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Expanding the SAR of Nontoxic Antiplasmodial Indolyl-3-ethanone Ethers and Thioethers

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Despite major strides in reducing *Plasmodium falciparum* infections, this parasite still accounts for roughly half a million annual deaths. This problem is compounded by the decreased efficacy of artemisinin combination therapies. Therefore, the development and optimisation of novel antimalarial chemotypes is critical. In this study, we describe our strategic approach to optimise a class of previously reported antimalarials, resulting in the discovery of 1-(5-chloro-1*H*-indol-3-yl)-2-[(4-cyanophenyl)thio]ethanone (**13**) and 1-(5-chloro-1*H*-indol-3-yl)-2-[(4-nitrophenyl)thio]ethanone (**14**), whose activity was equipotent to that of chloroquine against the *P. falciparum* 3D7 strain. Furthermore, these compounds were found to be nontoxic to HeLa cells as well as being non-haemolytic to uninfected red blood cells. Intriguingly, several of our most promising compounds were found to be less active against the isogenic NF54 strain, highlighting possible issues with long-term dependability of malarial strains. Finally compound **14** displayed similar activity against both the NF54 and K1 strains, suggesting that it inhibits a pathway that is uncompromised by K1 resistance.

Introduction

Plasmodium falciparum induced malaria remains one of the most prevalent parasitic disease worldwide.[1] The WHO reported 212 million new cases of malaria and 429000 deaths in 2015, with children under five years of age and severely disadvantaged populations being particularly vulnerable.[2–4] Chemotherapeutics have formed a cornerstone of efforts to decrease the burden of malaria, but have been hampered by the continuous emergence of resistance to first-line treatments.[5–8] The rapid and efficacious nature of artemisinin combination therapies (ACT) against multidrug-resistant parasites[9] coupled with widespread adoption of ACT as a first-line treatment in most malaria-endemic countries[10] has contributed to the significant progress made in decreasing the rates of malarial transmission.[11] However, reports of delayed parasite clearance after standard ACT dosing regimens indicate that the current trends in decreasing the malaria burden may be under threat,[12–14] and highlights the urgent need to discover chemotypes that disrupt vital malarial biochemical processes, either via new targets, or by overcoming resistance pathways in current validated targets. The post-genomic era of drug discovery has provided stunning insight into biological pathways and has facilitated target-based drug discovery against human derived diseases.[15] However, targeted screening approaches have proven more challenging against infectious disease.[16] Conversely, phenotypic screening takes advantage of the greater chemical space sampled in the complex cellular environment, thereby increasing the probability of unearthing new classes of biological targets, or the discovery of active compounds that act against multiple targets.[17–20]

Recent studies conducted by our research group, focusing on indole-based antimalarials,[21,22] identified a new class of compounds, two members of which (**1** and **2**, Figure 1) were found to possess good antimalarial activity in an in vitro phenotypic screening campaign against the chloroquine-sensitive

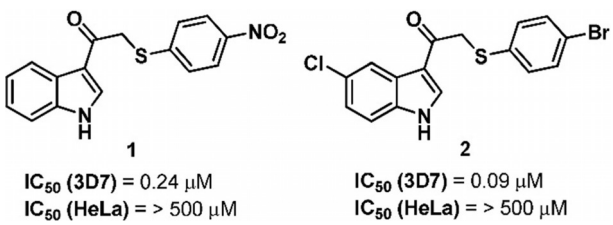

Figure 1. Promising early antimalarial compounds identified through phenotypic screening against the 3D7 *P. falciparum* strain.

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3D7 strain, with no observable mammalian cytotoxicity against a control HeLa cell line.

Accordingly, we rationalised a cohort of analogues (**3**–**22**) of compounds **1** and **2**, which were designed to answer specific questions pertaining to our structure–activity relationship (SAR). This study provided new insight into the SAR and pharmacophore of this class of compounds, which are discussed in detail below, and led to the discovery of two new compounds (**13** and **14**), which displayed potent activity against the 3D7 strain, similar to that of chloroquine, in the absence of notable HeLa cytotoxicity or haemolysis. Interestingly, biological assessment of our most active compounds against the chloroquine-sensitive NF54 strain resulted in a decrease in biological activity into the mid-nanomolar range, which was maintained against the multidrug-resistant K1 strain.

Results and Discussion

SAR strategy

SAR analysis from our initial study had provided suitable insight into positional substituents required for optimal activity (Figure 2). In this study, we sought to resolve greater detail of specific requirements at each position through strategic bioisosteric replacement of specific moieties, as well as combinations of promising substituents in order to enhance antimalarial potency without compromising mammalian toxicity.

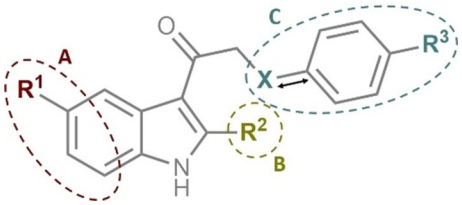


Figure 2. SAR analysis from our previous study identified three regions of interest for further investigation. Region A: C5 substituent at R^1 increases antimalarial activity, while C6 and C7 decreases activity. The physicochemical properties of the R^1 substituent which contribute toward activity is unresolved. Region B: Influence of R^2 substitution on activity unexplored. Region C: X = NH increases toxicity. Unclear whether an O or S in this region is superior for activity. Extension of chain between X and the phenyl ring not tolerated; *para* substitution on the ring is essential. Electron-donating groups at R^3 decrease activity and introduce toxicity. Strong electron-withdrawing groups increase activity.

Region A analogues had indicated a preference for C5 substitution; however, our C5-substituted analogues were limited to a chlorine or bromine derivative, providing limited information pertaining to what properties of the substituents are responsible for enhanced activity. We were particularly interested in whether the activity was possibly due to substituent size, enhanced lipophilicity, or the electron-withdrawing nature of the substituent. Halogen atoms are common substituents in medicinal chemistry, due to their contribution to hydrophobic interactions as well as halogen bond formation.[23] Moreover, their varying atomic radii allow subtle interrogation of substituent size limitations on biological activity. We therefore expanded our "halogenated set" through the incorporation of 5-fluoroindole analogues. The introduction of supposedly chemically inert methyl moieties does, on occasion, decrease the free energy required to desolvate the ligand in question, thereby energetically favouring binding in a hydrophobic pocket, in what is referred to as the "magic methyl effect".[24] Furthermore, melatonin features a C5-methoxylated indole, which has been implicated as a critical factor in malaria biology and exploited as an endogenous ligand for antimalarial drug design.[25–27] Consequently, we opted to synthesise analogues featuring a C5-methyl- or methoxyindole. In addition to this strategic structural modification, a C2-methylindole was incorporated to explore potential effects of this substitution.

The electronegativity of halogen atoms renders them net electron-withdrawing groups, which can potentially alter the electronic environment of the indole ring. We therefore sought to incorporate a bioisosteric nitrile moiety,[28,29] which has also demonstrated the capacity to displace bound waters for a net entropic gain.[30]

Region C analogues had focussed mainly on thiophenols, which had indicated firstly that *para*-substituted analogues are preferred for activity, with the strongly electron-withdrawing nitro group providing the greatest antimalarial activity. Electron-donating groups, particularly amines, negatively influenced activity and introduced significant mammalian cytotoxicity.

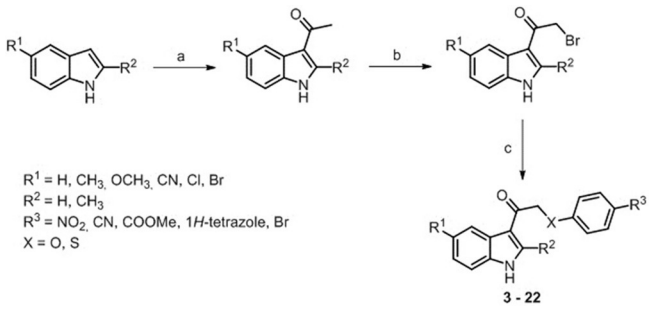
In addition, we had determined that extension of the chain between the sulfur atom and the aromatic ring was not well tolerated from an antimalarial and cytotoxicity perspective, as well as ruling out amides and α-aminocarbonyls as viable linker moieties between the indole and phenyl rings. However, it remained unresolved whether an α-oxo- or thiocarbonyl, is a preferable linker group. Accordingly, we generated a small cohort of oxygen- and sulfur-containing compounds featuring an electron-withdrawing nitro group or related bioisosteres.

Synthesis and biological evaluation

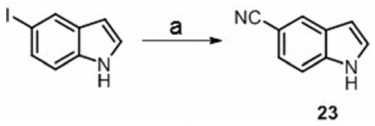
Our general synthetic pathway closely followed that of our original study,[21,22] whereby the appropriately substituted indole underwent a Friedel–Crafts acetylation, followed by selective bromination to yield the requisite α-bromoketone intermediates. These were then subjected to nucleophilic displacement with a suitable phenol or thiophenol in the presence of potassium carbonate to generate our final compounds at yields varying from poor to excellent (Scheme 1). Thiophenols featuring a strongly electron-withdrawing substituent have demonstrated the capacity to induce reductive dehalogenation of α-halogenated ketones.[31,32] However, this phenomenon was not observed to any significant extent in this study.

5-Cyano indole (**23**) was prepared in good yield from 5-iodoindole using a CuI/PPh_3 catalytic system with CuCN as a nitrile source (Scheme 2), while 3-acetyl-5-methoxyindole (**24**) was synthesised from 5-bromo-3-acetylindole via an Ullman-type nucleophilic displacement (Scheme 3).

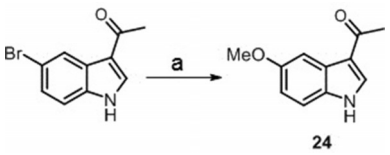
Tetrazole **25** was generated via a 1,3-dipolar cycloaddition of 4-cyanophenol as per the method of Koyama et al.[33]



Scheme 1. Reagents and conditions: a) Acetyl chloride, $SnCl_4$, CH_2Cl_2, CH_3NO_2, 0 8C–RT, 2 h, N_2; b) $CuBr_2$, $CHCl_3$/EtOAc, reflux, variable times; c) O or S nucleophile, K_2CO_3, acetone, reflux, 5 h.

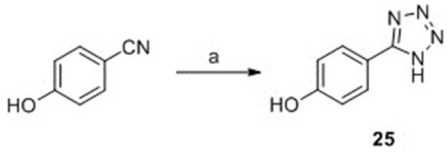


Scheme 2. Reagents and conditions: a) CuI, PPh_3, CuCN, DMF, 140 8C, 24 h, N_2.



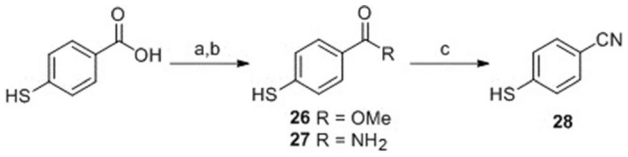
Scheme 3. Reagents and conditions: a) CuI, NaOMe, DMF, 6 h, reflux, N_2.

(Scheme 4). 4-Mercaptomethylbenzoate (**26**) was synthesised from 4-mercaptobenzoic acid via the acid chloride, while a similar method was used to generate the corresponding primary

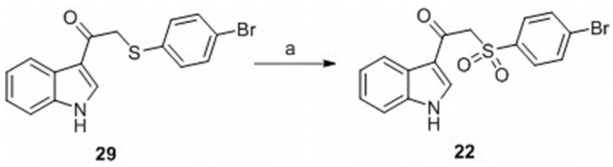


Scheme 4. Reagents and conditions: a) NaN_3, NH_4Cl, DMF, 125 8C, 7 h, N_2.

amide **27**, which was dehydrated into the desired 4-cyanothiophenol (Scheme 5). The remaining indoles, phenols, and thiophenols were all commercially available. Finally, we applied an *m*-CPBA oxidation method[34] to derive a sulfone analogue (**22**) of an antimalarial analogue **29**, previously reported by us[21] (Scheme 6).



Scheme 5. Reagents and conditions: a) $SOCl_2$, 70 8C, 2 h; b) NaOMe or NH_4OH; c) $POCl_3$, DMF, 0 8C, 2 h.



Scheme 6. Reagents and conditions: a) *m*-CPBA, CH_2Cl_2, RT, 2 h.

Compounds **3**–**22** were initially subjected to a single-concentration screen against both the 3D7 and HeLa lines at 20 mm (Figure 3). This screen identified several compounds that inhibited *P. falciparum* cell viability to below 15%. Compounds **6**, **11**, **17**, and **22** were unable to decrease 3D7 cell viability below 50% and were not subjected to further assessment. Compound **17** seemingly enhanced malarial proliferation, indicating possible assay interference. Importantly, none of the compounds inhibited HeLa cell viability to below 77%, indicating that these compounds display a high degree of selectivity. To refine our SAR understanding, compounds that inhibited 3D7 viability to below 50% were submitted for IC_{50} determination (Table 1).

Compounds **3**–**10** were designed as oxygen-containing analogues of compounds **1** and **2**. The negligible loss of biological activity observed through compounds **3** and **4** suggested that both an oxygen and nitrile moiety were relatively well tolerated. However, activity was not maintained with an electron-withdrawing tetrazole (**5**). Furthermore, the incorporation of a pyridine (**6**) resulted in a significant decrease in activity. Activity was somewhat restored upon incorporation of a hydrophobic 5-methyl moiety (**7**), and to a lesser extent with the 2-methyl (**8**), whereas compound **9**, which features a strongly electron-withdrawing nitrile moiety at the indole C5 position, possessed no observable activity. Finally, biological activity was significantly improved through the combination of a *para*-cyanophenol and a 5-chloroindole (**10**). We proceeded to prepare a series of sulfur-containing analogues (**11**–**22**). As a direct comparison of **1** and **4**, compound **11** resulted in a moderate yet notable improvement in biological activity, while replacement of the nitrile with a methyl ester (**12**) dramatically decreased activity. Compounds **13** and **14**, which combined design elements of compounds **1**, **2**, and **11** increased biological activity into the low nanomolar range, with activity similar to that of chloroquine.

Activity was reduced upon replacement of the C5 chlorine with fluorine (**15** and **16**), while the nitrile analogue of compounds **2** and **15** (compound **17**) was inactive. Compounds **18**–**20** were designed as sulfur analogues of **7**, **8**, and **9**, respectively. Compounds **18** and **20** in particular, showed a vast improvement in biological activity over their corresponding oxygen analogues. Interestingly, incorporation of a C5 methoxy substituent (**21**) showed a moderate but discernible improvement in biological activity relative to **18**.

The final compound tested in this series, a sulfone derivative (**22**) of a low-micromolar inhibitor from our original study, was found to be inactive. With respect to our study questions, these data provided several new elements to our SAR hypothesis. The differences in activity between compounds **7**–**10** and

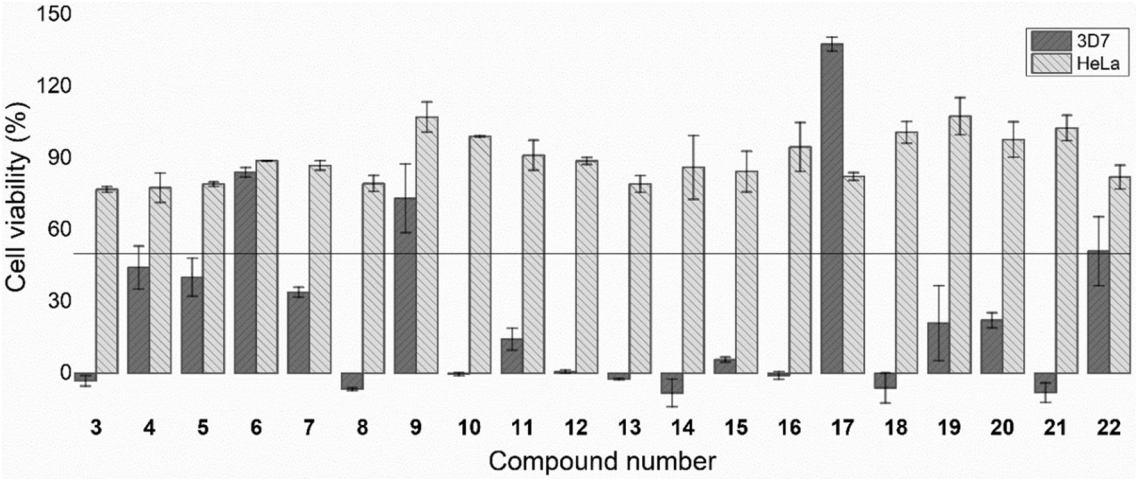
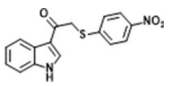
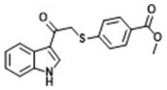
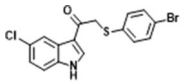
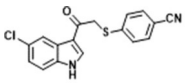
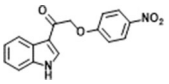
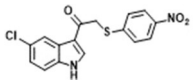
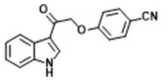
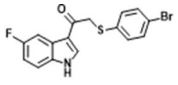
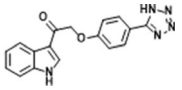
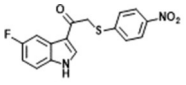
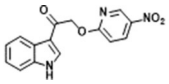
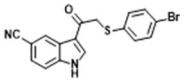
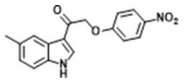
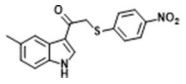
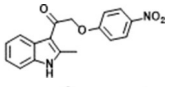
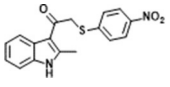
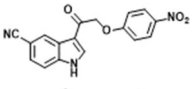
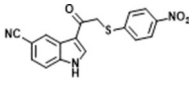
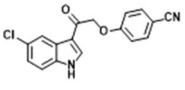
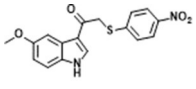
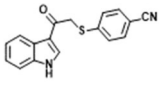
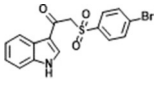


Figure 3. Percent viability of 3D7 and HeLa cell lines at 20 mm. Vales are the mean ± SD of experiments performed in duplicate. Compounds that decreased cell viability below the 50% threshold were submitted for IC_{50} determination. In general, low mammalian cytotoxicity was observed.

Table 1. IC_{50} values of compounds **1**–**22** against *Pf* strain 3D7.

No.	Structure	IC_{50} [mm][a]	No.	Structure	IC_{50} [mm][a]
1		0.20[b]	**12**		3.5 ± 0.7
2		0.090[b]	**13**		0.027 ± 0.0
3		0.33 ± 0.1	**14**		0.044 ± 0.0
4		0.41 ± 0.0	**15**		1.1 ± 0.2
5		8.3 ± 0.3	**16**		0.098 ± 0.0
6		ND[c]	**17**		ND[c]
7		0.87 ± 0.1	**18**		0.25 ± 0.0
8		2.2 ± 0.2	**19**		1.4 ± 0.3
9		ND[c]	**20**		0.54 ± 0.0
10		0.1 ± 0.0	**21**		0.13 ± 0.0
11		0.13 ± 0.0	**22**		ND[c]
				chloroquine	0.03 ± 0.0

[a] Values are the mean ± SD of biological repeat experiments performed in technical duplicate. [b] Values determined in a previous study.[21] [c] Not determined.

13, and **18**–**20** indicates that the sulfur moiety is seemingly superior to an oxygen in terms of 3D7 inhibition; however, oxidation of the sulfur, which is a possible metabolic pathway of this series, decreases activity, with a negligible effect on cytotoxicity. Strongly electron-withdrawing groups are beneficial for biological activity, but the relative loss of activity observed with the tetrazole and ester moieties suggests that this effect is not ubiquitous to all electron-withdrawing groups, and a size limitation may exist at the putative binding site. C2 methylation was seemingly detrimental to activity overall, while hydrophobicity is seemingly more important than electron-withdrawing capacity with respect to C5 substitution. Furthermore, the moderate decrease in activity observed between compounds **14** and **16** in addition to the moderate gain observed between **18** and **21**, point toward an optimal substituent size in this region, which is currently best exploited with a chlorine analogue. However, this may still be a result of a halogen bonding effect. Due to their encouraging activity against 3D7, compounds **13** and **14** were investigated for possible haemolysis, where they were found to be nonlytic at concentrations far exceeding the HC_{50} value of the haemolytic peptide gramicidin D (Figure 4).

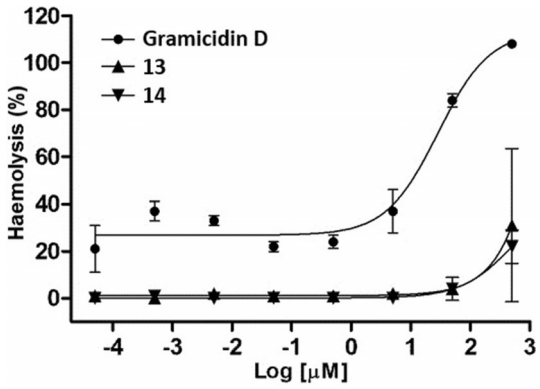


Figure 4. Haemolysis assay of compounds **13** and **14** versus gramicidin D, which indicates low levels of haemolysis at concentrations in excess of the HC_{50} value of gramicidin D. Values are the mean ± SD of biological repeat experiments performed in technical duplicate.

Compounds **10**, **11**, **13**, **14**, **18**, and **21** were selected for biological evaluation against the isogenic NF54 strain as well as the multidrug-resistant K1 strain (Table 2). In this screen, all compounds suffered from a dramatic decrease in biological activity against NF54, particularly **10**, **13**, and **18**. The remaining compounds **11**, **14**, and **21** all retained lower, albeit encouraging, mid-nanomolar activity. Furthermore, this strain was seemingly more susceptible to chloroquine than 3D7. Compound **14** was the most active of our compounds against the K1 stain, in the mid-nanomolar range, while notable improvements in susceptibility were observed with compounds **13** and **20**. The general consistency and/or improvement in compound activity between the NF54 and K1 strains suggests that these compounds do not inhibit a pathway that is compromised by K1 resistance, as shown by a >2-fold difference between the isolates. However, the question as to the major changes in biological activity between the isogenic chloroquine-sensitive strains is more perplexing.

Table 2. IC_{50} values of compounds **10**, **11**, **13**, **14**, **18**, and **21** against *Pf* strains NF54 and K1.

No.	IC_{50} [mM][a]	
	NF54	K1
10	0.77 ± 0.0	0.9 ± 0.1
11	0.5 ± 0.0	0.45 ± 0.1
13	>1	0.57 ± 0.1
14	0.28 ± 0.0	0.36 ± 0.0
18	0.9 ± 0.0	0.66 ± 0.0
21	0.65 ± 0.0	0.48 ± 0.1
chloroquine	0.0034 ± 0.0	
artesunate		0.0027 ± 0.0

[a] Values are the mean ± SEM of experiments performed in triplicate.

It must be noted that these assays were conducted independently of one another in separate laboratories, where subtle inter-lab variability may account for variations in the biological data. Furthermore, recent studies have demonstrated that phenotypic and genotypic characteristics of *P. falciparum* isolates are unstable in long-term culture, which alter the susceptibility of different *P. falciparum* strains to different drugs.[35,36] To illustrate, cultures of the 3D7 strain of *P. falciparum* have been found to express significantly higher levels of the adhesion protein encoding *var* genes than NF54,[37] which is particularly active in the ring stage.[38] While this specific alteration may not adequately answer our specific alterations in biological activity, it does highlight the challenges in antimalarial drug discovery, and the need for robust multifaceted datasets to adequately assess promising compounds.

Conclusions

In summary, this study sought to expand the SAR of a promising class of nontoxic antimalarial compounds through specific compound modifications. Through this strategy, we have refined our SAR hypothesis against a currently unknown target. Furthermore, compounds **13** and **14** were identified as potent non-haemolytic inhibitors of *P. falciparum* 3D7 with negligible cytotoxicity toward a HeLa cell line. However, this activity was decreased significantly when scrutinised against two further *P. falciparum* strains. Whilst the reasons for this remain unclear at this stage, compound **14** maintained encouraging activity against NF54 as well as K1, indicating that it inhibits a biological pathway independent of those compromised by resistance in K1, and it remains a promising compound in malaria drug discovery. Further studies are planned to elucidate the mechanism of action of this class as well as to fully elucidate the pharmacophore.

Experimental Section

Chemistry

General methods: NMR spectra were obtained with Bruker Fourier 300, 400, or 600 MHz Avance II spectrometers. Chemical shifts are

reported in ppm with residual reference solvent resonances as follows: [D$_6$]acetone d_H 2.05, d_C 29.8, 206.2; CD_3OD d_H 3.31, d_C 49.0; [D$_6$]DMSO d_H 2.50, d_C 39.5 ppm.[39] High-resolution mass spectrometry was performed on a Waters Synapt G2 TOF instrument with an ESI source. IR spectra were obtained using a PerkinElmer Spectrum 2000 FTIR instrument. Melting points were determined using a SMP30 Advanced Digital Melting Point apparatus. Flash column chromatography was performed using Kieselgel 60 (230–400 mesh) silica gel. All bulk solvents were distilled prior to use, while anhydrous solvents were prepared by standard procedures outlined by Perrin and Armarego.[40] Compounds **13** and **14** were determined to have a measured purity of ¨ 95% by LC–UV/Vis–MS.

5-Cyanoindole (23): CuI (80 mg, 0.41 mmol) was suspended in anhydrous *N*,*N*-DMF (20 mL) in a two-neck round-bottom flask under a nitrogen atmosphere. To this, 5-iodoindole (500 mg, 2.05 mmol), PPh_3 (107 mg, 0.41 mmol) and CuCN (552 mg, 6.17 mmol) were added, and allowed to stir for 24 h at 140 8C, after which time the reaction mixture was allowed to cool. Organic material was dissolved in EtOAc, and washed with saturated brine solution (3× 10 mL). The extract was dried over anhydrous $MgSO_4$ before concentrating in vacuo. Compound **23** was purified by flash column chromatography (hexane/EtOAc, 7:3) to yield a brown amorphous solid (77%): IR (film): $\tilde{\nu}_{max}$ = 3400, 2224, 1610, 1414, 769, 732 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 300 MHz): d_H = 11.65 (1H, brs, NH-1), 8.08 (1H, dd, *J* = 1.6, 0.7 Hz, H-4), 7.57–7.54 (2H, m, H-2, H-7), 7.41 (1H, dd, *J* = 8.4, 1.6 Hz, H-6), 6.58 ppm (1H, dd, *J* = 3.2, 0.7 Hz, H-3); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): d_C = 137.6, 128.1, 127.4, 125.7, 123.7, 120.8, 112.7, 102.1 ppm.

1-(5-Methoxy-1H-indol-3-yl)ethanone (24): Sodium methoxide (sodium 362 mg, methanol, 10 mL) was added to a stirring mixture of CuI (484 mg, 2.5 mmol), 1-(5-bromo-1*H*-indol-3-yl)ethanone (250 mg, 1.1 mmol) in anhydrous *N*,*N*-DMF (15 mL). The reaction was allowed to proceed for 6 h under reflux, following which the reaction mixture was allowed to cool before filtering. The crude organics were re-dissolved in EtOAc and washed with 10% NaOH (30 mL). The mixture was dried over $MgSO_4$, concentrated in vacuo and purified by flash column chromatography (CH_2Cl_2/EtOAc, 4:1) to yield a brown solid (20%): IR (film): $\tilde{\nu}$ = 3150, 2934, 1613, 1518, 1427, 804, 652, 587 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz): d_H = 8.08 (1H, s, H-2), 7.77 (1H, d, *J* = 2.4 Hz, H-4), 7.32 (1H, d, *J* = 8.9 Hz, H-7), 6.86 (1H, dd, *J* = 8.9, 2.4 Hz, H-6), 3.83 (3H, s, H-9), 2.50 ppm (3H, s, H-2'); ^{13}C NMR (CD_3OD, 75 MHz): d_C = 195.0, 156.2, 134.3, 131.9, 126.2, 116.9, 112.9, 112.1, 103.1, 54.6, 25.6 ppm.

The synthesis and characterisation of several 3-acetylindoles and corresponding a-bromoketones has previously been reported by us.[41,42] Below are compounds used in this study, which are yet to be reported by us.

1-(5-Methyl-1H-indol-3-yl)ethanone: (19%), brown solid; IR (film): $\tilde{\nu}_{max}$ = 3132, 2918, 1621, 1431, 1374, 943, 798, 757 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 300 MHz): d_H = 11.77 (1H, brs, NH-1), 8.22 (1H, s, H-2), 7.98–7.96 (1H, m, H-4), 7.34 (1H, d, *J* = 8.3 Hz, H-7), 7.02 (1H, dd, *J* = 8.3, 1.6 Hz, H-6), 2.42 (3H, s, H-2'), 2.39 ppm (3H, s, H-8); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): d_C = 193.0, 135.3, 134.6, 130.7, 126.0, 124.5, 121.4, 117.0, 112.1, 27.6, 21.7 ppm.

1-(2-Methyl-1H-indol-3-yl)ethanone: (43%), brown solid; IR (film): $\tilde{\nu}_{max}$ = 3162, 3050, 2970, 1609, 1528, 1444, 1413, 1390, 969, 905, 739 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): d_H = 11.81 (1H, brs, NH-1), 8.02–8.00 (1H, m, H-4), 7.37–7.35 (1H, m, H-7), 7.14–7.11 (2H, m, H-5, H-6), 2.67 (3H, s, H-8), 2.51 ppm (3H, s, H-2'); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): d_C = 193.5, 144.6, 135.1, 127.3, 122.2, 121.7, 121.0, 113.9, 111.6, 31.3, 15.4 ppm.

1-(5-Cyano-1H-indol-3-yl)ethanone: (46%), cream solid; IR (film): $\tilde{\nu}_{max}$ = 3109, 2220, 1739, 1628, 1615, 1444, 1376, 805, 655 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 300 MHz): d_H = 12.40 (1H, brs, NH-1), 8.52 (1H, dd, *J* = 1.6, 0.8 Hz, H-4), 8.51 (1H, s, H-2), 7.65 (1H, dd, *J* = 8.5, 0.8 Hz, H-7), 7.58 (1H, dd, *J* = 8.5, 1.6 Hz, H-6), 2.48 ppm (3H, s, H-2'); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): d_C = 192.9, 138.5, 136.6, 126.3, 125.7, 125.0, 120.2, 116.9, 113.7, 103.9, 27.3 ppm.

1-(5-Fluoro-1H-indol-3-yl)ethanone: (50%), yellow solid; ^{1}H NMR ([D$_6$]DMSO, 300 MHz): d_H = 11.96 (1H, s, NH-1); 8.36 (1H, s, H-2); 7.86–7.80 (1H, dd, *J* = 10.0, 2.6 Hz, H-4); 7.50–7.45 (1H, m, H-7); 7.10–7.02 (1H, m, H-6); 2.44 ppm (s, 3H, H-2'); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): d_C = 192.7, 160.6 (d, $J_{F,C}$ = 236 Hz), 135.9, 133.2, 125.8 (d, $J_{F,C}$ = 11.2 Hz), 116.8 (d, $J_{F,C}$ = 4.4 Hz); 113.4 (d, $J_{F,C}$ = 10.5 Hz), 110.9 (d, $J_{F,C}$ = 25.6 Hz), 106.0 ($J_{F,C}$ = 22.6 Hz); 27.1 ppm.

2-Bromo-1-(5-methyl-1H-indol-3-yl)ethanone: (19%), yellow solid; IR (film): $\tilde{\nu}_{max}$ = 3185, 2919, 1643, 1514, 1430, 1365, 801, 761, 672 cm^{-1}; ^{1}H NMR (CH_3OD, 300 MHz): d_H = 8.21 (1H, s, H-2), 8.03 (1H, s, H-4), 7.34 (1H, d, *J* = 8.3 Hz, H-7), 7.09 (1H, d, *J* = 8.3 Hz, H-6), 4.47 (2H, s, H-2'), 2.45 ppm (3H, s, H-8); ^{13}C NMR (CH_3OD, 75 MHz): d_C = 188.0, 135.4, 134.6, 131.9, 126.0, 124.8, 121.2, 113.6, 111.3, 31.1, 20.4 ppm.

2-Bromo-1-(2-methyl-1H-indol-3-yl)ethanone (44%), brown solid; IR (film): $\tilde{\nu}_{max}$ = 3302, 3114, 2946, 1715, 1624, 1454, 1411, 980, 890, 757, 589 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz): d_H = 7.97–7.94 (1H, m, H-4), 7.38–7.34 (1H, m, H-7), 7.21–7.17 (2H, m, H-5, H-6), 4.51 (2H, s, H-2'), 2.74 (3H, s, H-8); ^{13}C NMR (CD_3OD, 75 MHz): d_C = 188.1, 146.6, 135.3, 126.6, 122.2, 121.8, 120.5, 110.9, 34.4, 14.0 ppm.

3-(2-Bromoacetyl)-1H-indole-5-carbonitrile: Inseparable mixture with 1-(5-cyano-1*H*-indol-3-yl)ethanone, and was taken forward into the next step without further purification.

2-Bromo-1-(5-methoxy-1H-indol-3-yl)ethanone (37%), cream solid; IR (film): $\tilde{\nu}_{max}$ = 3186, 2928, 1639, 1514, 1426, 1029, 951, 920, 886, 743, 670 cm^{-1}; ^{1}H NMR ([D$_6$]acetone, 300 MHz): d_H = 11.07 (1H, brs, NH-1), 8.34 (1H, d, *J* = 3.3 Hz, H-2), 7.82 (1H, d, *J* = 2.6 Hz, H-4), 7.43 (1H, d, *J* = 8.9 Hz, H-7), 6.89 (1H, dd, *J* = 8.9, 2.6 Hz, H-6), 4.51 (2H, s, H-2'), 3.84 ppm (3H, s, H-9); ^{13}C NMR ([D$_6$]acetone, 75 MHz): d_C = 186.1, 156.4, 134.0, 131.8, 126.9, 114.1, 113.5, 112.8, 103.2, 54.9, 32.2 ppm.

2-Bromo-1-(5-fluoro-1H-indol-3-yl)ethanone (34%), cream solid; ^{1}H NMR ([D$_6$]DMSO, 300 MHz): d_H = 12.27 (1H, s, NH-1); 8.54 (1H, d, *J* = 3.31 Hz, H-2); 7.81 (1H, dd, *J* = 9.9, 2.5 Hz H-4); 7.54–7.49 (1H, m, H-7); 7.14–7.07 (1H, m, H-6); 4.65 ppm (2H, s, H-2"); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): d_C = 186.9, 157.4 (d, $J_{F,C}$ = 243 Hz), 136.8, 133.3, 126.2 (d, $J_{F,C}$ = 11.8 Hz), 115.5, 113.6 (d, $J_{F,C}$ = 10.5 Hz); 111.5 (d, $J_{F,C}$ = 25.7 Hz) 106. (d, $J_{F,C}$ = 26.9 Hz) 33.5 ppm.

4-(1H-Tetrazol-5-yl)phenol (25): NaN_3 (272 mg, 4.2 mmol) and ammonium chloride (224 mg, 4.2 mmol) were added to a stirring mixture of 4-cyanophenol (500 mg, 4.2 mmol) in anhydrous *N*,*N*-DMF (2 mL) under a nitrogenous atmosphere. The reaction proceeded for 7 h at 125 8C, following which time it was allowed to cool. NaOH (1 m, 10 mL) was added to the mixture and stirred for a further 30 min, followed by adjustment to pH 1 by the addition of small portions of a 32% HCl solution. The organic material was extracted with EtOAc (3×15 mL) prior to drying over $MgSO_4$. Solvent was removed in vacuo, and the crude organic sold was further dried at 80 8C for 24 h at low pressure. Compound **25** was purified by flash column chromatography (hexane/EtOAc, 1:1) followed by crystallisation from EtOAc and washing with $CHCl_3$ to yield a white crystalline solid (31%): IR (film): $\tilde{\nu}_{max}$ = 3414, 2843, 2620, 1614, 1599, 1415, 1279, 1249, 1079, 830, 751 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz):

d_H = 7.84 (2H, d, *J* = 8.7 Hz, H-2', H-6'), 6.95 (2H, d, *J* = 8.7 Hz, H-3', H-5'); ^{13}C NMR (CD_3OD, 100 MHz): d_C = 160.6, 155.4, 128.6, 115.8, 114.4 ppm.

Methyl 4-mercaptobenzoate (26): 4-Mercaptobenzoic acid (500 mg, 3.24 mmol) was dissolved in thionyl chloride (3 mL) under a nitrogenous atmosphere and heated at 70 °C for 2 h. Following cooling, sodium methoxide solution was added dropwise, allowing the fumes to purge. The reaction mixture was dissolved in EtOAc (45 mL) and washed with saturated brine (3×10 mL). Solvent was removed in vacuo affording a beige waxy solid, which required no further purification (43%): ^{1}H NMR ([D$_6$]DMSO, 300 MHz): d_H = 7.96–7.93 (2H, m, H-3); 7.67–7.64 (2H, m, H-2); 3.82 (3H, s, H-7) ppm; ^{13}C NMR ([D$_6$]DMSO, 75 MHz): d_C = 165.7, 141.3, 130.3, 128.5 126.5, 52.4 ppm. The same method was used to generate 4-mercaptobenzamide **27**, which was taken forward without full characterisation.

4-Mercaptobenzonitrile (28): To a stirred solution of 4-mercaptobenzamide (□ 3.55 mmol, 1 equiv) in DMF was added $POCl_3$ (8.88 mmol, 2.5 equiv) at 0 °C. The mixture was allowed to react for 2 h, following which the reaction mixture was dissolved in EtOAc (20 mL) and washed with saturated brine (3×10 mL). In vacuo solvent removal afforded a yellow waxy solid which required no further purification (93%): ^{1}H NMR ([D$_6$]DMSO, 300 MHz): d_H = 7.88–7.84 (2H, m, H-3); 7.73–7.69 ppm (2H, m, H-2); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): d_C = 141.5, 133.3, 126.6, 118.4, 109.9 ppm.

General synthetic procedure for compounds 3–22: A relevant phenol or thiophenol (2 equiv) was added to a stirring suspension of a corresponding 2-bromo-1-(1*H*-indol-3-yl)ethanone (1 equiv) and K_2CO_3 (2 equiv) in acetone. The reaction mixture was heated at reflux for 5 h, following which time the reaction mixture was cooled and dissolved in EtOAc. The organic phase was washed with water (3×15 mL) and saturated brine (10 mL). Solvent was removed in vacuo, and the resultant crude product was purified by normal-phase flash chromatography (hexane/EtOAc, 4:1) to yield a crystalline solid. The solid was re-dissolved in acetone and was allowed to crystallise through slow evaporation. These resultant crystals were washed with cold chloroform to yield the desired product.

1-(1H-Indol-3-yl)-2-(4-nitrophenoxy)ethanone (3): White crystalline solid, mp: 220–222 °C, (66%); IR (film): $\tilde{\nu}_{max}$ = 3172, 2922, 1635, 1581, 1505, 1428, 1340, 1235, 1111 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 600 MHz): d_H = 8.52 (1H, s, H-2); 8.24–8.18 (2H, m, H-6'); 8.14–8.11 (1H, m, H-4); 7.53–7.50 (1H, m, H-7); 7.27–7.14 (2H, m, H-5, H-6, H-5'); 5.55 ppm (2H, s, H-2'). ^{13}C NMR ([D$_6$]DMSO, 150 MHz): d_C = 188.3, 163.8, 141.0, 136.5, 134.3, 125.9, 125.4, 123.3, 122.2, 121.2, 115.3, 113.1, 112.4, 70.3 ppm; HRESMS *m/z* 297.0871 (calcd for $C_{16}H_{13}N_2O_4$ $[M+H]^+$ 297.0875).

1-(1H-Indol-3-yl)-2-(4-cyanophenoxy)ethanone (4): White crystalline solid, mp: 237–239 °C, (76%); $\tilde{\nu}_{max}$ = 3171, 2221, 1643, 1605, 1506, 1429, 1300, 1156, 938, 790 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): d_H = 12.12 (1H, s, NH-1); 8.51 (1H, s, H-2); 8.14–8.12 (1H, m, H-4); 7.79–7.75 (2H, m, H-6'); 7.52–7.49 (1H, m, H-7); 7.26–7.18 (2H, m, H-5, H-6); 7.15–7.11 (2H, m, H-5'); 5.47 ppm (s, 2H, H-2'); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): d_C = 188.5, 161.9, 136.4, 134.2 (2× CH), 125.3, 123.2, 122.1, 121.2, 119.2, 115.7, 113.2, 112.3, 102.9, 69.9 ppm; HRESMS *m/z* 277.0968 (calcd for $C_{17}H_{13}N_2O_2$ $[M+H]^+$ 277.0977).

1-(1H-Indol-3-yl)-2-(4-[1H-tetrazol-5-yl]phenoxy)ethanone (5): White needles, mp: 225–227 °C (20%); IR (film): $\tilde{\nu}_{max}$ = 3473, 3307, 2957, 2737, 1640, 1617, 1595, 1523, 1442, 1379, 1244, 1141, 836, 759 cm^{-1}; ^{1}H NMR ([D$_6$]acetone, 600 MHz): d_H = 8.57 (1H, s, H-2), 8.26–8.24 (1H, m, H-4), 8.02–7.99 (2H, m, H-6'), 7.59–7.57 (1H, m, H-7), 7.31–7.23 (2H, m, H-5, H-6), 7.01–6.99 (2H, m, H-5'), 6.27 (2H, s, H-2'); ^{13}C NMR ([D$_6$]acetone, 100 MHz): d_C = 185.3, 165.8, 160.2, 137.8, 134.7, 129.0, 126.6, 124.5, 123.3, 122.5, 120.1, 116.7, 115.0, 113.0, 58.9, ppm; HRESMS *m/z* 320.1142 (calcd for $C_{17}H_{14}N_5O_2$ $[M+H]^+$ 320.1147).

1-(1H-Indol-3-yl)-2-(5-nitropyridin-2-yloxy)ethanone (6): White crystalline solid, mp: 258–260 °C, (13%); IR (film): $\tilde{\nu}_{max}$ = 3403, 3126, 1602, 1458, 1418, 1280, 1167, 1108.6, 1027, 837, 745 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 300 MHz): d_H = 12.19 (s, 1H, NH-1); 9.22 (1H, d, *J* = 3.1 Hz, H-6'); 8.61 (1H, s, H-2); 8.22 (1H, dd, *J* = 10.0, 3.1 Hz, H-8'); 8.11–8.08 (1H, m, H-4); 7.54–7.51 (1H, m, H-7); 7.28–7.19 (2H, m, H-5, H-6); 6.55 (1H, d, *J* = 10.0 Hz, H-9'); 5.52 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): d_C = 186.2, 160.9, 143.1, 136.6, 134.5, 133.9, 129.8, 125.2, 123.2, 122.2, 121.0, 118.1, 113.5, 112.5, 55.2 ppm; HRESMS *m/z* 298.0824 (calcd for $C_{15}H_{12}N_3O_4$ $[M+H]^+$ 298.0828).

1-(5-Methyl-1H-indol-3-yl)-2-(4-nitrophenoxy)ethanone (7): Orange crystalline solid, mp: 235–237 °C, (82%); IR (film): $\tilde{\nu}_{max}$ = 3253, 2920, 2854, 1709, 1650, 1591, 1507, 1422, 1339, 1251, 1234, 1110, 820, 750, 689 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): d_H = 12.02 (1H, brs, NH-1), 8.44 (1H, s, H-2), 8.21–8.19 (2H, m, H-6'), 7.94 (1H, s, H-4), 7.39 (1H, d, *J* = 8.3 Hz, H-7), 7.18–7.15 (2H, m, H-5'), 7.07 (1H, d, *J* = 8.3 Hz, H-6), 5.52 (2H, s, H-2'), 2.39 ppm (3H, s, H-8); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): d_C = 188.6, 164.2, 141.4, 135.2, 134.5, 131.4, 126.2, 126.1, 125.1, 121.3, 115.7, 113.2, 112.4, 70.7, 21.7 ppm; HRESMS *m/z* 311.1025 (calcd for $C_{17}H_{15}N_2O_4$ $[M+H]^+$ 311.1032).

1-(2-Methyl-1H-indol-3-yl)-2-(4-nitrophenoxy)ethanone (8): Yellow crystalline solid, mp: 217–219 °C, (98%); IR (film): $\tilde{\nu}_{max}$ = 3274, 2923, 1637, 1590, 1508, 1427, 1333, 1250, 1171, 974, 838, 740 cm^{-1}; ^{1}H NMR ([D$_6$]acetone, 600 MHz): d_H = 11.08 (1H, brs, NH-1), 8.22–8.20 (2H, m, H-6'), 8.06–8.04 (1H, m, H-4), 7.45–7.43 (1H, m, H-7), 7.21–7.19 (2H, m, H-5, H-6), 7.19–7.17 (2H, m, H-5', H-9'), 5.56 (2H, s, H-2'), 2.81 ppm (3H, s, H-8); ^{13}C NMR ([D$_6$]acetone, 150 MHz): d_C = 188.3, 164.2, 145.0, 141.4, 135.3, 126.7, 125.5, 122.3, 121.9, 120.9, 115.1, 111.4, 111.3, 72.3, 14.6 ppm; HRESMS *m/z* 311.1026 (calcd for $C_{17}H_{15}N_2O_4$ $[M+H]^+$ 311.1032).

1-(5-Cyano-1H-indol-3-yl)-2-(4-nitrophenoxy)ethanone (9): Cream crystalline solid, mp: 254–256 °C, (17% over two steps); IR (film): $\tilde{\nu}_{max}$ = 3180, 3116, 2924, 2216, 1729, 1657, 1505, 1430, 1344, 1261, 1239, 945, 779 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): d_H = 8.63 (1H, s, H-2), 8.46 (1H, d, *J* = 1.4 Hz, H-4), 8.20–8.16 (2H, m, H-6'), 7.70 (1H, d, *J* = 8.5 Hz, H-7), 7.61 (1H, dd, *J* = 8.5, 1.4 Hz, H-6), 7.18–7.14 (2H, m, H-5'), 5.54 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): d_C = 189.1, 164.0, 141.5, 138.7, 136.9, 126.7, 126.6, 126.3, 125.5, 120.5, 115.7, 114.4, 113.7, 104.8, 70.8 ppm; HRESMS *m/z* 320.0676 (calcd for $C_{17}H_{10}N_3O_4$ $[M□H]^□$ 320.0671).

1-(5-Chloro-1H-indol-3-yl)-2-(4-cyanophenoxy)ethanone (10): White crystalline solid; mp: 229–231 °C, (36%); IR (film): $\tilde{\nu}_{max}$ = 3177, 2917, 2224, 1659, 1509, 1250, 1153, 946, 807 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): d_H = 12.32 (1H, s, NH-1); 8.57 (1H, s, H-2); 8.10 (1H, d, *J* = 2.0 Hz, H-4); 7.78–7.75 (2H, m, H-6'); 7.54 (1H, d, *J* = 8.7 Hz, H-7); 7.26 (1H, dd, *J* = 8.7, 2.0 Hz, H-6); 7.15–7.12 (2H, m, H-5'); 5.47 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): d_C = 188.7, 161.8, 135.5, 134.9, 134.2, 126.9, 126.5, 123.3, 120.3, 119.2, 115.8, 114.1, 112.8, 103.1, 69.9 ppm (CH_2, C2'); HRESMS *m/z* 311.0591 (calcd for $C_{17}H_{12}{}^{35}ClN_2O_2$ $[M+H]^+$ 311.0587).

1-(1H-Indol-3-yl)-2-[(4-cyanophenyl)thio]ethanone (11): Brown crystalline solid, mp: 204–206 °C, (22%); IR (film): $\tilde{\nu}_{max}$ = 3211, 2220, 1627, 1402, 1239, 1137, 1088, 947, 813 cm^{-1}; ^{1}H NMR ([D$_6$]acetone, 600 MHz): δ_H = 11.25 (1H, s, NH-1); 8.49 (1H, d, J = 3.1 Hz, H-2); 8.29–8.28 (1H, m, H-4); 7.67–7.65 (2H, m, H-5'); 7.58–7.56 (2H, m, H-6'); 7.54–7.53 (1H, m, H-7); 7.27–7.22 (2H, m, H-5, H-6); 4.61 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]acetone, 150 MHz): δ_C = 187.8, 144.8, 136.8, 133.7, 132.0, 126.8, 125.8, 123.2, 122.0, 121.7, 118.4, 115.4, 111.8, 107.8, 38.9 ppm; HRESMS m/z 293.0739 (calcd for $C_{17}H_{13}N_2OS$ [M + H]$^+$ 293.0749).

1-(1H-Indol-3-yl)-2-[(4-methylbenzoate)thio]ethanone (12): White crystalline solid, mp: 99–101 °C, (26%); IR (film): $\tilde{\nu}_{max}$ = 3261, 2920, 2850, 1713, 1628, 1518, 1432, 1274, 1274, 1109 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 600 MHz): δ_H = 12.11 (1H, s, NH-1); 8.53 (1H, d, J = 3.2 Hz, H-2); 8.13 (1H, d, J = 8.1 Hz, H-4); 7.85–7.82 (2H,m, Hz, H-6'); 7.50–7.46 (3H, m, H-5', H-7); 7.24–7.18 (2H, m, H-5, H-6); 4.61 (2H, s, H-2'); 3.81 ppm (3H, s, H-10'); ^{13}C NMR ([D$_6$]DMSO, 150 MHz): δ_C = 188.4, 165.9, 144.0, 136.6, 135.1, 129.5, 126.3, 125.9, 125.4, 123.1, 122.1, 121.2, 114.9, 112.3, 52.0, 39.2 ppm; HRESMS m/z 326.0842 (calcd for $C_{18}H_{16}NO_3S$ [M + H]$^+$ 326.0851).

1-(5-Chloro-1H-indol-3-yl)-2-[(4-cyanophenyl)thio]ethanone (13): White crystalline solid, mp: 249–251 °C, (36%); IR (film): $\tilde{\nu}_{max}$ = 3203, 2907, 2219, 1634, 1592, 1434, 1229, 1142, 1088, 952, 894 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 300 MHz): δ_H = 12.33 (1H, s, NH-1); 8.63 (1H, d, J = 2.7 Hz, H-2); 8.09 (1H, d, J = 2.1 Hz, H-4); 7.74–7.71 (2H, m, H-6'); 7.55–7.49 (3H, m, H-5', H-7); 7.26 (1H, dd, J = 8.6, 2.1 Hz, H-6); 4.66 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]DMSO, 75 MHz): δ_C = 188.5, 144.5, 136.5, 135.2, 132.4, 126.9, 126.7, 126.6, 123.3, 120.4, 119.0, 114.5, 114.1, 107.1, 39.8 ppm; HRESMS m/z 327.0353 (calcd for $C_{17}H_{12}{}^{35}ClN_2OS$ [M + H]$^+$ 327.0359).

1-(5-Chloro-1H-indol-3-yl)-2-[(4-nitrophenyl)thio]ethanone (14): Yellow crystalline solid, mp: 249–251 °C (46%); IR (film): $\tilde{\nu}_{max}$ = 3170, 3121, 2922, 1713, 1637, 1577, 1507, 1424, 1329, 1087, 843, 739, 680 cm^{-1}; ^{1}H NMR ([D$_6$]acetone, 600 MHz): δ_H = 11.37 (1H, brs, NH-1), 8.59 (1H, d, J = 2.4 Hz, H-2), 8.28 (1H, d, J = 2.1 Hz, H-4), 8.16–8.13 (2H, m, H-5'), 7.64–7.61 (2H, m, H-6'), 7.57 (1H, d, J = 8.7 Hz, H-7), 7.27 (1H, dd, J = 8.7, 2.1 Hz, H-6), 4.69 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]acetone, 150 MHz): δ_C = 188.7, 148.3, 145.9, 136.0, 135.8, 128.5, 127.9, 127.3, 124.5, 124.3, 121.9, 115.9, 114.5, 39.9 ppm; HRESMS m/z 347.0255 (calcd for $C_{16}H_{12}{}^{35}ClN_2O_3S$ [M + H]$^+$ 347.0257).

1-(5-Fluoro-1H-indol-3-yl)-2-[(4-bromophenyl)thio]ethanone (15): White crystalline solid, mp: 177–179 °C, (63%); IR (film): $\tilde{\nu}_{max}$ = 3197, 2921, 1714, 1612, 1467, 1430, 1168, 810, 706 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO-, 600 MHz): δ_H = 12.19 (1H, s, NH-1); 8.56 (1H, s, H-2); 7.79 (1H, dd, J = 9.8, 2.7 Hz, H-4); 7.51–7.46 (3H, m, H-7, H-5'); 7.34–7.32 (2H, m, H-6'); 7.10–7.07 (1H, m, H-6); 4.48 ppm (2H, s, C2'); ^{13}C NMR ([D$_6$]DMSO, 150 MHz): δ_C = 188.8; 158.7 (d, $J_{F,C}$ = 235 Hz); 136.4, 136.0, 133.2, 131.6, 129.8, 126.0 (d, $J_{F,C}$ = 11.1 Hz), 118.5, 114.9 (d, $J_{F,C}$ = 4.3 Hz), 113.5 (d, $J_{F,C}$ = 9.7 Hz), 111.2 (d, $J_{F,C}$ = 25.8 Hz), 106.0 (d, $J_{F,C}$ = 24.3 Hz), 39.8 ppm (CH_2, C2'); HRESMS m/z 363.9803 (calcd for $C_{16}H_{12}{}^{79}BrFNOS$ [M + H]$^+$ 363.9807).

1-(5-Fluoro-1H-indol-3-yl)-2-[(4-nitrophenyl)thio]ethanone (16): White crystalline solid, mp: 210–212 °C, (55%); IR (film): $\tilde{\nu}_{max}$ = 3171, 2917, 2850, 1727, 1613, 1505, 1174, 840, 737 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 600 MHz): δ_H = 12.25 (1H, s, NH-1); 8.65 (1H, d, J = 3.3 Hz, H-2); 8.14–8.12 (2H, m, H-6'); 7.79 (1H, dd, J = 9.8, 2.5 Hz, H-4); 7.59–7.57 (2H, m, H-5'); 7.53–7.51 (1H, m, H-7); 7.12–7.08 (1H, m, H-6); 4.73 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]DMSO, 150 MHz): δ_C = 187.8, 158.7 (d, $J_{F,C}$ = 236 Hz), 147.4, 144.4, 136.6, 133.2, 126.4, 125.9 (d, $J_{F,C}$ = 11.4 Hz), 125.9, 123.8, 114.8 (d, $J_{F,C}$ = 4.4 Hz), 113.6 (d, J_{FC} = 9.9 Hz), 111.3 (d, $J_{F,C}$ = 26.2 Hz), 106.0 (d, $J_{F,C}$ = 24.1 Hz), 38.7 ppm; HRESMS m/z 331.0545 (calcd for $C_{16}H_{12}FN_2O_3S$ [M + H]$^+$ 331.0553).

1-(5-Cyano-1H-indol-3-yl)-2-[(4-bromophenyl)thio]ethanone (17): Cream crystalline solid, mp: 242–244 °C, (6% over two steps); IR (film): $\tilde{\nu}_{max}$ = 3112, 2930, 2220, 1784, 1614, 1523, 1440, 1374, 1085, 805, 784 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ_H = 12.56 (1H, brs, NH-1), 8.67 (1H, s, H-2), 8.48 (1H, d, J = 1.5 Hz, H-4), 7.68 (1H, d, J = 8.4 Hz, H-7), 7.61 (1H, dd, J = 8.4, 1.5 Hz, H-6), 7.49–7.46 (2H, m, H-6'), 7.35–7.31 (2H, m, H-5'), 4.52 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ_C = 189.2, 138.5, 137.2, 135.8, 131.7, 129.9, 126.2, 126.0, 125.2, 120.0, 118.7, 115.1, 113.9, 104.3, 39.6 ppm;* HRESMS m/z 370.9849 (calcd for $C_{17}H_{12}{}^{79}BrN_2OS$ [M + H]$^+$ 370.9854. *Resolved by HSQC.

1-(5-Methyl-1H-indol-3-yl)-2-[(4-nitrophenyl)thio]ethanone (18): Yellow crystalline solid, mp: 218–221 °C (62%); IR (film): $\tilde{\nu}_{max}$ = 3159, 3112, 2914, 1703, 1611, 1502, 1431, 1338, 1088, 960, 800, 739 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ_H = 12.02 (1H, brs, NH-1), 8.52 (1H, m, H-2), 8.14–8.11 (2H, m, H-6'), 7.95 (1H, s, H-4), 7.60–7.56 (2H, m, H-5'), 7.37 (1H, m, H-7), 7.05 (1H, m, H-6), 4.70 (2H, s, H-2'), 2.39 ppm (3H, s, H-8); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ_C = 188.3, 148.1, 144.9, 135.6, 135.4, 131.4, 126.8, 126.1, 125.0, 124.2, 121.4, 114.9, 112.4, 39.2, 21.8 ppm; HRESMS m/z 327.0798 (calcd for $C_{17}H_{15}N_2O_3S$ [M + H]$^+$ 327.0803).

1-(2-Methyl-1H-indol-3-yl)-2-[(4-nitrophenyl)thio]ethanone (19): Orange crystalline solid, mp: 215–217 °C (69%); IR (film): $\tilde{\nu}_{max}$ = 3288, 2906, 1732, 1610, 1500, 1455, 1416, 1332, 980, 832, 738, 715 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ_H = 12.05 (1H, brs, NH-1), 8.15–8.12 (2H, m, H-6'), 8.06–8.02 (1H, m, H-4), 7.55–7.52 (2H, m, H-5'), 7.41–7.39 (1H, m, H-7), 7.19–7.16 (2H, m, H-5, H-6), 4.80 (2H, s, H-2'), 2.76 ppm (3H, s, H-8); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ_C = 187.7, 147.8, 145.6, 144.3, 134.8, 126.7, 126.5, 123.8, 122.2, 121.8, 120.8, 111.9, 111.4, 42.4, 15.3 ppm; HRESMS m/z 327.0801 (calcd for $C_{17}H_{15}N_2O_3S$ [M + H]$^+$ 327.0803).

1-(5-Cyano-1H-indol-3-yl)-2-[(4-nitrophenyl)thio]ethanone (20): Yellow crystalline solid, mp: 226–230 °C, (10% over two steps); IR (film): $\tilde{\nu}_{max}$ = 3182, 2920, 2219, 1637, 1580, 1513, 1434, 1335, 1080, 843, 742 cm^{-1}; ^{1}H NMR ([D$_6$]acetone, 300 MHz): δ_H = 11.65 (1H, brs, NH-1), 8.71 (1H, s, H-2), 8.64 (1H, d, J = 1.4 Hz, H-4), 8.17–8.13 (2H, m, H-6'), 7.76 (1H, d, J = 8.6 Hz, H-7), 7.65–7.61 (2H, m, H-5'), 7.59 (1H, dd, J = 8.6, 1.4 Hz, H-6), 4.72 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ_C = 188.8, 147.7, 145.0, 138.9, 137.9, 126.9, 126.6, 126.5, 125.0, 124.3, 120.4, 115.5, 114.4, 104.8, 39.3 ppm;* HRESMS m/z 336.0454 (calcd for $C_{17}H_{10}N_3O_3S$ [M H]$^+$ 336.0443). *Resolved by HSQC.

1-(5-Methoxy-1H-indol-3-yl)-2-[(4-nitrophenyl)thio]ethanone (21): Yellow crystalline solid, mp: 218–223 °C (87%); IR (film): $\tilde{\nu}_{max}$ = 3165, 3122, 2928, 1735, 1610, 1501, 1427, 1337, 1275, 842, 798, 739 cm^{-1}; ^{1}H NMR ([D$_6$]DMSO, 400 MHz): δ_H = 12.05 (1H, brs, NH-1), 8.53 (1H, d, J = 3.3 Hz, H-2), 8.14–8.12 (2H, m, H-6'), 7.63 (1H, d, J = 2.4 Hz, H-4), 7.59–7.57 (2H, m, H-5'), 7.39 (1H, d, J = 8.8 Hz, H-7), 6.86 (1H, dd, J = 8.8, 2.4 Hz, H-6), 4.70 (2H, s, H-2'), 3.76 ppm (3H, s, H-9); ^{13}C NMR ([D$_6$]DMSO, 100 MHz): δ_C = 188.3, 156.2, 148.1, 144.9, 135.7, 131.8, 126.8, 126.7, 124.2, 115.1, 113.5, 113.4, 103.3, 55.7, 39.1 ppm (CH_2, C2'); HRESMS m/z 343.0744 (calcd for $C_{17}H_{15}N_2O_4S$ [M + H]$^+$ 343.0753).

1-(1H-Indol-3-yl)-2-[(4-bromophenyl)sulfonyl]ethanone (22): A solution of m-CPBA (2.5 equiv) in CH_2Cl_2 (3 mL) was added dropwise to a solution of 1-(1H-indol-3-yl)-2-(4-bromophenyl)ethanone (100 mg, mmol) in THF (3 mL). The reaction mixture was allowed to stir for 3 h at room temperature, followed by the addition of a

saturated sodium bicarbonate solution, and extracted with EtOAc (3×15 mL). Solvent was removed in vacuo and crude organics were purified by normal-phase flash chromatography (hexane/EtOAc, 1:1) to yield a white solid (75%), ^{1}H NMR ([D$_6$]acetone, 600 MHz): d_H = 11.29 (1H, s, NH-1); 8.42 (1H, d, J = 3.1 Hz, H-2); 8.21 (1H, d, J = 7.7 Hz, H-4); 7.87–7.82 (4H, m, H-5', H-6'); 7.53 (1H, d, J = 7.8 Hz H-7); 7.29–7.22 (2H, m, H-5, H-6); 4.87 ppm (2H, s, H-2'); ^{13}C NMR ([D$_6$]acetone, 150 MHz): d_C = 181.5, 139.5, 137.1, 136.1, 132.3, 130.5, 128.3, 125.9, 123.7, 122.5, 121.8, 117.4, 112.2, 64.2 ppm; HRESMS m/z 377.9790 (calcd for $C_{16}H_{13}{}^{79}BrNO_3S$ $[M+H]^+$ 377.9800).

Biological assessment

Antiplasmodial assay (3D7): *P. falciparum* (3D7 strain) parasites were maintained at 37 8C under an atmosphere of 5% CO_2, 5% O_2, 90% N_2 in RPMI 1640 medium containing 25 mm HEPES, 22 mm glucose, 0.65 mm hypoxanthine, 0.5% (*w*/*v*) Albumax II, 0.05 mg mL^{-1} gentamicin, and 2–4% haematocrit human erythrocytes. For compound assessment, parasite cultures were adjusted to 2% parasitaemia and 1% haematocrit, distributed in 96-well plates and compound stocks of 20 mm in dimethyl sulfoxide (DMSO) added to a final concentration of 20 mm. Incubation was continued for 48 h, after which parasite levels in the culture wells were determined using a parasite lactate dehydrogenase (pLDH) assay.[43] Briefly, 20 mL of culture was removed from each well and transferred to a separate plate containing 125 mL per well of pLDH reagent (44 mm Tris, pH 9, containing 0.18 m l-lactic acid, 0.13 mm acetylpyridine adenine dinucleotide, 0.39 mm nitrotetrazolium blue chloride, 0.048 mm phenazineethosulfate, and 0.16% (*v*/*v*) Triton X-100). Colour development was monitored as absorbance at 620 nm and converted into percent parasite viability relative to untreated control wells. Dose–response assays were conducted as described above, except that parasite cultures were incubated in 96-well plates with threefold serial dilutions of test compounds. Plots of percent parasite viability versus log[compound] were used to derive IC_{50} values by nonlinear regression analysis using GraphPad Prism v.4.0 for Windows.

Antiplasmodial assay (NF54 and K1): Both parasite strains were cultured continuously in normal type A human erythrocytes (2% haematocrit) using a method modified from Trager and Jensen.[44] Parasite cultures were maintained in complete tissue culture medium of RPMI 1640 supplemented with 25 mm HEPES buffer, 20 mg mL^{-1} gentamicin, 27 mm sodium hydrogen carbonate, and 0.5% Albumax II serum supplement. They were incubated at 37 8C in an atmosphere of 3% O_2, 4% CO_2, and 91% N_2. In vitro antiplasmodial activity was again determined using a pLDH assay.[43] Starting from a concentration of 1000 ng mL^{-1}, test compounds were serially diluted twofold to 2 ng mL^{-1} in complete medium. Each dilution was distributed in a 96-well microtiter plate with parasite suspension in triplicate and incubated at 37 8C for 72 h. Cultures in each well were re-suspended carefully, and aliquots were removed and spectrophotometrically analysed at 620 nm for pLDH activity. The IC_{50} values were obtained by nonlinear dose–response curve-fitting analysis using GraphPad Prism v.4.0 for Windows.

Cytotoxicity assay: HeLa cells (Cellonex, South Africa) were cultured in DMEM containing 4 mm l-glutamine and 10% (*v*/*v*) fetal bovine serum at 37 8C and 5% CO_2. Cells were seeded at 2×10^4 cells per well in 96-well plates one day prior to the addition of test compounds to a final concentration of 20 mm. After a 24-h incubation, 0.54 mm resazurin in phosphate-buffered saline (PBS) was added to each well (20 mL per well) and conversion of resazurin into resorufin by viable cells was determined after 2–4 h by measuring fluorescence ($I_{ex}560/I_{ex}590$) in a Spectramax M3 plate reader (Molecular Devices). Fluorescence emission readings were converted into percent cell viability relative to readings obtained from wells containing untreated control cells.

Haemolysis assay: Tenfold serial dilutions of test compounds were incubated in a 96-well plate with uninfected human erythrocytes (1% haematocrit) in *P. falciparum* culture medium for 48 h at 37 8C; 20 mL of the supernatant in each well was removed, transferred to a separate plate containing 130 mL per well water and haemoglobin, and absorbance was read at 405 nm. As a control for 100% haemolysis, the culture medium supernatant of wells containing untreated erythrocytes was removed and replaced with water to induce hypotonic lysis.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: antimalarial agents · assay variability · indoles · *Plasmodium falciparum* · thioethers

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