# Design, synthesis and anti-plasmodial evaluation in vitro of new 4-aminoquinoline isatin derivatives

**ARTICLE** *in* BIOORGANIC & MEDICINAL CHEMISTRY · JUNE 2005

Impact Factor: 2.79 · DOI: 10.1016/j.bmc.2005.02.037 · Source: PubMed



**7 AUTHORS**, INCLUDING:

Idan Chiyanzu
North West University South Africa
**9** PUBLICATIONS **170** CITATIONS

SEE PROFILE

Peter Smith
University of Cape Town
**247** PUBLICATIONS **4,407** CITATIONS

SEE PROFILE

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 3249–3261
Bioorganic & Medicinal Chemistry

# Design, synthesis and anti-plasmodial evaluation in vitro of new 4-aminoquinoline isatin derivatives

Idan Chiyanzu,[a] Cailean Clarkson,[b] Peter J. Smith,[b] Julie Lehman,[c] Jiri Gut,[c] Philip J. Rosenthal[c] and Kelly Chibale[a,*]

[a]*Department of Chemistry, University of Cape Town, Rondebosch, 7701, South Africa*
[b]*Division of Pharmacology, Department of Medicine, University of Cape Town, K-45 OMB, Groote Schuur Hospital, Observatory, 7925, South Africa*
[c]*Department of Medicine, San Francisco General Hospital, University of California, San Francisco, Box 0811, San Francisco, CA 94143, USA*

Received 26 January 2005; revised 16 February 2005; accepted 16 February 2005
Available online 19 March 2005

**Abstract**—A new class of 4-aminoquinoline derivatives based on the natural product isatin scaffold were designed and synthesized for biological evaluation against three strains of the malaria parasite *Plasmodium falciparum*. These derivatives showed anti-plasmodial $IC_{50}$ values in the ranges of 1.3–0.079 and 2.0–0.050 μM against a chloroquine-sensitive (D10) and two resistant (K1 and W2) strains of *P. falciparum*, respectively. In order to determine potential targets for this class of compounds in *P. falciparum*, selected compounds were also tested against the parasitic cysteine protease falcipain-2. In terms of further development of this class of isatin derivatives, two of the compounds based on a flexible alkyl chain linker and a thiosemicarbazone moiety warrant further investigation as potential anti-plasmodial leads. These two derivatives showed good in vitro activity against K1 and W2 with $IC_{50}$ values of 51 and 54 nM, respectively, while retaining potency against the D10 strain with $IC_{50}$ values of 79 and 95 nM, respectively. Generally speaking, the inhibitory potency of all compounds in the series against the parasites did not strongly correlate with inhibitory potency against falcipain-2 for selected compounds tested, which at best was weak to moderate, suggesting other mechanisms of inhibition may also be involved or compounds may be selectively taken up by *Plasmodium falciparum*.


## 1. Introduction

*Plasmodium falciparum* malaria continues to be a global health problem, especially in developing countries such as sub-Saharan Africa. It is responsible for over 1 million deaths per year.[1] The rapid spread of resistance to available anti-malarial drugs, especially chloroquine and related quinoline-based agents, has highlighted the need to identify alternative anti-malarial compounds. Recent approaches aimed at decreasing and/or slowing down the potential for quinoline-based anti-malarial drug resistance has included the design and synthesis of quinoline-containing dual inhibitors or 'double drugs' that would potentially inhibit haemozoin formation and another target within *P. falciparum*. This approach is exemplified by cysteine protease inhibitors based on mefloquine and chloroquine[2] as well as quinoline-based inhibitors of a neutral zinc aminopeptidase,[3] primaquine-based plasmepsin inhibitors[4] and inhibitors of antioxidant enzymes, such as glutathione reductase, conjugated to a 4-aminoquinoline moiety.[5,6]

Amongst other objectives, our current medicinal chemistry programme is geared towards addressing the problem of drug resistance through the development of single agents that provide maximal anti-infective and/or anti-cancer activity by acting against multiple targets within the same disease-causing organism and/or cell.[7] Within this context, one of the approaches we have taken is to hybridize electrophilic moieties with known minimum structural requirements for biological activity, bioactiphores, from various anti-infective and/or anti-cancer agents. The goal of this approach is to target, amongst other things, cysteine residues of biomolecules by exploiting the strong nucleophilic character of the

*Keywords*: Isatins; Aminoquinolines; *Plasmodium falciparum*; Malaria; Anti-plasmodial activity; Falcipain-2; Mannich bases; Thiosemicarbazones.
* Corresponding author. Tel.: +27 21 650 2553; fax: +27 21 689 7499; e-mail: chibale@science.uct.ac.za

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.02.037

thiol (SH) group, as a thiolate, which allows S-alkylation/arylation and S-acylation/sulfonylation of cysteine residues. Indeed a vast number of cysteine protease inhibitors exploit this property of sulfur.[8–10]

In our hybridization approaches, we have embarked on the rational selection of a variety of electrophilic warheads and their chemical linkage to bioactiphores from a diverse range of anti-infective (anti-malarial, anti-tuberculosis, anti-trypanosomal and anti-HIV/AIDS) and anti-cancer agents. This paper describes the design and synthesis of 4-aminoquinoline-based isatin derivatives **1**–**4** (Fig. 1), in which the ketone and thiosemicarbazone moieties serve as electrophilic warheads, and reports on their in vitro anti-plasmodial activity against a chloroquine sensitive (D10) and two resistant (K1 and W2) strains of *P. falciparum*. A few non-quinoline Mannich bases **5** were also designed with a view to establishing preliminary structure–activity relationships with respect to the role of the 4-aminoquinoline moiety in the biological activity. In order to determine potential targets for this novel class of aminoquinolines, and in view of the fact that we have already reported on the inhibition by isatins of parasitic cysteine proteases from multiple protozoan parasites,[11] a few selected compounds were screened against a *P. falciparum*-derived cysteine protease, falcipain-2, a known target for anti-malarial agents that block haemoglobin degradation.[12]

The derivatives, **1**–**4**, were designed on the basis of a multi-therapeutic strategy exemplified for quinoline ethylene isatin derivatives **4** in Figure 2. It is postulated that quinoline anti-malarials accumulate in the parasite's acidic food vacuole and inhibit β-haematin formation. Toxic haem thus builds up in the vacuole subsequently killing the parasites.[13] Though the mode of action of these agents is still unclear, they remain potential sources of anti-malarial therapy. This is partly because *P. falciparum* has had difficulty developing rapid resistance to this class of compounds. For example simple modification of the lateral side chain of chloroquine has resulted in new derivatives, including metallocenes,[14,15] with activity against chloroquine-resistant strains.[13,16,17]

While the isatin unit is a privileged natural product scaffold onto which other bioactiphores can be appended, the thiosemicarbazone moiety has been selected to provide reactive sites (the imine and thiol carbonyl) for alkylation of the enzyme cysteine thiolate.[8,18] This moiety is also likely a metal chelator in which the hydrazinic nitrogen and sulfur atoms could be involved in binding to endogenous iron within *P. falciparum*. This has implications for metabolism within the parasite by, for example, inhibiting metal-dependent enzymes. The hydrazinic nitrogen could also assist in the accumulation within the acidic food vacuole of *P. falciparum*. The basic protonatable piperazine nitrogen of the Mannich bases **1** and **2** was envisaged to further increase accumulation of the molecule. The 7-chloroquinoline moiety has been proposed from earlier findings to bind to haematin in the parasite's acidic food vacuole, thus inhibiting

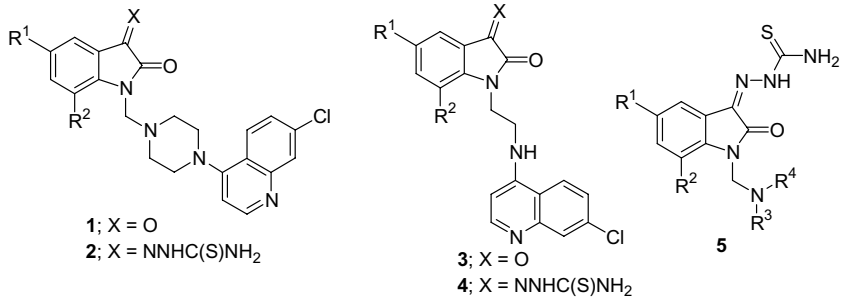



**Figure 1.** Chemical structures of aminoquinoline and Mannich base isatin derivatives **1**–**5**.

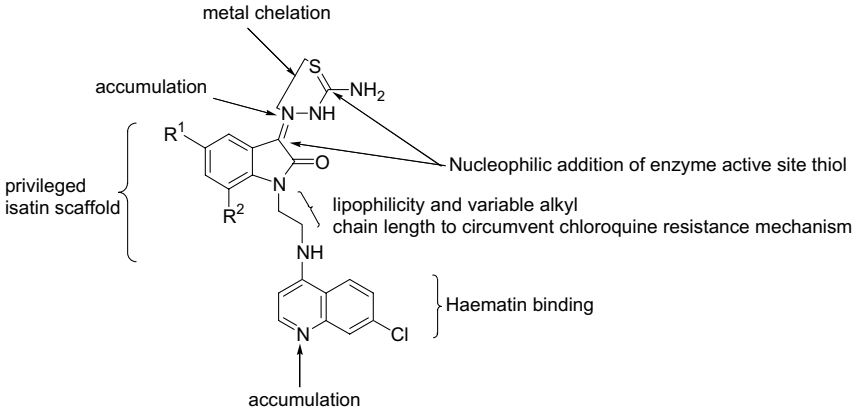



**Figure 2.** Multi-therapeutic strategy for quinoline–ethylene isatin derivatives **4**.

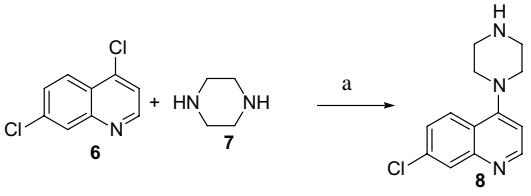
haemazoin formation, and also increases accumulation of the drug due to the protonatable quinoline nitrogen.[19] The variable alkyl chain in **3** and **4** is important not only for circumversion of the chloroquine resistance mechanism but also for lipophilicity, which is an important parameter in the effectiveness of iron chelating antimalarial agents.[20]

### 1.1. Synthesis of target compounds

Chloro-4-piperazin-1-yl-quinoline **8**, the precursor compound for Mannich bases was firstly synthesized in good yield (67%) from condensation of 4,7-dichloroquinoline **6** and piperazine **7** (excess) in *N*-methyl-2-pyrrolidine (NMP), to give the target compound **8** (Scheme 1).

Upon the preparation of the required secondary amine precursor **8**, we proceeded with the Mannich reaction. Mannich reactions can be performed via a one pot direct multi-component protocol requiring isatin, an aldehyde and an amine or via a preformed iminium ion. The latter approach was used in this work. Accordingly, paraformaldehyde (1.0 equiv) and chloro-4-piperazin-1-yl-quinoline **8** (1.0 equiv) were dissolved in ethanol and stirred for 30 min. The iminiun ion formed in situ was then reacted with selected isatins **9** (Scheme 2a) and isatin-3-thiosemicarbazones (Scheme 2b) in ethanol for 3 h at room temperature to furnish the desired *N*-Mannich isatin derivatives accomplished in low to excellent yields 26–93% (Table 1). The above mentioned approach was used in the preparation of non-quinoline based Mannich derivatives **5**.

The quinoline–ethylene isatin derivatives **3** and **4** listed in Table 2 were prepared according to Scheme 3. Reaction of excess 2-amino ethanol **11** and 4,7-dichloroquinoline **6** as reported previously[19] gave **12** in a good yield of 92%. This was followed by *o*-mesylation[21] in pyridine at 0 °C for 5 h to furnish mesylate **13** in a yield of 82%. Chemoselectivity in the mesylation of preformed alcohol **12** was not a problem in spite of the presence of the 4-amino (NH) group, which is poorly nucleophilic due to the conjugation of the lone pair of electrons into the quinoline nitrogen. The synthesis of quinoline–ethylene isatin derivatives (Scheme 3) employed commercially available isatins and sodium hydride as the base in *N*,*N*-dimethylformamide (DMF) as the solvent resulting in good yields (68–88%) of the products (Table 2). Selected precursors were used in the synthesis of thiosemicarbazones, (Scheme 3).



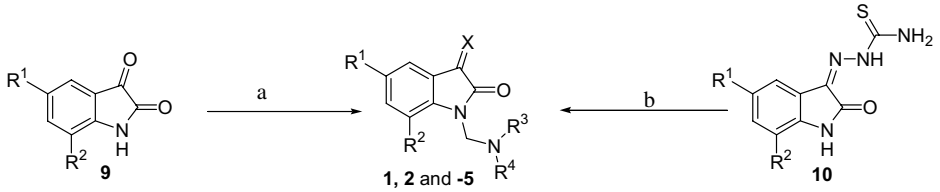

**Scheme 1.** Synthesis of chloro-4-piperazin-1yl quinoline **8**. Reagents and conditions: (a) $K_2CO_3$, $Et_3N$, NMP, 135 °C, 4 h, 67%.

### 1.2. Biological evaluation

$IC_{50}$ values against recombinant falcipain-2 were determined essentially as described previously.[22] Accordingly, equal amounts (~1 nM) each of recombinant protein were incubated with different concentrations of inhibitors added from 100× stocks in DMSO in 100 mM sodium acetate (pH 5.5), −10 mM dithiothreitol for 30 min at room temperature before addition of the substrate benzoxycarbonyl-Leu-Arg-7-amino-4-methyl-coumarin (final concentration, 25 μM). Fluorescence was continuously monitored for 30 min at room temperature in a Labsystems Fluoroskan II spectrofluorometer. $IC_{50}$ values were determined from plots of activity over inhibitor concentration with GraphPad Prism software.

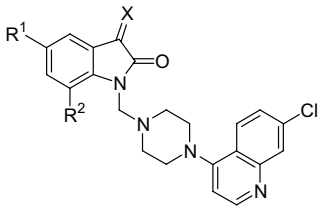
Parasites were cultured (D10 and K1) essentially as described by Trager and Jensen.[23] Initial 1 mg/mL stocks of the compounds were made up in DMSO, diluted with water and finally complete medium on the day of the experiment. The highest concentration of DMSO that the parasites were exposed to was 0.05%, which had no measurable effect on parasite viability. No attempt was made to determine $IC_{50}$ values greater than 1000 ng/mL. All experiments were performed in duplicate on a single occasion using a chloroquine-sensitive strain of *P. falciparum* (D10). The more active compounds were also tested against a chloroquine-resistant strain of *P. falciparum* (K1). After culture with inhibitors for 48 h, parasite lactate dehydrogenase (pLDH) activity was used to measure parasite viability as described by Makler et al.[24]

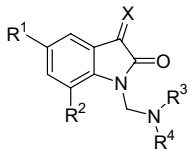
Ring stage *P. falciparum* strain W2 parasites were synchronized for 15 min at 37 °C 5% sorbitol, adjusted to 2% haematocrit 1% parasitaemia, and incubated at 37 °C under the atmosphere of 3% $O_2$, 5% $CO_2$, 91% $N_2$ in mediumRPMI-1640 supplemented with 10% human serum in the presence of various concentrations of inhibitors for 48 h without media change. Inhibitors

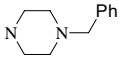
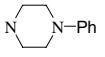


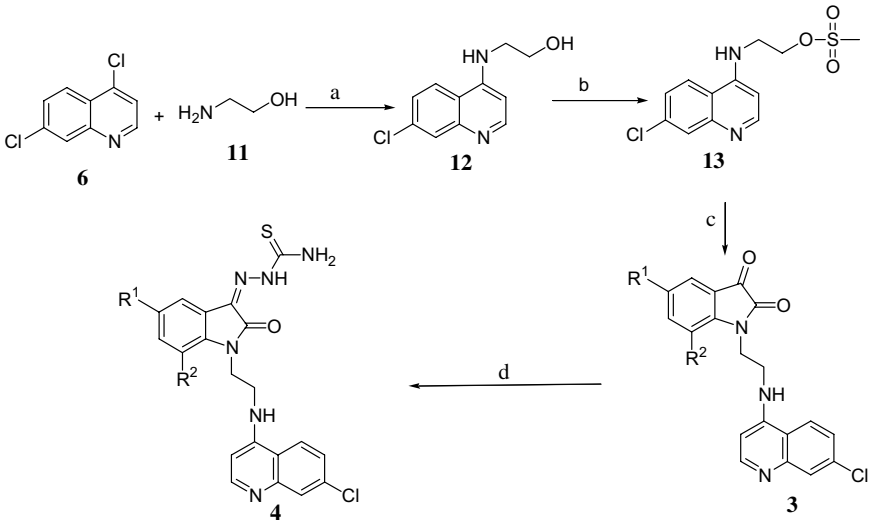

**Scheme 2.** Synthesis of Mannich bases **1**, **2** and **5**. Reagents and conditions: (a) $CH_2O$, **8**, EtOH, rt, 3 h, 26–93%; (b) $CH_2O$, secondary amine, EtOH, 45 °C, 3 h, 26–93%.

**Table 1.** Chemical and physical data of quinoline and non-quinoline Mannich based isatin derivatives





| Compound | $R^1$ | $R^2$ | $(R^3)R^4$ | X | Time (h) | % Yield | Mp (°C) |
|---|---|---|---|---|---|---|---|
| **1a** | H | H | — | O | 4 | 93 | 203–206 |
| **1b** | Me | Me | — | O | 4 | 85 | 209–213 |
| **1c** | Cl | H | — | O | 4 | 24 | 153–157 |
| **1d** | F | H | — | O | 4 | 94 | 110–117 |
| **1e** | Me | H | — | O | 4 | 55 | 202–203 |
| **1f** | I | H | — | O | 4 | 59 | 151–153 |
| **1g** | Br | H | — | O | 4 | 48 | 210–212 |
| **2a** | H | H | — | $NNHC(S)NH_2$ | 3 | 78 | 216–217 |
| **2b** | F | H | — | $NNHC(S)NH_2$ | 3 | 51 | 233–234 |
| **2c** | Cl | H | — | $NNHC(S)NH_2$ | 3 | 86 | 234–235 |
| **2d** | Br | H | — | $NNHC(S)NH_2$ | 3 | 73 | 236–237 |
| **2e** | Me | H | — | $NNHC(S)NH_2$ | 3 | 78 | 232–234 |
| **5a** | H | H | $NEt_2$ | $NNHC(S)NH_2$ | 3 | 54 | 134–135 |
| **5b** | H | H |  | $NNHC(S)NH_2$ | 3 | 95 | 179–181 |
| **5c** | H | H |  | $NNHC(S)NH_2$ | 3 | 90 | 181–184 |
| **5d** | H | H |  | $NNHC(S)NH_2$ | 3 | 92 | 208–210 |
| **5e** | H | H |  | $NNHC(S)NH_2$ | 3 | 62 | 187–188 |
| **5f** | H | H |  | $NNHC(S)NH_2$ | 3 | 76 | 188–191 |

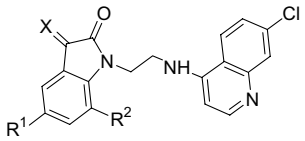


**Scheme 3.** Synthesis of quinoline–ethylene isatin derivatives **3** and **4**. Reagents and conditions: (a) 0.3 equiv of $K_2CO_3$, 0.3 equiv of $Et_3N$, reflux, 8 h, 92%; (b) 2.5 equiv of methanesulfonyl chloride, pyridine, 0 °C, 5 h, 83%; (c) NaH, isatin/5-substituted isatin, DMF, 60 °C, 24 h, 68–89%; (d) $H_2NNHC(S)NH_2$, ethanol, 3 h, 45 °C, 68–89%.

were added from DMSO dissolved stocks, maximum concentration of DMSO used was 0.1%. Two independent assays are performed in 200 μL final volume in flat bottom 96-well plates.

After 48 h the culture medium was removed and replaced with 1% formaldehyde in PBS pH 7.4, and fixed for 48 h at room temperature. Fixed parasites were transferred into 0.1% Triton-X-100 in PBS containing

**Table 2.** Chemical and physical data of a series of ethylene–quinoline isatin derivatives



**3a – 4c**

| Compound | $R^1$ | $R^2$ | X | Time (h) | %Yield | Mp (°C) |
|---|---|---|---|---|---|---|
| **3a** | H | H | O | 16 | 88 | 240–243 |
| **3b** | Me | H | O | 16 | 68 | 240–243 |
| **3c** | Cl | H | O | 16 | 69 | 221–223 |
| **3d** | Me | Me | O | 16 | 89 | 219 |
| **3e** | I | H | O | 16 | 70 | 200–201 |
| **4a** | H | H | $NNHC(S)NH_2$ | 3 | 70 | 167–171 |
| **4b** | Me | H | $NNHC(S)NH_2$ | 3 | 89 | 227–230 |
| **4c** | Cl | H | $NNHC(S)NH_2$ | 3 | 68 | 221–227 |

1 nM dye YOYO-1 (Molecular Probes). Parasitaemia was determined from dot plots (forward scatter vs Fluorescence FL-1) acquired on FACSort flow cytometer using CellQuest software (Beckton Dickinson).[25]

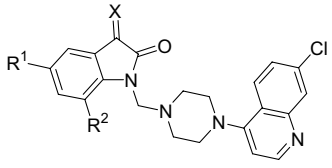
The 50% inhibitory concentration ($IC_{50}$) values for the compounds were obtained from the dose–response curves, using non-linear dose–response curve fitting analyses with GraphPad Prism v.3.00 software.

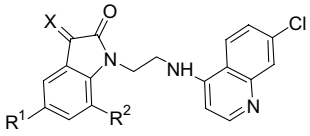
## 2. Results

The results of the biological evaluations are presented in Table 3. Quinoline thiosemicarbazone derivatives **2** and **4** generally showed better inhibition of falcipain-2 compared to the corresponding ketones **1** and **3**. It is noteworthy that all non-quinoline Mannich base thiosemicarbazones **5** were less active compared to the corresponding quinoline Mannich bases **4** ($IC_{50} > 20$ μM). This may suggest preference for larger hydrophobic groups in the S2 enzyme binding pocket.

Within the thiosemicarbazones, quinoline-Mannich base derivatives **1a**, **1c** and **1e** were more active against falcipain-2, with $IC_{50}$ values mostly less than 10 μM, compared to the ethylene quinolines **4a**–**b**. Considering compounds that were similar (**2a**–**d**) with respect to position 5 substituents, the general order of activity against falcipain-2 was Br > F > H. This trend is consistent with

**Table 3.** $IC_{50}$ values of compounds against chloroquine sensitive (D10), chloroquine resistant (K1 and W2) strains and falcipain-2





| Compound | $R^1$ | $R^2$ | X | $IC_{50}$, μM | | | | Clog *P* |
|---|---|---|---|---|---|---|---|---|
| | | | | D10[a] | K1[b] | W2[b] | FP-2[c] | |
| **1a** | H | H | O | 0.95 | ND[d] | 0.96 | >20 | 3.31 |
| **1c** | Cl | H | O | 1.07 | ND | ND | ND | 4.32 |
| **1d** | F | H | O | 0.72 | 0.72 | 0.91 | >20 | 3.75 |
| **1f** | I | H | O | 0.92 | ND | 0.79 | 20 | 4.61 |
| **2a** | H | H | $NNHC(S)NH_2$ | 1.37 | ND | 0.96 | 9.26 | 4.49 |
| **2b** | F | H | $NNHC(S)NH_2$ | 1.18 | ND | 1.19 | 7.99 | 4.63 |
| **2c** | Cl | H | $NNHC(S)NH_2$ | 1.37 | ND | 1.18 | ND | 5.20 |
| **2d** | Br | H | $NNHC(S)NH_2$ | 0.64 | 0.52 | 1.23 | 6.07 | 5.35 |
| **3a** | H | H | O | 0.90 | 0.99 | 0.12 | >20 | 3.88 |
| **3b** | Me | H | O | 0.55 | 0.73 | 0.49 | >20 | 4.38 |
| **3c** | Cl | H | O | 0.38 | 1.51 | ND | ND | 4.77 |
| **3d** | Me | Me | O | 0.33 | 0.45 | 0.23 | 20 | 4.88 |
| **4a** | H | H | $NNHC(S)NH_2$ | 0.32 | 0.71 | 0.24 | 14.65 | 5.15 |
| **4b** | Me | H | $NNHC(S)NH_2$ | 0.079 | 0.10 | 0.051 | 11.64 | 5.65 |
| **4c** | Cl | H | $NNHC(S)NH_2$ | 0.095 | 0.054 | ND | ND | 5.86 |
| **CQ** | | | | 0.033 | 0.312 | 0.240 | ND | 5.08 |

[a] Chloroquine-sensitive strain.
[b] Chloroquine-resistant strain.
[c] Falcipain-2.
[d] Not determined.

the trend previously observed for isatin-3-thiosemicarbazones in which a large (iodo) substituent at position 5 resulted in high potency against the enzyme.[11]

In the study of anti-malarial effects of compounds **1**–**4**, it is evident that thiosemicarbazones **4a**, **4b** and **4c** were generally superior compared to the corresponding ketone derivatives **3a**, **3b** and **3c**. This trend was, however, not observed for the Mannich bases **1** and **2**. Compounds **4b** and **4c** were particularly active against all three *P. falciparum* strains. These two compounds were even superior to chloroquine in the resistant W2 and K1 strains, with $IC_{50}$ values of 0.051 and 0.054 μM, respectively, while still retaining respectable activity against D10. Within the thiosemicarbazones, the ethylene-based derivatives were comparatively more potent than the Mannich bases. For example, compounds **4b** and **4c** had better $IC_{50}$ values, roughly all less than 1 μM. Removal of the quinoline ring system generally resulted in a significant loss of activity against all the three parasite strains (compounds **5a**–**f**; $IC_{50}$ values >10 μM for strain W2). Among the more active ethylene–quinoline thiosemicarbazones, methyl substitution at position 5 on the isatin ring resulted in better inhibitors of parasite growth in W2 strain. Compound **4b**, with $IC_{50}$ = 0.051 μM, was superior (5-fold) to chloroquine in this strain. Chloro substitution at position 5, as in **4c**, also showed excellent inhibitory activity against K1 with an $IC_{50}$ value of 0.054 μM, which was also superior (6-fold) to chloroquine. Considering compounds of series **4** only, the order of anti-malarial activity in K1 was generally Cl > Me > H. In all cases, the biological assays did not show the food vacuole abnormality that accompanies intracellular falcipain-2 inhibition, that is, swelling due to accumulation of undigested haemoglobin. The SAR study within this class of compounds clearly shows no strong correlation between inhibition of falcipain-2 and anti-malarial activity.

## 3. Discussion

We have shown that aminoquinoline thiosemicarbazone derivatives of isatin are potent inhibitors of *P. falciparum* growth. Considering inhibition of the protease falcipain-2, quinoline-Mannich base isatin derivatives (**2a**, **2b** and **2d**) blocked activity at $IC_{50}$ values of less than 10 μM. Compounds **4a**, **4b** and **4c** (quinoline–ethylene isatin derivatives) effectively blocked parasite development, but had modest activity against falcipain-2, suggesting a lack of strong correlation between the potencies of compounds against falcipain-2 and parasites. This suggests that the anti-malarial activity of inhibitors **4a**, **4b** and **4c** were not entirely due to inhibition of falcipain-2 and that other mechanisms, including selective uptake of the compounds by the parasite, are involved. Although falcipain-2 appears not to be the principal target for these compounds, it is possible that the compounds may be acting through the inhibition of another cysteine protease since there are many cathepsin L-like cysteine proteases in *P. falciparum*.[25–27] The possibility that these compounds may be exerting their anti-malarial effects through inhibition of another cysteine protease in *P. falciparum* is supported by the fact that isatins such as compounds **2a**, **2b**, **2d**, **4a** and **4b** can bind to and inhibit a cysteine protease.[11] The inhibitory activity (albeit modest) as demonstrated against falcipain-2 is a case in point. Alternatively compounds such as **4a**, **4b**, **4c** may be acting via a protease-independent mechanism. Similar conclusions have recently been made for tridentate chelating 2-acetylpyridine thiosemicarbazones.[28] Our results further suggest that compounds that share structural features of the potent compounds that we have identified, but also have improved intracellular inhibition of falcipain-2, should offer excellent anti-malarial activity.

The excellent inhibitory results against parasite development by compounds **4a**, **4b** and **4c** led to the speculation that improved potency could have been due to the contribution from specific moieties notably thiosemicarbazone and 4-aminoquinoline moieities, the ethylene linker and substituents at position 5 of the isatin scaffold (chloro and methyl substitution at position 5 on the aromatic ring of the isatin is favoured presumably for binding to the target). Thiosemicarbazones are potential metal-chelators in *P. falciparum*, which may exhibit biological activity by a number of mechanisms including: (a) metal-interactive inhibition of cysteine proteases;[29] (b) inhibition of metal-dependent enzymes such as falcilysin,[30] ribonucleotide reductase[31,32] and aminopeptidases;[3] (c) formation of lethal complexes, which are directly toxic to the parasite as a result of the induction of oxidative stress.[28]

Introduction of the ethylene linker in the molecule was generally more favoured compared to Mannich bases with a piperazinyl linker. The ethylene linker increases the lipophilicity of compounds **4a**, **4b**, **4c** compared to **1** and **2**. For example compounds **4a** and **4c** have higher Clog *P* values compared to the corresponding Mannich base compounds **2a** and **2c**, which probably aids their passage through parasite membranes on the way to their presumed site of action, the acidic food vacuole. If these compounds are also exerting their anti-malarial effects via chelation of iron in *P. falciparum*, then high lipophilicity is a major contributor. Lipophilicity is an important physical property of iron chelators since the iron withheld by chelators from *P. falciparum* most likely resides within the parasitic compartment of the malaria infected red blood cell. As such an effective anti-malarial iron chelator should have the ability to cross-lipid membranes and display high selective affinity for iron (II) or iron (III) compared to other endogenous transition metals. A correlation between the degree of lipophilicity of an iron chelator and its anti-plasmodial activity has been demonstrated.[20]

## 4. Conclusion

In summary we have designed compounds based on a multi-therapeutic strategy that were effective against three strains of the malaria parasite *P. falciparum* and with potential to inhibit *P. falciparum*-derived cysteine proteases. Two compounds were particularly effective

against chloroquine-resistant strains, thus validating their ability to evade the chloroquine resistance mechanism and cross-membranes to reach their site of action. These results indicate that multi-therapeutic strategies are promising strategies for identifying hits for subsequent optimization into potential leads against drug resistant parasites. Given the presumed multiple targets for the different moieties of these compounds, each mode of action needs to be determined with a view to establishing the mechanisms(s) most responsible for the observed anti-malarial activity.

## 5. Experimental

### 5.1. General

Proton nuclear magnetic resonance ($^1$H NMR) spectra were recorded at ambient temperatures using the following instruments: Varian Mercury (300 MHz) or Varian Unity Spectrometer (400 MHz) and TMS was used as an internal standard. The chemical shifts ($\delta$) are given in parts per million relative to TMS ($\delta = 0.00$). Carbon-13 nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded at 75 MHz or 100 MHz with the same internal standard. Diverse solvents were used in the determination of spectra for different compounds. The chemical shifts ($\delta$) are given in ppm relative to TMS ($\delta = 0.00$). Mass spectra were recorded by means of a VG micromass 16 F spectrometer at 70 eV with accelerating voltage 4 kV. Accurate masses were determined using a VG-70E spectrometer and VG (Micromass) 70-SE magnetic sector mass spectrometer. Infrared spectra were measured either in solution form using chloroform or as solids (KBr pellets) or Nujol mulls on a satellite FT-IR spectrophotometer. Micro (elemental) analysis was performed using a Fisons EA 1108 CHNS-O instrument. Melting points were determined by using a Reicher–Jung Thermovar (temperature range 0–350 °C) on cover slips and are uncorrected. Column chromatography and preparative layer chromatography (p.l.c), carried out on silica gel (Merck Kieselgel 60) were used in purification of samples. Reactions were monitored by thin-layer chromatography (TLC) using coated silica gel plates, detection by an ultra-violet lamp. The lipophilicity of the compounds was estimated via the Clog *P* values, which were calculated using ChemDraw Ultra 6.0.

**5.1.1. 7-Chloro-4-piperazin-1-yl-quinoline (8).** A mixture of 4,7-dichloroquinoline (2.0 g, 10.10 mmol), piperazine (4.35 g, 50.49 mmol), $K_2CO_3$ (0.419 g, 3.03 mmol), triethylamine (0.423 mL, 3.02 mmol) in *N*-methyl-2-pyrrolidinone (10 mL) was stirred under nitrogen at 135 °C for 4 h. After cooling to room temperature, the mixture was diluted with $CH_2Cl_2$ (50 mL) and washed with brine ($10 \times 50$ mL). The organic layer was separated, dried over sodium sulfate, filtered and concentrated. Column chromatography ($SiO_2$; MeOH/EtOAc, 2:80) yielded the amine **8** (1.87 g, 67%) as a white solid; mp 113–114 °C (from methanol); $R_f$ 0.34 (MeOH/EtOAc, 2:8); $\delta_H$ (300 MHz; $CDCl_3$); 8.70 (1H, d, *J* 5.1, H-2′), 8.02 (1H, d, *J* 2.1, H-8′), 7.93 (1H, d, *J* 9.0, H-5′), 7.40 (1H, dd, *J* 2.1 and 9.0, H-6′), 6.81 (1H, d, *J* 5.1, H-3′), 3.17–3.12 (8H, br s, H-2 and H-3); $\delta_C$ (75 MHz); 157.3, 151.9, 150.2, 134.8, 128.9, 126.1, 125.2, 121.9, 108.9, 53.5 ($2 \times$ C) and 46.1 ($2 \times$ C); LRMS (EI) *m*/*z* 247 [66%, $M^+$] and 205 [100%, $M-C_{11}H_{10}N_2Cl$]. Anal Calcd for $C_{13}H_{14}N_3Cl$: C 63.0%, H 5.7%, N 16.9%. Found: C 62.8%, H 5.6%, N 16.6%.

### 5.2. A. General procedure for preparing compounds 1a–g

To a solution of isatin or substituted isatin (0.2 g, 1.35 mmol) in 5 mL of 99.9% ethanol was added to a mixture of compound **8** (0.27 g, 1.35 mmol) and aqueous formaldehyde 37% (0.03 mL, 1.35 mmol) also dissolved in 10 mL of 99.9% ethanol. The reaction mixture was stirred for 3 h at room temperature, refrigerated for 48 h to form crystals. The crystalline products were separated by filtration, washed and vacuum dried. Recrystallization from ethanol rendered desired products in pure form.

**5.2.1. *N*$^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-1*H*-indole-2,3-dione (1a).** (0.51 g, 93%) obtained as reddish-orange crystals; mp 203–206 °C (from ethanol); IR (KBr) $\nu_{max}/cm^{-1}$ 1730 (keto C=O), 1617 (amide C=O), 1572 (C=N); $\delta_H$ (300 MHz; $CDCl_3$); 8.70 (1H, d, *J* 5.1, H-2″), 8.02 (1H, d, *J* 2.1, H-8″), 7.92 (1H, d, *J* 9.0, H-5″), 7.66 (1H, dd, *J*, 0.6 and 8.0, H-4), 7.62 (1H, ddd, *J* 0.6, 6.8 and 7.8, H-6), 7.43 (1H, dd, *J* 2.1 and 9.0, H-6″), 7.19 (1H, ddd, *J* 0.9, 6.8 and 8.0 and H-5), 7.10 (1H, d, *J* 7.8, H-7), 6.82 (1H, d, *J* 5.1, H-3″), 4.60 (2H, s, –NC*H*$_2$N–), 3.24 (4H, br s, –N(CH$_2$C*H*$_2$)$_2$NAr), 2.95 (4H, br s, –N(C*H*$_2$CH$_2$)$_2$NAr); $\delta_C$ (75 MHz); 182.1, 159.0, 156.6, 151.8, 151.5, 150.1, 138.4, 128.9, 128.4, 126.3, 125.4, 125.2, 124.0, 121.8, 117.4, 111.5, 109.1, 62.3, 51.8 ($2 \times$ C) and 50.6 ($2 \times$ C); HRMS (FAB) found *m*/*z* 407.12661 $[M+1]^+$, $C_{22}H_{19}O_2N_4Cl$ requires 407.12747. Anal Calcd for $C_{22}H_{19}O_2N_4Cl$ C 64.4%, H 4.7%, N 13.3%. Found: C 64.6%, H 4.5%, N 13.3%.

**5.2.2. *N*$^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5,7-dimethyl-1*H*-indole-2,3-dione (1b).** The conditions employed for the preparation of this compound were those described in general procedure **A**. However, 1.14 mmol of isatin and compound **8** were used.

(0.42 g, 85%) obtained as red crystals; mp 209–213 °C (from ethanol); IR ($CHCl_3$) $\nu_{max}/cm^{-1}$ 1739 (keto C=O), 1635 (amide C=O), 1542 (C=N); $\delta_H$ (400 MHz; $CDCl_3$); 8.71 (1H, d, *J* 5.2, H-2″), 8.02 (1H, d, *J* 2.0, H-8″), 7.93 (1H, d, *J* 9.0, H-5″), 7.43 (1H, dd, *J*, 2.0 and 9.0, H-6″), 7.31 (1H, d, *J* 2.0, H-4), 7.20 (1H, d, *J* 2.0, H-6), 6.80 (1H, d, *J* 5.2, H-3″), 4.51 (2H, s, –NC*H*$_2$N–), 3.23 (4H, br s, –N–CH$_2$C*H*$_2$NAr), 2.86 (4H, br s, –NC*H*$_2$CH$_2$NAr), 2.58 (3H, s, Ar–C*H*$_3$), 2.29 (3H, s, Ar–C*H*$_3$); $\delta_C$ (75 MHz); 182.7, 159.0, 157.7, 151.1, 150.1, 138.9, 134.9, 128.8, 128.7, 126.8, 125.9, 125.6, 125.0, 122.2, 118.4, 111.8, 109.8, 62.4, 52.8 ($2 \times$ C), 50.6 ($2 \times$ C), 20.1 and 19.1; LRMS (EI) *m*/*z* 434 [58%, $M^+$], 119 [100%, $M-C_8H_9N$]. Anal. Calcd for $C_{24}H_{23}O_2N_4Cl$: C 66.3%, H 5.3%, N 12.9%. Found: C 67.3%, H 5.5%, N 12.3%.

**5.2.3. $N^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-chloro-1*H*-indole-2,3-dione (1c).** The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.10 mmol of isatin and compound **8** were used.

(0.13 g, 24%) obtained as orange crystals; mp 153–157 °C (from ethanol); IR $\nu_{max}/cm^{-1}$ 1736 (keto C=O), 1617 (amide C=O), 1511 (C=N); $\delta_H$ (300 MHz; $CDCl_3$); 8.69 (1H, d, *J* 5.0, H-2″), 8.00 (1H, d, *J* 2.0, H-8″), 7.89 (1H, d, *J* 8.8, H-5″), 7.61 (1H, d, *J* 2.4, H-4), 7.57 (1H, dd, *J* 2.4 and 8.4, H-6), 7.42 (1H, dd, *J* 2.0 and 8.8, H-6″), 7.09 (1H, d, *J* 8.4, H-7), 6.80 (1H, d, *J* 5.0, H-3″), 4.57 (2H, s, –NC*H*$_2$N–), 3.21 (4H, br s, –NCH$_2$C*H*$_2$NAr), 2.91 (4H, br s, –NC*H*$_2$CH$_2$NAr); $\delta_C$ (75 MHz); 181.9, 158.3, 156.6, 151.7, 149.9, 149.5, 138.8, 128.9, 128.4, 126.3, 125.2, 124.9, 123.7, 121.7, 118.4, 112.9, 109.1, 62.4, 51.8 (2 × C) and 50.6 (2 × C); LRMS (EI) *m/z* 440 [3%, $M^+$], 260 [100%, M−$C_{14}H_{15}ClN_3$]. Anal. Calcd for $C_{22}H_{18}O_2N_4Cl_2{\cdot}H_2O$: C 57.6%, H 4.1%, N 12.2%. Found: C 57.6%, H 4.1%, N 11.9%.

**5.2.4. $N^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-fluoro-1*H*-indole-2,3-dione (1d).** The conditions employed for the preparation of this compound were those described in general procedure **A**. However, 1.21 mmol of isatin and compound **8** were used.

(0.49 g, 94%) obtained as orange crystals; mp 110–117 °C (from ethanol); IR ($CHCl_3$) $\nu_{max}/cm^{-1}$, 1744 (keto C=O), 1615 (amide C=O), 1562 (C=N); $\delta_H$ (400 MHz; $CDCl_3$); 8.70 (1H, d, *J* 5.2, H-2″), 8.03 (1H, d, *J* 2.0, H-8″), 7.90 (1H, d, *J* 9.0, H-5″), 7.42 (1H, dd, *J* 2.0 and 9.0, H-6″), 7.35 (1H, d, *J* 2.1, H-4), 7.31 (1H, dd, *J* 2.1 and 8.2, H-6), 7.11 (1H, d, *J* 8.2, H-7), 6.82 (1H, d, *J* 5.2, H-3″), 4.58 (2H, s, –NC*H*$_2$N–), 3.25 (4H, br s, –NCH$_2$C*H*$_2$NAr), 2.93 (4H, br s, –NC*H*$_2$CH$_2$NAr); $\delta_C$ (75 MHz); 181.9, 159.6, 156.7, 151.8, 150.1, 150.0, 136.7, 136.1, 128.8, 126.7, 125.3, 125.0, 123.6, 121.9, 119.3, 112.4, 109.2, 62.7, 52.1 (2 × C), 50.8 (2 × C); HRMS (FAB) *m/z* 425.11703 $[M+1]^+$ $C_{22}H_{18}ClFN_4O_2$ requires 425.11805. Anal. Calcd for $C_{22}H_{18}ClFN_4O_2{\cdot}H_2O$: C 60.1%, H 4.3%, N 11.8%. Found: C 60.4%, H 4.3%, N 11.5%.

**5.2.5. $N^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-methyl-1*H*-indole-2, 3-dione (1e).** The conditions employed for the preparation of this compound were those described in general procedure **A**. However, 1.24 mmol of isatin and compound **8** were used.

(0.29 g, 55%) obtained as reddish-orange crystals; mp 202–203 °C (from ethanol); IR ($CHCl_3$) $\nu_{max}/cm^{-1}$, 1729 (ketone C=O), 1609 (amide C=O), 1518 (C=N); $\delta_H$ (300 MHz; $CDCl_3$); 8.69 (1H, d, *J* 5.1, H-2″), 8.02 (1H, d, *J* 2.1, H-8″), 7.90 (1H, d, *J* 8.7, H-5″), 7.45 (1H, d, *J* 2.4, H-4), 7.43 (1H, dd, *J* 2.4 and 7.8, H-6), 7.38 (1H, dd, *J* 2.1 and 8.7, H-6″), 7.02 (1H, d, *J* 7.8, H-7), 6.81 (1H, d, *J* 5.1, H-3″), 4.58 (2H, s, –NC*H*$_2$N–), 3.24 (4H, br s, –NCH$_2$C*H*$_2$NAr), 2.94 (4H, br s, –NC*H*$_2$CH$_2$NAr), 2.35 (3H, s, Ar–C*H*$_3$); $\delta_C$ (75 MHz); 182.4, 159.1, 156.6, 151.5, 150.7, 150.2, 138.4, 128.8, 128.4, 126.4, 125.7, 125.6, 124.4, 121.8, 118.2, 110.5, 109.0, 62.3, 51.6 (2 × C), 50.6 (2 × C) and 21.2; HRMS (FAB) found *m/z* 421.14346 $[M+1]^+$ $C_{23}H_{21}ClN_4O_2$ requires 421.14312. Anal. Calcd for $C_{23}H_{21}ClN_4O_2{\cdot}H_2O$: C 62.8%, H 4.8%, N 12.8%. Found: C 63.0%, H 4.9%, N 12.9%.

**5.2.6. $N^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-iodo-1*H*-indole-2, 3-dione (1f).** The conditions employed for the preparation of this compound were those described in general procedure **A**. However, 0.73 mmol of isatin and compound **8** were used.

(0.23 g, 59%) obtained as red crystals; mp 151–153 °C (from ethanol); IR ($CHCl_3$) $\nu_{max}/cm^{-1}$ 1745 (keto C=O), 1602 (amide C=O), 1536 (C=N); $\delta_H$ (400 MHz; $CDCl_3$); 8.68 (1H, d, *J* 5.2, H-2″), 8.02 (1H, d, *J* 2.0, H-8″), 7.94 (1H, d, *J* 9.0, H-5″), 7.93 (1H, d, *J* 0.3, H-4), 7.90 (1H, dd, *J* 0.3 and 8.0, H-6), 7.43 (1H, dd, *J* 2.0 and 9.0, H-6″), 6.94 (1H, d, *J* 8.0, H-7), 6.81 (1H, d, *J* 5.2, H-3″), 4.58 (2H, s, –NC*H*$_2$N–), 3.22 (4H, br s, –NCH$_2$C*H*$_2$NAr), 2.92 (4H, br s, –NC*H*$_2$CH$_2$NAr); $\delta_C$ (75 MHz); 181.6, 158.6, 156.9, 151.8, 150.7, 150.0, 136.7, 135.8, 128.8, 126.4, 124.9, 124.5, 123.9, 121.8, 119.2, 113.7, 109.1, 62.4, 51.8 (2 × C) and 50.2 (2 × C); HRMS (FAB) found *m/z* 534.03212 $[M+1]^+$ $C_{22}H_{18}ClIN_4O_2$ requires 534.03195. Anal. Calcd for $C_{22}H_{18}ClIN_4O_2{\cdot}0.25H_2O$ : C 49.2%, H 3.4%, N 10.4%. Found: C 49.2%, H 3.1%, N10.3%.

**5.2.7. $N^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-bromo-1*H*-indole-2, 3-dione (1g).** The conditions employed for the preparation of this compound were those described in general procedure **A**. However, 0.88 mmol of isatin and compound **8** were used.

(0.21 g, 48%) obtained as orange crystals; mp 210–212 °C (from ethanol); IR ($CHCl_3$) $\nu_{max}/cm^{-1}$ 1736 (keto C=O), 1618 (amide C=O), 1502 (C=N); $\delta_H$ (300 MHz; $CDCl_3$); 8.70 (1H, d, *J* 5.1, H-2″), 8.02 (1H, d, *J* 2.1, H-8″), 7.90 (1H, d, *J* 9.0, H-5″), 7.72 (1H, d, *J* 2.1, H-4), 7.55 (1H, dd, *J* 2.1 and 8.4, H-6), 7.42 (1H, dd, *J* 2.1 and 9.0, H-6″), 7.05 (1H, d, *J* 8.4, H-7), 6.82 (1H, d, *J* 5.2, H-3″), 4.58 (2H, s, –NC*H*$_2$N–), 3.24 (4H, br s, –NC*H*$_2$CH$_2$NAr), 2.93 (4H, br s, –NCH$_2$C*H*$_2$NAr); $\delta_C$ (75 MHz); 182.2, 161.1, 156.6, 151.8, 150.8, 150.0, 136.1, 128.8, 128.4, 126.4, 126.0, 124.9, 124.0, 121.8, 118.1, 112.5, 109.0, 62.4, 51.8 (2 × C) and 50.6 (2 × C); LRMS (EI) *m/z* 486 [3%, $M^+$], 260 [100%, M−$C_{14}H_{15}ClN_3$]. Anal. Calcd for $C_{22}H_{18}O_2BrN_4Cl{\cdot}H_2O$: C 52.4%, H 3.6%, N 11.5%. Found: C 52.7%, H 3.5%, N 11.6%.

### 5.3. B. General procedure for the preparation of compounds 2a–e

To a solution of isatin or substituted isatin-3-thiosemicarbazone derivative (0.2 g, 1.36 mmol) in 5 mL of 99.9% ethanol was added a mixture of compound **8** (0.22 g, 1.36 mmol) and aqueous formaldehyde 37% (0.027 mL, 1.35 mmol) also dissolved in 10 mL ethanol (99.9%). The reaction mixture was stirred for 3 h at room temperature for isatins or 45 °C for isatin-3-thio-

semicarbazones, refrigerated for 48 h to form crystals. The crystalline product was separated by filtration, vacuum dried and recrystallized from ethanol.

**5.3.1. $N^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-1*H*-indole-2,3-dione 3-thiosemicarbazone (1a).** (0.29 g, 78%) obtained as orange crystals; mp 216–217 °C (from ethanol); IR (KBr): $\nu_{max}/cm^{-1}$ 3686 (N–H), 3036 (Ar–H), 1675 (C=O), 1511 (C=N), 1122 (C=S); $\delta_H$ (300 MHz; DMSO-$d_6$); 12.40 (1H, s, NNH), 9.04 (1H, s, N*H*H), 8.69 (1H, s, NH*H*), 8.65 (1H, d, *J* 5.2, H-2″), 7.96 (1H, d, *J* 9.0, H-5″), 7.91 (1H, d, *J* 2.4, H-8″), 7.66 (1H, dd, *J* 1.2 and 7.6, H-4), 7.48 (1H, dd, *J* 2.4 and 9.0, H-6″), 7.42 (1H, ddd, *J* 1.2, 7.2 and 8.0, H-6), 7.32 (1H, d, *J* 7.2, H-7), 7.15 (1H, ddd, *J* 0.4, 7.6 and 8.0, H-5), 6.61 (1H, d, *J* 5.2, H-3″), 4.60 (2H, s, –NC$H_2$N–), 3.13 (4H, br s, –NC$H_2$CH$_2$NAr), 2.87 (4H, br s, –NCH$_2$C$H_2$NAr); $\delta_C$ (75 MHz); 179.4, 162.6, 156.9, 152.8, 150.3, 144.5, 134.2, 131.8, 131.7, 128.9, 126.8, 126.4, 123.1, 122.1, 121.3, 120.1, 111.7, 110.2, 61.7, 52.3 (2 × C) and 50.7 (2 × C); HRMS (FAB) *m/z* 480.13677 $[M+1]^+$, $C_{23}H_{22}ClN_7OS$ requires 480.13732. Anal. Calcd for $C_{23}H_{22}ClO_5N_7$: C 57.6%, H 4.6%, N 20.4%, S 6.7%. Found: C 57.6%, H 4.7%, N 20.4%, S 6.8%.

**5.3.2. $N^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-5-chloro-1*H*-indole-2,3-dione 3-thiosemicarbazone (2c).** The conditions employed for the preparation of this compound were those described in general procedure **B**. However, 0.37 mmol of compound **1c** and thiosemicarbazide were used.

(0.35 g, 86%) obtained as orange crystals; mp 234–235 °C (from ethanol); IR (KBr): $\nu_{max}/cm^{-1}$ 3687 (N–H), 1675 (C=O), 1518 (C=N), 1120 (C=S); $\delta_H$ (300 MHz; $CDCl_3$); 12.3 (1H, s, NNH), 9.10 (1H, s, N*H*H), 8.71 (1H, s, NH*H*), 8.68 (1H, d, *J* 5.4, H-2″), 8.01 (1H, d, *J* 2.0, H-8″), 7.88 (1H, d, *J* 8.8, H-5″), 7.61 (1H, d, *J* 2.1, H-4), 7.59 (1H, dd, *J* 2.1 and 8.4, H-6), 7.42 (1H, dd, *J* 2.0 and 8.8, H-6″), 7.11 (1H, d, *J* 8.4, H-7), 6.81 (1H, d, *J* 5.4, H-3″), 4.58 (2H, s, –NC$H_2$N–), 3.21 (4H, br s, –NCH$_2$C$H_2$NAr), 2.91 (4H, br s, –NC$H_2$CH$_2$NAr); $\delta_C$ (75 MHz); 178.7, 162.6, 158.3, 156.7, 151.7, 150.1, 149.5, 138.9, 128.9, 128.4, 126.4, 125.2,123.9, 123.7, 121.7, 119.1, 111.9, 110.0, 62.4, 51.9 (2 × C), 51.2 (2 × C). Anal. Calcd for $C_{23}H_{21}Cl_2N_7OS$: C 53.8%, H 4.1%, N 18.6% S 5.8%. Found: C 53.7%, H 4.1%, N 19.0%, S 6.2%; HRMS (FAB) *m/z* 514.09889 $[M+1]^+$ $C_{23}H_{21}Cl_2N_7OS$ requires 514.09836.

**5.3.3. $N^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-5-bromo-1*H*-indole-2,3-dione3-thiosemicarbazone (2d).** The conditions employed for the preparation of this compound were those described in general procedure **B**. However, 0.67 mmol of compound **1d** and thiosemicarbazide were used.

(0.27 g, 73%) obtained as orange crystals; mp 236–237 °C (from ethanol); IR (KBr): $\nu_{max}/cm^{-1}$ 3688 (N–H), 1628 (C=O), 1515 (C=N), 1122 (C=S); $\delta_H$ (300 MHz; DMSO-$d_6$); 12.24 (1H, s, NNH), 9.14 (1H, s, N*H*H), 8.87 (1H, s, NH*H*), 8.69 (1H, d, *J* 5.4, H-2″), 7.97 (1H, d, *J* 9.0, H-5″), 7.91 (1H, d, *J* 2.4, H-8″), 7.62 (1H, d, *J*, 2.1, H-4), 7.50 (1H, dd, *J* 2.1 and 7.8, H-6), 7.39 (1H, dd, *J* 2.4 and 9.0, H-6″), 7.35 (1H, d, *J* 7.8, H-7), 6.69 (1H, d, *J* 5.4, H-3″), 4.67 (2H, s, –NC$H_2$N–), 3.15 (4H, br s, –NCH$_2$C$H_2$NAr), 2.89 (4H, br s, –NC$H_2$CH$_2$N–); $\delta_C$ (75 MHz); 179.5, 161.8, 156.9, 152.1, 149.5, 143.7, 133.5, 131.6, 131.0, 130.9, 127.9, 126.1, 125.7, 122.9, 121.3, 119.3, 111.0, 109.4, 60.9, 52.5 (2 × C) and 49.9 (2 × C); HRMS (FAB) *m/z* 558.04865 $[M+1]^+$ $C_{23}H_{21}BrClN_6OS$ requires 558.04784. Anal. Calcd for $C_{23}H_{21}BrClN_7OS$: C 49.4%, H 3.8%, N 17.0%, S 5.7%. Found: C 50.2%, H 3.9%, N 17.2%, S 5.1%.

**5.3.4. $N^1$-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-methyl-1*H*-indole-2,3-dione-3-thiosemicarbazone (2e).** The conditions employed for the preparation of this compound were those described in general procedure **B**. However, 0.85 mmol compound **1e** and thiosemicarbazide were used.

(0.33 g, 78%) obtained as yellowish-orange crystals; mp 232–234 °C (from ethanol); IR (KBr): $\nu_{max}/cm^{-1}$ 3687 (N–H), 1675 (C=O), 1518 (C=N), 1122 (C=S); $\delta_H$ (300 MHz; DMSO-$d_6$); 12.37 (1H, s, NNH), 9.17 (1H, s, N*H*H), 8.91 (1H, s, NH*H*), 8.68 (1H, d, *J* 5.4, H-2″), 7.99 (1H, d, *J* 9.0, H-5″), 7.94 (1H, d, *J* 2.1, H-8), 7.78 (1H, d, *J* 2.1, H-4), 7.55 (1H, dd, *J* 2.1 and 8.7, H-6), 7.48 (1H, d, *J* 2.1 and 9.0, H-6″), 7.34 (1H, d, *J* 8.7, H-7), 6.69 (1H, d, *J* 5.4, H-3″), 4.81 (2H, s, –NC$H_2$N–), 3.16 (4H, br s, –NCH$_2$C$H_2$NAr), 2.90 (4H, br s, –NC$H_2$CH$_2$NAr), 2.27 (3H, s, Ar–C$H_3$); $\delta_C$ (75 MHz); 179.4, 161.7, 156.2, 152.8, 149.5, 144.0, 142.4, 134.6, 132.0, 130.8, 127.9, 126.1, 125.0, 123.6, 121.3, 120.0, 112.3, 109.4, 63.1, 51.6 (2 × C), 49.5 (2 × C) and 21.3; HRMS (FAB) *m/z* 494.15187 $[M+1]^+$ $C_{24}H_{24}ClN_7OS$ requires 494.15297. Anal. Calcd for $C_{24}H_{24}ClN_7OS$: C 58.4%, H 4.9%, N 19.8%, S 6.5%. Found: C 58.4%, H 4.9%, N19.7%, S 6.2%.

### 5.4. C. General procedure for preparation of compounds 5a–f

Equimolar quantities of isatin-3-thiosemicarbazone (0.20 g, 0.91 mmol), amine (0.09 mL, 0.91 mmol) and paraformaldehyde (0.08 g, 0.90 mmol) were dissolved in warm 99.9% ethanol. The mixture was continuously stirred at 25 °C for 6 h. After standing for approximately 24 h in the fridge, a crystalline product was formed. The product was then separated by filtration, vacuum dried and recrystallized from ethanol.

**5.4.1. $N^1$-Diethylaminomethyl-indole-2,3-dione-3-thiosemicarbazone (5a).** (0.15 g, 54%) obtained as yellow crystals; mp 134–135 °C (ethanol) (lit. 135 °C);[33] $\delta_H$ (300 MHz; DMSO-$d_6$); 12.10 (1H, s, NNH), 9.05 (1H, s, N*H*H), 8.69 (1H, s, NH*H*), 7.71 (1H, dd, *J* 0.9 and 7.5, H-4), 7.44 (1H, ddd, *J* 0.9, 6.6 and 8.0, H-6), 7.17 (1H, ddd, *J* 0.9, 6.6 and 7.5, H-5), 7.19 (1H, d, *J* 8.0, H-7), 4.52 (2H, s, –NC$H_2$N–), 2.65 (4H, q, *J* 6.9, –NC$H_2$CH$_3$), 1.06 (6H, t, *J* 6.9, –NCH$_2$C$H_3$).

**5.4.2. $N^1$-Piperidin-1-ylmethyl-indole-2,3-dione-3-thiosemicarbazone (5b).** (0.27 g, 95%); obtained as yellow solids; mp 179–180 °C (from ethanol) (lit. 174 °C);[33] $\delta_H$ (300 MHz, DMSO-$d_6$); 12.38 (1H, s, NNH), 9.05 (1H, s, N*H*H), 8.69 (1H,s, NH*H*), 7.71 (1H, dd, *J* 0.9 and 7.5, H-4), 7.41 (1H, ddd, *J* 0.9, 7.8 and 8.1, H-6), 7.26 (1H, d, *J* 7.8, H-7), 7.17 (1H, ddd, *J* 1.2, 7.5 and 8.1, H-5), 4.47 (2H, s, –NC$H_2$N–), 2.54 (4H, t, *J* 4.8, H-2′), 1.46 (4H, m, H-3′ 3′), 1.33 (2H, m, H-4′ 4′).

**5.4.3. $N^1$-Pyrrolidin-1-ylmethyl-indole-2,3-dione-3-thiosemicarbazone (5c).** (0.25 g, 90%) obtained as yellow crystals; mp 181–184 °C (from ethanol) (lit. 177–178 °C);[33] $\delta_H$ (400 MHz, DMSO-$d_6$); 12.29 (1H, s, NNH), 9.03 (1H, s, N*H*H), 8.68 (1H, s, NH*H*), 7.68 (1H, dd, *J* 1.2 and 7.6, H-4), 7.42 (1H, ddd, *J* 1.2, 7.2 and 8.0, H-6), 7.23 (1H, d, *J* 8.0, H-7), 7.12 (1H, ddd, *J* 0.9, 7.2 and 7.6, H-5), 4.61 (2H, s, –NC$H_2$N–), 2.60 (4H, br t, *J* 3.2, H-2′), 1.65 (4H, m, H-3).

**5.4.4. $N^1$-Morpholin-4-ylmethyl-indole-2,3-dione-3-thiosemicarbazone (5d).** (0.27 g, 92%); as yellow crystals; mp 208–210 °C (from ethanol) (lit. 215–216 °C);[33] $\delta_H$ (300 MHz; DMSO-$d_6$); 12.37 (1H, s, NNH), 9.05 (1H, s, N*H*H), 8.70 (1H, s, NH*H*), 7.72 (1H, dd, *J* 0.6 and 7.2, H-4), 7.42 (1H, ddd, *J* 0.9, 7.5 and 8.0, H-6), 7.27 (1H, d, *J* 8.0, H-7), 7.15 (1H, ddd, *J* 0.6, 7.2 and 7.5, H-5), 4.49 (2H, s, –NC$H_2$N–), 3.54 (4H, br s, H-3′), 2.57 (4H, br s, H-2′).

**5.4.5. $N^1$-4-Benzyl-piperazin-1-ylmethyl-indole-2,3-dione-3-thiosemicarbazone (5e).** (0.23 g, 63%); obtained as yellow crystals; mp 187–188 °C; IR (KBr): $\nu_{max}$/cm$^{-1}$ 3680 (N–H), 3428 (N–H), 1637 (C=O), 1505 (C=N), 1123 (C=S); $\delta_H$ (300 MHz, DMSO-$d_6$); 12.38 (1H, s, NNH), 9.06 (1H, s, N*H*H), 8.69 (1H, s, NH*H*), 7.71 (1H, dd, *J* 0.9 and 7.5, H-4), 7.43 (1H, ddd, *J* 0.9, 7.2 and 7.8, H-6), 7.25–7.23 (5H, m, H-2″, H-3″, H-4″, H-5″ and H-6″), 7.19 (1H, d, *J* 7.8, H-7), 7.17 (1H, ddd, *J* 0.6, 7.2 and 7.5, H-5), 4.52 (2H, s, –NC$H_2$N–), 3.43 (2H, s, –NC$H_2$Ar), 2.59 (4H, br s, –NC$H_2$CH$_2$NCH$_2$Ar), 2.34 (4H, br s, –NCH$_2$C$H_2$-NCH$_2$Ar); $\delta_C$ (75 MHz); 178.7, 161.6, 143.7, 138.2, 131.0, 130.9, 128.6 (2 × C), 128.0, 126.8 (2 × C), 122.8, 120.5, 119.2, 111.1, 61.8, 52.3 (2 × C), 50.1 (2 × C) and 49.9. Anal. Calcd for $C_{21}H_{24}N_6OS$: C 62.1%, H 6.6%, N 20.1%, S 6.6. Found: C 61.7%, H 5.9%, N 20.6%, S 6.6%; HRMS (FAB) *m/z* 409.18109 [M+1]$^+$, $C_{21}H_{24}N_6OS$ requires 409.18106.

**5.4.6. $N^1$-4-Phenyl-piperazin-1-ylmethyl-indole-2,3-dione-3-thiosemicarbazone (5f).** (0.27 g, 76%) obtained yellowish crystals; 188–191 °C; IR (KBr): $\nu_{max}$/cm$^{-1}$ 3686 (N–H), 3575 (N–H), 1694 (C=O), 1502 (C=N), 1127 (C=S); $\delta_H$ (300 MHz, DMSO-$d_6$); 12.39 (1H, s, NNH), 9.04 (1H, s, N*H*H), 8.69 (1H, s, NH*H*), 7.79 (1H, dd, *J* 0.9 and 7.6, H-4), 7.45 (1H, ddd, *J* 0.9, 6.6 and 8.0, H-6), 7.18 (1H, d, *J* 8.0, H-7), 7.21–7.13 (5H, m, H-2″, H-3″ and 4″), 7.10 (1H, ddd, *J* 0.6, 6.6 and 7.6, H-5), 4.59 (2H, s, –NC$H_2$N–), 3.09 (4H, br s, –NCH$_2$C$H_2$NAr), 2.76 (4H, br s, –NC$H_2$CH$_2$NAr); $\delta_C$ (75 MHz); 178.7, 161.7, 151.0, 143.7, 131.1, 130.9, 128.8, 122.0, 120.6 (2 × C), 120.5, 119.3 (2 × C), 119.2, 111.1, 60.9, 50.1 (2 × C) and 48.1 (2 × C). Anal. Calcd for $C_{20}H_{22}N_6OS$: C 60.9%, H 5.6%, N 21.3%, S 8.1%. Found: C 61.3%, H 6.3%, N 18.7%, S 5.7%; HRMS (FAB) *m/z* 395.16544 [M+1]$^+$, $C_{20}H_{22}N_6$ OS requires 395.16541.

### 5.5. D. General procedure for the preparation of compounds 4

Equimolar quantities of ethyl-quinoline isatin derivative (0.1 g, 0.284 mmol) and thiosemicarbazide (0.038 g, 0.284 mmol) were dissolved in warm 99.9% ethanol. The mixture was then continuously stirred at 45 °C for 5–6 h. After standing for approximately 24 h at room temperature, a crystalline product was formed. The product was then separated by filtration, vacuum dried and recrystallized from acetonitrile.

**5.5.1. $N^1$-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-1*H*-indole-2,3-dione-3-thiosemicarbazone (4a).** (0.08 g, 70%) obtained as orange crystals; mp 167–171 °C (from acetonitrile); IR (KBr): $\nu_{max}$/cm$^{-1}$ 3680 (N–H), 3404 (N–H), 3012 (Ar-H), 1635 (C=O), 1516 (C=N), 1140 (C=S); $\delta_H$ (400 MHz, DMSO-$d_6$); 12.31 (1H, s, NNH), 9.02 (1H, s, N*H*H), 8.67 (1H, s, NH*H*), 8.40 (1H, d, *J* 5.4, H-2″), 7.98 (1H, d, *J* 9.0, H-5″), 7.76 (1H, d, *J* 2.0, H-8″), 7.67 (1H, dd, *J* 0.6 and 7.4, H-4), 7.38 (1H, dd, *J* 2.0 and 9.0, H-6″), 7.28 (1H, ddd, *J* 0.6, 7.6 and 7.8, H-6), 7.10 (1H, ddd, *J* 0.9, 7.4 and 7.6, H-5), 7.02 (1H, d, *J* 7.8, H-7), 6.63 (1H, d, *J* 5.4, H-3″), 4.01 (2H, t, *J* 5.6, –C$H_2$CH$_2$NHAr), 3.65 (2H, t, *J* 5.6, -CH$_2$C$H_2$NHAr); $\delta_C$ (75 MHz); 179.4, 161.8, 152.6, 150.5, 149.7, 149.1, 143.7, 138.6, 134.1, 128.2, 124.9, 124.5, 123.7, 123.1, 118.2, 117.3, 110.3, 104.5, 74.9 and 42.4; HRMS (FAB) found *m/z* 425.09419 [M+1]$^+$, $C_{20}H_{17}ClN_6OS$] requires 425.09513. Anal. Calcd for $C_{20}H_{17}ClN_6OS{\cdot}0.5H_2O$: C 55.4%, H 3.9%, N 18.8%, S 6.9%. Found: C 55.3%, H 4.0%, N 18.8%, S 6.9%.

**5.5.2. $N^1$-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-5-methyl-1*H*-indole-2,3-dione-3-thiosemicarbazone (4b).** The conditions employed for the preparation of this compound were those described in general procedure **D**. However, 0.27 mmol of compound **1b** and thiosemicarbazide were used.

(0.11 g, 89%) obtained as yellow-orange crystals; mp 227–230 °C (from ethanol); IR (KBr): $\nu_{max}$/cm$^{-1}$ 3632 (N–H), 3378 (N–H), 1665 (C=O), 1506 (C=N), 1127 (C=S); $\delta_H$ (400 MHz; DMSO-$d_6$); 12.29 (1H, s, NNH), 8.98 (1H, s, N*H*H), 8.63 (1H, s, NH*H*), 8.40 (1H, d, *J* 5.6, H-2″), 8.01 (1H, d, *J* 9.0, H-5″), 7.76 (1H, d, *J* 2.0, H-8″), 7.49 (1H, d, *J* 2.0, H-4), 7.39 (1H, dd, *J* 2.0 and 9.0, H-6″), 7.08 (1H, dd, *J* 2.0 and 7.4, H-6), 7.00 (1H, d, *J* 7.4, H-7), 6.60 (1H, d, *J* 5.6, H-3″), 3.98 (2H, t, *J* 5.4, –NC$H_2$CH$_2$NHAr), 3.62 (2H, t, *J* 5.4, –NCH$_2$C$H_2$NHAr), 2.31 (3H, s, Ar–C$H_3$); $\delta_C$ (75 MHz); 179.4, 161.8, 152.5, 150.6, 149.7 149.0, 141.6, 138.9, 134.2, 128.1, 125.3, 124.9, 124.5, 121.9, 118.2, 118.1, 110.9, 110.6, 74.9, 41.8 and 21.2; LRMS (FAB) *m/z* 439 [5%, (M+1)$^+$], and 307 [30%, M−$C_{18}H_{14}ClN_3$]. Anal. Calcd for $C_{21}H_{19}ClN_6OS$: C

57.5%, H 4.4%, N 19.3%, S 7.3%. Found: C 57.1%, H 4.3%, N 18.8%, S 7.3%.

**5.5.3. $N^1$-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-5-chloro–1*H*-indole-2,3-dione 3-thiosemicarbazone (4c).** The conditions employed for the preparation of this compound were those described in general procedure **D**. However, 0.26 mmol of compound **3c** and thiosemicarbazide were used.

(0.16 g, 68%) obtained as orange crystals; mp 221–227 °C (from ethanol); IR (KBr): $\nu_{max}/cm^{-1}$ 3688 (N–H), 3478 (N–H), 1665 (C=O), 1510 (C=N), 1121 (C=S); $\delta_H$ (400 MHz, DMSO-$d_6$); 12.29 (1H, s, NNH), 8.98 (1H, s, N*H*H), 8.64 (1H, s, NH*H*), 8.40 (1H, d, *J* 5.0, H-2″), 8.01 (1H, d, *J* 9.0, H-5″), 7.76 (1H, d, *J* 2.1, H-8″), 7.49 (1H, d, *J* 2.0, H-4), 7.39 (1H, dd, *J* 2.0 and 9.0, H-6″), 7.09 (1H, dd, *J* 2.0 and 7.4, H-6), 7.01 (1H, d, *J* 7.4, H-7), 6.60 (1H, d, *J* 5.0, H-3″), 3.98 (2H, t, *J* 6.4, –NC*H*$_2$CH$_2$NHAr), 3.62 (2H, t, *J* 6.4, –NCH$_2$C*H*$_2$NHAr); $\delta_C$ (75 MHz); 178.3, 158.2, 151.9, 150.8, 149.8, 149.0, 142.1, 136.7, 127.5, 127.3, 124.8, 124.2, 123.5, 121.9, 117.6, 117.0, 109.7, 104.7, 74.7 and 42.3; HRMS (FAB) found *m/z* 459.05659 $[M+1]^+$, $C_{20}H_{16}Cl_2N_6OS$ required 459.05615. Anal. Calcd for $C_{20}H_{16}Cl_2N_6OS$: C 57.5%, H 4.4%, N 19.3%, S 7.3%. Found: C 57.1%, H 4.3%, N 19.0%, S 7.2%.

**5.5.4. 2-(7-Chloro-quinolin-4-ylamino)-ethanol (12).** A mixture of 4,7-dichloroquinoline (4.0 g, 20.19 mmol), ethanolamine (24.42 mL, 403.9 mmol) and triethylamine (0.84 mL, 6.06 mmol) was stirred under reflux for 8 h. The reaction mixture was then cooled to room temperature during which a white precipitate formed. The precipitate was filtered, washed with water (3 × 50 mL) and recrystallized from methanol to obtain (4.10 g, 92%) as cream-white crystals, mp 213–215 °C (from methanol) (lit. 214 °C);[19] $R_f$ 0.42 (MeOH/DCM, 2:8); $\delta_H$ (300 MHz; $CD_3OD$); 8.35 (1H, d, *J* 6.0, H-2′), 8.09 (1H, d, *J* 9.0, H-5′), 7.78 (1H, d, *J* 2.1, H-8′), 7.40 (1H, dd, *J* 2.1 and 9.0, H-6′), 6.57 (1H, d, *J* 6.0, H-3′), 3.86 (2H, t, *J* 5.7, ArNHCH$_2$C*H*$_2$–), 3.52 (2H, t, *J* 5.7, ArNHC*H*$_2$–); $\delta_C$ (75 MHz); 151.8, 150.1, 149.0, 133.3, 127.4, 123.9, 117.4, 108.6, 58.7 and 45.0 ; LRMS (EI) *m/z* 223.1 $[M^+]$. Anal. Calcd: C 59.3%, H 4.9%, N 12.6%. Found: C 59.0%, H 4.9%, N 12.6%.

**5.5.5. Methanesulfonic acid 2-(7-chloro-quinolin-4-ylamino)-ethyl ester (13).** A solution of compound **12** (8.0 g, 36 mmol) in 24 mL of pyridine was stirred at 0 °C for 30 min. Whilst at 0 °C methanesulfonyl chloride (6.97 mL, 90.0 mmol) in 22 mL of pyridine was added slowly to the above solution, and the resultant mixture stirred for 5 h. The reaction mixture was then diluted with 17% aqueous ammonia solution (25 mL), extracted with $CH_2Cl_2$ (3 × 50 mL), dried over anhydrous $MgSO_4$, concentrated and recrystallized from methanol/water to obtain yield (8.96 g, 83%) obtained as white crystals, mp 138–140 °C (from MeOH/$H_2O$), $R_f$ 0.76 (MeOH/DCM, 2:8); IR (KBr) $\nu_{max}/cm^{-1}$ ; 3218 (N–H), 1579 (C=N), 1442 (C=C); $\delta_H$ (300 MHz; $CD_3OD$); 8.39 (1H, d, *J* 5.7, H-2′), 8.08 (1H, d, *J* 9.0, H-5′), 7.79 (1H, d, *J* 2.1, H-8′), 7.41 (1H, dd, *J* 2.1 and 9.0, H-6′), 6.61 (1H, d, *J* 5.7, H-3′), 4.48 (2H, t, *J* 5.4, ArNHCH$_2$C*H*$_2$–), 3.74 (2H, t, *J*, 5.4, ArNHC*H*$_2$–) 3.07 (3H, s, –OSO$_2$–C*H*$_3$); $\delta_C$ (100 MHz); 151.9, 150.2, 149.1, 133.2, 127.8, 124.1, 123.9, 117.9, 108.0, 66.8, 42.4 and 37.8; HRMS (EI) found *m/z* 300.03487 $[M]^+$, $C_{12}H_{13}O_3ClN_2S$ requires 300.03354. Anal. Calcd for $C_{12}H_{13}O_3N_2SCl$: C 47.9%, H 4.4%, N 9.3%, 10.7%. Found: C 48.1%, H 4.4%, N 9.2%, S 10.3%.

### 5.6. E. General procedure for preparation compounds 3a–e

To a mixture of isatin or substituted isatin (0.1 g, 0.68 mmol) in 10 mL of anhydrous DMF at 0 °C was added NaH (0.02 g, 0.815 mmol) was then added to this mixture followed by Compound **13** (0.27 g, 0.88 mmol) warming to room temperature with stirring over 1 h. The temperature was then raised to 60 °C and stirring continued for a further 12 h. Ice-cold water was added to the reaction and a coloured precipitate formed. The solid was filtered, washed with water (2 × 25 mL), petroleum ether (1 × 10 mL) and dried under vacuum to give the desired product.

**5.6.1. $N^1$-[2-(7-Chloro-quinolin-4′-ylamino)-ethyl]-1*H*-indole-2,3-dione (3a).** (0.21 g, 88%) obtained as orange crystals; mp 240–243 °C (from methanol); $R_f$ 0.43 (MeOH/EtOAc, 2:8); IR ($CHCl_3$) $\nu_{max}/cm^{-1}$ 3236 (N–H), 1734 (keto C=O), 1600 (amide C=O), 1514 (C=N); $\delta_H$ (300 MHz; DMSO-$d_6$); 8.41 (1H, d, *J* 5.4, H-2″), 7.97 (1H, d, *J* 9.0, H-5″), 7.76 (1H, d, *J* 2.4, H-8″), 7.52 (1H, dd, *J* 0.6 and 7.2, H-4), 7.48 (1H, ddd, *J* 0.6, 6.6 and 7.4, H-6), 7.39 (1H, dd, *J* 2.4, 9.0, H-6″), 7.07 (1H, ddd, *J* 0.9, 6.6 and 7.2, H-5), 7.03 (1H, dd, *J* 0.9 and 7.4, H-7), 6.62 (1H, d, *J* 5.4, H-3″), 3.93 (2H, t, *J* 5.1, ArNHCH$_2$C*H*$_2$–), 3.60 (2H, t, *J* 5.1, ArNHC*H*$_2$–); $\delta_C$ (75 MHz); 183.3, 158.3, 151.9, 150.6, 149.7, 149.0, 137.8, 133.3, 127.4, 124.3, 124.0, 123.7, 122.9, 117.4, 117.0, 110.2, 104.6, 74.9 and 42.0; HRMS (FAB) found *m/z* 352.08494 $[M+1]^+$, $C_{19}H_{14}O_2N_3Cl$] requires 352.08527. Anal. Calcd for $C_{19}H_{14}O_2N_3Cl$: C 64.8%, H 4.0%, N 11.9%. Found: C 64.4%, H 3.9%, N 11.2%.

**5.6.2. $N^1$-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-5-methyl-1H-indole-2,3-dione (3b).** The conditions employed for the preparation of this compound were those described in general procedure **E.** However, 0.62 mmol of isatin and 0.81 mmol of compound **13** were used.

0.62 mmol (0.16 g, 68%) obtained as red crystals; mp 240–243 °C (from methanol); $R_f$ 0.28 (MeOH/EtOAc; 2:8); IR (KBr) $\nu_{max}/cm^{-1}$ 3236 (N–H), 1737 (keto C=O), 1600 (amide C=O), 1514 (C=N); $\delta_H$ (300 MHz; $CDCl_3$); 8.53 (1H, d, *J* 5.4, H-2″), 7.93 (1H, d, *J* 2.4, H-8″), 7.68 (1H, d, *J* 9.0, H-5″), 7.43 (1H, d, *J* 2.0, H-4), 7.39 (1H, dd, *J* 2.0 and 7.2, H-6), 7.37 (1H, dd, *J* 2.4 and 9.0, H-6″), 6.81 (1H, d, *J* 7.2, H-7), 6.62 (1H, d, *J* 5.4, H-3″), 4.15 (2H, t, *J* 5.4, ArNHCH$_2$C*H*$_2$–), 3.67 (2H, t, *J* 5.4, ArNHC*H*$_2$–), 2.32 (3H, s, Ar–C*H*$_3$); $\delta_C$ (75 MHz), 183.6, 158.2, 152.0, 150.5, 149.7, 149.1, 137.7, 133.4, 126.8, 124.6, 124.2, 123.4, 123.1, 117.5, 116.9, 111.2, 104.6, 74.6, 42.1 and 20.3; LRMS (EI) *m/z* 365.0 [16%, $M^+$], 191

[100%, M−$C_{10}H_8ClN_2$]. Anal. Calcd for $C_{20}H_{16}O_2N_3Cl$: C 65.7%, H 4.4%, N 10.5%. Found: C 65.3%, H 4.3%, N 10.9%.

**5.6.3. 5-Chloro-$N^1$-[2-(7-chloro-quinolin-4′-ylamino)-ethyl]-1*H*-indole-2,3-dione (3c).** The conditions employed for the preparation of this compound were those described in general procedure **E**. However, 0.55 mmol of isatin and 0.72 mmol of compound **13** were used.

(0.15 g, 69%) obtained as orange crystals; mp 221–223 °C (from ethanol); $R_f$ 0.46 (MeOH; EtOAc, 2:8); IR ($CHCl_3$) $\nu_{max}/cm^{-1}$, 3237 (N–H), 1734 (C=O), 1606 (amide C=O), 1519 (C=N); $\delta_H$ (300 MHz; DMSO-$d_6$); 8.43 (1H, d, *J* 5.4, H-2″), 7.94 (1H, d, *J* 8.4, H-5″), 7.78 (1H, d, *J* 2.1, H-8″), 7.59 (1H, d, *J* 2.4, H-4), 7.40 (1H, dd, *J* 2.4 and 8.7, H-6), 7.31 (1H, dd, *J* 2.1 and 8.4, H-6″), 7.07 (1H, d, *J* 8.7, H-7), 6.64 (1H, d, *J* 5.4, H-3″), 3.96 (2H, t, *J* 5.4, ArNHC$H_2$C$H_2$–), 3.64 (2H, t, *J* 5.4, ArNHC$H_2$–); $\delta_C$ (75 MHz); 182.3, 158.2, 151.9, 150.7, 149.8, 149.0, 136.7, 127.4, 127.2, 124.8, 124.2, 123.4, 121.9, 117.5, 117.0, 109.7, 104.7, 74.6 and 42.3; HRMS (FAB) found *m/z* 386.04642 $[M+1]^+C_{19}H_{13}O_2N_3Cl_2$ requires 386.04630. Anal. Calcd for $C_{19}H_{13}O_2N_3Cl_2$: C 59.1%, H 3.4%, N 10.9%. Found: C 59.0%, H 3.4%, N 10.8%.

**5.6.4. $N^1$-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-5,7-dimethyl-1*H*-indole-2,3-dione (3d).** The conditions employed for the preparation of this compound were those described in general procedure **E**. However, 0.57 mmol of isatin and 0.74 mmol of compound **13** were used.

(0.19 g, 89%) obtained as orange crystals; mp 219 °C (from ethanol); $R_f$ 0.53; IR ($CHCl_3$) $\nu_{max}/cm^{-1}$ 3236 (N–H), 1736 (keto C=O), 1607 (amide C=O), 1519 (C=N); $\delta_H$ (300 MHz; DMSO-$d_6$); 8.40 (1H, d, *J* 5.4, H-2″), 8.03 (1H, d, *J* 9.0, H-5″), 7.78 (1H, d, *J* 2.1, H-8″), 7.44 (1H, d, *J* 2.4, H-4), 7.40 (1H, d, *J* 2.4, H-6), 7.39 (1H, dd, *J* 2.1 and 9.0, H-6″), 6.62 (1H, d, *J* 5.4, H-3″), 4.12 (2H, t, *J* 6.3, ArNHC$H_2$C$H_2$–), 3.64 (2H, t, *J* 6.3, ArNHC$H_2$–), 2.40 (3H, s, Ar–C$H_3$), 2.22 (3H, s, Ar–C$H_3$); $\delta_C$ (75 MHz); 182.2, 158.6, 151.8, 150.4, 149.8, 149.0, 136.3, 127.5, 127.4, 124.1, 123.7, 122.7, 121.2, 117.8, 117.4, 110.6, 104.6, 74.3, 42.7, 19.8 and 18.2; HRMS (FAB) found *m/z* 380.11717 $[M+1]^+$ $C_{21}H_{18}O_2N_3Cl$ requires 380.11657. Anal. Calcd for $C_{21}H_{18}O_2N_3Cl$: C 66.4%, H 4.7%, N 11.1%. Found: C 66.3%, H 4.6%, N 10.9%.

## Acknowledgements

This material is based upon work supported by the South African National Research Foundation under grant number 2053362 (K.C.). We would also like to thank the University of Cape Town and the South African Medical Research Council for additional support. Work in the laboratories of P.J.R. was supported by grants from the National Institutes of Health (AI AI35800 and AI35707) and the Medicines for Malaria Venture. P.J.R. is a Doris Duke Charitable Foundation Distinguished Clinical Scientist.

## References and notes

1. World Health Organisation Fact Sheet No. 94. Revised Oct. WHO information. http://www.int/inf~fs/en/fact094.html 1998.
2. Lima, P. D. C.; Barreiro, E. J. Avery, M. A. *Abstracts of Papers*, 223rd ACS National Meeting, Orlando, FL, USA, April 7–11, 2002; American Chemical Society: Washington, DC, 2002; MEDI-176.
3. Flipo, M.; Florent, I.; Grellier, P.; Sergheraert, C.; Deprez-Poulain, R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2659.
4. Romeo, S.; Dell'Agli, M.; Parapini, S.; Rizzi, L.; Galli, G.; Mondani, M.; Sparatore, A.; Taramelli, D.; Bosisio, E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2931.
5. Biot, C.; Dessolin, J.; Davioud-Charvet, E. *Redox Rep.* **2003**, *8*, 280.
6. Davioud-Charvet, E.; Delarue, S.; Biot, C.; Schwobel, B.; Boehme, C. C.; Mussigbrodt, A.; Maes, L.; Sergheraert, C.; Grellier, P.; Schirmer, R. H.; Becker, K. *J. Med. Chem.* **2001**, *44*, 4268.
7. Musonda, C. C.; Taylor, D.; Lehman, J.; Gut, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3901.
8. Lecaille, F.; Kaleta, J.; Bromme, D. *Chem. Rev.* **2002**, *102*, 4459.
9. Sajid, M.; McKerrow, J. H. *Mol. Biochem. Parasitol.* **2002**, *120*, 1.
10. Marquis, R. W. *Ann. Rep. Med. Chem.* **2000**, *35*, 309.
11. Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3527.
12. Rosenthal, P. J.; Sijwali, P. S.; Singh, A.; Shenai, B. R. *Curr. Pharm. Des.* **2002**, *8*, 1659.
13. Egan, T. J. *Drug Des. Rev.-Online* **2004**, *1*, 93.
14. Biot, C. *Curr. Med. Chem.-Anti-Infect. Agents* **2004**, *3*, 135.
15. Chibale, K.; Moss, J. R.; Blackie, M.; van Schalkwyk, D.; Smith, P. J. *Tetrahedron Lett.* **2000**, *41*, 6231.
16. Ridley, R. G.; Hofheinz, W.; Matile, H.; Jaquet, C.; Dorn, A.; Masciadri, R.; Jolidon, S.; Richter, W. F.; Guenzi, A.; Girometta, M.-A.; Urwyler, H.; Huber, W.; Thaithong, S.; Peters, W. *Antimicrob. Agents Chemother.* **1996**, *40*, 1846.
17. De, D.; Krogstad, F. M.; Byers, L. D.; Krogstad, D. J. *J. Med. Chem.* **1998**, *42*, 4918.
18. Du, X.; Guo, C.; Hansell, E.; Doyle, P. S.; Caffrey, C. R.; Holler, T. P.; McKerrow, J. H.; Cohen, F. E. *J. Med. Chem.* **2002**, *45*, 2695.
19. Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Walden, J. *J. Med. Chem.* **2000**, *43*, 283.
20. Pradines, B.; Rolain, J. M.; Ramiandrasoa, F.; Fusai, T.; Mosnier, J.; Rogier, C.; Daries, W.; Baret, E.; Kunesch, G.; Le Bras, J.; Parzy, D. *J. Antimicrob. Chemother.* **2002**, *50*, 177.
21. Lundt, L.; Madsen, R. *Synthesis* **1992**, 1129.
22. Semenov, A.; Olson, J. E.; Rosenthal, P. J. *Antimicrob. Agents Chemother.* **1998**, *42*, 2254.
23. Trager, W.; Jensen, J. *Science* **1976**, *193*, 673.
24. Makler, M. T.; Piper, R. C.; Milhous, W. K. *Parasitol. Today* **1998**, *14*, 376.
25. Sijwali, P. S.; Kato, K.; Seydel, K. B.; Gut, J.; Lehman, J.; Klemba, M.; Goldberg, D. E.; Miller, L. H.; Rosenthal, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8721.
26. Greenbaum, D. C.; Baruch, A.; Grainger, M.; Bozdech, Z.; Medzihradszky, K. F.; Engel, J.; Derisi, J.; Holder, A. A.; Bogyo, M. *Science* **2002**, *298*, 2002.

27. Eksi, S.; Czesny, B.; Greenbaum, D. C.; Bogyo, M.; Williamson, K. C. *Mol. Microbiol.* **2004**, *53*, 243.
28. Greenbaum, D. C.; Mackey, Z.; Hansell, E.; Doyle, P.; Gut, J.; Caffrey, C. R.; Lehman, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. *J. Med. Chem.* **2004**, *47*, 3212.
29. Sweeney, D.; Raymer, M. L.; Lockwood, T. D. *Biochem. Pharmacol.* **2003**, *66*, 663.
30. Eggleson, K. K.; Duffin, K. L.; Goldberg, D. E. *J. Biol. Chem.* **1999**, *274*, 411.
31. Moormann, A. M.; Hossler, P. A.; Meshnick, S. R. *Mol. Biochem. Parasitol.* **1999**, *98*, 283.
32. Fritsch, G.; Sawatzki, G.; Treumer, J.; Jung, A.; Spira, D. T. *Exp. Parasitol.* **1997**, *63*, 1.
33. Rajendra, S. V.; Wobles, L. W. *J. Med. Chem.* **1967**, *10*, 972.