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Original article

Preparation and antimalarial activity of a novel class of carbohydrate-derived, fused thiochromans

Henok H. Kinfe [a, *], Paseka T. Moshapo [a], Felix L. Makolo [a], David W. Gammon [b], Martin Ehlers [c], Carsten Schmuck [c]

[a] Department of Chemistry, University of Johannesburg, Auckland Park 2006, South Africa
[b] Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa
[c] Institute of Organic Chemistry, University of Duisburg-Essen, Universitätstrasse 7, 45141 Essen, Germany

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ABSTRACT

A novel class of fused thiochroman derivatives has been prepared by an efficient and versatile synthetic procedure involving nucleophilic displacement of the side-chain iodo substituent in 2-deoxy-2-*C*-iodomethyl glucosides by thiophenolate ions, and subsequent intramolecular *C*-glycoside formation. A range of aromatic substituents is tolerated, and the subsequent facile selective oxidation of the sulfur to the sulfoxide or sulfone level expands the range and molecular diversity of the series of compounds. A selection of the sulfoxide and sulfone derivatives bearing lipophilic substituents on the aromatic portion were found to have antimalarial activities in the low micromolar range.

1. Introduction

Thiochromans, 3,4-dihydro-2*H*-1-benzothiopyrans and their derivatives, continue to attract significant interest from organic and medicinal chemists due to their presence as key components in biologically active compounds that have shown anti-cancer [1,2], anti-HIV [3], anti-bacterial [4] and anti-fungal [5] activity, as well as being employed in the treatment of depression [6], schizophrenia, Parkinson's [7] and Alzheimer's diseases [8,9]. Moreover, the enhanced biological activity exhibited by thiochromans in comparison to their corresponding oxygen analogues [7,10] and the ease of derivatizing them into sulfoxides, sulfones, and Pummerer products, thus generating libraries of compounds for structure activity relationship studies, highlights their potential in medicinal chemistry programmes. However, despite their wide spectrum of biological and medicinal importance, the evaluation of thiochroman derivatives for their antimalarial activity has been overlooked. According to our literature survey, the last antimalarial activity study conducted on thiochroman derivatives was four decades ago. In 1978 Razdan et al. from the company *Arthur D. Little Inc*. reported that some derivatives of thiochromans were found to be active and were curative at dose levels of 160–360 mg/kg against *Plasmodium berghei* in mice [11]. Although these results were encouraging, there have been no further reports on any studies conducted to improve the efficacy and cytotoxicity (if any) of such thiochroman derivatives in relation to their antimalarial activity.

The reliability, effectiveness, limited host toxicity as well as low cost of chloroquine as an antimalarial drug in the past decades might have discouraged research and development of alternative antimalarials until the emergence of chloroquine-resistant malaria strains [12,13]. Currently, artemisinin-based combination therapy is World Health Organization's standard treatment against *Plasmodium falciparum*, the most lethal species that causes malaria in humans, in which the regimen uses a double or triple combination therapy with the aim of either delaying or preventing the development of drug resistance [14–16]. Although there are preventative and curative measures that have been adopted and with no clinical resistance having been reported against artemisinin, recent indications from South-East Asia are increasingly pointing towards tolerance which may eventually lead to resistance against this class of drugs [17–19]. Malaria accounts for over half-a-million deaths per annum worldwide and thus still constitutes a global health risk. Spitzmüller and Mestres have recently highlighted that while there is an urgent need to map the macromolecular drug target space in *P. falciparum*, there is also a need to expand the range of chemotypes with potential antimalarial activity [20]. Research in these

* Corresponding author.
E-mail address: hhkinfe@uj.ac.za (H.H. Kinfe).

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directions will increase the chances of discovery and development of new antimalarial drugs with novel mechanisms of action [21].

Our interest in the stereoselective synthesis of carbohydrate based thiochroman derivatives and expansion of the range of antimalarial chemotypes, prompted us to prepare novel thiochroman derivatives and evaluate their antimalarial activities. Herein we report the *in vitro* antimalarial activity of thiochroman derivatives **3**–**9**, differing in their sulfur oxidation states, aromatic substituents, anomeric configurations and nature of protecting groups.

2. Results and discussion

2.1. Chemistry: preparation of thiochromans

Thiochroman derivatives (**3**–**4**) were synthesized starting with an α,β-anomeric mixture of 2-*C*-iodomethyl-glucosyl acetates **1** as outlined in Scheme 1 following our recently reported protocol [22,23]. Treatment of the anomeric mixture **1** with a range of aryl sodium thiolates that were each freshly prepared in DMF yielded sulfides (**2**) which then underwent Lewis acid catalysed Friedel–-Crafts alkylation to give the α-*C*-glycosides (**3**–**4**) as single isomers. Efforts to debenzylate thiochromans **3** using traditional hydrogenolysis using H_2 and catalytic Pd/C to obtain hydrophilic thiochroman derivatives were unsuccessful; however, acetylated thiochroman **3g** could be readily hydrolysed under basic conditions to afford the more hydrophilic derivative **4** (Scheme 1).

Oxidation of thiochromans **3(a–f)** in the presence of cerium ammonium nitrate, wet silica gel and catalytic amounts of potassium bromide formed a diastereomeric 2:1 mixture of sulfoxides without over-oxidation to sulfones. The diastereomers were separated by column chromatography to afford **5(a–f)** and **6(a–f)**. Efforts to identify the stereochemistry of each sulfoxide by growing crystals of the respective compounds with a view to obtain single crystal X-ray diffraction were unsuccessful and the configurations are tentatively assigned. The chemical structure of the sulfoxides was confirmed by a combination of common techniques such as IR, NMR and HRMS (Scheme 2) [24].

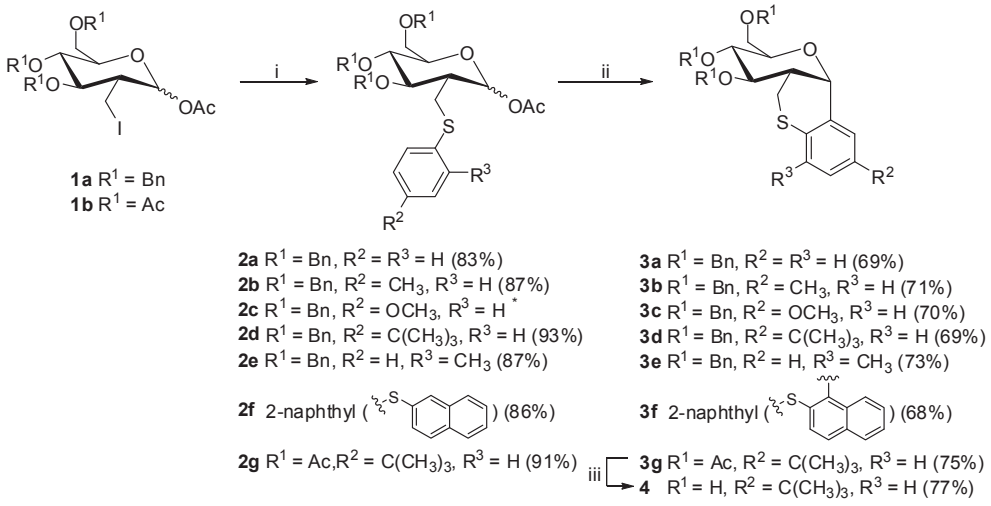
Oxidation of sulfides **3** with excess OXONE® produced sulfones **7**. The acetylated thiochroman **7g** was further hydrolysed to afford the triol **8**. Portions of **7(a–e)** were then epimerized upon treatment with NaH to provide the β-C-glycosides **9(a–e)** in excellent yields following our recently reported protocol (Scheme 3) [25].

2.2. Antimalarial activity of synthetic thiochromans

After the successful synthesis of the thiochroman derivatives, these were evaluated for their activity against the chloroquine-sensitive 3D7 and chloroquine-resistant FCR3 strains of the malaria parasite, *P. falciparum*, by measuring parasite survival using a parasite lactate dehydrogenase (pLDH) assay. Compounds that exhibited parasite survival rates of 0–15% at a single concentration of 10 μM were screened further for dose–response to determine the IC_{50} values and the results are summarized in Table 1 (refer to the supporting information for detailed data).

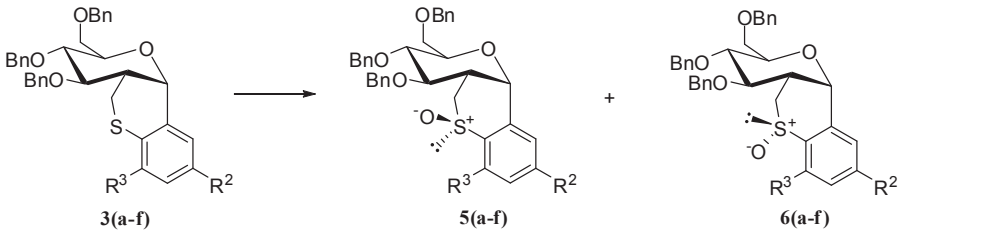
The structural parameters explored for SAR correlation were oxidation state of the sulfur (sulfide, sulfoxide and sulfone), nature and position of the substituent on the aromatic ring of the thiochroman motif, stereochemistry of the sulfoxide group, anomeric configuration of the sugar moiety (and consequent geometry of the ring fusion), nature of protecting group and hydrophilicity.

The lack of high potency and activity of thiochromans **3a–f** and thiochroman derivatives **5a**, **6a**, and **7a**, in comparison to the other analogues, indicates the requirement for sulfur in higher oxidation states and the presence of a substituent on the aromatic ring of the thiochroman moiety to impart activity/potency. In agreement with the literature report, the sulfone functional group imparted higher antimalarial activity than the corresponding sulfides (compare **3a–f** vs **7b–f** and the epimerized sulfones in Table 1) [26]. With regard to the sulfoxides, there is only one instance of significant difference in the activity of the diastereomeric sulfoxides, *viz*. the case of **5b** and **6b**, with **5b** essentially inactive against strain 3D7,



[a]Reagents and conditions: i) 60% NaH, Aryl thiol, DMF, rt, 5 min; ii) $BF_3{\cdot}Et_2O$, DCM, 0 °C, 5 min; iii) K_2CO_3, MeOH, rt, 3 h. *Sulfide **2c** was unstable on standing and thus it was immediately cyclized without purification.

Scheme 1. Diastereoselective synthesis of thiochroman derivatives **3-4**[a].

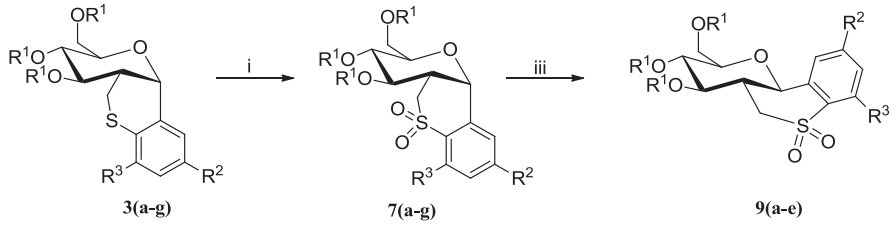


5a $R^2 = R^3 = H$ (40%)
5b $R^2 = CH_3$, $R^3 = H$ (44%)
5c $R^2 = OCH_3$, $R^3 = H$ (49%)
5d $R^2 = C(CH_3)_3$, $R^3 = H$ (39%)
5e $R^2 = H$, $R^3 = CH_3$ (43%)
5f 2-naphthyl (26%)

6a $R^2 = R^3 = H$ (27%)
6b $R^2 = CH_3$, $R^3 = H$ (24%)
6c $R^2 = OCH_3$, $R^3 = H$ (23%)
6d $R^2 = C(CH_3)_3$, $R^3 = H$ (33%)
6e $R^2 = H$, $R^3 = CH_3$ (28%)
6f 2-naphthyl (18%)

[b]Reagents and conditions: CAN, KBr, 50% wet silica gel, CH_3CN:DCM (8:2), rt, 30 min.

Scheme 2. Oxidation of thiochroman derivatives **3** to their diastereomeric sulfoxides **5** and **6**[b].



7a R^1 = Bn, $R^2 = R^3 = H$ (69%)
7b R^1 = Bn, $R^2 = CH_3$, $R^3 = H$ (71%)
7c R^1 = Bn, $R^2 = OCH_3$, $R^3 = H$ (70%)
7d R^1 = Bn, $R^2 = C(CH_3)_3$, $R^3 = H$ (69%)
7e R^1 = Bn, $R^2 = H$, $R^3 = CH_3$ (73%)
7f 2-naphthyl (43%)
ii → **7g** R^1 = Ac, $R^2 = C(CH_3)_3$, $R^3 = H$ (76%)
ii → **8** $R^1 = H$, $R^2 = C(CH_3)_3$, $R^3 = H$ (68%)

9a R^1 = Bn, $R^2 = R^3 = H$ (87%)
9b R^1 = Bn, $R^2 = CH_3$, $R^3 = H$ (88%)
9c R^1 = Bn, $R^2 = OCH_3$, $R^3 = H$ (92%)
9d R^1 = Bn, $R^2 = C(CH_3)_3$, $R^3 = H$ (94%)
9e R^1 = Bn, $R^2 = H$, $R^3 = CH_3$ (85%)

[c]Reagents and conditions: i) Wet Al_2O_3, OXONE®, DCM, rt, 12 h; ii) K_2CO_3, MeOH, rt, 3h; iii) NaH, DMF, rt, 15 min.

Scheme 3. Base-promoted epimerization of α-*C*-glycosides **7**(**a**–**f**) to β-*C*-glycosides **9**(**a**–**e**)[c].

while **6b** has activity comparable to the other sulfoxides. Moreover, the activity of the sulfones was dependent on the anomeric configuration of the sugar moiety (or ring-fusion geometry), with a 1,2-*cis* isomer found to be more potent than the corresponding 1,2-*trans* isomer (compare **7c** vs **9c**, **7d** vs **9d, 7e** vs **9e** and **7f** vs **9f**). The structural feature with the highest impact on the potency is a bulky, lipophilic *tert*-butyl substituent at the *para*-position of the aromatic ring in both the sulfoxide and sulfone variants of the thiochroman, as is evident from the lowest recorded IC_{50} values (<0.4 μM for **5d** and **7d**) in the entire series. A similar observation was noted by Bachi et al. where a free hydroxyl group was protected to reduce polarity and resulted in a higher potency [26]. Thus, fine tuning the balance between polarity and lipophilicity could be a crucial factor in the antimalarial activity of the test compounds.

Unlike the activity against the 3D7 strains, the oxidation state of sulfur was not crucial for activity against the FCR3 strain: thiochromans **3b**–**f** did not show any activity against 3D7 but the same compounds (with the exception of **3d**) exhibited activity against FCR3 (Entries 2–6 in Table 1). This also suggests the strain specificity of the tested compounds.

Almost all the compounds tested against the FCR3 strain exhibited activity with the exception of **3d**, **6a** and **9f**. In contrast to the activity profile against 3D7, activity against FCR3 could not be correlated with structural features. However, as for the activity against the 3D7 strain, the sulfoxides and sulfones having a lipophilic *tert*-butyl group exhibited the highest antimalarial potency.

To assess the effect of the protecting group on the sugar moiety and water solubility of the analogues, acetate protected analogues **3g** and **7g** as well as the water soluble analogues **4** and **8** were assayed for their antimalarial activity against both the 3D7 and FCR3 strains. However, both sets of compounds were found to be inactive against both strains of the *P. falciparum* confirming the importance of the balance between lipophilicity and polarity.

Finally, to investigate the selectivity of all of the active compounds, they were assayed for cytotoxicity towards human fetal lung fibroblast (WI-38) cell line and the results are summarized in Table 1. All tested compounds except **6c** were found to be non-toxic.

Table 1
In vitro antimalarial activities of the thiochroman derivatives against chloroquine-sensitive (3D7) and chloroquine-resistant (FCR3) *Plasmodium falciparum* strains, and their cytotoxicities.

Entry	S-oxidation	Tested compound	3D7 IC_{50} (μM)	FCR3 IC_{50} (μM)	Cytotoxicity IC_{50} (μM)
1	Sulfide	**3a** R^1 = Bn, $R^2 = R^3$ = H	*3.46*	*2.05*	>100
2		**3b** R^1 = Bn, R^2 = CH_3, R^3 = H	**>100**	*3.37*	>100
3		**3c** R^1 = Bn, R^2 = OCH_3, R^3 = H	**>100**	*1.73*	>100
4		**3d** R^1 = Bn, R^2 = $C(CH_3)_3$, R^3 = H	**>100**	**>100**	n.d.[a]
5		**3e** R^1 = Bn, R^2 = H, R^3 = CH_3	**>100**	*2.26*	>100
6		**3f** 2-naphthyl	**>100**	*7.11*	>100
7		**3g** R^1 = Ac, R^2 = $C(CH_3)_3$, R^3 = H	**>100**	>100	n.d.
8		**4** R^1 = H, R^2 = $C(CH_3)_3$, R^3 = H	**>100**	>100	n.d.
9	Sulfoxide 1	**5a** $R^2 = R^3$ = H	**>100**	10.34	>100
10		**5b** R^2 = CH_3, R^3 = H	**>100**	*4.11*	>100
11		**5c** R^2 = OCH_3, R^3 = H	*2.75*	*2.62*	>100
12		**5d** R^2 = $C(CH_3)_3$, R^3 = H	*0.30*	*0.33*	>100
13		**5e** R^2 = H, R^3 = CH_3	*4.13*	*2.76*	>100
14		**5f** 2-naphthyl	*3.55*	*0.52*	>100
15	Sulfoxide 2	**6a** $R^2 = R^3$ = H	**>100**	**>100**	n.d.
16		**6b** R^2 = CH_3, R^3 = H	*2.57*	*2.91*	>100
17		**6c** R^2 = OCH_3, R^3 = H	*2.21*	*2.07*	**8.76**
18		**6d** R^2 = $C(CH_3)_3$, R^3 = H	*1.33*	*0.33*	>100
19		**6e** R^2 = H, R^3 = CH_3	*6.29*	*4.34*	>100
20		**6f** 2-naphthyl	*2.55*	*2.17*	>100
21	Sulfone	**7a** R^1 = Bn, $R^2 = R^3$ = H	**>100**	*3.95*	>100
22		**7b** R^1 = Bn, R^2 = CH_3, R^3 = H	*2.32*	*1.93*	>100
23		**7c** R^1 = Bn, R^2 = OCH_3, R^3 = H	*1.80*	*1.70*	>100
24		**7d** R^1 = Bn, R^2 = $C(CH_3)_3$, R^3 = H	*0.39*	*0.28*	>100
25		**7e** R^1 = Bn, R^2 = H, R^3 = CH_3	*2.34*	*1.62*	>100
26		**7f** 2-naphthyl	*2.09*	*3.83*	>100
27		**7g** R^1 = Ac, R^2 = $C(CH_3)_3$, R^3 = H	*>100*	*>100*	n.d.
28		**8** R^1 = H, R^2 = $C(CH_3)_3$, R^3 = H	*>100*	*>100*	n.d.
29	Epimerized	**9a** $R^1 = R^2$ = H	*4.14*	*3.21*	>100
30	sulfone	**9b** R^1 = CH_3, R^2 = H	*2.34*	*1.69*	>100
31		**9c** R^1 = OCH_3, R^2 = H	*2.50*	*1.95*	>100
32		**9d** R^1 = $C(CH_3)_3$, R^2 = H	**>100**	*0.70*	>100
33		**9e** R^1 = H, R^2 = CH_3	3.40	*2.14*	>100
34		**9f** 2-naphthyl	>100	>100	n.d.
35		Chloroquine	0.016	0.064	

[a] n.d. = not determined.

3. Conclusion

Thirty four carbohydrate-fused thiochromans and their respective sulfoxide and sulfone derivatives were synthesised and evaluated for their antimalarial activity against both the chloroquine-sensitive 3D7 and chloroquine-resistant FCR3 strains. Sulfoxide and sulfone derivatives of the carbohydrate based thiochromans possessing a bulky and lipophilic *tert*-butyl group at the *para*-position of the aromatic ring of the thiochroman moiety exhibited the highest potency, with IC_{50} values in the range of 0.3–0.4 μM against both strains. Although these fall short of the nanomolar activity of chloroquine (6.77 nM–9.32 nM), their moderate to good activity, low cytotoxicity and the fact that they represent a hitherto under-represented class of compounds with antimalarial activity, suggests they are worthy of further study. These observations therefore provide a platform for preparation and evaluation of modified sulfoxide and sulfone derivatives bearing alternative and perhaps bulkier lipophilic substituents. Since thiochromans have rarely been reported as having antimalarial activity, an investigation on the possible mode of action and bioassay of the active compounds in combination with known antimalarials is underway and the results will be reported in due course.

4. Experimental protocols

4.1. General methods

All the solvents used were freshly distilled. Dichloromethane was distilled over phosphorous pentoxide in a condenser fitted with a drying tube containing calcium chloride. Other solvents were dried by appropriate techniques. The 2-*C*-iodomethyl-glucosyl acetates **1**, sulfides **2** and **3**, sulfoxides **5** and **6** and sulfones **7** and **9** were synthesized according to literature methods previously reported and their experimental data were in agreement with the literature [22–25]. The synthesis of the acetate analogues (**2g**, **3g**, **4**, **7g**) and the triol **8** is discussed below. All reagents were purchased from Sigma Aldrich. All reactions were monitored by thin layer chromatography (TLC) on aluminum-backed Merck silica gel 60 F_{254} plates using an ascending technique. The plates were visualized by spraying with a 1:1 solution of 5% *p*-anisaldehyde in ethanol and 10% sulfuric acid in ethanol baking at 150 °C. Gravity column chromatography was done on Merck silica gel 60 (70–230 mesh). Melting points were determined using a Reichert-Jung Thermovar hot-stage microscope and are uncorrected. Optical rotations were determined on a Perkin–Elmer 141 polarimeter in chloroform solutions at 25 °C. The concentration *c* refers to g/100 mL. Infrared spectra were recorded using Tensor 27 Bruker and Perkin–Elmer FT-IR spectrum BX. All proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded as deuteriochloroform solutions using tetramethylsilane as an internal standard on a Bruker Ultrashield (400 MHz) spectrometer. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on the same instrument at 100 MHz using tetramethylsilane as an internal standard. All chemical shifts are reported in ppm. Anomeric ratios are calculated from the ^{1}H NMR spectroscopy of the crude product. Mass spectrometers were recorded on a Walters API Quattro Micro spectrometer at the University of Stellenbosch, South Africa.

4.1.1. 1,3,4,6-tetra-O-acetyl-2-deoxy-2-C-(4-tert-butylphenyl)thiomethyl-D-glucopyranosyl (*2g*)

To a solution of 4-*tert*-butylbenzenethiol (219 μL 1.27 mmol) in DMF (10 mL), sodium hydride (60% dispersion on oil, 50.8 mg, 1.62 mmol) was added and the mixture was vigorously stirred at room temperature for 5–10 min under nitrogen. A solution of iodoacetate **1b** (500 mg, 1.06 mmol) in DMF (2 mL) was then added and after 5 min stirring of the reaction mixture, methanol (3 mL) was added dropwise and the resulting clear solution was concentrated *in vacuo*. The solution was directly submitted to silica gel chromatography (ethyl acetate/hexane, 2:8) to give the corresponding sulfide **2g**: 495 mg, 91% yield; 1:0.2 α:β anomeric mixture, colourless syrup; IR (neat cm^{-1}) 2961, 1743, 1430, 1210, 1008, 821; ^{1}H NMR (400 MHz, $CDCl_3$)δ: **major anomer**: 7.39–7.18 (m, 4H, Ar), 6.39 (d, J = 3.2 Hz, 1H, H-1), 5.25 (t, J = 10.2 Hz, 1H, H-3), 4.99 (t, J = 9.6 Hz, 1H, H-4), 4.36 (dd, J = 4.4 Hz and 12.8 Hz, 1H, H-6_a), 3.96 (m, 2H, H-6_b and H-5), 3.02 (dd, J = 3.8 Hz and 13.6 Hz, 1H, H-7_a), 2.63 (dd, J = 10.8 Hz and 13.6 Hz, 1H, H-7_b), 2.40–2.25 (m, 1H, H-2), 2.15–1.95 (m, 12H, 4× $-OCOCH_3$), 1.28 (s, 9H, $-C(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$)δ: 170.7 ($-O\underline{C}OCH_3$), 170.6 ($-O\underline{C}OCH_3$), 170.1

(−OCOCH$_3$), 168.6 (−OCOCH$_3$), 150.2 (Ar), 130.0 (Ar), 126.3 (Ar), 91.2 (C-1), 71.6 (C-3), 69.5 (C-5), 68.7 (C-4), 61.7 (C-6), 42.8 (C-2), 34.5 (−C(CH$_3$)$_3$), 31.2 (C-7), 29.7 (−C(CH$_3$)$_3$), 20.8 (−OCOCH$_3$), 20.7 (−OCOCH$_3$), 20.6 (−OCOCH$_3$); ^{1}H NMR (400 MHz, CDCl$_3$)δ: **Minor anomer**: 5.62 (d, J = 8.8 Hz, 1H, H-1), 5.34 (t, J = 10.2 Hz, 1H, H-3), 1.26 (s, 9H, −C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, CDCl$_3$)δ: 169.9 (−OCOCH$_3$), 169.7 (−OCOCH$_3$), 168.3 (−OCOCH$_3$), 131.0 (Ar), 130.6 (Ar), 126.2 (Ar), 92.8 (C-1), 72.3 (C-3), 71.9 (C-5), 68.9 (C-4), 61.7 (C-6), 44.8 (C-2), 31.6 (C-7), 20.8 (−OCOCH$_3$), 20.6 (−OCOCH$_3$). HRMS (ES + -TOF) m/z: [M + NH$_4$]$^+$ Calcd for $C_{25}H_{38}NO_9S$ 528.2267; Found: 528.2269.

4.1.2. 2-(Acetoxymethyl)-9-tert-butyl-2,3,4,4a,5,10b-hexahydrothiochromeno[4,3-b]pyran-3,4-diyl diacetate (**3g**)

Sulfide **2g** (100 mg, 0.19 mmol), was dissolved in dry dichloromethane (3 mL) under an atmosphere of nitrogen and stirred together with 3 Å molecular sieves at room temperature for 1 h. The mixture was cooled down to 0 °C and then treated dropwise with $BF_3{\cdot}Et_2O$ (414 μL, 0.783 mmol) of 48% BF_3 solution in diethylether. After stirring at this temperature for 5 min, Et_3N (0.7 mL) was added and the solids removed by filtration through a pad of Celite® bed. The solution was then diluted with water (10 mL) and the aqueous phase was extracted with dichloromethane (3 × 10 mL). The combined organic phases were successively washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane, 2:8) to yield the corresponding thiochroman **3g**: 66 mg, 75% yield; white solid, Mp 215–217 °C; IR (neat cm^{-1}) 2963, 1744, 1362, 1219, 1031, 831; $[\alpha]_D$ (c 0.1, $CHCl_3$) +89.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55 (dd, J = 0.4 Hz and 2.0 Hz, 1H, Ar), 7.16 (ddd, J = 0.5 Hz, 2.3 Hz and 8.2 Hz, 1H, Ar), 7.00 (d, J = 8.4 Hz, 1H, Ar), 5.50 (dd, J = 9.0 Hz, and 11.0 Hz, 1H, H-3), 5.17 (d, J = 6.0 Hz, 1H, H-1), 5.04 (dd, 1H, J = 9.2 Hz and 10.0 Hz, H-4), 4.26 (dd, J = 2.9 Hz and 12.2 Hz, 1H, H-6$_a$), 4.04 (dd, J = 2.0 Hz, and 12.0 Hz, 1H, H-6$_b$), 3.75–3.63 (m, 1H, H-5), 3.33 (dd, J = 2.4 Hz and 13.6 Hz, 1H, H-7$_a$), 2.84 (dd, J = 4.4 Hz and 13.6 Hz, 1H, H-7$_b$), 2.78–2.65 (m, 1H, H-2), 2.10 (s, 3H, −OCOCH$_3$), 2.04 (s, 3H, −OCOCH$_3$), 1.95 (s, 3H, −OCOCH$_3$), 1.27 (s, 9H, −C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, CDCl$_3$)δ: 170.7 (−OCOCH$_3$), 170.2 (−OCOCH$_3$), 170.0(−OCOCH$_3$), 148.4 (Ar), 130.7 (Ar), 129.4 (Ar), 126.5 (Ar), 125.4 (Ar), 124.2 (Ar), 72.4 (C-1), 70.7 (C-4), 70.0 (C-3), 69.6 (C-5), 63.1 (C-6), 36.7 (C-2), 34.4 (−C(CH$_3$)$_3$), 31.2 (−C(CH$_3$)$_3$), 25.9 (C-7), 20.8 (−OCOCH$_3$), 20.7 (−OCOCH$_3$). HRMS (ES + -TOF) m/z: [M + NH$_4$]$^+$ Calcd for $C_{23}H_{34}NO_7S$ 468.2056; Found: 468.2054.

4.1.3. 9-tert-Butyl-2-(hydroxymethyl)-2,3,4,4a,5,10b-hexahydrothiochromeno[4,3-b]pyran-3,4-diol (**4**)

To a solution of thiochroman **3g** (92.0 mg, 0.18 mmol) in methanol (5 mL) was added anhydrous K_2CO_3 (2.49 mg, 0.018 mmol) and the mixture was stirred at room temperature for 3 h upon which the reaction indicated disappearance of starting material on TLC. The solvent was removed under reduced pressure and the residue was submitted for purification by silica gel column chromatography (100% ethyl acetate) to afford the thiochroman **4**: 45 mg, 77% yield; white solid, Mp 222–224 °C; IR (neat cm^{-1}) 3268, 2909, 1078, 869, 778; $[\alpha]_D$ (c 0.1, CH_3OH) +105.5; ^{1}H NMR (400 MHz, CD_3OD)δ: 7.62 (dd, J = 0.8 Hz and 2.0 Hz, 1H, Ar), 7.06 (ddd, J = 0.7 Hz, 2.3 Hz and 8.4 Hz, 1H, Ar), 6.88 (d, J = 8.0 Hz, 1H, Ar), 4.99 (d, J = 5.6 Hz, 1H, H-1), 3.80–3.71 (m, 2H, H-3, H-6$_a$), 3.61 (dd, J = 6.2 Hz and 11.8 Hz, 1H, H-6$_b$), 3.34–3.22 (m, 2H, H-4, H-7$_a$), 3.18–3.12 (m, 1H, H-5) 3.09 (dd, J = 4.0 Hz and 13.2 Hz, 1H, H-7$_b$), 2.31–2.20 (m, 1H, H-2), 1.21 (s, 9H, −C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, CD_3OD)δ: 148.9 (Ar), 132.5 (Ar), 132.3 (Ar), 126.8 (Ar), 125.8 (Ar), 125.6 (Ar), 76.0 (C-5), 73.5 (C-1 and C-4) 71.3 (C-3), 63.3 (C-6), 40.0 (C-2), 35.3 (−C(CH$_3$)$_3$), 31.7 (−C(CH$_3$)$_3$), 27.1 (C-7). HRMS (ES + -TOF) m/z: [M+H]$^+$ Calcd for $C_{17}H_{25}O_4S$ 325.1474; Found: 325.1487.

4.1.4. 2-(Acetoxymethyl)-9-tert-butyl-2,3,4,4a,5,10b-hexahydro-S-S-dioxothiochromeno[4,3-b]pyran-3,4-diyl diacetate (**7g**)

Sulfide **3g** (126 mg, 0.280 mmol) was added to a vigorously stirring suspension of wet alumina (852 mg wetted with 90 μL of water) and OXONE (1.4 g, 2.28 mmol) in DCM (5 mL). The reaction mixture was stirred to completion at room temperature for 12 h. The reaction mixture was then filtered to remove the adsorbent. Evaporation of the solvent and flash chromatographic purification on silica gel (ethyl acetate/hexane, 3:7) afforded the title sulfone: 103 mg, 76% yield; white solid, Mp 183–185 °C; IR (neat cm^{-1}) 2955, 1745, 1218, 1035, 786; $[\alpha]_D$ (c 0.1, CH_3OH) +26.5; ^{1}H NMR (400 MHz, CD_3OD)δ 7.82 (d, J = 8.4 Hz, 1H, Ar), 7.61–7.55 (m, 2H, Ar), 5.33–5.23 (m, 2H, H-1, H-3), 4.83 (t, J = 6.0 Hz, 1H, H-4), 4.73 (dd, J = 8.0 Hz and 12.0 Hz, 1H, H-6$_a$), 4.05 (dd, J = 3.0 Hz and 12.2 Hz, 1H, H-6$_b$), 4.06–4.00 (m, 1H, H-5), 3.77 (dd, J = 8.6 Hz and 14.2 Hz, 1H, H-7$_a$), 3.36 (dd, J = 3.4 Hz and 14.2 Hz, 1H, H-7$_b$), 3.10–3.00 (m, 1H, H-2), 2.12 (s, 3H, −OCOCH$_3$), 2.09 (s, 3H, −OCOCH$_3$), 2.01 (s, 3H, −OCOCH$_3$), 1.32 (s, 9H, (−C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, CDCl$_3$)δ: 170.6 (−OCOCH$_3$), 169.5 (−OCOCH$_3$), 169.0 (−OCOCH$_3$), 157.0 (Ar), 135.9 (Ar), 132.5 (Ar), 127.3 (Ar), 126.0 (Ar), 123.7 (Ar), 73.0 (C-5), 69.4 (C-3), 68.1 (C-4), 67.5 (C-1), 61.2 (C-6), 48.4 (C-7), 37.1 (C-2), 35.2 (−C(CH$_3$)$_3$), 30.9 (−C(CH$_3$)$_3$), 20.8 (−OCOCH$_3$), 20.7 (−OCOCH$_3$). HRMS (ES + -TOF) m/z: [M+H]$^+$ Calcd for $C_{23}H_{31}O_9S$ 483.1689; Found: 483.1686.

4.1.5. 9-tert-Butyl-2-(hydroxymethyl)-2,3,4,4a,5,10b-hexahydro-S-S-dioxothiochromeno[4,3-b]pyran-3,4-diol (**8**)

To a solution of thiochroman **7g** (80.0 mg, 0.177 mmol) in methanol (5 mL) was added anhydrous K_2CO_3 (2.45 mg, 0.0177 mmol) and the mixture was stirred at room temperature for 3 h upon which the reaction indicated disappearance of starting material on TLC. The solvent was removed under reduced pressure and the residue was submitted for purification by silica gel column chromatography (100% ethyl acetate) to afford the thiochroman **8**: 43 mg, 68% yield; white solid, Mp 218–220 °C; IR (neat cm^{-1}) 3331, 2922, 1600, 1350, 1112, 788; $[\alpha]_D$ (c 0.1, CH_3OH) +51.0; ^{1}H NMR (400 MHz, CD_3OD)δ: 7.76 (t, J = 1.2 Hz, 1H, Ar), 7.67 (d, J = 8.4 Hz, 1H, Ar), 7.52 (ddd, J = 0.8 Hz, 2.0 Hz and 8.4 Hz, 1H, Ar), 5.21 (d, J = 5.6 Hz, 1H, H-1), 3.88–3.73 (m, 3H, H-3, H-6$_a$ and H-7$_a$), 3.68–3.55 (m, 2H, H-6$_b$ and H-7$_b$), 3.28–3.12 (m, 2H, H-4 and H-5), 2.63–2.50 (m, 1H, H-2), 1.26 (s, 9H, −C(CH$_3$)$_3$); ^{13}C NMR (100 MHz, CD_3OD)δ: 158.1 (Ar), 138.0 (Ar), 135.8 (Ar), 127.3 (Ar), 125.9 (Ar), 124.4 (Ar), 77.3 (C-4), 73.4 (C-5), 72.1 (C-1), 71.1 (C-3), 63.2 (C-6), 50.6 (C-7), 42.6 (C-2), 36.3 (−C(CH$_3$)$_3$), 31.4 (−C(CH$_3$)$_3$). HRMS (ES + -TOF) m/z: [M+H]$^+$ Calcd for $C_{17}H_{25}O_6S$ 357.1372; Found: 357.1363.

4.2. In vitro antimalarial assay

The *in vitro* antimalarial activity of test samples against the 3D7 and FCR3 strains of the malaria parasite, *P. falciparum*, is measured by parasite survival using parasite lactate dehydrogenase (pLDH) assay. This enzymatic assay involves the parasite lactate dehydrogenase, which is distinguishable from the host lactate dehydrogenase. Lactate dehydrogenase is an enzyme found in all the cells and catalyses the formation of pyruvate from lactate reducing a co-enzyme NAD (nicotinamide adenine dinucleotide) to NADH. In parasites, the NAD analogue APAD (3-acetylpyridine adenine nucleotide) is reduced to APADH and upon this reduction the yellow NBT/PES (nitro blue tetrazolium + phenazine ethosulphate) is converted to purple formazan crystals. The absorbance is read at 620 nm using a multiwell spectrophotometer (Infinite F500). The

formation of these crystals indicates the pLDH activity and therefore the survival of parasites.

The percentage survival of parasites is a measure of their inhibitory activity against *P. falciparum*. This inhibitory activity is determined by the IC_{50} and is measured by making 10 three-fold serial dilutions of the test samples in triplicates in a transparent 96-well flat bottom plate (Netstar). The plate is put in an airtight box, gassed and incubated for 48 h followed by developing with NBT/PES reagent. The IC_{50} are expressed as the % parasite survival relative to the control, calculated from fitted sigmoidal dose–response curves. The dose–response curves were obtained by plotting percentage parasite survival against the logarithm of the concentration using the GraphPad Prism software package (GraphPad software, Inc, California, USA). IC_{50}s were calculated graphically by interpolation from these curves. The Z'-factor for all the tests were found to be between 0.5 and 1.0.

4.3. In vitro cytotoxicity assay

The WI-38 cell line – normal Human Fetal Lung Fibroblast from ECACC was routinely maintained as a monolayer cell culture at 37 °C, 5% CO_2, 95% air and 100% relative humidity in EMEM containing 10% fetal bovine serum, 2 mM L-glutamine and 50 μg/ml gentamicin. For screening experiment, the cells (21-50 passages) were inoculated in a 96-well microtiter plates at plating densities of 10 000 cells/well and were incubated for 24 h. After 24 h the cells were treated with the experimental drugs which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations. Cells without drug addition served as control. The blank contains complete medium without cells. Parthenolide was used as a standard. The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein-bound dye was extracted with 10 mM Tris base for optical density determination at the wavelength 540 nm using a multiwell spectrophotometer. Data analysis was performed using GraphPad Prism software. 50% of cell growth inhibition (IC_{50}) was determined by non-linear regression.

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Appendix A. Supplementary data

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