = 15.5 Hz), 7.91 (1H, d, J = 15.5 Hz), 7.69 (1H, d, J = 8.5 Hz), 7.61 (1H, dt, J = 7 Hz), 7.54 (2H, t, J = 7.5 Hz), 7.48 (1H, t, J = 7.5 Hz), 7.34 (1H, dd, J = 8.5, 1.5 Hz), 7.21 (1H, dd, J = 7.5, 1.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) 190.5, 152.4, 151.1, 142.8, 138.2, 137.8, 137.0, 133.2, 128.8 (2C), 128.7 (2C), 128.6, 126.9, 122.0, 117.8, 110.6. UV (MeOH) λ_{max} (log ε) 337 (4.14), 294 (4.50), 206 (4.40) nm; ESIMS m/z 298.0842 (calcd for $C_{18}H_{13}NO_2Na$, 298.0844).

5.1.2. General procedure for oxidation of 8-hydroxyquinolines to quinoline-quinones with Fremy's salt (potassium nitrosodisulfonate)

Typically, a solution of Fremy's salt (1 g, 4 mmol) and potassium dihydrogen phosphate (400 mg, 3 mmol) in water (75 mL) was stirred at RT for 10 min then the quinolinol (6 mmol) in acetone (70 mL) was added. After stirring for 30 min, a second solution of Fremy's salt (1 g) and potassium dihydrogenphosphate (400 mg) in water (30 mL) was added, then a third equal amount after another 30 min. After stirring for a further 2 h the mixture was extracted into dichloromethane, dried and evaporated in vacuo to give the product as an orange gum. Purification by column chromatography over silica gel eluting with ethyl acetate (0–40%) in dichloromethane gave the products as orange solids.

Due to the instability of the quinones, these were mostly used directly in the subsequent hypotaurine addition reaction.

5.1.2.1. 5,8-Dihydro-5,8-dioxoquinoline (RN 858471-89-3). 8-Hydroxyquinoline (1 g, 6.9 mmol) to give quinone (0.48 g, 44%).[17] ^{1}H NMR (300 MHz, $CDCl_3$) 9.06 (1H, dd, J = 4.2, 1.5 Hz), 8.42 (1H, dd, J = 7.8, 1.5 Hz), 7.71 (1H, dd, J = 7.8, 4.8 Hz), 7.16 (1H, d, J = 10.5 Hz), 7.06 (1H, d, J = 10.5 Hz); EIMS m/z 160.0403 (calcd for $C_9H_6NO_2$, 160.0399); EIMS m/z 182.0216 (calcd for $C_9H_5NO_2Na$, 182.0218).

5.1.2.2. 2-Methyl-5,8-dihydro-5,8-dioxoquinoline (RN 90800-33-2). 2-Methyl-8-hydroxyquinoline (1 g, 6.9 mmol) to give quinone (0.45 g, 41%).[17] ^{1}H NMR (300 MHz, $CDCl_3$) 8.22 (1H, d, J = 8.1 Hz), 7.50 (1H, d, J = 8.1 Hz), 7.05 (1H, d, J = 10.5 Hz), 6.96 (1H, d, J = 10.5 Hz), 2.71 (3H, s); EIMS m/z 173.04739 (calcd for $C_{10}H_7NO_2$, 173.04768).

5.1.2.3. 2-Hydroxymethyl-5,8-dihydro-5,8-dioxoquinoline (RN 251652-78-5). 2-Hydroxymethyl-8-hydroxyquinoline (300 mg, 1.7 mmol) to give quinone (265 mg, 82%).[17] ^{1}H NMR (300 MHz, CD_3CN) 8.37 (1H, d, J = 8.4 Hz), 7.86 (1H, dd, J = 8.1, 0.6 Hz), 7.07 (1H, dd, J = 10.5, 0.6 Hz), 7.01 (1H, dd, J = 10.5, 0.6 Hz), 4.80 (2H, s), 3.78 (1H, br s); ESIMS m/z 212.0323 (calcd for $C_{10}H_7NO_3Na$, 212.0318).

5.1.2.4. 5,8-Dihydro-5,8-dioxoquinoline-2-carboxaldehyde (RN 326801-24-5). 8-Hydroxyquinoline-2-carboxaldehyde (200 mg, 1.2 mmol) to give the quinone (160 mg, 74%).[17] ^{1}H NMR (300 MHz, $CDCl_3$) 10.29 (1H, s), 8.61 (1H, d, J = 7.8 Hz), 8.31 (1H, d, J = 7.8, Hz), 7.26 (1H, d, J = 10.5 Hz), 7.15 (1H, d, J = 10.5 Hz), EIMS m/z 187.02693 (calcd for $C_{10}H_5NO_3$, 187.02694).

5.1.2.5. 5,8-Dihydro-5,8-dioxoquinoline-2-carbonitrile (RN 326801-23-4). 8-Hydroxyquinoline-2-carbonitrile (1 g, 6.9 mmol) to give the quinone (0.25 g, 24%).[17] ^{1}H NMR (300 MHz, $CDCl_3$) 8.60 (1H, d, J = 8.1 Hz), 8.07 (1H, d, J = 7.8, Hz), 7.27 (1H, d, J = 9.6 Hz), 7.17 (1H, d, J = 10.5 Hz), EIMS m/z 184.0270 (calcd for $C_{10}H_4N_2O_2$, 184.0273).

5.1.2.6. 5,8-Dihydro-5,8-dioxoquinoline-*N*,*N*-dimethyl-2-carboxamide (RN880137-63-3). 8-Hydroxyquinoline-*N*,*N*-dimethyl-2-carboxamide (160 mg, 0.74 mmol) to give the quinone (140 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) 8.51 (1H, d, J = 8.1 Hz), 8.03 (1H, d,

J = 8.1, Hz), 7.18 (1H, d, *J* = 10.5 Hz), 7.08 (1H, d, *J* = 10.5 Hz), 3.15 (3H, s), 3.12 (3H, s). ESIMS *m/z* 253.0577 (calcd for $C_{12}H_{10}N_2O_3Na$, 253.0589).

5.1.2.7. (*E*)-3-(5,8-Dihydro-5,8-dioxoquinolin-2-yl)-1-phenylprop-2-en-1-one (RN880137-65-5). (*E*)-3-(8-Hydroxyquinolin-2-yl)-1-phenylprop-2-en-1-one (60 mg, 0.22 mmol) to give the quinone (50 mg, 58%). ^{1}H NMR ($CDCl_3$) 8.45 (1H, d, *J* = 8.1 Hz), 8.27 (1H, d, *J* = 15.6 Hz), 8.08 (2H, dt, *J* = 8.1, 1.2 Hz), 7.85 (1H, d, *J* = 15.3 Hz), 7.85 (1H, d, *J* = 7.8 Hz), 7.61 (1H, m), 7.52 (2H, m), 7.17 (1H, d, *J* = 10.2 Hz), 7.08 (1H, d, *J* = 10.5 Hz). ESIMS *m/z* 312.0657 (calcd for $C_{18}H_{11}NO_3Na$, 312.0631).

5.1.2.8. Octyl-5,8-dihydro-5,8-dioxoquinoline-2-carboxylate (RN 880137-66-6). To a stirred solution of [bis(trifluoroacetoxy)iodo]-benzene (PIFA) (258 mg, 0.6 mmol) in MeCN/water 2:1 (3 mL) at 0 °C was added octyl 8-hydroxyquinoline-2-carboxylate (71 mg, 0.24 mmol) in CH_2Cl_2 (1 mL). The solution was stirred for 20 min, poured into CH_2Cl_2 (20 mL), washed with water and dried in vacuo to give octyl 5,8-dioxo-5,8-dihydroquinoline-2-carboxylate (69 mg, 90%) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) 8.58 (1H, d, *J* = 8.1 Hz), 8.45 (1H, d, *J* = 8.1 Hz), 7.24 (1H, d, *J* = 10.5 Hz), 7.14 (1H, d, *J* = 10.5 Hz), 4.46 (2H, t, *J* = 7.0 Hz), 1.83 (2H, m), 1.45-1.28 (10H, m), 0.88 (3H, t, *J* = 6.7 Hz). ^{13}C NMR ($CDCl_3$) 183.7, 181.9, 163.8, 152.6, 147.0, 139.5, 138.0, 136.1, 130.1, 128.4, 66.9, 31.6, 29.1, 29.0, 28.4, 25.7, 22.5, 14.0. FABMS *m/z* 316.1555 (calcd for $C_{18}H_{22}NO_4$, 316.1549).

5.1.3. General procedure for addition of hypotaurine

Typically, a solution of hypotaurine (220 mg, 2 mmol) in water (4 mL) was added to the quinone (2.9 mmol) in acetonitrile (10 mL) and ethanol (10 mL). The reaction mixture was stirred at room temperature for 18 h then the solvents were removed in vacuo to give an orange solid. The crude product was purified by one of two methods: *Isolation method 1:* Column chromatography on silica gel eluting with methanol/chloroform (0:1 to 1:1) gave the product as an orange solid. *Isolation method2:* Methanol was added, the mixture sonicated for 1 min then the orange solid isolated by filtration, then washed with further methanol.

5.1.3.1.Octyl-2H-pyrido[2,3-*g*][1,4]benzothiazine-5,10-dione, 3,4-dihydro-1,1-dioxo-7-carboxylate (9) (RN 880137-44-0). Octyl-5,8-dihydro-5,8-dioxoquinoline-2-carboxylate (140 mg) with hypotaurine (96 mg) isolated by method 1 to give a bright yellow solid (92 mg, 49%). ^{1}H NMR (d_6-DMSO) 9.44 (1H, s, NH), 8.53 (1H, d, *J* = 8 Hz, H-9), 8.39 (1H, d, *J* = 8 Hz, H-8), 4.36 (2H, t, *J* = 6.7 Hz, H_2-3'), 3.89 (2H, m, H_2-3), 3.41 (2H, t, *J* = 6.0 Hz, H_2-2), 1.75 (2H, m, H_2-4'), 1.40-1.26 (10H, m, H_2-5'-9'), 0.85 (3H, t, *J* = 6.6 Hz, H_3-10'). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} ($\log\varepsilon$) 422 (3.19), 297 (3.70), 274 (3.76), 237 (4.10) nm. HPLC 12.2 min. FABMS *m/z* 421.1419 (calcd for $C_{20}H_{25}N_2O_6S$ 421.1433).

5.1.3.2. 2H-Pyrido[2,3-*g*][1,4]benzothiazine-5,10-dione, 3,4-dihydro-1,1-dioxo-7-carbonitrile (4) (RN 880137-45-1). 5,8-Dihydro-5,8-dioxoquinoline-2-carbonitrile (200 mg, 1.1 mmol) with hypotaurine (80 mg) isolated by method 2 to give an orange solid (94 mg, 30%). ^{1}H NMR (d_6-acetone) 8.78 (1H, d, *J* = 8 Hz, H-9), 8.73 (1H, br s, NH), 8.51 (1H, d, *J* = 8 Hz, H-8), 4.27 (2H, m, H-3) and 3.52 (2H, m, H-2). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} ($\log\varepsilon$) 397 (3.59), 273 (4.22), 232 (4.21) nm. HPLC 4.96 min. EIMS *m/z* 289.0152 (calcd for $C_{12}H_7N_3O_4S$, 289.0157).

5.1.3.3. 2H-Pyrido[2,3-*g*][1,4]benzothiazine-5,10-dione, 3,4-dihydro-7-methyl-1,1-dioxide(3) (RN880137-62-2) and 1H-pyrido[3,2-*g*][1,4]benzothiazine-5,10-dione, 2,3-dihydro-7-methyl-1,1-dioxide(22) (RN880137-50-8). 2-Methyl-5,8-dihydro-5,8-dioxoquinoline (200 mg, 1.2 mmol) with hypotaurine (130 mg) isolated by method 1 gave **3** as an orange solid (50 mg, 16%). ^{1}H NMR (d_6-DMSO) 9.38 (1H, s, NH), 8.36 (1H, d, *J*= 8 Hz, H-9), 7.84 (1H, d, *J* = 8 Hz, H-8), 3.97 (2H, m, H_2-3), 3.51 (2H, m, H_2-2) and 2.75 (3H, s, H_3-1'). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} ($\log\varepsilon$) 423 (3.26), 291 (3.95), 266 (4.13), 234 (4.23) nm. HPLC 4.9 min. EIMS M^+ *m/z* 278.0352 (calcd for $C_{12}H_{10}N_2O_4S$, 278.0361).

Compound **22** as a yellow solid (40 mg, 13%). ^{1}H NMR (d_6-DMSO) 9.29 (1H, s, NH), 8.37 (1H, d, *J* = 8 Hz, H-9), 7.76 (1H, d, *J* = 8 Hz, H-8), 3.98 (2H, m, H_2-3), 3.52 (2H, m, H_2-2) and 2.79 (3H, s, H_3-1'). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} ($\log\varepsilon$) 421(3.32), 293(4.04), 270(4.09), 235(4.28) nm. HPLC 5.3 min. EIMS MH^+ *m/z* 279.0435 (calcd for $C_{12}H_{11}N_2O_4S$, 279.0440).

Crystallographic data (excluding structure factors) for the structure of compound **3** in this paper have been deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication No. CCDC 695838. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Crystal data. $C_{12}H_{10}N_2O_4S$, *M* 278.28, triclinic, space group *P*-1 *a* 6.4790(2), *b* 7.7900(2), *c* 11.4908(3) Å, α 77.9930(10) β 85.9670(10) γ 84.4930(10)°, *V* 563.89(3) Å^3, *Z* 2, *F*(000) 288, λ 0.71073 Å, μ(Mo Kα) 0.300 cm^{-1}, *T* 90(2) K, 1841 unique reflections. Refinement of 173 parameters converged at final R_1 = 0.0355, wR_2(all data) = 0.0949.

5.1.3.4. 2H-Pyrido[2,3-*g*][1,4]benzothiazine-5,10-dione, 3,4-dihydro-1,1-dioxide (2) (RN 880137-51-9). 5,8-Dihydro-5,8-dioxoquinoline (30 mg, 0.19 mmol) with hypotaurine (21 mg) isolated by method 2 gave **2** as an orange solid (20 mg, 40%). ^{1}H NMR (d_6-DMSO) 9.43 (1H, s, NH), 9.06 (1H, dd, *J* = 1, 3 Hz, H-9), 8.49 (1H, dd, *J* = 1, 6 Hz, H-7), 7.97 (1H, dd, *J* = 3, 6 Hz, H-8), 3.99 (2H, m, H_2-3) and 3.50 (2H, m, H_2-2). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} ($\log\varepsilon$) 421 (3.29), 265 (4.07), 232 (4.17) nm; HPLC 5.65 min. EIMS *m/z* 264.0201 (calcd for $C_{11}H_8N_2O_4S$, 264.0205).

5.1.3.5. 2H-Pyrido[2,3-*g*][1,4]benzothiazine-5,10-dione, 3,4-dihydro-*N*,*N*-dimethyl-1,1-dioxo-7-carboxamide (10) (RN 880137-52-0) and 1H-Pyrido[3,2-*g*][1,4]benzothiazine-5,10-dione, 2,3-dihydro-*N*,*N*-dimethyl-1,1-dioxo-7-carboxamide (21) (RN 880137-53-1). 5,8-Dihydro-5,8-dioxoquinoline-*N*,*N*-dimethyl-2-carboxamide (140 mg, 0.61 mmol) with hypotaurine (90 mg) isolated by method 1 gave one regioisomer as an orange solid (52 mg, 26%) and the later eluting **21** as an orange solid (15 mg, 8%). Compound **10**: ^{1}H NMR (d_6-DMSO) 9.48 (1H, s, NH), 8.57 (1H, d, *J* = 8 Hz, H-9), 8.07 (1H, d, *J* = 8 Hz, H-8), 4.00 (2 H, m, H_2-3), 3.51 (2H, m, H_2-2), 3.17 (3H, s, N-Me) and 3.04 (3H, s, N-Me). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} ($\log\varepsilon$) 422 (3.02), 296 (3.65), 236 (3.96) nm. HPLC 4.48 min. EIMS *m/z* 335.0561 (calcd for $C_{14}H_{13}N_3O_5S$, 335.0576).

Compound **21**: ^{1}H NMR (d_6-DMSO) 9.38 (1H, s, NH), 8.57 (1H, d, *J*= 8 Hz, H-9), 7.99 (1H, d, *J* = 8 Hz, H-8), 3.99 (2H, m, H_2-3), 3.52 (2H, m, H_2-2), 3.18 (3H, s, N–Me) and 3.04 (3H, s, N-Me). UV (MeOH) λ_{max} ($\log\varepsilon$) 422 (3.29), 297 (3.94), 268 (4.01), 236 (4.21) nm. HPLC 4.21 min. EIMS MNa^+ *m/z* 358.0475 (calcd for $C_{14}H_{13}N_3O_5SNa$, 358.0474).

5.1.3.6. (*E*)-1-Phenyl-3-(2H-pyrido[2,3-*g*][1,4]benzothiazine-5,10-dione, 3,4-dihydro-1,1-dioxide)prop-2-en-1-one (6) (RN 880137-57-5) and (*E*)-1-phenyl-3-(1H-pyrido[3,2-*g*][1,4]benzothiazine-5,10-dione, 3,4-dihydro-1,1-dioxide)prop-2-en-1-one 3804 (19). (*E*)-3-(5,8-Dihydro-5,8-dioxoquinolin-2-yl)-1-phenylprop-2-en-1-one (50 mg, 0.17 mmol) with hypotaurine (30 mg) isolated by method 1 gave **6** as an orange solid (15 mg, 22%) and the later eluting **19** also as an orange solid (15 mg, 22%). Compound **6**: ^{1}H NMR (d_6-DMSO) 9.35 (1H, s, NH), 8.46 (1H, d, *J* = 8 Hz, H-9), 8.42 (1H, d, *J* = 8 Hz, H-8), 8.26 (1H, d,

J = 16 Hz, H-2'), 8.14 (2H, d, J = 7 Hz, H-5'), 7.80 (1H, d, J = 16 Hz, 1'), 7.73 (1H, t, J = 7 Hz, H-7'), 7.63 (2H, t, J = 7 Hz, H-6'), 3.91 (2H, m, H_2-3) and 3.42 (2H, m, H_2-2). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} (log ε) 313 (4.36), 274 (4.36), 244 (4.47) nm. HPLC 10.6 min. EIMS m/z 394.0549 (calcd for $C_{20}H_{14}N_2O_5S$ 394.0623).

Compound **19**: ^{1}H NMR (d_6-DMSO) 9.38 (1H, s, NH), 8.57 (1H, d, J = 8 Hz, H-9), 8.42 (1H, d, J = 8 Hz, H-8), 8.43, d, J = 15 Hz, H-2'), 8.24 (2H, dd, J = 7.5, 1 Hz, H-5'), 7.92 (1H, d, J = 15 Hz, 1'), 7.85 (1H, d, J = 8 Hz, H-7'), 7.75 (2H, t, J = 7.5 Hz, H-6'), 4.00 (2H, m, H_2-2) and 3.54 (2H, t J = 6 Hz, H_2-3). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} (log ε) 326 (4.43), 203 (4.29) nm. HPLC 8.71 min. EIMS m/z 395.0731 (calcd for $C_{20}H_{15}N_2O_5S$ 395.0702).

5.1.3.7. 2H-Pyrido[2,3-*g*][1,4]benzothiazine-5,10-dione, 3,4-dihydro-7-hydroxymethyl- 1,1-dioxide (7) and 1H-pyrido[3,2-*g*][1,4]benzothiazine-5,10-dione,3,4-dihydro-7-hydroxymethyl-1,1-dioxide (20).

5,8-Dihydro-2-(hydroxymethyl)-5,8-dioxoquinoline (54 mg, 0.29 mmol) with hypotaurine (22 mg) isolated by method 1 gave adduct **7** as an orange solid (19 mg, 23%) and the later eluting **20** as a orange solid (14 mg, 17%). ^{1}H NMR (d_6-DMSO) 9.28 (1H, s, NH), 8.38 (1H, d, J = 8.5 Hz, H-9), 7.92 (1H, d, J = 8 Hz, H-8), 5.74 (1H, t, J = 6 Hz, OH), 4.70 (2H, d, J = 5.5 Hz, H-1'), 3.86 (2H, t, J = 6 Hz H-2) and 3.38 (2H, t, J = 6 Hz H-3). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} (log ε) 421 (3.01), 290 (3.71), 266 (3.88), 259 (3.87), 234 (4.01), 200 (3.91). HPLC 3.41 min. ESIMS m/z 317.0196 (calcd for $C_{12}H_{10}N_2O_5SNa$, 317.0208).

Compound **20**: ^{1}H NMR (d_6-DMSO) 9.20 (1H, s, NH), 8.39 (1H, d, J = 8.5 Hz, H-9), 7.85 (1H, d, J = 8 Hz, H-8), 5.77 (1H, t, J = 6 Hz, OH), 4.70 (2H, d, J = 5.5 Hz, H-1'), 3.86 (2H, m, H-2) and 3.38 (2H, m, H-3). UV (MeOH) λ_{max} (log ε) 424 (3.11), 291 (3.72), 267 (3.83), 236 (4.06), 192 (3.95). HPLC 4.27 min. ESIMS m/z 382.1073 (calcd for $C_{16}H_{20}N_3O_6S$, 382.1211).

5.1.4. 2H-Pyrido[2,3-*g*][1,4]benzothiazine-5,10-dione, 3,4-dihydro-1,1-dioxo-7-carbaldehyde (5) (RN 880137-59-7)

A mixture of compound **3** (50 mg, 0.18 mmol) and selenium dioxide (80 mg, 0.72 mmol) in dioxane (4 mL) and water (0.5 mL) was stirred under nitrogen at 90°C for 14 h. Further selenium dioxide (160 mg) was added in batches whilst heating over 12 h. Separation by column chromatography over silica gel eluting with 10–100% MeOH in $CHCl_3$ gave the aldehyde **5** as an orange solid (18 mg, 34%). ^{1}H NMR (d_6-DMSO) 10.23 (1H, s, C*H*O), 9.62 (1H, s, NH), 8.70 (1H, d, J = 8 Hz, H-9), 8.40 (1H, d, J = 8 Hz, H-8), 4.02 (2H, m, H_2-3) and 3.53 (2H, m, H_2-2). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} (log ε) 421 (3.14), 289 (3.77), 267 (3.90), 234 (4.07) nm. HPLC 4.66 min.EIMS m/z 292.0147 (calcd for $C_{12}H_8N_2O_5S$, 292.0154).

5.1.5. 2H-Pyrido[2,3-*g*][1,4]-benzothiazine-5,10-dione, 3,4-dihydro-7-(hydromethoxymethyl)-1,1-dioxide (13) (RN 880137-60-0)

A sample of aldehyde **5** (5 mg, 0.02 mmol) was left in methanol (1 mL) overnight then the solvents removed to give the hemi acetal (5 mg, 95%) as a yellow solid. ^{1}H NMR (CD_3OD) 8.53 (1H, d, J = 8 Hz, H-9), 8.02 (1H, d, J = 8 Hz, H-8), 5.64 (1H, s, H-1'), 4.04 (2H, m, H_2-3), 3.59 (3H, m, H_4-3') and 3.43 (2H, m, H_2-2). UV (MeOH) λ_{max} (log ε) 416 (2.95), 267 (3.72), 233 (3.91) nm. HPLC 4.65 min. EIMS m/z 325.0490 (calcd for $C_{13}H_{13}N_2O_6S$ 325.0494).

5.1.6. 4H-Pyrido[2,3-*g*][1,4]benzothiazine-5,10-dione-7-methyl-1,1-dioxide (25) (RN 880137-62-2)

Compound **3** (10 mg, 0.036 mmol) was stirred in 1M KOH (2 mL) for 3 h. The resultant red orange solution was passed down a short column of weak cationic ion exchange resin (Amberlite IRC 86, 2 g) to give a yellow solution which was freeze dried to give the pure product **25** as a yellow solid (9 mg, 91%). ^{1}H NMR (d_6-DMSO) 8.41 (1H, d, J = 8 Hz, H-9), 7.87 (1H, d, J = 8 Hz, H-8), 7.26 (1H, d, J = 9 Hz, H-3), 6.64 (1H, d, J = 9 Hz, H-2) and 2.78 (3H, s, H_3-1'). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} (log ε) 421(3.09), 267(3.94), 238(3.97), 210(3.90) nm. HPLC 4.11 min. EIMS m/z 276.0197 (calcd for $C_{12}H_8N_2O_4S$, 276.0205).

5.1.7. 2-Methyl-6-methylsulfonyl-7-methylamino-5,8-dihydro-5,8-dioxoquinoline (15)

To a solution of 2-methyl-quinoline-quinone (400 mg, 2.3 mmol) in acetonitrile (10 ml) and ethanol (5 ml) was added sodium methanesulfinate (236 mg, 2.31 mmol) in water (5 ml). The mixture was left at RT for 2 h, then the solvents removed in vacuo. Purification by column chromatography over silica gel, eluting with EtOAc in DCM gave the intermediate methyl-6-methylsulfonyl-5,8-dihydro-5,8-dioxoquinoline as an off white solid (320 mg, 55%). ^{1}H NMR ($CDCl_3$) 8.52 (1H, d, J = 8 Hz, H-9), 7.40 (1H, d, J = 8 Hz, H-8), 7.19 (1H, s, H-3), 3.16 (3H, s, S-Me) and 2.75 (3H, s, C-Me). To a solution of methyl-6-methylsulfonyl-5,8-dihydro-5,8-dioxoquinoline (220 mg, 0.88 mmol) in acetonitrile (5 ml) and methanol (5 ml) was added methylamine (40% in water, 1 ml). The resultant yellow solution was evaporated in vacuo. Purification over silica gel eluting with 5% MeOH in $CHCl_3$ gave the crude product as an orange solid (75 mg, 31%), crystallisation from methanol gave **15** as an orange crystalline solid (25 mg, 10%).^{1}H NMR ($CDCl_3$) 9.72 (1H, br s, NH), 8.34 (1H, d, J = 8 Hz, H-9), 7.56 (1H, d, J = 8 Hz, H-8), 3.46 (3H, d, J = 6 Hz, H-3), 3.42 (3H, s, H-2) and 2.75 (3H, s, Me). ^{13}C NMR in Table 1. UV (MeOH) λ_{max} (log ε) 421 (3.29), 292 (3.86), 268 (3.95), 259 (3.96), 237 (4.15), 199 (3.96) nm. HPLC 6.35 min. EIMS m/z 303.0408 (calcd for $C_{12}H_{12}N_2O_4SNa$, 303.0415).

5.1.8. 2H-Pyrido[2,3-*g*][1,4]-benzothiazine-5,10-dioxo-3,4-dihydro-*N*-(2-dimethylaminoethyl)-1,1-dioxo-7-carboxamide (11)

To a solution of acid **1** (62 mg 0.20 mmol) in DMF (10 mL) was added dimethylethylenediamine (0.026 mL, 0.24 mmol) followed by *N*,*N*-diisipropylethylamine (DIPEA, 0.105 mL, 0.60 mmol). The mixture was cooled to 0 °C and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, 135 mg, 0.26 mmol) was added, then stirred at room temperature for 3 h. The solvents were removed in vacuo. The product was purified by reverse phase (C18) column, followed by crystallization from MeCN and EtOAc twice then preparative HPLC Mobile phase A (TFA/H_2O, pH 2.5) and B (90% MeCN/H_2O): t_0 = 1% B, t_{10} = 1% B, t_{11} = 15% B, t_{19} = 15% B, t_{20} = 1% B, t_{22} = 1% B. Flow rate 13 mL/min. t_R = 15.25 min. The product was freeze dried to give the TFA salt of **11** as a yellow solid (30 mg, 30%). ^{1}H NMR (d_6-DMSO): δ 9.35 (s, 1H, H-4), 9.29 (br, 1H, H-15), 8.96 (br, t, 1H, H-15), 8.57 (d, J = 8.1 Hz, 1H, H-9), 8.43 (d, J = 8.1 Hz, 1H, H-8), 3.91 (br, 2H, H-3), 3.70 (br, q, J = 5.8 Hz, 2H, H-13), 3.42 (t, J = 6.0 Hz, 2H, H-2), 3.30 (2H, H-14, overlapped by water), 2.80 (s, 6H, H-16) ppm. ^{1}H NMR (d_4-MeOH): δ 8.55 (d, J = 8.0 Hz, 1H, H-9), 8.47 (d, J = 8.0 Hz, 1H, H-8), 4.09 (br, t, J = 5.9 Hz, 2H, H-3), 3.86 (t, J = 5.8 Hz, 2H, H-13), 3.52 (t, J = 5.9 Hz, 2H, H-2), 3.43 (t, J = 5.8 Hz, 2H, H-14), 2.99 (s, 6H, H-16) ppm. ^{13}C NMR in Table 1. FABMS m/z 379.1076 (calcd for $C_{16}H_{19}N_4O_5S$, 379.1076).

5.1.9. (2-Dimethylaminoethyl)-2H-pyrido[2,3-*g*][1,4]-benzothiazine-5,10-dioxo-3,4-dihydro-1,1-dioxo-7-carboxylate (12)

To a suspension of 3-dimethylaminopropionic acid hydrochloride (230 mg, 1.5 mmol) in MeCN (20 mL) was added one drop of DMF followed by oxalyl chloride (1.29 mL, 15.0 mmol) slowly. The mixture was stirred for 4 h before all solvents were removed. The resultant red brown solid was dissolved in DMF (10 mL), then added to a solution of **7** (147 mg, 0.50 mmol), DMAP (6 mg, 0.05 mmol) and DIPEA (522 μL, 3.0 mmol) in DMF (10 mL). The mixture was stirred for 16 h then the solvents were removed in vacuo. The product was purified by C18 column chromatography, then washed with a little MeCN and EtOAc, and then further purified by preparative HPLC Mobile phase A (TFA/H_2O, pH 2.5) and B (90% MeCN/H_2O) iso-

cratic 9% B, flow rate 13 mL/min, t_R = 11.11 min. The product was freeze-dried to give the TFA salt of **12** as a brown solid (10 mg, 4%). ^{1}H NMR (D_2O): δ 8.47 (d, J = 8.2 Hz, 1H, H-9), 7.92 (d, J = 8.2 Hz, 1H, H-8), 5.42 (s, 2H, H-11), 4.14 (br t, J = 6.0 Hz, 2H, H-3), 3.61 (br t, J = 6.0 Hz, 2H, H-2) 3.53 (t, J = 6.7 Hz, 2H, H-15), 3.10 (t, J = 6.7 Hz, 2H, H-14) and 2.94 (s, 6H, H-17) ppm. ^{13}C NMR in Table 1. FABMS m/z 394.1073 (calcd for $C_{17}H_{20}N_3O_6S$, 394.1073).

5.2. Biological assays

5.2.1. Reagents and chemicals

Polymorphprep (Axis-Shield, Norway) was obtained through Medica Pacifica Ltd. (New Zealand). WST-1 and mPMS (1-methoxy-5-methylphenazinium methylsulfate) were from Dojindo Laboratories (Kumamoto, Japan). Cell culture reagents were obtained from Invitrogen (New Zealand). All other reagents were obtained through Sigma–Aldrich (New Zealand) unless otherwise stated.

5.2.2. Preparation of monosodium urate crystals (MSU)

Uric acid (250 mg) was added to 45 mL of ddH_2O containing 300 μL of 5 M NaOH and the solution boiled until the uric acid was dissolved. The solution was passed through a 0.2 μM filter and 1 mL of 5 M NaCl was added. The hot solution was allowed to cool then left at 26 °C for 7 days to allow crystal formation. The resulting MSU crystals were washed with ethanol and acetone. The resulting MSU crystals were needle shaped, 5–25 μm in length, and were birefringent to polarised light. All MSU crystals were tested as endotoxin free by LAL assay (<0.01 EU/10 mg).

5.2.3. Neutrophil superoxide assay

Human neutrophils were isolated from anti-coagulated whole human blood using Polymorphprep (density 1.113 g/mL, 500 g, 35 min). Neutrophils (resuspended in HBSS) were plated out in 96-well flat-bottomed plates at 1×10^6 cells/well. Neutrophils were treated with different concentrations of the test compound (1% DMSO/HBSS) for 30 min prior to addition of the detection dye WST-1 (final concentration 0.35 mM). The respiratory burst was triggered by addition of PMA (final concentration 0.295 μM), and dye reduction was monitored (450 nm) for 20 min at 37 °C. Superoxide production was calculated as the rate of dye reduction over time compared to buffer-treated cell controls.

5.2.4. Anti-proliferative assay

HL60 cells (suspended in RPMI1640/5%FCS) were plated out in 96-well flat-bottomed plates at 2×10^4 cells/well. The cells were treated with different concentrations of the test compound (dissolved in 1% DMSO/RPMI1640/5% FCS) and cultured for 48 hours (37 °C, 5% CO_2). MTT was then added to each well (final concentration 1.10 mM) and the cells were incubated for 2 h. Lysing buffer (10% w/v SDS, 45% v/v DMF/H_2O, pH 4) was added to each well and the plates were incubated overnight to ensure complete lysis. Proliferative activity was determined by reading the absorbance of the samples at 570 nm compared to buffer-treated cell controls.

5.2.5. In vivo assay

C57BL/J6 male mice were bred and housed in a conventional animal facility at the Malaghan Institute of Medical Research. All animals used for the experiments were aged between 8 and 10 weeks. Experimental procedures were approved by the Victoria University Animal Ethics Committee (New Zealand) and carried out in accordance with the VUAEC guidelines for the care of animals. Mice were injected intraperitoneally (i.p.) with monosodium urate (MSU) crystals (3 mg) suspended in PBS (500 μL). Test compounds were administered by oral gavage immediately following administration of MSU. Control mice received PBS. Four hours after MSU crystal administration, mice were euthanased by CO_2. The peritoneal cavity was washed with PBS (3 mL) containing 25 U/mL heparin. Total cell numbers were counted and the sample volume adjusted to 1×10^6 cells/mL for subsequent analysis of superoxide production.

5.2.6. Detection of superoxide production by peritoneal neutrophils

Peritoneal neutrophils were tested for superoxide production as described above. To determine the number of neutrophils in each sample, a small aliquot (100 μL) of each cell sample was spun onto a glass slide and stained with the Diff-Quick Staining Kit (Dade Behring, Newark, USA). The percentage of neutrophils in the peritoneal wash was determined microscopically using standard histological criteria. Superoxide production was then corrected for the number of neutrophils/mL in each sample.

5.2.7. Serum toxicity markers

Blood was collected from the heart of euthanased mice by cardiac puncture. Whole blood samples were shipped over ice for analysis (Gribbles Analytical Laboratories, Hamilton, New Zealand) to determine the levels of liver enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase) and the renal marker creatinine in the serum.

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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.2008.09.052.

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