Ferrocene–pyrimidine conjugates: Synthesis, electrochemistry, physicochemical properties and antiplasmodial activities

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JULY 2015

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2015.05.043

5 AUTHORS, INCLUDING:

Carmen Abriette de Kock

University of Cape Town

42 PUBLICATIONS **339** CITATIONS

SEE PROFILE

Peter Smith

University of Cape Town

247 PUBLICATIONS **4,407** CITATIONS

SEE PROFILE

Kamaljit Singh

Guru Nanak Dev University

113 PUBLICATIONS **1,452** CITATIONS

SEE PROFILE

European Journal of Medicinal Chemistry 100 (2015) 1–9

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Ferrocene-pyrimidine conjugates: Synthesis, electrochemistry, physicochemical properties and antiplasmodial activities

Rakesh Chopra [a], Carmen de Kock [b], Peter Smith [b], Kelly Chibale [c], Kamaljit Singh [a, *]

[a] Department of Chemistry, UGC-Centre for Advanced Studies-II, Guru Nanak Dev University, Amritsar 143005, India
[b] Division of Pharmacology, Department of Medicine, University of Cape Town, Observatory 7925, South Africa
[c] Department of Chemistry, South African Medical Research Council Drug Discovery and Development Research Unit, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 701, South Africa

ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form
26 May 2015
Accepted 28 May 2015
Available online 30 May 2015

Keywords:
Ferrocene conjugates
Ferrocene/ferrocenium redox couple
Huisgen 1,3-dipolar cycloaddition
Antiplasmodial studies
Lipophilicity

ABSTRACT

The promise of hybrid antimalarial agents and the precedence set by the antimalarial drug ferroquine prompted us to design ferrocene-pyrimidine conjugates. Herein, we report the synthesis, electrochemistry and anti-plasmodial evaluation of ferrocenyl-pyrimidine conjugates against chloroquine susceptible NF54 strain of the malaria parasite *Plasmodium falciparum*. Also their physicochemical properties have been studied.

1. Introduction

Ferroquine (SSR97193) **1** is an effective 4-aminoquinoline-ferrocene conjugate antimalarial agent [1–6], which is active *in vitro* as well as *in vivo* against both chloroquine resistant (CQ^R) as well as chloroquine susceptible (CQ^S) *Plasmodium falciparum* strains. Although ferrocene itself lacks any antimalarial activity [7], the antimalarial activity of ferroquine presumably takes advantage of the affinity of the *P. falciparum* for the metal (Fe^{2+}) center of ferrocene core. Also the redox (Fe^{2+}/Fe^{3+}) behavior of the ferrocene based compounds is known to influence the bioactivity of the ferrocenyl derivatives [8–11]. Since the antimalarial activity of **1** is superior to that of chloroquine **2** (Fig. 1), this may suggest that the mode of action of **1** is not essentially same as that of **2**. Within the context of the mode of action of **1**, it has also been suggested that **1** makes a strong complex with hematin and blocks the hemozoin based detoxification process of the parasite [5] or even act as a resistance reversing agent through blocking of the *P. falciparum* transmembrane protein (*Pf*CRT) [12] by virtue of its lipophilic properties. A carbosilane analog (**3**) of ferroquine (Fig. 1) with potent antiplasmodial activity has been recently reported [13,14].

Malaria has posed a serious threat to the human health as nearly 1 million people face death due to malaria every year [15]. Besides, being epidemic in sub-Saharan Africa, the disease has slowly gained its ground in Asia and Latin America also. In fact, on the borders of Thailand, *P. falciparum* has become resistant to almost all the available antimalarial drugs [16–18]. The weak immune system of pregnant women and/or children below the age of 5 exposes them to greater risk from this killer disease. The activity of the one-time most active antimalarial drugs (mostly quinoline based) has been compromised owing to growing drug resistance [19,20]. Resistance to the artemisinin class of drugs as well as to primaquine has been reported [21–23]. Thus, the burden of malaria is further expected to rise, which necessitates an urgent need for effective new drugs [24–28] or drug combinations to combat the growing resistance.

Hybrid drugs have been portrayed as drugs of the future owing to the potential advantages over single drug and/or multicomponent combination therapy. As part of our continuing interest in the synthesis, biology, structure-activity relationship and mode of action studies of hybrid antimalarials [29–34], recently we have reported [35–37] a series of pyrimidine-quinoline hybrids, with antiplasmodial activity against both CQ^s as well as CQ^R strains of *P. falciparum*. We now report the synthesis of new hybrids in which

* Corresponding author.
E-mail address: kamaljit.chem@gndu.ac.in (K. Singh).

http://dx.doi.org/10.1016/j.ejmech.2015.05.043
0223-5234/

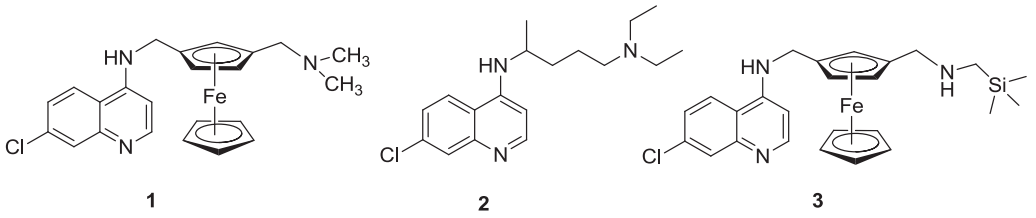


Fig. 1. Antimalarial agents: ferroquine **1**, chloroquine **2** and carbosilane analog **3**.

ferrocene and pyrimidine moieties are linked through five-membered heterocyclic units. The choice of these heterocycles was guided by the fact that many of these systems are present in molecules, which possess antimalarial activity [13,14,38]. Additionally, we discuss the electrochemical behavior, physico-chemical and antiplasmodial properties of the title compounds.

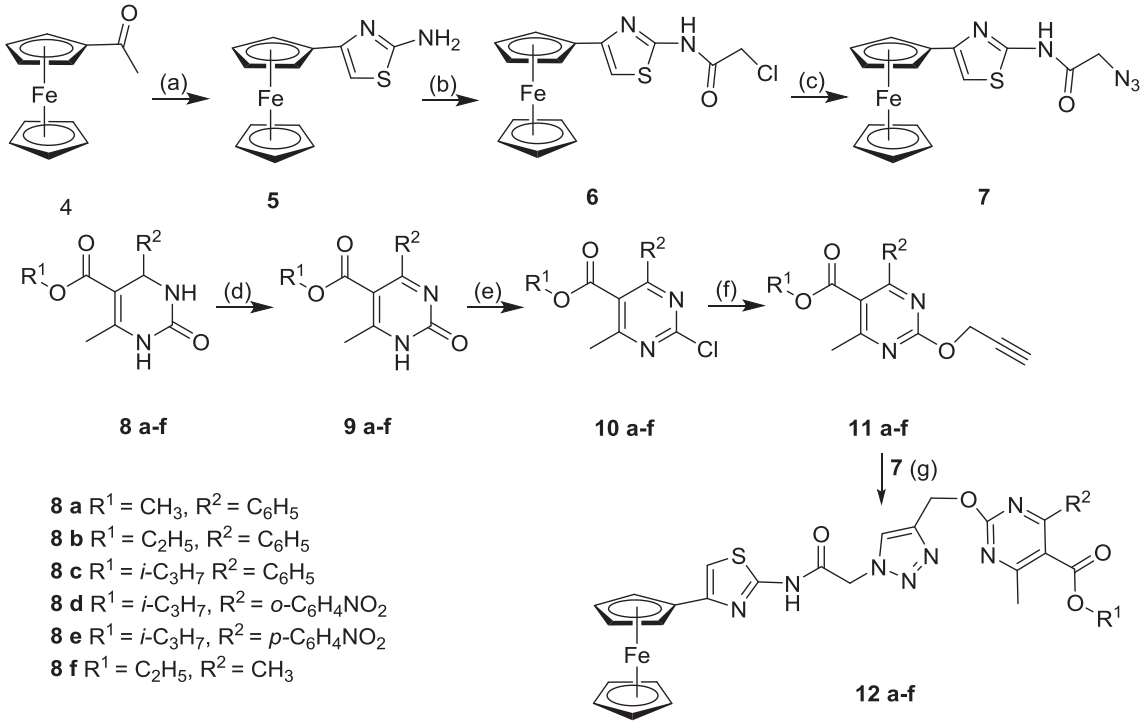
2. Results and discussion

2.1. Synthesis of pyrimidine-ferrocene conjugates

The synthetic pathway to pyrimidine-ferrocenyl conjugates **12a**–**f** commences with the synthesis of 2-amino-4-ferrocenylthiazole **5** from acetylferrocene [39]. Chloroacetylation of **5** upon refluxing with chloroacetyl chloride in dry toluene furnished **6** (Scheme 1), which upon a nucleophilic substitution reaction with NaN_3 in dry DMF at 60 °C gave **7**. On the other hand, C2-propargylated pyrimidines were synthesized by carrying out pyridinium chlorochromate (PCC) mediated dehydrogenation [40] of compound **8** prepared via conventional Bignelli condensation [41] to yield oxidized intermediate **9**. Subsequent chlorination of **9** using $POCl_3$ under reflux provided chloropyrimidines **10** [42]. Base-catalyzed nucleophillic substitution of **10** with propargyl alcohol led to the formation of C2-propargylated pyrimidines **11**. Finally, copper assisted azide-alkyne Huisgen 1,3-dipolar cycloaddition reaction between **7** and appropriate **11a**–**f** delivered click [43,44] condensed products **12a**–**f** in a synthetically useful manner (Table 2). All the compounds were characterized using spectroscopic techniques and microanalytical data and depicted correct spectral data (Fig. S1–S30). Additionally, purity of the hybrids was checked using HPLC (Fig. S31–S36).

2.2. Electrochemical properties

The redox behavior of the hybrids **12a**–**f** was determined using cyclic voltammetry (CV). The measurements were performed in nitrogen gas purged solutions of **12a**–**f** in anhydrous dichloromethane. The CV plots were recorded using Ag/Ag^+ (0.01 M n-$Bu_4N^+PF_6^-$) as a reference electrode, which was calibrated by a ferrocene/ferrocenium (Fc/Fc^+) redox couple. The hybrids depicted a single electron reversible oxidation peaks similar to ferrocene and the electrochemical data are presented in Table 1. As could be seen from the entries in Table 1, the hybrids show a one electron redox change. However, there is insignificant change in the redox behavior



8 a $R^1 = CH_3$, $R^2 = C_6H_5$
8 b $R^1 = C_2H_5$, $R^2 = C_6H_5$
8 c $R^1 = i\text{-}C_3H_7$ $R^2 = C_6H_5$
8 d $R^1 = i\text{-}C_3H_7$, $R^2 = o\text{-}C_6H_4NO_2$
8 e $R^1 = i\text{-}C_3H_7$, $R^2 = p\text{-}C_6H_4NO_2$
8 f $R^1 = C_2H_5$, $R^2 = CH_3$

Scheme 1. Synthetic route (a–c) to azidoacetamidoferrocenyl thiazole **7** and hybrids **12** (d–g): (a) thiourea, iodine 100 °C overnight, 88%, (b) chloroacetyl chloride, dry toluene, reflux, 85%, (c) NaN_3, dry DMF Δ 60 °C, 89%, (d) PCC, DCM, 24 h, 65%, (e) $POCl_3$, 105 °C, 95%, (f) 2-propyn-1-ol (propargyl alcohol), K_2CO_3, CH_3CN, 94%, (g) $CuSO_4 \cdot 5H_2O$, sodium-L-ascorbate, EtOH: H_2O (9:1 v/v), 92%.

Table 1
Electrochemical properties of hybrids **12a**–**f**.

Hybrid	$E_{p,a}$ (V)	$E_{p,c}$ (V)	$E_{1/2}{}^{0+}$ (V)[a,b]	ΔE_p (V)[c]
12a	0.588	0.496	0.542	0.092
12b	0.581	0.494	0.537	0.087
12c	0.575	0.489	0.532	0.086
12d	0.580	0.488	0.534	0.092
12e	0.575	0.489	0.532	0.096
12f	0.578	0.490	0.534	0.088

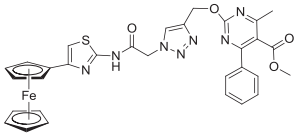
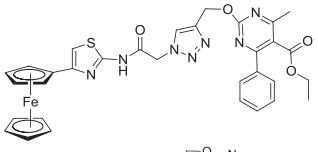
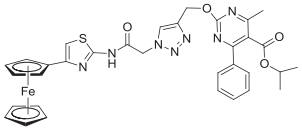
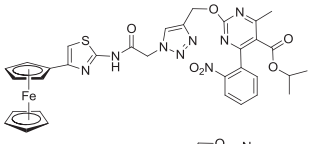
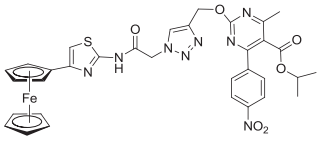
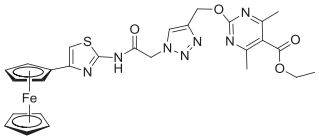
[a] Recorded in dichloromethane (1×10^{-4} M).
[b] $E_{1/2}{}^{0/+} = (E_{p,a} + E_{p,c})/2$.
[c] $\Delta E_p = |E_{p,a} - E_{p,c}|$.

of **12a**–**f** as expected, since in all these hybrids, the ferrocene unit is attached at the C-4 position of the thiazole ring and is insulated from the electronic effects of the substituents on the pyrimidine ring. Thus, compounds **12a**–**f** would exert an identical magnitude of redox action. Cyclic voltammograms for **12a**–**f** are presented in ESI (Fig. S37– S42). Further, the hybrids **12a**–**f** adopted identical molecular geometry as visualized from their energy minimized structures (Fig. S43–S48).

2.3. Anti-plasmodial activities

The *in vitro* antiplasmodial activities of the hybrids **12a**–**f** against CQ^S NF54 strain are summarized in Table 2. Inspection of Table 2 reveals that replacing a methyl ester group at the C-5 position of the pyrimidine ring in **12a** with an ethyl or *iso*-propyl group, to produce **12b** and **12 c** in that order (increasing lipophilicity) results in enhancement of anti-plasmodial activity [IC_{50} (μM): 28.19 (**12a**), 17.65 (**12b**), 7.68 (**12c**), Table 2] of the hybrid compounds. Similarly, replacing C-4 phenyl group of the ethyl ester hybrid **12b** [IC_{50} (μM): 17.65] with methyl group to form less lipophilic **12f** [IC_{50} (μM): 22.46] recorded a marginal decrease in the antiplasmodial activity. The data reported in Table 2 also suggested superior antimalarial activity of the *iso*-propyl ester hybrids compared to methyl or ethyl ester counterparts as well as indicated greater activity of the C-4 phenyl group appended hybrids. Thus, retaining these structural features, additional hybrids were synthesized, wherein a polar nitro group was introduced at either *o*- or *p*-position of the C-6 phenyl ring of the pyrimidine core. However, compared to **12c**, it was found that the antiplasmodial activity decreases in the order **12c** > **12d** > **12e** (Table 2).

Table 2
Molecular weights, yield (%) and *in vitro* antiplasmodial activities of hybrids **12a**–**f**.

Hybrid	Structure	IC_{50} (μM)[a,b,c]	Mol. Wt.	cLogP[d]	Yield[e] (%)
12a		28.19 ± 1.80	649	4.01	97
12b		17.65 ± 2.40	663	4.54	97
12 c		7.68 ± 0.50	677	4.85	96
12d		14.54 ± 0.70	722	4.59	94
12e		15.92 ± 4.90	722	4.59	91
12f		22.46 ± 3.30	601	2.94	97
CQ[f]		0.006 ± 0.002			
ART[g]		<2 ± ND			

[a] Against CQ^S NF54 strain.
[b] Activity in μM.
[c] Mean of three independent experiments.
[d] ClogP calculated from ChemDraw Ultra 11.0 plus.
[e] Isolated yield.
[f] Chloroquine.
[g] Artesunate.

2.4. Physicochemical parameters

Pharmacokinetics of a drug in human body is often estimated by utilizing physico-chemical parameters such as aqueous solubility (S_W) and lipophilicity. A good balance of hydrophobic and hydrophilic character ensures efficient drug permeability through lipid membranes as well as blood serum for drug action. Drugs with oral dosage formulations are adequately soluble in both water and *n*-octanol [45–47]. The cLogP values (Table 2) of the hybrids **12a**–**12e** are identical, indicating similar hydrophilicity, although the C-4 methyl substituted hybrid **12f** is significantly hydrophilic compared to corresponding C-4 aryl substituted analogs. Thus, the hybrids **12a**–**12e** are expected to possess identical adsorption or cell permeation in biological environment. Various physicochemical parameters such as aqueous solubility, S_w and the distribution coefficient (logD) and pKa of all compounds were evaluated and results are summarised in Table 3. The solubility in *n*-octanol (S_{OC}) was evaluated from the experimental S_W and logD data using the relation: log S_{OC} = log D + log S_W. The aqueous solubility (S_W) in PBS (phosphate saline buffer) buffer and logD in *n*-octanol/PBS buffer mixture was evaluated using HPLC [48] at the cytoplasmic pH 7.4 of the parasite [49]. Data in Table 3 shows that **12a**–**f** exhibit moderate to poor S_W in μM range, while have comparatively higher solubility in the organic solvent. The observed trend in the antiplasmodial activity (Table 2) of **12a**–**f** could be related to the calculated distribution coefficients. LogD increases from **12a**–**c**, as also the hydrophobicity as well as antiplasmodial activity. Moving from **12b** to **12f** also shows corresponding decrease in logD in line with the observed order of antiplasmodial activity (**12b** > **12f**). Similar to the redox potentials, **12a**–**f** do not depict significant variation in the p*K*a values, as the thiazole and the amide linker units are remote from both ferrocene as well as pyrimidine cores. However, the higher magnitude of the p*K*a values suggests higher basic character of these hybrids and is thus expected to accumulate in the food vacuole of the parasite.

3. Conclusions

In summary, new ferrocenyl-pyrimidine derivatives **12a**–**f** have been synthesized and structure activity relationship for *in vitro* anti-plasmodial activity against chloroquinoline susceptible strain NF54 is presented. The compound bearing aromatic substituent at C-4 position of the pyrimidine core and isopropyl ester groups at C-5 position is found be the most active of this series of hybrids. The hybrids depicted a single electron reversible oxidation behavior similar to ferrocene.

4. Experimental

4.1. Materials and reagents

All liquid reagents were dried/purified following recommended drying agents and/or distilled over 4 Å molecular sieves. CH_3CN was dried by refluxing over P_2O_5. DMF was dried overnight by storing over 4 Å molecular sieves. Acetylferrocene [50] was prepared according to literature reported procedure. Chloroacetyl chloride, propargyl alcohol, iodine and thiourea were bought from Spectrochem. K_2CO_3 was dried overnight in furnace.

4.2. Instrumentation

UV–visible studies were carried out using UV-1800 SHIMADZU UV-Spectrophotometer. The pH titrations were carried out using Equip-Tronics Digital pH meter model –EQ 610 and electrode was calibrated using standard buffers of pH 4.0, 7.0 and 9.2. The pH titrations were performed by addition of aliquots of acid and base to 50 ml solution of compounds **12a**–**f**. Electrochemical studies were carried out by cyclic voltammetry on CHI 660C instrument. Phosphate buffer of pH 7.4 consists of KH_2PO_4, $Na_2HPO_4 \cdot 7H_2O$ and NaCl. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Biospin Avance III HD at 500 MHz, with TMS as internal standard using $CDCl_3$ as deuterated solvent. Data are reported as follows: chemical shift in ppm (δ), integration, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant *J* (Hz). Mass spectrum (HRMS) was recorded on Bruker HRMS MICROTOF II spectrometer. The HPLC system consisted of a Waters 2489 HPLC separations module equipped with Waters 2489 photodiode array detector, Waters 515 HPLC pump, and Empower software (Waters Corporation, Milford, MA, USA) for system control, data collection, and processing. Liquid chromatography was carried out using Symmetry (4.6 mm × 75 mm), 3.5 μm C-18 reverse phase column. The mobile phase consisted of premixed HPLC grade methanol and water in the varying ratio and degassed prior to operating under isocratic conditions at a flow rate of 0.5 ml/min with 20 μL standard sample injections. A calibration plot of the peak area *versus* compound concentration for each compound showed excellent linearity ($0.99 < r^2 < 1$) over the concentration range (0–10 μM) employed for the assays. The HPLC data was recorded at the absorption maximum (300 nm) for compounds **12a**–**f**. The retention time (tR) is expressed in minutes (min). The purity of the products was checked by HPLC analytical methods. IR spectrum was recorded on Perkin–Elmer FTIR-C92035 Fourier-transform spectrophotometer in range 400–4000 cm^{-1} using KBr and dry DCM as medium. All reported yields are isolated yields. Melting points were determined in open capillaries and are uncorrected. For column chromatography silica gel (60–120 mesh) was employed and eluents were ethyl acetate/hexane mixtures.

4.3. Determination of aqueous solubility

The aqueous solubility values of solid compounds **12a**–**f** were obtained by preparing saturated solutions in PBS (10 mM) at pH 7.4. The slurries were stirred with magnetic bars in a water bath at 32 °C

Table 3
Physicochemical properties of **12a**–**f**.

Hybrid	pK_{a1}[a,b]	pK_{a2}[a,b]	S_w (μM)[c]	logD[c]	S_{oc} (μM)[d]
12a	7.95	10.45	4.3 ± 0.99	0.50 ± 0.01	13.5
12b	8.05	10.45	10.3 ± 0.13	0.56 ± 0.08	37.3
12c	8.70	10.95	1.03 ± 0.09	1.13 ± 0.39	13.8
12d	8.10	10.80	1.92 ± 0.08	0.92 ± 0.07	15.9
12e	8.10	10.60	3.48 ± 0.75	0.58 ± 0.09	13.2
12f	7.65	9.80	11.4 ± 0.89	0.48 ± 0.04	34.4

[a] The pK_a data represents mean of the three determinations obtained at 298.1 K.
[b] pK_{a1} corresponds to the pK_a of the triazole nitrogen atom and pK_{a2} represents the pK_a of the amide NH of the bridge.
[c] Mean ±SD was determined from three independent measurements.
[d] Solubility in n-octanol (S_{oc}) at pH 7.4 was calculated from experimental aqueous solubility (S_w) and distribution coefficient log D (n-octanol/PBS buffer) at pH 7.4 using log S_{OC} = log D + log S_w.

for 24 h. The saturated solution was filtered and the filtrate was analyzed using HPLC. Further solutions of known concentrations of compounds **12a**–**f** were prepared in DMSO and were analyzed using HPLC. Calibration curve (1×10^{-3} to 1×10^{-7} M) for concentration vs response was obtained. Concentrations of saturated aqueous solutions of **12a**–**f** were deduced from the calibration curve by measuring the response obtained for aqueous saturated solutions of **12a**–**f**. Concentration thus obtained corresponds to the limit of solubility of the compound under the experimental conditions used [48,51].

4.4. Experimental determination of partition coefficient (log D)

n-Octanol and PBS buffer solutions (pH 7.4) were mixed in equal volumes and were saturated with each other by stirring for 24 h. Accurately weighed compounds **12a**–**f** (0.002 g) were dissolved in 0.75 ml of presaturated *n*-octanol in graduated (2 ml) test tube and sonicated for 10 min. To the above sonicated solutions 0.75 ml presaturated PBS buffer solution was added and solutions were sonicated for 1 h then centrifuged for 30 min at 4000 rpm. Final volume was close to 1 ml. Aqueous and organic phases were separated and concentration of **12a**–**f** in both the phases was measured by HPLC using response obtained and calibration (response vs concentration) curve for compounds **12a**–**f**. Log of concentration ratios in two phases corresponds to log D values for **12a**–**f** [52].

4.5. Determination of pKa

The stock solution (10 mM) of compounds **12a**–**f** was prepared in DMSO. The working concentration (30 μM) was obtained by diluting stock solution with distilled water so that overall DMSO corresponds to 30% *v/v* in solution. The pH of the solution was adjusted to 3 by adding HCl solution (0.1 M). The pH of solutions was adjusted in the range 3–12 using NaOH (0.1 M). The UV–Visible spectra were recorded in the order of increasing pH (3–12) using a Shimadzu 1601 PC spectrophotometer and a 1 cm quartz cuvette. All measurements were carried out at 298.1 K. Plots of absorption at 300 nm *versus* apparent pH values of sample solution furnished sigmoid curves. The p*K*a values were determined for the samples **12a**–**f** as center point of their respective titration curves [53].

5. Synthesis of 2-amino-4-ferrocenylthiazole 5

Acetylferrocene **4** (2 g, 8.7 mmol), thiourea (1.3 g, 17.5 mmol) and resublimed iodine (2.2 g, 8.7 mmol) were taken in round bottom flask, reaction mixture was heated neat at 100 °C for 12 h. The resultant dark brown solid was dissolved in hot water and reaction mixture was neutralized by adding K_2CO_3 and extracted with ethyl acetate (3 × 20 ml). The organic phase was separated dried over anhydrous sodium sulfate. Finally, the solvent was removed under reduced pressure to obtain crude **5**, which was purified by column chromatography over silica 60–120 using 30:80 (ethyl acetate/hexane) as eluent to yield analytically pure **5** as orange solid (92%). Rf: (0.3, 50% ethyl acetate/hexane). Mp 201–203 °C (DCM/hexane). IR (KBr): ν_{max} 1088, 1177, 1392, 1510, 1656, 2907, 3079, 3235 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 4.03 (s, 5H, Fc), 4.18 (s, 2H, Fc), 4.55 (s, 2H, Fc), 5.00 (s, 2H, NH_2), 6.27 (s, 1H, thiazole C–5H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 65.59, 67.49, 68.38, 79.22, 98.98 and 149.99. HRMS calcd for $C_{13}H_{12}FeN_2S$ $(M)^+$ 284.0071, found 284.0044.

5.1. Synthesis of 2-chloro-N-(4-ferrocenylthiazol-2-yl)acetamide 6

To a solution of 4-ferrocenylthiazole-2-amine **5** (2 g, 7 mmol) in dry toluene was added dry chloroacetyl chloride (1.3 ml, 14 mmol) and reaction mixture was refluxed overnight. After completion of reaction (TLC), toluene was removed under reduced pressure. The residue was dissolved in DCM (30 ml) and washed with cold water (20 ml). The organic phase was dried with anhydrous sodium sulfate and solvent was removed under reduced pressure to obtain the chloroacetylated thiazole **6**, which was purified by column chromatography over silica 60–120 using 20:80 (ethyl acetate/hexane) as eluent to yield analytically pure **6** as red solid (92%). Rf: (0.5, 50% ethyl acetate/hexane). Mp 120–122 °C (DCM/hexane). IR (KBr): ν_{max} 1088, 1177, 1392, 1510, 1656, 2907, 3079, 3235 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 4.03 (s, 5H, Fc), 4.16 (s, 2H, Fc), 4.26 (s, 2H, Fc), 4.64 (s, 2H, CH_2), 6.77 (s, 1H, thiazole C–5H), 9.85 (s, br, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 29.61, 52.13, 66.75, 68.85, 69.46, 79.63, 105.69, 149.72, 155.99 and 164.48. HRMS calcd for $C_{15}H_{13}FeN_2OSCl$ $(M + 1)^+$ 360.9820, found 360.1478.

5.2. Synthesis of 2-azido-N-(4-ferrocenylthiazol-2-yl)acetamide 7

Chloroacetylated thiazole **6** (1.5 g, 4.1 mmol) was dissolved in dry DMF and NaN_3 (0.41 g, 6.2 mmol) was added and the reaction mixture was heated at 60 °C overnight. After completion of reaction (TLC), DMF was removed under reduced pressure. The residue was dissolved DCM (30 ml) and the organic layer was washed with water (2 × 20 ml). Upon drying the organic extract with anhydrous sodium sulfate, solvent was removed to obtain crude **7**, which was purified by column chromatography over silica 60–120 using 20:80 (ethyl acetate/hexane) as eluent to yield analytically pure **7** as orange solid (92%). Rf: (0.3, 50% ethyl acetate/hexane). Mp 115–118 °C (DCM/hexane). IR (KBr): ν_{max} 1105, 1283, 1419, 1546, 1675, 2111, 2979, 3097, 3299 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 4.07 (s, 5H, Fc), 4.20 (s, 2H, Fc), 4.30 (s, 2H, Fc), 4.68 (s, 2H, CH_2), 6.81 (s, 1H, thiazole C–5H), 9.89 (br, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 29.70, 52.22, 66.84, 68.94, 69.55, 79.72, 105.78, 149.81, 156.08 and 164.57. HRMS calcd for $C_{15}H_{13}FeON_5S$ $(M + 1)^+$ 368.0224, found 368.0024.

5.3. Synthesis of alkyl 4-methyl-6-aryl-2-(prop-2-yn-1-yloxy) pyrimidine-5-carboxylates 11a–f

To a solution of appropriate **10** (0.5 g, 2.56 mmol) in dry CH_3CN (30 ml), dry K_2CO_3 (1.06 g, 7.69 mmol) and propargyl alcohol (0.2 ml, 3.84 mmol) were added in that sequence and reaction mixture was refluxed overnight. After completion of reaction (TLC), CH_3CN was removed under vacuum. Water was then added to the residue and the product extracted with DCM (2 × 20 ml). Solvent was removed to obtain crude **11**, which was purified by column chromatography over silica 60–120 using 20:80 (ethyl acetate/hexane) as eluent to isolate analytically pure **11a**–**f**. The characteristic data of compounds obtained using this procedure is given below.

5.3.1. Methyl-4-methyl-6-phenyl-2-(prop-2-yn-1-yloxy) pyrimidine-5-carboxylate 11a

White solid, Yield: 94%. Rf: (0.5, 30% ethyl acetate/hexane). Mp 69–73 °C (DCM/hexane) IR (KBr): ν_{max} 1533, 1552, 1708, 2985, 3250 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 2.48 (t, $J = 2.5$ Hz, 1H, CCH), 2.58 (s, 3H, C-6 CH_3), 3.69 (s, OCH_3), 5.09 (d, $J = 5$, 2H, OCH_2), 7.43–7.50 (m, 3H, ArH), 7.64–7.69 (m, 2H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): 22.70, 52.43, 55.10, 74.91, 78.24, 120.09, 128.32, 128.43, 130.31, 137.64, 163.09, 166.21, 168.55, 168.94. HRMS calcd for $C_{16}H_{14}N_2O_3$ $(M)^+$ 282.1004, found 282.1008.

5.3.2. Ethyl-4-methyl-6-phenyl-2-(prop-2-yn-1-yloxy)pyrimidine-5-carboxylate ***11b***

White solid, Yield: 94%. Rf: (0.5, 30% ethyl acetate/hexane). Mp 65–67 °C (DCM/hexane). IR (KBr): ν_{max} 1533, 1552, 1708, 2985, 3250 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 1.07 (t, J = 5.0 Hz, 3H, OCH_3), 2.5 (t, J = 2.5 Hz, 1H, CCH), 2.61 (s, 3H, C-6 CH_3), 4.18 (q, J = 10.0 Hz, 2H, OCH_2), 5.11 (d, J = 2.5 Hz, C-2 OCH_2), 7.44–7.69 (m, 5H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 13.62, 22.77, 55.15, 61.75, 74.76, 78.29, 120.53, 128.36, 128.43, 130.25, 137.54, 163.06, 166.36, 168.13, 168.97. HRMS calcd for $C_{17}H_{16}N_2O_3$ $(M)^+$ 296.1161, found 296.1489.

5.3.3. Isopropyl-4-methyl-6-phenyl-2-(prop-2-yn-1-yloxy) pyrimidine-5-carboxylate ***11c***

Colorless oil. Yield: 94%. Rf: (0.5, 30% ethyl acetate/hexane). IR (Dry DCM): ν_{max} 1556, 1585, 1713, 2939, 2981, 3047, 3255 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 1.09 (d, J = 10.0 Hz, 6H, $CH(CH_3)_2$), 2.49 (t, J = 2.5 Hz, 1H, CCH), 2.59 (s, 3H, C-6 CH_3), 5.05–5.10 (m, 3H, CH & C-2 OCH_2), 7.43–7.49 (m, 3H, ArH), 7.67–7.69 (m, 2H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 21.29, 22.67, 55.11, 69.64, 74.75, 78.33, 121.05, 128.43, 130.19, 137.52, 162.99, 166.18, 167.61 and 168.65. HRMS calcd for $C_{18}H_{18}N_2O_3$ $(M)^+$ 310.1317, found 310.1314.

5.3.4. Isopropyl-4-methyl-6-(2-nitrophenyl)-2-(prop-2-yn-1-yloxy)pyrimidine-5-carboxylate ***11d***

White solid, Yield: 94%. Rf: (0.5, 30% ethyl acetate/hexane). Mp 98–100 °C (DCM/hexane). IR (KBr): ν_{max} 1532, 1552, 1708, 2938, 3041, 3250 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 0.94 (d, J = 5.0 Hz, 6H, $CH(CH_3)_2$), 2.47 (t, J = 2.5 Hz, 1H, CCH), 2.70 (s, 3H, C-6 CH_3), 4.91–4.96 (m, 1H, OCH), 5.02 (d, J = 2.5 Hz, 2H, C-2 OCH_2), 7.62 (t, J = 10.0 Hz, 1H, ArH), 7.69 (t, J = 5.0 Hz, 1H, ArH), 7.60–7.70 (m, 2H, C_6H_5), 8.20 (d, J = 10.0 Hz, 1H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 21.36, 24.13, 55.70, 69.57, 75.28, 77.97, 119.92, 124.72, 130.01, 130.34, 133.46, 134.56, 147.51, 162.98, 165.44, 166.79 and 171.02. HRMS calcd for $C_{18}H_{17}N_3O_5$ $(M)^+$ 355.1168, found 355.1160.

5.3.5. Isopropyl-4-methyl-6-(4-nitrophenyl)-2-(prop-2-yn-1-yloxy)pyrimidine-5-carboxylate ***11e***

White solid. Yield: 94%. Rf: (0.5, 30% ethyl acetate/hexane). Mp 96–98 °C (DCM/hexane). IR (KBr): ν_{max} 1520, 1554, 1606, 1721, 2937, 2985, 3084, 3109, 3279 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 1.12 (d, J = 5.0 Hz, 6H, $CH(CH_3)_2$), 2.50 (t, J = 2.5 Hz, 1H, CCH), 2.62 (s, 3H, C-6 CH_3), 5.08–5.12 (m, 3H, OCH & C-2 OCH_2), 7.83 (d, J = 5.0 Hz, 2H, ArH), 8.31 (d, J = 10.0 Hz, 2H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 21.35, 22.91, 55.40, 70.13, 75.04, 77.97, 121.18, 123.57, 129.56, 143.54, 148.72, 163.06, 163.97, 166.74 and 169.60. HRMS calcd for $C_{18}H_{17}N_3O_5$ $(M)^+$ 355.1168, found 355.1172.

5.3.6. Ethyl-4,6-dimethyl-2-(prop-2-yn-1-yloxy)pyrimidine-5-carboxylate ***11f***

Colorless oil. Yield: 94%. Rf: (0.5, 30% ethyl acetate/hexane). IR (DCM): ν_{max} 1533, 1552, 1708, 2985, 3250 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 1.40 (t, J = 7.5 Hz, 3H, CH_3), 2.48 (t, J = 2.5 Hz, 1H, CCH), 2.52 (s, 6H, C-4 and C-6 CH_3), 4.41 (q, J = 5.0 Hz, 2H, OCH_2), 5.03 (d, J = 5.0 Hz, 2H, C-2 OCH_2). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 14.12, 23.17, 52.68, 54.90, 61.55, 74.67, 75.62, 78.19, 120.90, 162.75, 167.32 and 168.29. HRMS calcd for $C_{12}H_{14}N_2O_3$ $(M)^+$ 234.1004, found 234.1012.

6. Synthesis of alkyl 4-methyl-6-aryl-2-((1-(2-oxo-2-((4-ferrocenylthiazol-2-yl)amino)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)pyrimidine-5-carboxylate hybrids 12a–f

A solution of appropriate **11a–f** (0.1 g, 0.35 mmol) and **7** (0.13 g, 0.34 mmol) in EtOH: H_2O (8:2, *v/v*) (10 ml), $CuSO_4 \cdot 5H_2O$ (0.5 g, 0.02 mmol) and sodium ascorbate (0.1 g, 0.1 mmol) were added. Reaction mixture was stirred at ambient temperature. After completion of reaction (TLC), solvent was removed under vacuum. Water was then added to the residue and the product extracted with DCM (2 × 20 ml). The organic phase was dried over anhydrous sodium sulfate. Subsequently, solvent was removed under vacuum to obtain crude **12a–f**, which was purified by column chromatography over silica 60–120 using 60:40 (ethyl acetate/hexane) as eluents to yield analytically pure **12a–f**. The characteristic data of compounds obtained using this procedure is given below.

6.1. Methyl 4-methyl-2-((1-(2-oxo-2-((4-ferrocenylthiazol-2-yl) amino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-phenylpyrimidine-5-carboxylate ***12a***

Orange solid. Yield: 92%. Rf: (0.3, 50% ethyl acetate/hexane). Mp 262–264 °C (DCM/hexane). IR (KBr): ν_{max} 1420, 1553, 1588, 1732, 2947, 3081, 3150 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 2.62 (s, 3H, C-6 CH_3), 3.71 (s, 3H, OCH_3), 4.07 (s, 5H, Fc), 4.29 (s, 2H, Fc), 4.67 (s, 2H, Fc), 5.32 (s, 2H, $-COCH_2$), 5.75 (s, 2H, C-2 OCH_2), 6.81 (s, 1H, thiazole C-5 H), 7.45–7.50 (m, 3H, ArH), 7.68 (d, J = 5.0 Hz, 2H, ArH), 7.92 (s, 1H, triazolyl H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 22.84, 29.70, 52.57, 53.43, 60.83, 66.85, 68.96, 69.55, 79.15, 105.82, 119.97, 120.05, 125.23, 128.19, 128.25, 128.59, 130.45, 137.37, 144.20, 163.01, 163.50, 166.42, 168.68 and 168.90. HPLC [Methanol/H_2O, 25:75, 0.5 ml/min, 300 nm] t_R = 0.942 min. HRMS calcd for $C_{31}H_{27}FeO_4N_7S$ $(M)^+$ 649.1195, found 649.1274.

6.2. Ethyl 4-methyl-2-((1-(2-oxo-2-((4-ferrocenylthiazol-2-yl) amino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-phenylpyrimidine-5-carboxylate ***12b***

Orange solid. Yield: 92%. Rf: (0.3, 50% ethyl acetate/hexane). Mp 222–224 °C (DCM/hexane). IR (KBr): ν_{max} 1423, 1555, 1587, 1722, 2942, 2980, 2989, 3145, 3185 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 1.05 (t, J = 7.5 Hz, 3H, CH_3), 2.60 (s, 3H, C-6 CH_3), 4.06 (s, 5H, Fc), 4.16 (q, J = 10 Hz, 2H, OCH_2), 4.28 (s, 2H, Fc), 4.65 (s, 2H, Fc), 5.30 (s, 2H, $-COCH_2$), 5.73 (s, 2H, C-2 OCH_2), 6.78 (s, 1H, thiazole C-5 H), 7.45–7.47 (m, 3H, ArH), 7.65–7.67 (m, 2H, ArH), 7.90 (s, 1H, triazolyl H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 13.63, 22.81, 29.70, 52.54, 61.02, 61.78, 66.96, 69.04, 69.67, 105.90, 120.56, 125.26, 128.32, 128.50, 130.30, 137.51, 144.23, 149.54, 156.45, 163.02, 163.41, 166.64, 168.08 and 169.06. HPLC [Methanol/H_2O, 25:75, 0.5 mL/min, 300 nm] t_R = 0.969 min. HRMS calcd for $C_{32}H_{29}FeO_4N_7S$ $(M)^+$ 663.1351, found 663.1143.

6.3. Isopropyl 4-methyl-2-((1-(2-oxo-2-((4-ferrocenylthiazol-2-yl) amino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-phenylpyrimidine-5-carboxylate ***12c***

Orange solid. Yield: 92%. Rf: (0.3, 50% ethyl acetate/hexane). Mp 158–160 °C (DCM/hexane). IR (KBr): ν_{max} 1425, 1454, 1552, 1718, 2941, 2981, 3090, 3151, 3185 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 1.10 (d, J = 5 Hz, 6H, $-CH(CH_3)_2$), 2.62 (s, 3H, C-6 CH_3), 4.05 (s, 5H, Fc), 4.27 (s, 2H, Fc), 4.66 (s, 2H, Fc), 5.07–5.12 (m, 1H, OCH), 5.31 (s, 2H, $-COCH_2$), 5.75 (s, 2H, C-2 OCH_2), 6.81 (s, 1H, thiazole C-5 H), 7.44–7.48 (m, 3H, ArH), 7.68 (d, J = 10 Hz, 2H, ArH), 7.93 (s, 1H, triazolyl H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 21.32, 22.71, 29.70, 52.47, 60.99, 66.92, 68.92, 69.56, 69.68, 79.60, 105.88, 121.07, 125.38, 128.39, 128.49, 130.23, 137.49, 144.04, 149.49, 156.67, 163.23, 163.35, 166.46, 167.55 and 168.74. HPLC [Methanol/H_2O, 25:75, 0.5 mL/min, 300 nm] t_R = 0.940 min. HRMS calcd for $C_{33}H_{31}FeO_4N_7S$ $(M)^+$ 677.1508, found 677.1607.

6.4. Isopropyl 4-methyl-2-((1-(2-oxo-2-((4-ferrocenylthiazol-2-yl) amino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(2-nitrophenyl) pyrimidine-5-carboxylate **12d**

Orange solid. Yield: 92%. Rf: (0.3, 50% ethyl acetate/hexane). Mp 202–206 °C (DCM/hexane). IR (KBr): ν_{max} 1423, 1463, 1529, 1557, 1584, 1730, 2944, 2981, 3076, 3145, 3187 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 0.96 (d, J = 10 Hz, 6H, $-CH(CH_3)_2$), 2.72 (s, 3H, C-6 CH_3), 4.07 (s, 5H, Fc), 4.3 (s, 2H, Fc), 4.67 (s, 2H, Fc), 4.93–4.98 (m, 1H, OCH), 5.31 (s, 2H, $-COCH_2$), 5.67 (s, 2H, C-2 OCH_2), 6.81 (s, 1H, thiazole C-5 H), 7.36 (d, J = 5.0 Hz, 1H, ArH), 7.63–7.70 (m, 2H, ArH), 7.84 (s, 1H, triazolyl H), 8.19 (d, J = 5.0 Hz, 1H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 11.44, 14.13, 14.20, 22.66, 23.29, 25.29, 29.05, 29.70, 52.65, 60.85, 61.67, 66.82, 68.93, 69.53, 79.49, 105.78, 120.99, 125.18, 144.36, 163.17, 167.38 and 168.52. HPLC [Methanol/H_2O, 25:75, 0.5 mL/min, 300 nm] t_R = 0.971 min. HRMS calcd for $C_{33}H_{30}FeO_6N_8S$ $(M)^+$ 722.1358, found 722.1241.

6.5. Isopropyl 4-methyl-2-((1-(2-oxo-2-((4-ferrocenylthiazol-2-yl) amino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-6-(4-nitrophenyl) pyrimidine-5-carboxylate **12e**

Orange solid. Yield: 92%. Rf: (0.3, 50% ethyl acetate/hexane). Mp 240–242 °C (DCM/hexane). IR (KBr): ν_{max} 1432, 1526, 1561, 1719, 2949, 2980, 3085, 3148 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 1.13 (d, J = 5 Hz, 6H, $-CH(CH_3)_2$), 2.65 (s, 3H, C-6 CH_3), 4.07 (s, 5H, Fc), 4.3 (s, 2H, Fc), 4.67 (s, 2H, Fc), 5.09–5.14 (m, 1H, OCH), 5.32 (s, 2H, $-COCH_2$), 5.74 (s, 2H, C-2 OCH_2), 6.82 (s, 1H, thiazole C-5 H), 7.83 (d, J = 5.0 Hz, 2H, ArH), 7.94 (s, 1H, triazolyl H), 8.32 (d, J = 10 Hz, 2H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 21.36, 22.96, 29.70, 52.56, 61.19, 66.89, 68.98, 69.57, 70.13, 79.42, 105.93, 121.15, 123.62, 125.25, 129.55, 143.55, 143.92, 148.72, 149.48, 163.43, 164.41, 166.71 and 169.65. HPLC [Methanol/H_2O, 25:75, 0.5 mL/min, 300 nm] t_R = 0.969 min. HRMS calcd for $C_{33}H_{30}FeO_6N_8S$ $(M)^+$ 722.1358, found 722.1237.

6.6. Ethyl 4,6-dimethyl-2-((1-(2-oxo-2-((4-ferrocenylthiazol-2-yl) amino)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)pyrimidine-5-carboxylate **12f**

Orange solid. Yield: 92%. Rf: (0.3, 50% ethyl acetate/hexane). Mp 120–122 °C (DCM/hexane). IR (KBr): ν_{max} 1428, 1566, 1632, 1720, 2947, 2980, 3086, 3151 cm^{-1}. ^1H (500 MHz, $CDCl_3$, 25 °C): δ 1.43 (t, J = 7.5 Hz, 3H, CH_3), 2.57 (s, 6H, C-4 and C-6 CH_3), 4.07 (s, 5H, Fc), 4.3 (s, 2H, Fc), 4.43 (q, J = 10 Hz, 2H, OCH_2), 4.67 (s, 2H, Fc), 5.32 (s, 2H, $-COCH_2$), 5.68 (s, 2H, C-2 OCH_2), 6.81 (s, 1H, thiazole C-5 H), 7.92 (s, 1H, triazolyl H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 14.20, 23.29, 29.70, 52.62, 60.85, 61.67, 66.83, 68.92, 69.53, 105.79, 120.88, 125.20, 163.41, 167.38 and 168.52. HPLC [Methanol/H_2O, 25:75, 0.5 mL/min, 300 nm] t_R = 0.991 min. HRMS calcd for $C_{27}H_{27}FeO_4N_7S$ $(M)^+$ 601.1195, found 601.1191.

7. Materials and methods

7.1. In vitro anti-plasmodial assay

The test samples were tested in triplicate on one or two separate occasions against CQ^S strain of *Plasmodium falciparum* (NF54). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen [54]. Quantitative assessment of anti-plasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler [55].

The test samples were prepared to a 20 mg/ml stock solution in 100% DMSO. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at −20 °C. Further dilutions were prepared on the day of the experiment. CQ and artesunate (ART) were used as the reference drugs in all experiments. A full dose-response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value). Test samples were tested at a starting concentration of 100 μg/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 μg/ml. The same dilution technique was used for all samples. Active compounds were retested at a starting concentration of 10 μg/ml or 1000 ng/ml. Reference drugs were tested at a starting concentration of 1000 ng/ml. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The IC_{50} values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software.

7.2. Electrochemical studies

Electrochemical studies were performed on CHI 660C cyclic voltammeter with three-electrode configuration consisting of working (2 mm dia.) and counter electrodes of platinum and Ag/AgCl as the reference electrode. The solutions of the samples in dichloromethane (10^{-4} M) containing 0.01 M tetrabutylammoniumhexafluorophosphate (n-$Bu_4N^+PF_6^-$) as the supporting electrolyte were purged with nitrogen at room temperature for at least 10 min before recording the cyclic voltammograms and the working electrode was cleaned after each run. The voltammograms were recorded at a scan rate of 100 mV s^{-1}.

7.3. Computational studies

All theoretical calculations were carried out by using the Gaussian 09 suite of programs [56]. The molecular geometries of **12a**–**f** were optimized using the density functional theory (DFT) using B3LYP 6-31G as the basis set [57]. The first 50 excited states were calculated by using time-dependent density functional theory (TD-DFT) calculations. The molecular orbital contours were visualized (Fig. S43–S48) using Gauss view 5.0.9.

Acknowledgments

The authors are thankful to CSIR, New Delhi (Project No. 01(2687)12 EMR-II) for financial assistance and for SRF to Rakesh Chopra and GNDU for research facilities under UPE. The University of Cape Town, South African Medical Research Council, and South African Research Chairs Initiative of the Department of Science and Technology, administered through the South African National Research Foundation are gratefully acknowledged for support (K.C).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.05.043.

References

[1] C. Atteke, J.M. Ndong, A. Aubouy, L.A. Maciejewski, J.S. Brocard, J. Lebibi, P. Deloron, In vitro susceptibility to a new antimalarial organometallic analogue, ferroquine, of *plasmodium falciparum*, isolates from the Haut-Ogooue region of Gabon, J. Antimicrob. Chemother. 51 (2005) 1021–1024.
[2] C. Biot, L. Delhaes, H. Abessolo, O. Domarle, L.A. Maciejewski, M. Mortuaire, P. Delcourt, P. Deloron, D. Camus, D. Dive, J.S. Brocard, Novel metallocenic compounds as antimalarial agents. Study of the position of ferrocene in chloroquine, J. Organomet. Chem. 589 (1999) 59–65.
[3] C. Biot, L. Delhaes, C.M. Diaye, L.A. Maciejewski, D. Camus, D. Dive, J.S. Brocard, Synthesis and antimalarial activity in vitro of potential metabolites of

ferrochloroquine and related compounds, Bioorg. Med. Chem. 7 (1999) 2843–2847.
[4] F. Dubar, T.J. Egan, B. Pradines, D. Kuter, K.K. Ncokazi, The antimalarial ferroquine: role of the metal and intramolecular hydrogen bond in activity and resistance, ACS Chem. Biol. 6 (2011) 275–287.
[5] C. Biot, D. Taramelli, I. Forfar-Bares, L.A. Maciejewski, M. Boyce, G. Nowogrocki, J.S. Brocard, N. Basilico, P. Olliaro, T. Egan, Insights into the mechanism of action of ferroquine. Relationship between physicochemical properties and anti-plasmodial activity, Mol. Pharm. 2 (2005) 185–193.
[6] C. Supan, G. Mombo-Ngoma, M.P. Dal-Bianco, C.L. Ospina Salazar, S. Issifou, F. Mazuir, A. Filali-Ansary, C. Biot, D. Ter-Minassian, M. Ramharter, P.G. Kremsner, B. Lell, Pharmacokinetics of ferroquine, a Novel 4-aminoquinoline, in asymptomatic carriers of *Plasmodium falciparum* infections, Antimicrob. Agents Chemother. 56 (2012) 3165–3173.
[7] O. Domarle, G. Blampain, H. Agnaniet, T. Nzadiyabi, J. Lebibi, J. Brocard, L. Maciejewski, C. Biot, A.J. Georges, P. Millet, In vitro antimalarial activity of a new organometallic analog, ferrocene-chloroquine, Antimicrob. Agents Chemother. 42 (1998) 540–544.
[8] C. Biot, G. Glorian, L.A. Maciejewski, J.S. Brocard, O. Domarle, G. Blampain, P. Millet, A.J. Georges, H. Abessolo, D. Dive, Synthesis and antimalarial activity in vitro and in vivo of a new ferrocene-chloroquine analogue, J. Med. Chem. 40 (1997) 3715–3718.
[9] F. Dubar, C. Solmianny, J. Khalife, D. Dive, H. Kalamou, Y. Guerardel, P. Grellier, C. Biot, The ferroqiune antimalarial conundrum: redox activation and reinvasion inhibition, Angew. Chem. Int. Ed. 52 (2013) 7690–7693.
[10] D. Dive, C. Biot, Ferroquine as an oxidative shock antimalarial, Curr. Top. Med. Chem. 14 (2014) 1684–1692.
[11] N. Chavain, H. Vezin, D. Dive, N. Touati, J.-F. Paul, E. Buisine, C. Biot, Investigation of the redox behavior of ferroquine, a new antimalarial, Mol. Pharm. 5 (2008) 710–716.
[12] W. Daher, C. Biot, T. Fandeur, H. Jouin, L. Pelinski, E. Viscogliosi, L. Fraisse, B. Pradines, J. Brocard, J. Khalife, D. Dive, Assessment of *Plasmodium falciparum* resistance to ferroquine (SSR97193) in field isolates and in W2 strain under pressure, Malar. J. 5 (2006) 11.
[13] Y. Li, C.D. Kock, P.J. Smith, H. Guzgay, D.T. Hendricks, K. Naran, V. Mizrahi, D.F. Warner, K. Chibale, G.S. Smith, Synthesis, characterization, and pharmacological evaluation of silicon-containing aminoquinoline organometallic complexes as antiplasmodial, antitumor, and antimycobacterial agents, Organometallics 32 (2013) 141–150.
[14] Y. Li, C.D. Kock, P.J. Smith, K. Chibale, G.S. Smith, Synthesis and evaluation of a carbosilane congener of ferroquine and its corresponding half-sandwich ruthenium and rhodium complexes for antiplasmodial and β-hematin inhibition activity, Organometallics 33 (2014) 4345–4348.
[15] T.N.C. Wells, P.L. Alonso, W.E. Gutteridge, New medicines to improve control and contribute to the eradication of malaria, Nature 8 (2009) 879–891.
[16] M. Reacher, C.C. Campbell, J. Freeman, E.B. Doberstyn, A.D. Brandling-Bennett, Drug therapy for *Plasmodium falciparum* malaria resistant to pyrimethamine-sulfadoxine (Fansidar). A study of alternate regimens in Eastern Thailand, 1980, Lancet 2 (1981) 1066–1068.
[17] N.J. White, Antimalarial drug resistance: the pace quickens, J. Antinicorb. Chemother. 30 (1992) 571–585.
[18] M. Barends, A. Jaidee, N. Khaohirun, P. Singhasivanon, F. Nosten, In vitro activity of ferroquine (SSR 97193) against *Plasmodium falciparum* isolates from the Thai-Burmese border, Malar. J. 6 (2007) 81.
[19] R.E. Martin, R.V. Marchetti, A.I. Cowan, S.M. Howitt, S. Broer, K. Kirk, Chloroquine transport via the malaria parasite's chloroquine resistance transporter, Science 325 (2009) 1680–1682.
[20] T.E. Wellems, *Plasmodium* chloroquine resistance and the search for a replacement antimalarial drug, Science 298 (2002) 124–126.
[21] A.M. Dondrop, F. Nosten, W. Hanpithakpong, S.J. Lec, P. Ringwald, K. Silamut, M. Imwong, K. Chotivanich, P. Lim, T.A.S. Herdman, S. Yeung, P. Singhasivanon, N.P. Day, N. Lindegardh, D. Socheat, N.J. White, Artemisinin resistance in *Plasmodium falciparum* malaria, N. Engl. J. Med. 361 (2009) 455–467.
[22] H. Noedl, D. Socheat, W. Satimal, Artemisinin-resistant malaria in Asia, N. Engl. J. Med. 36 (2009) 540–541.
[23] D. Fernando, C. Rodrigo, S. Rajapakse, Primaquine in vivax malaria: an update and review on management issues, Malar. J. 10 (2011) 351.
[24] D.G. Carberra, C.L. Manach, F. Douelle, Y. Younis, T.-S. Feng, T. Paquet, A.T. Nchinda, L.J. Street, D. Taylor, C.D. Kock, L. Wisener, S. Duffy, K.L. White, M. Zabiulla, Y. Sambandan, S. Bashyam, D. Waterson, M.J. Witty, S.A. Charman, V.M. Avery, S. Wittlin, K. Chibale, 2,4-Diaminothienopyrimidines as orally active antimalarial agents, J. Med. Chem. 57 (2014) 1014–1022.
[25] S. Hameed, P.M. Chinnapattu, G. Shanbag, P. Manjrekar, K. Koushik, A. Raichurkar, V. Patil, S. Jatheendranath, S.S. Rudrapatna, S.P. Barde, N. Rautela, D. Awasthy, S. Morayya, C. Narayan, S. Kavanagh, R. Saralaya, S. Bharath, P. Viswanath, K. Mukherjee, B. Bandodkar, A. Srivastava, V. Panduga, J. Reddy, K.R. Prabhakar, A. Sinha, M.B.J. Díaz, M. S.Martínez, I.A. Barturen, S. Ferrer, L.M. Sanz, F.J. Gamo, S. Duffy, V.M. Avery, P.A. Magistrado, A.K. Lukens, D.F. Wirth, D. Waterson, V. Balasubramanian, P.S. Iyer, S. Narayanan, V. Hosagrahara, V.K. Sambandamurthy, S. Ramachandran, Aminoazabenzimidazoles, a novel class of orally active antimalarial agents, J. Med. Chem. 57 (2014) 5702–5713.
[26] R. Oliveira, R.C. Guedes, P. Meireles, I.S. Albuquerque, L.M. Goncalves, E. Pires, M.R. Bronze, J. Gut, P.J. Rosenthal, M. Prudencio, R. Moreira, P.M. O'Neill, F. Lopes, Tetraoxane–pyrimidine nitrile hybrids as dual stage antimalarials, J. Med. Chem. 57 (2014) 4916–4923.
[27] Y. Younis, L.J. Street, D. Waterson, M.J. Witty, Kelly Chibale, cell-based medicinal chemistry optimization of high throughput screening hits for orally active antimalarials. Part 2: hits from soft focus kinase and other libraries, J. Med. Chem. 56 (2013) 7750–7754.
[28] D.G. Cabrera, F. Douelle, T.-S. Feng, A.T. Nchinda, Y. Younis, K.L. White, Q. Wu, E. Ryan, J. Burrows, D. Waterson, M.J. Witty, S. Wittlin, S.A. Charman, K. Chibale, Novel orally active antimalarial thiazoles, J. Med. Chem. 54 (2011) 7713–7719.
[29] I. Chiyanzu, C. Clarkson, P.J. Smith, J. Lehman, J. Gut, P.J. Rosenthal, K. Chibale, Design, synthesis and anti-plasmodial evaluation in vitro of new 4-aminoquinoline isatin derivatives, Bioorg. Med. Chem. 13 (2005) 3249–3261.
[30] A. Sparatore, N. Basilico, S. Parapini, S. Romeo, F. Novelli, F. Sparatore, D. Taramelli, 4-Aminoquinoline quinolizidinyl- and quinolizidinylalkyl-derivatives with antimalarial activity, Bioorg. Med. Chem. 13 (2005) 5338–5345.
[31] C. Singh, H. Malik, S.K. Puri, Orally active amino functionalized antimalarial 1,2,4-trioxanes, Bioorg. Med. Chem. Lett. 14 (2004) 459–462.
[32] O. Dechy-Cabaret, F. Benoit-Vical, A. Robert, B. Meunier, Preparation and antimalarial activities of "Trioxaquines", new modular molecules with a trioxane skeleton linked to a 4-Aminoquinoline, ChemBioChem. 1 (2000) 281–283.
[33] O. Dechy-Cabaret, F. Benoit-Vical, C. Loup, A. Robert, H. Gornitzka, A. Bonhoure, H. Vial, J.F. Magnaval, J.P. Seguela, B. Meunier, Synthesis and antimalarial activity of trioxaquine derivatives, Chem. Eur. J. 10 (2004) 1625–1636.
[34] N.C.P. Araujo, V. Barton, M. Jones, P.A. Stocks, S.A. Ward, J. Davies, P.G. Bray, A.E. Shone, M.L.S. Cristiano, P.M. O'Neill, Semi-synthetic and synthetic 1,2,4-trioxaquines and 1,2,4-trioxolaquines: synthesis, preliminary SAR and comparison with acridine endoperoxide conjugates, Bioorg. Med. Chem. Lett. 19 (2009) 2038–2043.
[35] K. Singh, H. Kaur, P. Smith, C.D. Kock, K. Chibale, J. Balzarini, Quinoline–pyrimidine hybrids: synthesis, antiplasmodial activity, SAR, and mode of action studies, J. Med. Chem. 57 (2014) 435–448.
[36] K. Singh, H. Kaur, K. Chibale, J. Balzarini, Synthesis of 4-aminoquinoline–pyrimidine hybrids as potent antimalarials and their mode of action studies, Eur. J. Med. Chem. 66 (2013) 314–323.
[37] K. Singh, H. Kaur, K. Chibale, J. Balzarini, S. Little, P.V. Bharatam, 2-Aminopyrimidine based 4-aminoquinoline anti-plasmodial agents. Synthesis, biological activity, structure–activity relationship and mode of action studies, Eur. J. Med. Chem. 52 (2012) 82–97.
[38] F. Mjambili, M. Njoroge, K. Naran, C.D. Kock, P.J. Smith, V. Mizrahi, D. Warner, K. Chibale, Synthesis and biological evaluation of 2-aminothiazole derivatives as antimycobacterial and antiplasmodial agents, Bioorg. Med. Chem. Lett. 24 (2014) 560–564.
[39] R.M. Dodson, L.C. King, The reaction of ketones with halogens and thiourea, J. Am. Chem. Soc. 67 (1945) 2242–2243.
[40] K. Singh, K. Singh, An efficacious protocol for the oxidation of 3, 4-dihydropyrimidin-2(1H)-ones using pyridiniumchlorochromate as catalyst, Aust. J. Chem. 61 (2008) 910–913.
[41] P. Bignelli, Aldureids of ethylic acetoacetate and ethylic oxaloacetate, Gazz. Chim. Ital. 23 (1893) 360–416.
[42] K. Singh, K. Singh, B. Wan, S. Franzblau, K. Chibale, J. Balzarini, Facile transformation of Biginelli pyrimidin-2(1*H*)-ones to pyrimidines. In vitro evaluation as inhibitors of Mycobacterium tuberculosis and modulators of cytostatic activity, Eur. J. Med. Chem. 46 (2011) 2290–2294.
[43] V.O. Rodionov, S.I. Presolski, D.D. Díaz, V.V. Fokin, M.G. Finn, Ligand-accelerated Cu-catalyzed azide–alkyne cycloaddition: a mechanistic report, J. Am. Chem. Soc. 129 (2007) 12705–12712.
[44] H.C. Kolb, M.G. Finn, K.B. Sharpless, Click chemistry: diverse chemical function from a few good reactions, Angew. Chem. Int. Ed. 40 (2001) 2004–2021.
[45] G. Caron, F. Reymond, P.A. Carrupt, H.H. Girault, B. Testa, Combined molecular lipophilicity descriptors and their role in understanding intramolecular effects, Pharm. Sci. Technol. Today 2 (1999) 327–335.
[46] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (2001) 3–26.
[47] A. Avdeef, Physicochemical profiling (solubility, permeability and charge state), Curr. Top. Med. Chem. 1 (2001) 277–351.
[48] M. Steyn, D.D. N'Da, J.C. Breytenbach, P.J. Smith, S. Meredith, W.J. Breytenbach, Synthesis and antimalarial activity of ethylene glycol oligomeric ethers of artemisinin, J. Pharm. Pharmacol. 63 (2011) 278–286.
[49] D.C. Warhurst, J.C. Craig, I.S. Adagu, D.J. Meyer, S.Y. Lee, The relationship of physico-chemical properties and structure to the differential antiplasmodial activity of the cinchona alkaloids, Malar. J. 2 (2003) 26.
[50] M.M. Hale, Creative Commons Attribution License (CC-BY 2.0).
[51] L. Pan, Q. Ho, K. Tsutsui, L. Takahashi, comparison of chromatographic and spectroscopic methods used to rank compounds for aqueous solubility, J. Pharm. Sci. 4 (2001) 521–529.
[52] S.I. Pretorius, W.J. Breytenbach, C.D. Kock, P.J. Smith, D.D. N'Da, Synthesis, characterization and antimalarial activity of quinoline–pyrimidine hybrids, Bioorg. Med. Chem. 21 (2013) 269–277.
[53] T. Ossowski, M.O.F. Goulart, F.C. de Abreu, A.E.G. Sant'Ana, P.R.B. Miranda,

C.O. Costa, A. Liwo, P. Falkowski, D. Zarzeczanska, Determination of the pKa values of some biologically active and inactive hydroxyquinones, J. Braz. Chem. Soc. 19 (2008) 175–183.
[54] W. Trager, J.B. Jensen, Human malaria parasite in continuous culture, Science 193 (1976) 673–675.
[55] M.T. Makler, J.M. Ries, J.A. Williams, J.E. Bancroft, R.C. Piper, B.L. Gibbins, D.J. Hinrichs, Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity, Am. Soc. Trop. Med. Hyg. 48 (1993) 739–741.
[56] M.J. Frisch, et al., Gaussian 09, Revision B.01, Gaussian, Inc., Wallingford, CT, 2010. Complete reference is given in supporting information.
[57] L.J. Bartolotti, K. Fluchick, in: K.B. Lipkowitz, B.D. Boyd (Eds.), Reviews in Computational Chemistry, vol. 7, VCH, New York, 1996, p. 187.