

Synthesis and biological evaluation of phenolic Mannich bases of benzaldehyde and (thio)semicarbazone derivatives against the cysteine protease falcipain-2 and a chloroquine resistant strain of *Plasmodium falciparum*

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Abstract—A targeted series of phenolic Mannich bases of benzaldehyde and (thio)semicarbazone derivatives were synthesized and evaluated in vitro against the malarial cysteine protease falcipain-2 and a chloroquine resistant strain (W2) of *Plasmodium falciparum*. A novel series of 4-aminoquinoline semicarbazones were the most effective inhibitors of falcipain-2 (most potent inhibitor had $IC_{50} = 0.63 \mu\text{M}$) while a bisquinoline semicarbazone compound **8f** was the most potent antimalarial compound with an IC_{50} of $0.07 \mu\text{M}$ against W2. Compound **8f** also weakly inhibited falcipain-2, with an IC_{50} of $3.16 \mu\text{M}$, although its principal antiparasitic activity did not appear to be due to inhibition of this enzyme.

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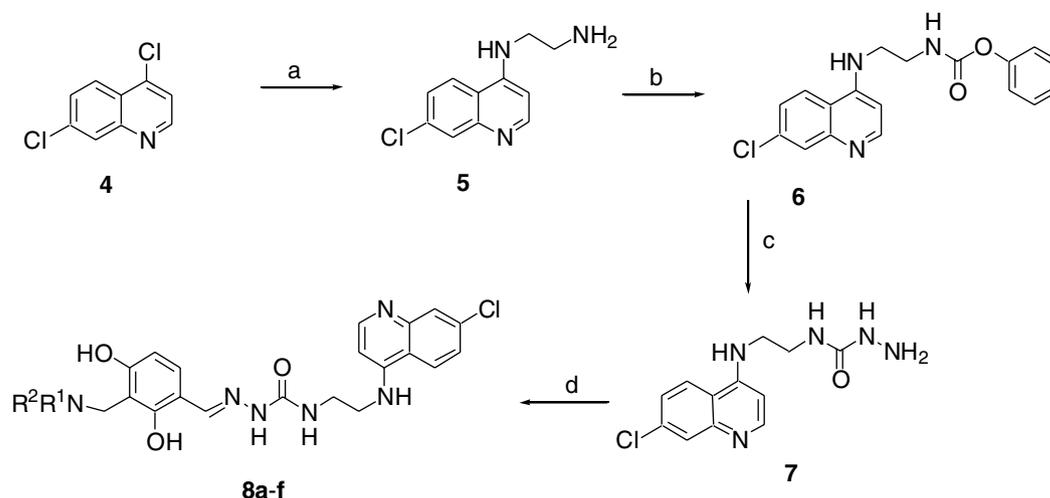
1. Introduction

Malaria is one of the world's most prevalent infectious diseases and ranks among the major health and developmental challenges facing large parts of the world, including many of the poorest countries. Despite the fact that effective antimalarial drugs exist, drug resistance, particularly to chloroquine, has become an enormous problem.¹ This has necessitated the search for novel and cost-effective drugs with high structural variation. A potential strategy for the treatment of parasitic diseases is to design compounds which selectively inhibit enzymes that are pivotal for survival and are part of biochemical pathways that are specific to the parasite.² In this regard the cysteine protease falcipain-2 is an attractive target enzyme because of its key role in haemoglobin hydrolysis.³

A number of compounds containing the phenolic Mannich base component have been synthesized and shown to possess significant anticancer and antimalarial properties.^{4,5} The bioactivities have been attributed partially to the α,β -unsaturated ketones liberated from the Mannich bases by deamination. These α,β -unsaturated ketones have markedly greater affinity for thiols over amino and hydroxy nucleophiles.^{6,7} This preferential affinity may result in a lack of mutagenicity and carcinogenicity, which are associated with certain alkylating agents due to presumed interaction with nucleic acids. On the other hand, compounds containing a thiosemicarbazone component have shown a broad spectrum of chemotherapeutic properties, including antimalarial,⁸ antitumour,⁹ antibacterial,¹⁰ antitrypanosomal,¹¹ and antiviral¹² activity. The compounds are also members of a class of iron-chelators that are Schiff bases. Since iron (Fe) is essential for the biological activity of a number of plasmodial proteins, including the rate-limiting enzyme of DNA synthesis, ribonucleotide reductase, withholding it inhibits the growth of the malaria parasite.^{13,14} In vitro studies have shown that iron-chelating agents inhibit parasite growth and proliferation.¹⁵ Recently, thiosemicarbazones have been synthesized

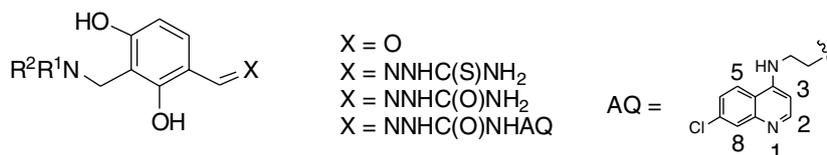
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Scheme 2. Reagents and conditions: (a) 5.0 equiv of $\text{NH}_2(\text{CH}_2)_2\text{NH}_2$, reflux, 6 h, 82%; (b) 1.0 equiv of chloroformate, 1.0 equiv of Et_3N , DMF/DCM (1:1), 0 °C, 1 h; (c) 10 equiv of $\text{H}_2\text{NNH}_2 \cdot \text{H}_2\text{O}$, MeOH, reflux, 12 h; (d) 1.0 equiv of **45**, 0.5 equiv of *p*-TsOH, MeOH, rt, 12 h.

Table 1. Effects of Mannich bases of aldehyde, (thio)semicarbazone and 4-aminoquinoline semicarbazone derivatives on the activity of falcipain-2 and development of *Plasmodium falciparum* W2 strain



Compound	NR^2R^1	X	IC_{50} (μM)	
			Falcipain-2	W2
2a	NEt_2	O	>20	>10
2b	Pyrrolidino	O	>20	>10
2c	Piperidino	O	>20	>10
2d	Morpholino	O	>20	>10
2e	Methylpiperazino	O	>20	>10
2f	4-(7-Cl-Quinoliny)-piperazino	O	>20	1.07
3a	NEt_2	NNHC(S)NH_2	11.07	>10
3b	Pyrrolidino	NNHC(S)NH_2	5.85	>10
3c	Piperidino	NNHC(S)NH_2	3.80	>10
3d	Morpholino	NNHC(S)NH_2	>20	>10
3e	Methylpiperazino	NNHC(S)NH_2	18.22	>10
3f	4-(7-Cl-Quinoliny)-piperazino	NNHC(S)NH_2	2.25	3.75
3g	4-(7-Cl-Quinoliny)-piperazino	NNHC(O)NH_2	>20	0.25
3h	NMB ^a	NNHC(S)NH_2	>20	>10
8a	NEt_2	NNHC(O)NHAQ	2.60	0.44
8b	Pyrrolidino	NNHC(O)NHAQ	0.72	1.07
8c	Piperidino	NNHC(O)NHAQ	0.63	0.27
8d	Morpholino	NNHC(O)NHAQ	>20	0.11
8e	Methylpiperazino	NNHC(O)NHAQ	>20	0.38
8f	4-(7-Cl-Quinoliny)-piperazino Chloroquine	NNHC(O)NHAQ	3.16	0.077 0.24

^a NMB, no Mannich base.

centration used. As expected for a 4-aminoquinoline, compound **2f** showed good activity (low-micromolar IC_{50} value) against the W2 strain of *P. falciparum* but exhibited poor inhibitory activity against falcipain-2. Thiosemicarbazones **3b**, **3c**, and **3f** were active against falcipain-2 with IC_{50} values less than 10 μM . Since the corresponding aldehyde precursors were ineffective

against falcipain-2, it is reasonable to suppose that the thiosemicarbazone moiety plays a key role in the inhibition of falcipain-2.¹⁶ This statement is further supported by the lack of activity displayed by semicarbazone **3g** which surprisingly showed superior antimalarial activity against W2 compared to **3f**, a thiosemicarbazone. However, of these thiosemicarbazones, only aminoquinoline

3f showed antimalarial activity with an IC_{50} value less than $10 \mu\text{M}$. The antimalarial activity may be due to the haem-binding aminoquinoline moiety. This is further supported by **2f** (aldehyde precursor) and **3g** (semicarbazone), both aminoquinolines, which also showed antimalarial activity despite exhibiting poor inhibitory activity against falcipain-2.

The only thiosemicarbazone from this limited series which showed activity against both falcipain-2 and cultured parasites with IC_{50} values of 2.25 and $3.75 \mu\text{M}$, respectively, is compound **3f**.

Thiosemicarbazones **3b**, **3c**, and **3f** and to a lesser extent semicarbazone **3g**, are potential metal chelators and may be acting as iron chelators in *P. falciparum*. The antimalarial action of chelators is dictated by three factors: iron(III)-binding capacity, chelator ingress into parasitized erythrocytes and chelator egress from parasites after treatment. Various iron chelators have been shown to improve drug lipophilicity leading to increased access of the drug to intracellular parasites and to faster speed of action.^{23,24} For example, desferrioxamine **9** (Fig. 2) does penetrate the infected red blood cell, and its antimalarial activity is dependent on this.²⁵ It would be predicted that an effective antimalarial iron chelator would have the ability to cross lipid membranes well, have a high affinity for iron, selectively bind iron as compared with other trace metals and selectively bind iron(III) rather than iron(II).²⁶

Since compound **3h** was ineffective against both falcipain-2 and cultured parasites at the maximum tested concentration, this suggests that the activity of compounds **3a**, **3b**, **3c**, and **3f** is not due to the thiosemicarbazone side chain alone. Coupled with the data for phenolic Mannich benzaldehyde derivatives, with an exception of **2f**, it may be concluded that the inhibitory activity of phenolic Mannich bases of thiosemicarbazones is due to the combined effects of both Mannich base and thiosemicarbazone components.

The results for aminoquinoline semicarbazones (Table 1) indicate that all compounds exhibited significant antiparasitodal activity. Compounds **8a**, **8b**, **8c**, and **8f** were active against both falcipain-2 and cultured parasites. There was generally no correlation between the ability of these compounds to inhibit falcipain-2 and their antiparasitodal activity against W2 in vitro. As such, the mechanism of action of these aminoquinoline semicarbazones is unclear. Most likely, their antimalarial effects are due to a combination of mechanisms, which include

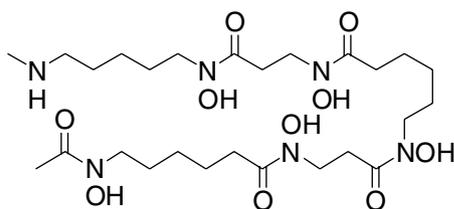


Figure 2. Chemical structure of desferrioxamine **9**.

inhibition of falcipain-2. In any case, it is evident from the results that the aminoquinolinyl moiety plays a significant role in determining the antimalarial activity of the tested aminoquinoline semicarbazones, and this is in agreement with results obtained with the phenolic Mannich bases of benzaldehyde and (thio)semicarbazone. The most potent compound against the W2 strain is **8f**, a bisquinoline, with an IC_{50} value of $0.077 \mu\text{M}$. Like chloroquine and other antimalarial bisquinolines, compound **8f** may exert its antimalarial properties by inhibiting haemazoin formation.^{27–29} Although compounds **8a**, **8b** and **8c** were slightly less active compared to chloroquine in the W2 strain, reliable comparative studies may only be made upon screening against a wide range of parasite strains of varying degrees of resistance and/or sensitivity. This is because it is now well accepted that drug resistance in malaria is often compound specific and strain-dependent. Compound **8c** was almost equipotent with chloroquine, whereas **8d** and **8f** were more active, with **8f** being 3 times more active than chloroquine. In light of the results obtained, this series of compounds particularly bisquinoline **8f**, which is a novel compound based on the semicarbazone scaffold, warrants further investigation

In conclusion, we have synthesized, via simple and straightforward routes, phenolic Mannich bases of benzaldehyde and, (thio)semicarbazone derivatives and identified novel potential antimalarial agents. Future research efforts should be directed towards aminoquinoline semicarbazones since a vast improvement in the activity of these antimalarial potential cysteine protease inhibitors is possible by a rational modification of the basic structure.

4. Experimental data

4.1. General method A for the preparation of compounds 2a–f

Amine (10.86 mmol) was treated with paraformaldehyde (10.86 mmol) in methanol (7.5 ml) at 65°C for 1 h. To the reaction mixture, a solution of 2,4-dihydroxybenzaldehyde (10.86 mmol) in methanol (5.0 ml) was then added. After stirring for an additional 1 h, the reaction mixture was cooled, concentrated and then subjected to column chromatography on silica gel.

4.1.1. 3-Dimethylaminomethyl-2,4-dihydroxy-benzaldehyde 2a. The conditions employed for the preparation of this compound were those described in General Method E. Column chromatography (ethyl acetate) afforded **2a** as a dark brown solid (1.75 g, 72%); mp $74–76^\circ\text{C}$ (from chloroform–hexane); $R_f = 0.40$ (MeOH:DCM 1:9); IR ν_{max} (Nujol)/ cm^{-1} : 1050 (C–O), 1150 (C–N), 1640 (C=O), 3450 (OH); δ_{H} (400 MHz; CDCl_3) 1.16 (6H, t, J 7.2, H–N[CH₂CH₃]₂), 2.71 (4H, q, J 7.2, N[CH₂CH₃]₂), 3.92 (2H, s, PhCH₂N–), 6.40 (1H, d, J 8.4, H-5), 7.30 (1H, d, J 8.4, H-6), 9.58 (1H, s, CHO); δ_{C} (100 MHz; CDCl_3) 10.8 (2C), 46.6, 49.3 (2C), 106.7, 110.1, 113.2, 134.5, 161.5, 169.2, 190.7; Anal. Found: C, 64.29; H, 7.79; N, 6.18% Calcd for

$C_{12}H_{17}NO_3$: C, 64.55; H, 7.67; N, 6.27%; HRMS (EI) m/z Found: $[M+H]^+$, 224.12861 Calcd for $C_{12}H_{18}NO_3$: M, 224.12866.

4.2. 2,4-Dihydroxy-3-pyrrolidin-1-ylmethyl-benzaldehyde 2b

The conditions employed for the preparation of this compound were those described in General Method A. Column chromatography (ethyl acetate) followed by MeOH–DCM (1:9) afforded **2b** as a brown oil (1.91 g, 79%); $R_f = 0.28$ (MeOH–DCM 1:9); IR ν_{max} (Nujol)/ cm^{-1} : 1060 (C–O), 1210 (C–N), 1670 (C=O) 3450 (OH); δ_H (400 MHz; $CDCl_3$) 1.91 (4H, m, $N[CH_2CH_2]_2$), 2.71 (4H, m, $N[CH_2CH_2]_2$), 3.99 (2H, s, $PhCH_2N$), 6.40 (2H, d, J 8.4, H-5), 7.31 (1H, d, J 8.4, H-6), 9.60 (1H, s, CHO); δ_c (100 MHz; $CDCl_3$) 23.6 (2C), 50.9, 53.4 (2C), 107.4, 109.9, 113.4, 134.6, 161.2, 168.6, 193.8; HRMS (EI) m/z Found: $[M+H]^+$, 222.11270 Calcd for $C_{12}H_{15}NO_3$: M, 222.11301.

4.2.1. 2,4-Dihydroxy-3-piperidin-1-ylmethyl-benzaldehyde 2c. The conditions employed for the preparation of this compound were those described in General Method A. Column chromatography (ethyl acetate) afforded **2c** as a light brown solid (2.26 g, 88%); mp 107–109 °C (from chloroform–hexane); $R_f = 0.40$ (MeOH–DCM 1:9); IR ν_{max} (Nujol)/ cm^{-1} : 1050 (C–O), 1220 (C–N), 1660 (C=O), 3400 (OH); δ_H (400 MHz; $CDCl_3$) 1.67 (10H, m, $HN[CH_2CH_2]_2CH_2$), 3.82 (2H, s, $PhCH_2N$), 6.40 (2H, d, J 8.4, H-5), 7.29 (1H, d, J 8.4, H-6), 9.59 (1H, s, CHO); δ_c (100 MHz; $CDCl_3$) 23.6, 25.5 (2C), 53.7, 60.0 (2C), 106.5, 109.9, 113.4, 134.6, 161.6, 168.5, 193.8; Anal. Found: C, 66.09; H, 7.36; N, 5.82% Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.96%; HRMS (EI) m/z Found: $[M+H]^+$, 236.12860 Calcd For $C_{13}H_{18}NO_3$: M, 236.12866.

4.2.2. 2,4-Dihydroxy-3-morpholin-1-ylmethyl-benzaldehyde 2d. The conditions employed for the preparation of this compound were those described in General Method A. Column chromatography (MeOH–DCM 1:9) afforded **2d** as a cream white solid (2.48 g, 96%); mp 150–152 °C (from chloroform–hexane); $R_f = 0.62$ (MeOH–DCM 1:9); IR ν_{max} (Nujol)/ cm^{-1} : 1050 (C–O), 1250 (C–N), 1660 (C=O) 3440 (OH); δ_H (300 MHz; $CDCl_3$) 2.63 (4H, m, $N[CH_2CH_2]_2O$), 3.71 (4H, m, $N[CH_2CH_2]_2O$), 3.84 (2H, s, $PhCH_2N$), 6.43 (1H, d, J 8.4, H-5), 7.32 (1H, d, J 8.4, H-6), 9.62 (1H, s, CHO); δ_c (75 MHz; $CDCl_3$) 52.8 (2C), 53.5, 66.6 (2C), 106.5, 109.6, 114.0, 134.8, 161.8, 166.8, 194.2; Anal. Found: C, 64.29; H, 7.79; N, 6.18% Calcd for $C_{12}H_{15}NO_4$: C, 64.55; H, 7.67; N, 6.27%; HRMS (EI) m/z Found: $[M+H]^+$, 238.10793 Calcd for $C_{12}H_{16}NO_4$: M, 238.10799.

4.2.3. 2,4-Dihydroxy-3-(4-methyl-piperazin-1-ylmethyl)-benzaldehyde 2e. The conditions employed for the preparation of this compound were those described in General Method A. Column chromatography (ethyl acetate) afforded **2e** as a pale brown solid (2.35 g, 86%); mp 106–108 °C (from chloroform–hexane); $R_f = 0.42$ (MeOH–DCM 1:9); IR ν_{max} (Nujol)/ cm^{-1} ;

1060 (C–O), 1130 (C–N), 1665 (C=O), 3400 (OH); δ_H (300 MHz; $CDCl_3$) 1.67 (3H, m, $HN[CH_2CH_2]_2NCH_3$), 2.60 (8H, m, $HN[CH_2CH_2]_2NCH_3$), 3.82 (2H, s, $PhCH_2N$), 6.40 (2H, d, J 8.7, H-5), 7.29 (1H, d, J 8.7, H-6), 9.60 (1H, s, CHO); δ_c (75 MHz; $CDCl_3$) 45.8, 52.4 (2C), 53.1, 54.7 (2C), 106.7, 109.7, 113.8, 134.7, 161.7, 167.3, 194.1; Anal. Found: C, 61.41; H, 7.45; N, 10.82% Calcd for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.25; N, 11.19%; HRMS (EI) m/z Found: $[M+H]^+$, 251.13958 Calcd for $C_{13}H_{19}N_2O_3$: M, 251.13956.

4.2.4. 3-[4-(7-Chloro-quinolin-4-yl)piperazin-1-ylmethyl]-2,4-dihydroxy benzaldehyde 2f. The conditions employed for the preparation of this compound were those described in General Method A. Column chromatography (ethyl acetate) afforded **2f** as a cream white solid (4.06 g, 94%); mp 167–169 °C (from chloroform–hexane); $R_f = 0.62$ (MeOH–DCM 1:9); IR ν_{max} (Nujol)/ cm^{-1} : 1040 (C–O), 1540 (C=N), 1600 (C=C), 1660 (C=O), 3350 (OH); δ_H (400 MHz; $CDCl_3$) 2.94 (4H, br s, $N[CH_2CH_2]_2NCH$), 3.30 (4H, br s, $N[CH_2CH_2]_2NCH$), 3.99 (2H, s, $PhCH_2N$), 6.47 (1H, d, J 8.7, H-5), 6.85 (1H, d, J 5.1, H-3'), 7.36 (1H, d, J 8.7, H-6), 7.43 (1H, dd, J 2.1, 9.3, H-6'), 7.91 (1H, d, J 9.3, H-5'), 8.15 (1H, d, J 2.1, H-8'), 8.74 (1H, d, J 5.1, H-2'), 9.65 (1H, s, CHO); δ_c (100 MHz; $CDCl_3$) 51.8 (2C), 52.4 (2C), 53.0, 106.2, 109.2, 109.6, 114.0, 122.2, 124.7, 126.5, 129.0, 134.9, 150.5, 151.9, 156.2, 160.5, 166.6, 194.2; Anal. Found: C, 63.30; H, 5.25; N, 10.31% Calcd for $C_{21}H_{20}ClN_3O_3$: C, 63.40; H, 5.07; N, 10.56%; HRMS (EI) m/z Found: $[M+H]^+$, 398.12734 Calcd for $C_{21}H_{21}ClN_3O_3$: M, 398.12714.

4.3. General Method B for the preparation of compounds 3a–h

A mixture of aldehyde (1.65 mmol) and thiosemicarbazide/semicarbazide (1.65 mmol) was dissolved in dry methanol (10 ml) under nitrogen. The resultant heated at reflux for 3 h until the reaction was completed (monitored by TLC). The solvent was removed under reduced pressure, and resulting solid was recrystallised from methanol to provide title compounds **3a–h**.

4.4. Compound 3h

The conditions employed for the preparation of this compound were those described in General Method B, which gave **3h** as a cream white solid (2.90 g, 89%); mp 237–239 °C (from methanol); $R_f = 0.50$ (17% NH_4OH –MeOH–DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} : 1150 (C=S), 1260 (C–N), 1540 (C=S), 1580 (C=C), 3150 (N–H), 3325 (N–H) 3450 (O–H/N–H); δ_H (300 MHz; $DMSO-d_6$) δ 6.26 (1H, dd, J 2.4, 8.4, H-5), 6.30 (1H, d, J 2.4, H-3), 7.65 (1H, d, J 8.4, H-6), 7.72 (1H, br s, $NHCSNH_2$), 7.92 (1H, s, $NHCSNH_2$), 8.24 (1H, s, $PhCH=NNH$), 9.72 (2H, s, OH), 11.14 (1H, s, $PhCH=NNH$); δ_c (75 MHz; $DMSO-d_6$) 102.3, 107.7, 111.7, 128.4, 140.0, 158.0, 160.4, 177.1; Anal. Found: C, 45.51; H, 4.34; N, 19.97; S, 15.59% Calcd for $C_8H_9N_3O_2S$: C, 45.49; H, 4.29; N, 19.89; S, 15.18%; HRMS (EI) m/z Found: $[M+H]^+$, 212.04980 Calcd for $C_8H_{10}N_3O_2S$: M, 212.14937.

4.5. Compound 3a

The conditions employed for the preparation of this compound were those described in General Method B, which gave **3a** as a yellow solid (0.46 g, 87%) (recrystallised from methanol); decomposes above 180 °C; $R_f = 0.60$ (17% NH_4OH – MeOH – DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} 1050 (C–O), 1140 (C=S), 1580 (C=C), 3130 (N–H), 3245 (N–H) 3400 (O–H/N–H); δ_{H} (300 MHz; CDCl_3) 1.05 (6H, t, J 7.2, $\text{N}[\text{CH}_2\text{CH}_3]_2$), 2.58 (4H, t, J 7.2, $\text{N}[\text{CH}_2\text{CH}_3]_2$), 3.80 (2H, s, PhCH_2N), 6.29 (1H, d, J 8.4, H-5), 7.55 (1H, d, J 8.4, H-6), 7.70 (1H, br s, NHCSNH_2), 7.91 (1H, br s, NHCSNH_2), 8.25 (1H, br s, $\text{PhCH}=\text{NNH}$), 12.34 (1H, s, $\text{PhCH}=\text{NNH}$); δ_{C} (75 MHz; CDCl_3) 10.8 (2C), 45.8 (2C), 49.2, 107.5, 111.7, 113.1, 126.1, 141.0, 158.9, 160.4, 177.0; LRMS (EI) m/z , M^+ 332; Anal. Found: C, 51.45; H, 6.76; N, 18.88; S, 11.24% Calcd for $\text{C}_{13}\text{H}_{20}\text{N}_4\text{SO}_2$: C, 52.68; H, 6.80; N, 18.90; S, 10.82%; HRMS (EI) m/z Found: $[\text{M}+\text{H}]^+$, 297.13858 Calcd for $\text{C}_{13}\text{H}_{21}\text{N}_4\text{SO}_2$: M, 297.13852.

4.6. Compound 3b

The conditions employed for the preparation of this compound were those described in General Method B, which gave **3b** as a yellow solid (0.39 g, 76%) (recrystallised from methanol); decomposes above 180 °C; $R_f = 0.56$ (17% NH_4OH – MeOH – DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} 1060 (C–O), 1150 (C=S), 1580 (C=C), 3400 (OH/N–H); δ_{H} (300 MHz; CDCl_3) 1.77 (4H, m, $\text{N}[\text{CH}_2\text{CH}_2]_2$), 2.58 (4H, m, $\text{N}[\text{CH}_2\text{CH}_2]_2$), 3.84 (2H, s, PhCH_2N), 6.29 (1H, d, J 8.4, H-5), 7.53 (1H, d, J 8.4, H-6), 7.69 (1H, br s, NHCSNH_2), 7.89 (1H, br s, NHCSNH_2), 8.24 (1H, br s, $\text{PhCH}=\text{NNH}$), 11.54 (1H, s, $\text{PhCH}=\text{NNH}$); δ_{C} (75 MHz; CDCl_3) 23.9 (2C), 51.6 (2C), 53.5, 107.8, 110.7, 112.3, 127.1, 159.3, 161.3, 177.7; Anal. Found: C, 53.02; H, 6.22; N, 19.05; S, 10.82% Calcd for $\text{C}_{13}\text{H}_{18}\text{N}_4\text{SO}_2$: C, 53.04; H, 6.16; N, 19.03; S, 10.89%; HRMS (EI) m/z Found: $[\text{M}+\text{H}]^+$, 295.12291 Calcd for $\text{C}_{13}\text{H}_{19}\text{N}_4\text{SO}_2$: M, 295.12287.

4.7. Compound 3c

The conditions employed for the preparation of this compound were those described in General Method B, which gave **3c** as a yellow solid (0.40 g, 78%) (recrystallised from methanol); decomposes above 126 °C; $R_f = 0.48$ (17% NH_4OH – MeOH – DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} ; 1030 (C–O), 1130 (C–N), 1590 (C=C), 3150 (N–H), 3250 (N–H), 3400 (OH/N–H); δ_{H} (400 MHz; $\text{DMSO}-d_6$) 1.43 (2H, m, $\text{N}[\text{CH}_2\text{CH}_2]_2\text{CH}_2$), 1.52 (4H, m, $\text{N}[\text{CH}_2\text{CH}_2]_2\text{CH}_2$), 2.48 (4H, m, $\text{N}[\text{CH}_2\text{CH}_2]_2\text{CH}_2$), 3.69 (2H, s, PhCH_2N), 6.28 (1H, d, J 8.8, H-5), 7.53 (1H, d, J 8.8, H-6), 7.70 (1H, br s, NHCSNH_2), 7.90 (1H, br s, NHCSNH_2), 8.23 (1H, br s, $\text{PhCH}=\text{NNH}$), 11.52 (1H, s, $\text{PhCH}=\text{NNH}$); δ_{C} (100 MHz; $\text{DMSO}-d_6$) 23.6 (2C), 25.5, 53.3, 54.2 (2C), 107.1, 111.1, 113.0, 125.3, 140.5, 158.7, 159.0, 177.2; HRMS (EI) m/z Found: $[\text{M}]^+$, 308.13071 Calcd For $\text{C}_{14}\text{H}_{20}\text{N}_4\text{SO}_2$: M, 308.13070.

4.8. Compound 3d

The conditions employed for the preparation of this compound were those described in General Method B, gave **3d** as a yellow solid (0.43 g, 84%) (recrystallised from methanol); decomposes above 200 °C; $R_f = 0.48$ (17% NH_4OH – MeOH – DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} 1060 (C–O), 1150 (C=S), 1590 (C=O), 3150 (N–H), 3260 (N–H), 3400 (O–H/N–H); δ_{H} (400 MHz; $\text{DMSO}-d_6$) 2.48 (4H, br s, $\text{N}[\text{CH}_2\text{CH}_3]_2\text{O}$), 3.60 (4H, br s, $\text{N}[\text{CH}_2\text{CH}_3]_2\text{O}$), 3.80 (2H, s, PhCH_2N), 6.20 (1H, d, J 8.4, H-5), 7.55 (1H, d, J 8.4, H-6), 7.69 (1H, br s, NHCSNH), 7.89 (1H, br s, NHCSNH), 8.24 (1H, s, $\text{PhCH}=\text{NNH}$), 11.16 (1H, s, $\text{PhCH}=\text{NNH}$); δ_{C} (100 MHz; $\text{DMSO}-d_6$) 53.1, 54.0 (2C), 66.7 (2C), 107.8, 108.1, 112.5, 127.6, 141.9, 158.7, 159.3, 177.; Anal. Found: C, 50.88; H, 6.06; N, 17.96; S, 9.53% Calcd for $\text{C}_{13}\text{H}_{18}\text{N}_4\text{SO}_3$: C, 50.31; H, 5.85; N, 18.05; S, 10.33%; HRMS (EI) m/z Found: $[\text{M}+\text{H}]^+$, 311.11802 Calcd for $\text{C}_{13}\text{H}_{19}\text{N}_4\text{SO}_3$: M, 311.11778.

4.9. Compound 3e

The conditions employed for the preparation of this compound were those described in General Method B, which gave **3e** as a yellow solid (0.52 g, 95%) (recrystallised from methanol); decomposes above 200 °C; $R_f = 0.52$ (17% NH_4OH – MeOH – DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} ; 1060 (C–O), 1140 (C=S), 1590 (C=C), 3150 (N–H), 3300 (N–H), 3400 (O–H/N–H); δ_{H} (300 MHz; $\text{DMSO}-d_6$) 2.19 (3H, s, $\text{N}(\text{CH}_2\text{CH}_2)_2\text{NCH}_3$), 2.38 (4H, br s, $\text{N}[\text{CH}_2\text{CH}_2]_2\text{NCH}_3$), 2.49 (4H, br s, $\text{N}[\text{CH}_2\text{CH}_2]_2\text{NCH}_3$), 3.74 (2H, s, PhCH_2N), 6.32 (1H, d, J 8.4, H-5), 7.58 (1H, d, J 8.4, H-6), 7.71 (1H, br s, NHCSNH), 7.91 (1H, br s, NHCSNH), 8.26 (1H, s, $\text{PhCH}=\text{NNH}$), 11.17 (1H, s, $\text{PhCH}=\text{NNH}$); δ_{C} (75 MHz; $\text{DMSO}-d_6$) 45.4 (2C), 51.8, (2C), 53.1, 54.4, 107.1, 107.2, 111.7, 126.6, 140.9, 158.2, 158.4, 177.1; Anal. Found: C, 50.86; H, 6.44; N, 17.99; S, 9.55% Calcd for $\text{C}_{14}\text{H}_{21}\text{N}_5\text{O}_2\text{S}$: C, 51.99; H, 6.54; N, 21.65; S, 9.91%; HRMS (EI) m/z Found: $[\text{M}+\text{H}]^+$, 324.14950 Calcd for $\text{C}_{14}\text{H}_{22}\text{N}_5\text{O}_2\text{S}$: M, 324.14941.

4.10. Compound 3f

The conditions employed for the preparation of this compound were those described in General Method B, which gave **3f** as a yellow solid (075 g, 96%) (recrystallised from methanol); decomposes above 240 °C; $R_f = 0.56$ (17% NH_4OH – MeOH – DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} (C–O), 1150 (C=S), 1560 (C=N), 1600 (C=C), 3140, 3275 (N–H), 3450 (O–H/N–H); δ_{H} (400 MHz; $\text{DMSO}-d_6$) 2.81 (4H, br s, $\text{N}[\text{CH}_2\text{CH}_2]_2\text{NCH}$), 3.24 (4H, br s, $\text{N}[\text{CH}_2\text{CH}_2]_2\text{NCH}$), 3.86 (2H, s, PhCH_2N), 6.36 (1H, d, J 8.4, H-5), 7.03 (1H, d, J 4.8, H-3'), 7.54 (1H, dd, J 2.0, 9.2, H-6'), 7.58 (1H, d, J 8.4, H-6), 7.73 (1H, s, NHCSNH_2), 7.93 (1H, s, NHCSNH_2), 7.97 (1H, d, J 2.0, H-8'), 8.02 (1H, d, J 9.2, H-5'), 8.27 (1H, s, $\text{PhCH}=\text{NNH}$), 8.70 (1H, d, J 4.8, H-2'), 11.20 (1H, s, $\text{PhCH}=\text{NNH}$); δ_{C} (100 MHz; $\text{DMSO}-d_6$) 52.3 (2C), 52.6 (2C), 53.5, 108.2, 109.0, 110.4, 112.5, 122.1, 126.6, 126.7, 127.7, 128.8, 134.3, 141.9, 150.3, 152.9, 156.7, 158.7, 159.3, 177.8; Anal.

Found: C, 55.96; H, 5.10; N, 17.71; S, 6.26% Calcd for $C_{22}H_{23}ClN_6O_2S$: C, 56.10; H, 4.92; N, 17.84; S, 6.81%; HRMS (EI) m/z Found: $[M]^+$, 470.12910 Calcd for $C_{22}H_{23}ClN_6SO_2Cl$: M, 470.129174.

4.11. Compound 3g

The conditions employed for the preparation of this compound were those described in General Method B, which gave **3g** as a yellow solid (0.69 g, 92%) (recrystallised from methanol); decomposes above 240 °C; $R_f = 0.08$ (17% NH_4OH –MeOH–DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} 1060 (C–O), 1560 (C=N), 1600 (C=C), 1650 (C=O), 3150 (N–H), 3300 (N–H), 3450 (O–H/N–H); δ_H (400 MHz; DMSO- d_6) 2.80 (4H, br s, $N[CH_2CH_2]_2NCH$), 3.22 (4H, br s, $N[CH_2CH_2]_2NCH$), 3.85 (2H, s, $PhCH_2N$), 6.24 (2H, s, $CONH_2$), 6.35 (1H, d, J 8.8, H-5), 7.02 (1H, d, J 4.8, H-3'), 7.40 (1H, d, J 8.8, H-6), 7.53 (1H, dd, J 2.0, 9.2, H-6'), 7.97 (1H, d, J 2.0, H-8'), 8.02 (1H, d, J 9.2, H-5'), 8.03 (1H, s, $PhCH=NNH$), 8.69 (1H, d, J 4.8, H-2'), 9.97 (1H, s, $PhCH=NNH$); δ_c (100 MHz; DMSO- d_6) 52.3 (2C), 52.7 (2C), 53.3, 108.0, 108.3, 110.3, 112.6, 122.1, 126.5, 126.7, 127.9, 128.8, 134.3, 140.0, 150.3, 152.9, 156.7, 157.1, 157.8, 158.9; Anal. Found: C, 58.05; H, 4.68; N, 18.55% Calcd for $C_{22}H_{23}ClN_6O_3$: C, 58.09; H, 5.10; N, 18.47%. HRMS (EI) m/z Found: $[M+H]^+$, 455.15970 Calcd for $C_{22}H_{24}ClN_6O_3$: M, 455.15984.

4.12. General Method C for the preparation of compounds 8a–f

To a solution of semicarbazide (0.54 mmol) in methanol (10 ml) was added a mixture of *p*-toluenesulfonic acid and aldehyde. The resultant was stirred at room temperature for 16 h. The reaction mixture was diluted with chloroform (20 ml). The organic layer was separated, washed with 5% aqueous sodium carbonate (3 × 10 ml), brine (2 × 10 ml), dried ($MgSO_4$) and concentrated under reduced pressure to afford a solid residue, which was column chromatographed on silica gel.

4.13. Compound 8a

The conditions employed for the preparation of this compound were those described in General Method C. Column chromatography (MeOH–DCM 1:9 followed by 17% NH_4OH –MeOH–DCM 1:2:2) afforded **8a** pale green solid (0.16 g, 61%) (recrystallised from methanol); decomposes above 150 °C; $R_f = 0.30$ (17% NH_4OH –MeOH–DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} 1060 (C–O), 1570 (C=N), 1650 (C=O), 3380 (N–H); δ_H (300 MHz; DMSO- d_6) 1.06 (6H, t, J 7.2, $N[CH_2CH_2]_2$), 2.64 (4H, t, J 7.2, $N[CH_2CH_2]_2$), 3.40 (2H, t, J 5.4, $CONHCH_2CH_2N$), 3.45 (2H, t, J 5.4, $CONHCH_2CH_2N$), 3.84 (2H, s, $PhCH_2N$), 6.32 (1H, d, J 8.7, H-5), 6.60 (1H, d, J 5.4, H-3'), 7.09 (1H, br s, $NHNCONH$), 7.37 (1H, d, J 8.7, H-6), 7.43 (1H, dd, J 2.1, 8.7, H-6'), 7.66 (1H, br s, $NHNCONH$), 7.79 (1H, d, J 8.7, H-5'), 8.04 (1H, s, $PhCH=NNH$), 8.24 (1H, d, J 2.1, H-8'), 8.40 (1H, d, J 5.7, H-2'); δ_c (100 MHz; DMSO- d_6) 11.3 (2C), 46.8, 49.4 (2C), 52.4, 54.6, 98.3, 106.9, 107.7, 108.0, 116.8, 121.8, 125.0, 127.6, 130.7,

134.4, 145.6, 148.8, 151.2, 152.0, 156.9, 157.5, 162.1. Anal. Found: C, 57.90; H, 6.14; N, 15.09% Calcd for $C_{24}H_{29}ClN_6O_3$: C, 57.30; H, 6.17; N, 16.72%; HRMS (EI) m/z Found: $[M]^+$, 484.19892 Calcd for $C_{24}H_{29}ClN_6O_3$: M, 484.19897.

4.14. Compound 8b

The conditions employed for the preparation of this compound were those described in General Method C. Column chromatography (MeOH–DCM 1:9 followed by 17% NH_4OH –MeOH–DCM 1:2:2) afforded **8b** as a brown solid (0.14 g, 52%) (recrystallised from methanol); decomposes above 100 °C; $R_f = 0.30$ (17% NH_4OH –MeOH–DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} 1060 (C–O), 1580 (C=N), 1650 (C=O), 3400 (N–H); δ_H (300 MHz; $CDCl_3$) 1.87 (4H, m, $N[CH_2CH_2]_2$), 2.69 (4H, m, $N[CH_2CH_2]_2$), 3.42 (2H, t, J 5.4, $CONHCH_2CH_2N$), 3.79 (2H, t, J 5.4, $CONHCH_2CH_2N$), 3.95 (2H, s, $PhCH_2N$), 6.01 (1H, br s, $NHNCONH$), 6.30 (1H, d, J 5.4, H-3'), 6.38 (1H, d, J 8.4, H-5), 6.83 (1H, br s, $NHNCONH$), 6.95 (1H, d, J 8.4, H-6), 7.28 (1H, dd, J 2.1, 9.0, H-6'), 7.77 (1H, d, J 9.0, H-5'), 7.85 (1H, s, $PhCH=NNH$), 7.88 (1H, d, J 2.1, H-8'), 8.42 (1H, d, J 5.4, H-2'); δ_c (75 MHz; $CDCl_3$) 23.3 (2C), 48.5, 50.0 (2C), 52.0, 54.0, 98.3, 106.9, 107.8, 108.0, 114.8, 121.8, 125.2, 128.0, 130.9, 135.8, 145.8, 148.6, 150.2, 151.8, 156.7, 157.4, 163.; Anal. Found: C, 54.56; H, 5.85; N, 14.75% Calcd for $C_{24}H_{27}ClN_6O_3$: C, 54.54; H, 5.87; N, 15.90%; HRMS (EI) m/z Found: $[M+H]^+$, 483.18312 Calcd for $C_{24}H_{28}ClN_6O_3$: M, 483.18331.

4.15. Compound 8c

The conditions employed for the preparation of this compound were those described in General Method C. Column chromatography (MeOH–DCM 1:9 followed by 17% NH_4OH –MeOH–DCM 1:2:2) afforded **8c** as a greenish yellow solid (0.14 g, 54%) (recrystallised from methanol); decomposes above 200 °C; $R_f = 0.30$ (17% NH_4OH –MeOH–DCM 1:2:2); IR ν_{max} (Nujol)/ cm^{-1} 1050 (C–O), 1580 (C=N), 1650 (C=O), 3370 (N–H); δ_H (400 MHz; CD_3OD) 1.42 (2H, m, $N[CH_2CH_2]_2CH_2$), 1.58 (4H, m, $N[CH_2CH_2]_2CH_2$), 2.42 (2H, m, $N[CH_2CH_2]_2CH_2$), 3.30 (2H, t, J 5.2, $CONHCH_2CH_2N$), 3.68 (2H, t, J 5.2, $CONHCH_2CH_2N$), 3.98 (2H, s, $PhCH_2N$), 6.13 (1H, d, J 5.2, H-3'), 6.16 (1H, br s, $NHNCONH$), 6.23 (1H, d, J 8.4, H-5), 6.84 (1H, d, J 8.4, H-6), 6.85 (1H, br s, $NHNCONH$), 7.05 (1H, dd, J 2.0, 8.8, H-6'), 7.60 (2H, d, J 8.8, H-5'), 7.68 (1H, d, J 2.0, H-8'), 7.80 (1H, s, $PhCH=NNH$), 8.25 (1H, d, J 5.2, H-2'); δ_c (100 MHz; $CDCl_3$) 23.8, 25.5 (2C), 26.3 (2C), 53.4, 54.3, 54.5, 99.6, 107.1, 108.8, 111.8, 116.2, 124.5, 126.8, 129.9, 132.2, 136.7, 146.5, 148.5, 151.5, 153.3, 158.7, 159.2, 162.2; HRMS (EI) m/z Found: $[M+H]^+$, 497.20614 Calcd for $C_{25}H_{29}ClN_6O_3$: M, 497.20679.

4.16. Compound 8d

The conditions employed for the preparation of this compound were those described in General Method C.

Column chromatography (MeOH–DCM 1:9 followed by 17% NH₄OH–MeOH–DCM 1:2:2) afforded **8d** a cream white solid (0.18 g, 69%) (recrystallised from methanol); decomposes above 220 °C; $R_f = 0.33$ (17% NH₄OH–MeOH–DCM 1:2:2); IR ν_{\max} (Nujol)/cm⁻¹ 1050 (C–O), 1570 (C=N) 1660 (C=O), 3350 (N–H); δ_H (300 MHz; CDCl₃) 2.62 (4H, br s, N[CH₂CH₂]₂O), 3.50 (2H, t, J 5.1, CONHCH₂CH₂N), 3.58 (2H, t, J 5.1, CONHCH₂CH₂N), 3.65 (4H, br s, N[CH₂CH₂]₂O), 3.81 (2H, s, PhCH₂N), 6.11 (1H, br s, NHNCONH), 6.38 (1H, d, J 8.7, H-5), 6.89 (1H, d, J 6.9, H-3'), 6.84 (1H, br s, NHNCONH), 7.11 (1H, d, J 9.0, H-5'), 7.39 (1H, d, J 8.7, H-6), 7.68 (1H, dd, J 2.4, 9.0, H-6'), 7.88 (1H, d, J 2.4, H-8'), 8.05 (1H, s, PhCH=NNH), 8.51 (1H, d, J 6.9, H-2'); δ_C (75 MHz; CDCl₃) 38.4, 44.5, 53.1 (2C), 53.3, 66.3 (2C), 99.4, 107.4, 107.9, 112.5, 116.5, 121.8, 126.1, 128.7, 129.9, 137.7, 141.7, 145.8, 155.2, 156.8, 157.9, 159.0, 162.0; Anal. Found: C, 54.82; H, 5.18; N, 12.57% Calcd for C₂₄H₂₇ClN₆O₄: C, 54.75; H, 5.70; N, 15.97%; HRMS (EI) m/z Found: [M+]⁺, 498.17798 Calcd for C₂₄H₂₇ClN₆O₄: M, 498.17823.

4.17. Compound 8e

The conditions employed for the preparation of this compound were those described in General Method C. Column chromatography (MeOH–DCM 1:9 followed by 17% NH₄OH–MeOH–DCM 1:2:2) afforded **8e** as a cream white solid (0.22 g, 80%) (recrystallised from methanol); decomposes above 140 °C; $R_f = 0.33$ (17% NH₄OH–MeOH–DCM 1:2:2); IR ν_{\max} (Nujol)/cm⁻¹ 1060 (C–O), 1130 (C–N), 1580 (C=N), 1650 (C=O) 3400 (N–H); δ_H (400 MHz; CDCl₃) 2.30 (3H, s, NCH₃), 2.60 (8H, br s, N[CH₂CH₂]₂N), 3.39 (2H, m, CONHCH₂CH₂N), 3.80 (2H, m, CONHCH₂CH₂N), 3.83 (2H, s, PhCH₂N), 6.11 (1H, br s, NHNCONH), 6.24 (1H, d, J 4.8, H-3'), 6.37 (1H, d, J 8.4, H-5), 6.84 (1H, br s, NHNCONH), 6.96 (1H, d, J 8.4, H-6), 7.17 (1H, d, J 2.0, 8.8, H-6'), 7.68 (1H, d, J 8.8, H-5'), 7.79 (1H, s, PhCH=NNH), 7.86 (1H, d, J 2.0, H-8'), 8.42 (1H, d, J 4.8, H-2'); δ_C (100 MHz; CDCl₃) 39.2, 45.8, (2C), 46.0 (2C), 52.6, 53.6, 54.8, 98.2, 107.1, 109.0, 109.4, 117.2, 121.8, 125.2, 128.0, 131.1, 134.8, 147.2, 148.6.

4.18. Compound 8f

The conditions employed for the preparation of this compound were those described in General Method C. Column chromatography (MeOH–DCM 1:9 followed by 17% NH₄OH–MeOH–DCM 1:2:2) afforded **8f** as a cream white solid (0.26 g, 74%) (recrystallised from methanol); decomposes above 140 °C; $R_f = 0.13$ (17% NH₄OH–MeOH–DCM 1:2:2); IR ν_{\max} (Nujol)/cm⁻¹ 1060 (C–O), 1580 (C=N) 1650 (C=O), 3400 (N–H); δ_H (300 MHz; DMSO-*d*₆) 2.82 (4H, br s, N[CH₂CH₂]₂CH₂Ph), 3.45 (4H, br s, N[CH₂CH₂]₂CH₂Ph), 3.41 (2H, t, J 5.4, CONHCH₂CH₂N), 3.47 (2H, t, J 5.4, CONHCH₂CH₂N), 3.87 (2H, s, PhCH₂N), 6.38 (1H, d, J 8.4, H-5), 6.62 (1H, d, J 5.1, H-3'), 7.04 (1H, d, J 5.1, H-3?), 7.15 (1H, br s, NHNCONH), 7.43 (1H, d, J 8.4, H-6), 7.50 (1H, dd, J 2.1, 9.0, H-6'), 7.55 (1H, dd, J 2.1, 9.0, H-6?), 7.59 (1H, br s,

NHNCONH), 7.79 (1H, d, J 2.1, H-8'), 7.98 (1H, d, J 2.1, H-8?), 8.02 (1H, d, J 9.0, H-5'), 8.07 (1H, s, PhCH=NNH), 8.21 (1H, d, J 9.0, H-5''), 8.41 (1H, d, J 5.1, H-2'), 8.71 (1H, d, J 5.1, H-2''); δ_C (75 MHz; DMSO-*d*₆) 37.7, 40.3, 51.5 (2C), 51.9, 52.2 (2C), 98.6, 107.2, 107.5, 109.5, 111.9, 117.2, 121.3, 123.8, 124.1, 125.7, 125.9, 127.1, 128.0, 133.4, 133.5, 139.8, 148.5, 149.6, 150.3, 151.4, 152.1, 155.9, 156.1, 156.2, 157.1, 158.1; Anal. Found C 54.76, H 4.71, N 14.70% Calc. For C₃₃H₃₂Cl₂N₈O₃: C 54.79, H 5.39, N 15.49%; HRMS (EI) m/z Found: M⁺, 658.19784 Calc. For C₃₃H₃₂Cl₂N₈O₃: M, 658.19744.

4.19. N'-(7-Chloro-quinolin-4-yl)-ethane-1,2-diamine 5

A mixture of 1,3-diaminoethane (5.00 g, 25.0 mmol), 4,7-dichloroquinoline (2.25 g, 5.0 mmol), K₂CO₃ (0.52 g, 3.78 mmol), triethylamine (0.50 ml, 3.78 mmol) and 1-methyl pyrrolidinone (9.0 ml) under nitrogen was heated at 135 °C for 4 h. The reaction mixture was cooled to room temperature and then aqueous sodium hydroxide (1 M, 50 ml) was added. The resultant mixture was extracted with hot ethyl acetate (3× 100 ml). The combined extracts were washed with brine, dried (MgSO₄) and concentrated to give a cream white solid (2.06 g, 82%) which recrystallised from methanol to give **5** as a white crystalline powder, mp 137–139 °C (from methanol) (Lit. mp 137–139 °C);²⁶⁷ $R_f = 0.35$ (MeOH–DCM 1:4); δ_H (300 MHz; CDCl₃) 3.46 (2H, t, J 5.6, NCH₂CH₂NH₂), 3.71 (2H, t, J 5.6, NCH₂CH₂NH₂), 6.40 (1H, d, J 5.2, H-3), 7.36 (1H, dd, J 2.4, 8.8, H-6), 7.77 (1H, d, J 2.4, H-8), 7.97 (1H, d, J 8.8, H-5), 8.34 (1H, d, J 5.2, H-2).

4.20. 1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-carbamic acid phenyl ester 6

Phenyl chloroformate (1.41 g, 9.02 mmol) was added to a stirred and cooled (0 °C) solution of N'-(7-chloro-quinolin-4-yl)-ethane-1,2-diamine (2.00 g, 9.02 mmol) and triethylamine (1.26 ml, 9.02 mmol) in DMF (10 ml). The mixture was stirred at room temperature for 45 min, diluted with water (50 ml) and extracted with chloroform (3× 50 ml). The combined organic layers were washed with water (3× 50 ml), brine (50 ml), dried (MgSO₄) and concentrated to give a yellow residue. Column chromatography on silica (MeOH–DCM 1:19) afforded **6** as a white solid (2.22 g, 72%) which was recrystallised from chloroform–hexane. mp 125–127 °C; $R_f = 0.43$ (MeOH–DCM 1:9); IR ν_{\max} (Nujol)/cm⁻¹; 1060 (C–O), 1580 (C=N), 1720 (C=O), 3225 (N–H); ¹H NMR δ_H (300 MHz; CDCl₃) 3.47 (2H, q, J 6.0, NCH₂CH₂NHCO), 3.70 (2H, q, J 6.0, NCH₂CH₂NHCO), 6.02 (1H, br s, HNCH₂CH₂NHCO), 6.21 (1H, br s, HNCH₂CH₂NHCO), 6.40 (1H, d, J 5.1, H-3), 7.22 (5H, m, Ph), 7.26 (1H, dd, J 2.1, 9.0, H-6), 7.66 (1H, d, J 9.0, H-5), 7.93 (1H, d, J 2.1, H-8), 8.34 (1H, d, J 5.1, H-2); δ_C (75 MHz; CDCl₃) 40.0, 45.1, 98.5, 117.2, 121.5, 121.6 (2C), 125.5, 125.7, 128.3 (2C), 126.6, 135.2, 148.8, 150.0, 150.8, 151.7, 156.9; Anal. Found: C, 62.64; H, 4.74; N, 12.15%. Calcd for C₁₈H₁₆ClN₃O₂: C, 63.25; H, 4.72; N, 12.29%; HRMS (EI) m/z Found: [M+H]⁺, 342.100089 Calcd for C₁₈H₁₆ClN₃O₂: M, 342.10093.

4.21. Compound 7

To a solution of [2-(7-chloro-quinolin-4-ylamino)-ethyl]-carbamic acid phenyl ester (1.26 g, 3.70 mmol) in dry methanol (10 ml) was added hydrazine monohydrate (1.85, 37 mmol) and the resulting mixture was stirred at 90 °C for 12 h. The reaction mixture was concentrated to give a white residue, which was subjected to column chromatography on silica gel (MeOH–DCM 1:9 followed by 17% NH₄OH–MeOH–DCM 1:2:2) to give **7** as white crystals (0.87 g, 84%); mp 177–179 °C (from methanol); R_f = 0.12 (MeOH–DCM 1:9); IR ν_{\max} (Nujol)/cm⁻¹; 1080 (C–O), 1140 (C–N), 1550 (C=N), 1600 (C=C), 1660 (C=O), 3150 (N–H), 3325 (N–H); δ_H (400 MHz; CD₃OD) 3.41 (2H, t, J 6.0, NCH₂CH₂NHCO), 3.52 (2H, t, J 6.0, NCH₂CH₂NHCO), 6.52 (1H, d, J 5.4, H-3), 7.35 (1H, dd, J 2.4, 8.8, H-6), 7.74 (1H, d, J 2.4, H-8), 7.99 (1H, d, J 8.8, H-5), 8.32 (1H, d, J 5.4, H-2); δ_C (100 MHz; CD₃OD) 38.1, 44.3, 98.3, 117.2, 123.0, 124.9, 126.3, 135.2, 148.3, 151.2, 151.6, 162.8; HRMS (EI) m/z Found: [M+H]⁺, 280.09658 Calcd for C₁₂H₁₅ClN₅O: M, 280.09651.

4.21.1. In vitro activities of compounds against falcipain-2. IC₅₀ values against the recombinant enzyme (falcipain-2) were determined as described by Greenbaum and coworkers.^{16a} Thus, an equal amount of recombinant protein (~1 nM) was incubated with different concentrations of inhibitors (added from 100× stock solutions 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 Fluoroscan® Ascent spectrofluorometer. IC₅₀ values were determined from plots of activity over inhibitor concentration with GraphPad Prism® software.

4.21.2. In vitro activities of compounds against W2 P. falciparum. W2-strain *P. falciparum* parasites (1% parasitaemia, 2% haematocrit) were cultured in 0.5 ml of medium in 48-well culture dishes. Appropriate inhibitors from 10 mM stocks in DMSO were added to cultured parasites to a final concentration of 20 μM. From 48-well plates, 125 μM of culture was transferred to two 96-well plates (duplicates). Serial dilutions (1%) of inhibitors were made to final concentrations of 10 μM, 2 μM, 0.4 μM, 80, 16, and 3.2 nM. Cultures were maintained at 37 °C for 2 days after which the parasites were washed and fixed with 1% formaldehyde in PBS. After two days, parasitaemia was measured by flow cytometry using the DNA stain YOYO-1 as a marker for cell survival.

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References and notes

- Payne, D. *Parasitol. Today* **1987**, *3*, 241.
- Rosenthal, P. J.; McKerrow, J. H.; Aikawa, M.; Nagasawa, H.; Leech, J. H. *J. Clin. Invest.* **1988**, *82*, 1560.
- Rosenthal, P. J. *Int. J. Parasitol.* **2004**, *34*, 1489.
- Dimmock, J. R.; Kumar, P. *Curr. Med. Chem.* **1997**, *4*, 1.
- Dimmock, J. R.; Vashishtha, S. C.; Quail, J. W.; Pugazhenthii, U.; Zimpel, Z.; Sudom, A. M.; Allen, T. M.; Kao, G. Y.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1998**, *41*, 4012.
- Mutus, B.; Wagner, J. D.; Talpas, C. J.; Dimmock, J. R.; Phillips, O. A.; Reid, R. S. *Anal. Biochem.* **1989**, *177*, 237.
- Dimmock, J. R.; Raghavan, S. K.; Logan, B. M.; Bigam, G. E. *Eur. J. Med. Chem.* **1983**, *18*, 248.
- Klayman, D. L.; Bartosevich, J. F.; Griffin, T. S.; Mason, C. J.; Scovill, J. P. *J. Med. Chem.* **1979**, *22*, 1367.
- Klayman, D. L.; Scovill, J. P.; Bartosevich, J. F.; Bruce, J. *J. Med. Chem.* **1983**, *26*, 35.
- Dobek, A. S.; Klayman, D. L.; Dickson, E. J., Jr.; Scovill, J. P.; Tramont, E. C. *Antimicrob. Agents Chemother* **1980**, *18*, 27.
- Casero, J. R. A.; Klayman, D. L.; Childs, G. E.; Scovill, J. P.; Desjardins, R. E. *Antimicrob. Agents Chemother* **1980**, *18*, 317.
- Shipman, J. C.; Smith, S. H.; Drach, J. C.; Klayman, D. L. *Eur. J. Med. Chem.* **1981**, *19*, 682.
- Cabantchik, Z. I.; Moody-Haupt, S.; Gordeuk, V. R. *FEMS Immunol. Med. Microbiol.* **1999**, *26*, 289.
- Richardson, D. R. *Exp. Opin. Invest. Drugs* **1999**, *8*, 2141.
- Walcourt, A.; Loyevsky, M.; Lovejoy, D. B.; Gordeuk, V. R.; Richardson, D. R. *Int. J. Biochem. Cell. Biol.* **2004**, *36*, 401.
- (a) 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*, 3212–3219; (b) Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3527; (c) Fujii, N.; Mallari, J. P.; Hansell, E. J.; Mackey, Z.; Doyle, P.; Zhou, Y. M.; Gut, J.; Rosenthal, P. J.; McKerrow, J. H.; Guy, R. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 121.
- (a) Hutchins, S. M.; Chapman, K. T. *Tetrahedron Lett.* **1994**, *35*, 4055; (b) Hutchins, S. M.; Chapman, K. T. *Tetrahedron Lett.* **1995**, *36*, 2583; (c) Burgess, K.; Linthicum, D. S.; Shin, H. *Angew. Chem. Int. Ed.* **1995**, *34*, 907; (d) Kim, J. M.; Bi, Y.; Paikoff, S. J.; Schultz, P. G. *Tetrahedron Lett.* **1996**, *37*, 5305; (e) Burgess, K.; Ibarzo, J.; Linthicum, D. S.; Russel, D. H.; Shin, H.; Shitangkoon, A.; Totani, A. J. *J. Am. Chem. Soc.* **1997**, *119*, 1556.
- Lam, P. Y. S.; Jadhav, P. K.; Eyermaun, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, Y. N.; Chang, C. H.; Weber, P. C.; Jackson, D. A.; Sharpe, T. R.; Viitanen, E. S. *Science* **1994**, *263*, 380.
- Chorev, M.; Goodman, M. *Acc. Chem. Res.* **1993**, *26*, 266.
- (a) Zhao, X.; Chang, Y. L.; Fowler, F. W.; Lauher, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 6627; (b) Chang, Y. L.; West, M. A.; Fowler, F. W.; Lauher, J. W. *J. Am. Chem. Soc.* **1993**, 5991; (c) Nowick, J. S.; Abdi, M.; Bellamo, K. A.; Love, J. A.; Martinez, E. J.; Noronha, G.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 89.
- (a) Coe, S.; Kane, J. J.; Nguyen, T. L.; Todedo, L. M.; Wininger, E.; Fowler, F. W.; Lauher, J. W. *J. Am. Chem. Soc.* **1991**, *119*, 86; (b) Nowick, J. S.; Powell, A.; Martinez, E. J.; Smith, E. M.; Noronha, G. *J. Org. Chem.* **1992**, *57*, 3763.

22. (a) Musonda, C. C.; Taylor, D.; Lehman, J.; Gut, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3901; (b) Musonda, C. C.; Gut, J.; Rosenthal, P. J.; Yardley, V.; Carvalho de Souza, R. C.; Chibale, K. *Bioorg. Med. Chem.* **2006**, *14*, 5605; (c) Chiyanzu, I.; Clarkson, C.; Smith, P. J.; Lehman, J.; Gut, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem.* **2005**, *13*, 3249–3261.
23. Lytton, S. D.; Mester, B.; Libman, J.; Shanzer, A.; Cabantchik, Z. I. *Blood* **1994**, *84*, 910.
24. Lytton, S. D.; Mester, B.; Dayan, I.; Glickstein, H.; Libman, J.; Shanzer, A. *Blood* **1993**, *81*, 214.
25. Scott, M. D.; Ranz, A.; Kuypers, F. A.; Lubin, B. H.; Meshnick, S. R. *Br. J. Haematol.* **1990**, *75*, 598.
26. Hershko, C.; Theanacho, E. N.; Spira, D. T.; Peter, H. H.; Dobbin, P.; Hider, R. C. *Blood* **1991**, *77*, 637.
27. Dorn, A.; Vippagunta, S. R.; Matile, H.; Jaquet, C.; Vennerstrom, J. L.; Ridley, R. G. *Biochem. Pharmacol.* **1998**, *55*, 727.
28. Scott, M. D.; Yang, L.; Ulrich, P.; Shupe, T. *Redox Rep.* **1997**, *3*, 159.
29. Singh, S.; Puri, S. K.; Singh, S. K.; Srivastava, R.; Gupta, R. C.; Pandey, V. C. *J. Biol. Chem.* **1997**, *272*, 13506.