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FULL PAPER

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New thiazolidine-2,4-dione derivatives combined with organometallic ferrocene: Synthesis, structure and antiparasitic activity

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Favourable physicochemical properties of an organometallic ferrocene and antiplasmodial potency of compounds containing the thiazolidine-2,4-dione framework (TZD-4) prompted us to explore compounds containing both the thiazolidine-2,4-dione core and the ferrocenyl unit with the primary aim of identifying compounds with promising antiprotozoal activities. Thus, a new series of rationally designed ferrocene-based thiazolidine-2,4-dione derivatives, containing a selection of secondary cyclic amines, was synthesised and fully characterised using standard spectroscopic techniques. The resulting compounds were screened for their antiplasmodial and antitrypanosomal activities against both the chloroquine-resistant (Dd2) strain of *Plasmodium falciparum* and the Nagana *Trypanosoma brucei brucei* 427. The general trend that emerged indicated that the target compounds were more selective towards *T. b. brucei* compared to the *P. falciparum* parasite. Moreover, the analogues bearing methylpiperazine (**8a**) and piperidine (**8b**) rings were more active against *T. b. brucei* compared to hit compound TZD-4. Except compound **8b**, which appeared promising, none of the synthesised compounds showed better activity than TZD-4 against the *P. falciparum* parasite. All the synthesised compounds were non-toxic and often showed >90% viability of the HeLa cell line screened.

KEYWORDS
ferrocene, *Plasmodium falciparum*, thiazolidine-2,4-dione, *Trypanosoma brucei brucei*, trypanosomiasis

1 | INTRODUCTION

The global decline in malaria burden in the past 15 years has been attributed to the availability of key intervention tools which include chemotherapy, vector control programmes and rapid diagnostic testing.[1] Artemisinin-based combination therapies (ACTs) are presently the mainstream of global malaria treatment.[2] Despite the effectiveness of ACTs over the last several decades, there are reported incidences of resistance to artemisinin, a fast-acting key partner of ACTs, in South East Asia. The treatment failures in western Cambodia[1,3] are a clear indication that the current global gains to eliminate malaria are at risk of being reversed. If the ongoing battle against malaria is to be won, there is an urgent need to continuously search for new classes of compounds with novel mechanisms of action.

Similarly, human African trypanosomiasis (HAT) or sleeping sickness is a serious public health disease that affects a significant proportion of the population in Africa. Although remarkable results have been achieved towards the elimination of HAT as a public health problem over the past 15 years,[4] the current clinically approved drugs suffer from several drawbacks that include associated difficulty with treatment regime and toxicity, reduced efficacy, emerging drug resistance and lack of a single agent to address both stages of the disease.[5] Thus, the continuing search for alternative therapeutic agents that are effective for the treatment of both stages 1 and 2 is justified.

The fusion of two dissimilar bioactive pharmacophores or structural units into a single molecule is an appealing approach that has attracted great attention in the field of medicinal chemistry.[6] A typical example of this approach is ferroquine, the first novel organometallic drug candidate to enter clinical trials[7] for treatment of malaria despite some reports indicating that free ferrocene by itself lacks antimalarial activity.[8] This unique chemical compound is particularly highly active against CQ^R clones of *Plasmodium falciparum*[9–11] and, more importantly, it shows no cross-resistance with other quinoline antimalarials.[11] Although the exact mechanism of action of ferroquine is still unresolved and remains controversial, its activity in part has been attributed to the production of toxic hydroxyl radicals[10] and largely due to direct inhibition of hemozoin formation.[12] Since the initial report on antiplasmodial activity of ferrochloroquine by Biot *et al.*,[10] several reports involving the development of ferrocene-based antimalarial derivatives have emerged and they received greater attention.[13–18] Besides antiplasmodial properties, the ferrocene scaffold has attractive attributes that include its robust stability, favourable electrochemical properties, low toxicity and ease of use, making it a suitable candidate for numerous biological applications.[19,20]

In an effort to identify new chemical entities with a high probability of binding to falcipain 2 (FP-2) and inhibiting *P. falciparum*, Mugumbate and co-workers conducted ligand- and structural-based virtual screening of a library of compounds from the ZINC database.[21] Amongst the hits identified included compounds containing the thiazolidine-2,4-dione (TZD-4) framework (Figure 1), which showed inhibitory activity against FP-2 with promising growth inhibition of *P. falciparum*.

Previously, Li and co-workers, also using a virtual screening campaign approach, identified several compounds with modest *in vitro* inhibitory activity, $IC_{50} = 10.9$ μM, including compound **A** as an FP-2 inhibitor (Figure 1).[22] Subsequently, Sharma *et al.*[23] explored the structure–activity relationship around the **TZD-4** hit, compounds that possess the thiazolidine-2,4-dione pharmacophoric unit for the inhibition of growth of *P. falciparum*. The *in vitro* biological evaluation of these compounds against FP-2 and *P. falciparum* revealed several compounds with IC_{50} values in the low micromolar range. With our interest in ferrocene-based antimalarial agents[16,24] coupled with the reported biological activity profile of thiazolidine-2,4-dione derivatives, we rationally designed our target compounds by amalgamation of these structural motifs into a single molecule with the intention of generating new compounds (Figure 1) with improved pharmacological properties. To the best of our knowledge, there have been no reports focusing on ferrocenyl-derived thiazolidine-2,4-diones as antiprotozoal agents.

2 | EXPERIMENTAL

2.1 | Materials and Methods

All chemicals and solvents used in this study were sourced from Sigma-Aldrich Ltd and/or Merck Ltd, and were used without further purification. The progress of the reactions was monitored by TLC using Merck F_{254} silica gel plates (supported on aluminium), which were

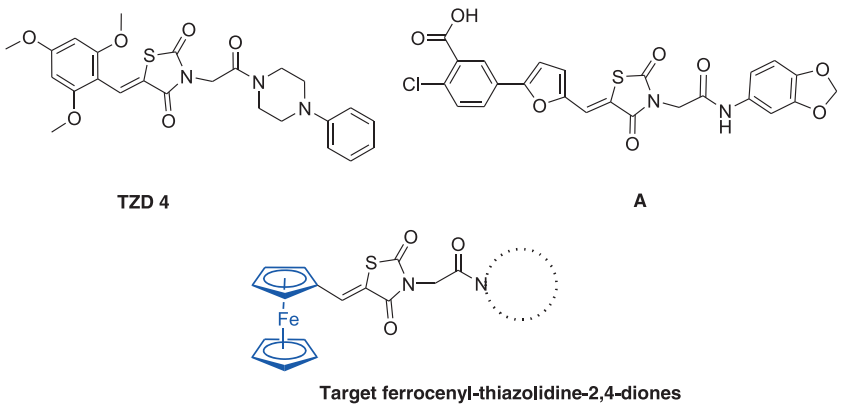


FIGURE 1 Chemical structures of thiazolidine-2,4-diones and target series

visualised under UV (254 and 366 nm) light or, where necessary, stained in iodine flask. The crude compounds were purified by flash column chromatography using Merck Kieselgel 60 Å, 70–230 silica gel mesh or by preparative TLC using Merck $60GF_{254}$ silica gel coated on glass plates (2.0 mm × 200 mm × 200 mm). ^{1}H NMR and ^{13}C NMR spectra were recorded in designated solvents with Bruker Fourier 300 MHz, Bruker Avance III HD 400 MHz or Bruker Avance II 600 MHz spectrometers and were internally referenced to the solvent peaks. The spectra were processed using MestReNova software. The chemical shifts were recorded in parts per million (ppm) and the *J*-coupling constants in herts (Hz). The abbreviations used to describe signal multiplicities are: s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet and m = multiplet. The melting points were determined with a Reichert 281313 hot-stage apparatus, and are uncorrected. High-resolution electrospray ionisation accurate mass spectrometry (HRMS) measurements were recorded in positive or negative mode with a Waters Synapt G2 (Central Analytical Facility, University of Stellenbosch). Infrared (IR) spectra were recorded with a PerkinElmer FT-IR Spectrum 100 spectrometer (neat).

2.2 | General Procedure: Preparation of Ferrocene-Containing Derivatives 5a–d, 7 and 11[23]

A mixture of ferrocenecarboxaldehyde (1.0 eq.) and an appropriate thaizolidin-2,4-dione derivative or 2-(phenylimino)thiazolidin-4-one (1 eq.) in the presence of piperidine (1.5 eq.) in ethanol (10 ml) was heated at 60°C for 6 to 24 h. After completion of the reaction, as indicated by TLC, the solvent was removed *in vacuo* to give crude products, which were purified by silica gel column chromatography to afford the desired compounds as solids.

2.2.1 | (*Z*)-5-((Ferrocenyl)methylene)-3-(2-oxo-2-(pyrrolidin-1-yl)ethyl)thiazolidine-2,4-dione (5a)

Red solid; 0.03 g (89%); m.p. > 286°C. R_f (hexane–EtOAc, 60:40%): 0.21. IR (ν_{max}, cm^{-1}): 1257 (η^5-C_5H_4), 3086 (vinyl C—H), 1732, 1682, 1652 (C═O), 1605 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.79 (1H, s, vinyl C—H), 4.73 (2H, s, η^5-C_5H_4), 4.66 (2H, s, η^5-C_5H_4), 4.44 (2H, s, —NCH_2CO—), 4.25 (5H, s, η^5-C_5H_5), 3.52 (2H, t, J = 6.7 Hz, CH_2), 3.29 (2H, t, J = 6.8 Hz, CH_2), 1.97–1.88 (2H, m, CH_2), 1.84–1.75 (2H, m, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 167.1, 164.6, 162.9, 136.2, 116.5, 76.3, 72.2 (2C), 70.6 (2C), 69.9 (5C), 45.8, 45.0, 43.0, 25.6, 23.6. HRMS: *m/z* (ESI) found 425.0622 $[M + H]^+$, calcd for $C_{20}H_{20}FeN_2O_3S$, 424.2960. Anal. Calcd for $C_{20}H_{20}FeN_2O_3S{\cdot}2CH_3OH$ (%): C, 54.10; H, 5.78; N, 5.74; S, 6.57. Found (%): C, 54.58; H, 5.47; N, 5.95; S, 6.29.

2.2.2 | (*Z*)-5-((Ferrocenyl)methylene)-3-(2-morpholino-2-oxoethyl)thiazolidine-2,4-dione (5b)

Red solid; 0.18 g (53%); m.p. 216–218°C. R_f (hexane–EtOAc, 50:50%): 0.24. IR (ν_{max}, cm^{-1}): 1236 (η^5-C_5H_4), 3091 (vinyl C—H), 1738, 1658 (C═O), 1602 (C═C). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.78 (1H, s, vinyl C—H), 4.57 (4H, s, η^5-C_5H_4), 4.49 (2H, s, —NCH_2CO—), 4.22 (5H, s, η^5-C_5H_5), 3.88 (2H, br s, CH_2), 3.77 (2H, br s, CH_2), 2.73 (2H, br s, CH_2), 2.64 (2H, br s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 168.1, 165.5, 163.3, 137.1, 117.0, 76.5, 72.4 (2C), 70.8 (2C), 70.2 (5C), 47.7, 45.1, 42.3, 27.8, 27.3. HRMS: *m/z* (ESI) found 441.0568 $[M + H]^+$, calcd for $C_{20}H_{20}FeN_2O_4S$, 440.2950. Anal. Calcd for $C_{20}H_{20}FeN_2O_4S{\cdot}CH_3OH$ (%): C, 53.40; H, 5.12; N, 5.93; S, 6.79. Found (%): C, 53.73; H, 5.11; N, 6.16; S, 6.75.

2.2.3 | (*Z*)-5-((Ferrocenyl)methylene)-3-(2-oxo-2-thiomorpholinoethyl)thiazolidine-2,4-dione (5c)

Red solid; 0.10 g (52%); m.p. 210–214°C. R_f (hexane–EtOAc, 70:30%): 0.30. IR (ν_{max}, cm^{-1}): 1253 (η^5-C_5H_4), 3098 (vinyl C—H), 1740, 1669, 1652 (C═O), 1603 (C═C). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.78 (1H, s, vinyl C—H), 4.57 (4H, s, η^5-C_5H_4), 4.49 (2H, s, —NCH_2CO—), 4.22 (5H, s, η^5-C_5H_5), 3.88 (2H, br s, CH_2), 3.77 (2H, br s, CH_2), 2.73 (2H, br s, CH_2), 2.64 (2H, br s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 168.1, 165.5, 163.3, 137.1, 117.0, 76.5, 72.4 (2C), 70.8 (2C), 70.2 (5C), 47.7, 45.1, 42.3, 27.8, 27.3. HRMS: *m/z* (ESI) found 457.0343 $[M + H]^+$, calcd for $C_{20}H_{20}FeN_2O_3S_2$, 456.3560. Anal. Calcd for $C_{20}H_{20}FeN_2O_3S_2{\cdot}½CH_3OH$ (%): C, 52.12; H, 4.69; N, 5.93; S, 13.58. Found (%): C, 52.39; H, 4.15; N, 5.85; S, 13.17.

2.2.4 | (*Z*)-5-((Ferrocenyl)methylene)-3-(2-oxo-2-(4-phenylpiperazin-1-yl)ethyl) thiazolidine-2,4-dione (5d)

Red solid; 0.02 g (95%); m.p. 186–188°C. R_f (hexane–EtOAc, 70:30%): 0.30. IR (ν_{max}, cm^{-1}): 1226 (η^5-C_5H_4), 3098 (C—H), 1737, 1671, 1652 (C═O), 1599 (C═C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.80 (1H, s, vinyl

C—H), 7.31 (2H, t, J = 7.4 Hz, ArH), 6.98–6.93 (3H, m, ArH), 4.58 (2H, s, —NCH_2CO—), 4.57 (4H, s, η^5-C_5H_4), 4.23 (5H, s, η^5-C_5H_5), 3.81 (2H, br s, CH_2), 3.69 (2H, br s, CH_2), 3.28 (2H, br s, CH_2), 3.21 (2H, br s, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 168.1, 165.5, 163.3, 137.1, 129.5 (2C), 121.2, 117.2, 117.1 (2C), 76.6, 72.3 (2C), 70.8 (2C), 70.2 (5C), 49.9, 49.7, 44.9, 42.3 (2C). HRMS: m/z (ESI) found 516.1044 $[M + H]^+$, calcd for $C_{26}H_{25}FeN_3O_3S$, 515.4090. Anal. Calcd for $C_{26}H_{25}FeN_3O_3S{\cdot}½CH_3CO_2CH_2CH_3$ (%): C, 60.11; H, 5.22; N, 7.51; S, 5.73. Found (%): C, 60.07; H, 5.94; N, 7.06; S, 5.01.

2.2.5 | (*Z*)-(5-((Ferrocenyl)methylene)-2,4-dioxothiazolidin-3-yl)acetic acid (7)

Red solid; 0.03 g (83%); m.p. > 286°C. R_f (DCM–2 M methanolic NH_3, 86:14%): 0.41. IR (ν_{max}, cm^{-1}): 3196 (OH, br), 1715, 1677, 1658 (C═O), 1599 (C═C), 1254 (η^5-C_5H_4). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.81 (1H, s, vinyl C—H), 4.72 (2H, s, η^5-C_5H_4), 4.66 (2H, s, η^5-C_5H_4), 4.35 (2H, s, —NCH_2CO—), 4.25 (5H, s, η^5-C_5H_5). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 167.0, 164.4, 136.4, 116.2, 76.1, 72.2 (2C), 70.6 (2C), 69.8 (5C). HRMS: m/z (ESI) found 369.9825 $[M - H]^-$, calcd for $C_{16}H_{13}FeNO_4S$, 371.1880.

2.2.6 | (5*Z*)-5-((Ferrocenyl)methylene)-2-(phenylimino)thiazolidin-4-one (11)

Red solid; 0.17 g (86%). m.p. 233–235°C. R_f (hexane–EtOAc, 70:30%): 0.38. IR (ν_{max}, cm^{-1}): 1248 (η^5-C_5H_4), 3057 (vinyl C—H), 1706, 1638 (C═O), 1586 (C═C), 1604 (C═C). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.60 (1H, s, vinyl C—H), 7.46–7.41 (2H, m, ArH), 7.24–7.22 (3H, m, ArH), 4.51 (2H, s, η^5-C_5H_4), 4.47 (2H, s, η^5-C_5H_4), 4.16 (5H, s, η^5-C_5H_5). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 169.3, 156.3, 145.7, 133.9, 129.6, 125.7, 122.2, 118.9, 76.9, 71.8 (2C), 70.5 (2C), 69.9 (5C). HRMS: m/z (ESI) found 389.0170 $[M + H]^+$, calcd for $C_{20}H_{16}FeN_2OS$, 388.2660. Anal. Calcd for $C_{20}H_{16}FeN_2OS$ (%): C, 61.87; H, 4.15; N, 7.22; S, 8.26. Found (%): C, 61.65; H, 4.66; N, 7.07; S, 7.69.

2.3 | Preparation of Ferrocene-Containing Thiazolidine-2,4-dione Derivatives 8a and 8b

A solution of **8** (1.0 eq.) and 1-methylpiperazine or phenylpiperazine (1 eq.) in dry dimethylformamide (DMF; 5 ml) was cooled to 0°C. *N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl; 1 eq.), 1-hyroxybenzotriazole hydrate (HOBt·H_2O; 1 eq.) and DIPEA (2 eq.) were added and the resulting mixture allowed to stir for 24 h at room temperature. Water (40 ml) was added to the resulting reaction product, which was extracted with ethyl acetate (3 × 40 ml). The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution (30 ml), brine (30 ml), dried over $MgSO_4$ and concentrated *in vacuo* to give the crude product, which was purified with preparative TLC using DCM–methanolic ammonia solution to afford desired products.

2.3.1 | (*Z*)-5-((Ferrocenyl)methylene)-3-(2-(4-methylpiperazin-1yl)-2-oxoethyl) thiazolidin-2,4-dione (8a)

Red solid; 117 mg (44%); m.p. 220–222°C. R_f (DCM–2 M methanolic NH_3, 96:4%): 0.15. IR (ν_{max}, cm^{-1}): 1231 (η^5-C_5H_4), 3088 (C═C—H), 1742, 1672, 1652 (C═O), 1603 (C═C). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.77 (1H, s, vinyl C—H), 4.55 (4H, d, J = 2.2 Hz, η^5-C_5H_4), 4.50 (2H, s, —NCH_2CO—), 4.21 (5H, s, η^5-C_5H_5), 3.65–3.62 (2H, m, CH_2), 3.53–3.50 (2H, m, CH_2), 2.50–2.47 (2H, m, CH_2), 2.42–2.39 (2H, m, CH_2), 2.32 (3H, s, —NCH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 168.2, 165.5, 163.1, 136.9, 117.2, 76.5, 72.3 (2C), 70.7 (2C), 70.1 (5C), 54.7, 54.5, 46.1, 44.7, 42.2 (2C). HRMS: m/z (ESI) found 454.0888 $[M + H]^+$, calcd for $C_{21}H_{23}FeN_3O_3S$, 453.3380. Anal. Calcd for $C_{21}H_{23}FeN_3O_3S{\cdot}CH_3OH$ (%): C, 54.44; H, 5.61; N, 8.66; S, 6.61. Found (%): C, 54.17; H, 5.67; N, 8.67; S, 6.31.

2.3.2 | (*Z*)-5-((Ferrocenyl)methylene)-3-(2-oxo-2-(piperidin-1-yl)ethyl)thiazolidine-2,4-dione (8b)

Red solid; 106 mg (61%); m.p. 162–163°C. R_f (*n*-hexane–EtOAc, 70:30%): 0.27. IR (ν_{max}, cm^{-1}): 1253 (η^5-C_5H_4), 3097 (C═C—H), 1725, 1657 (C═O), 1597 (C═C). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.77 (1H, s, vinyl C—H), 4.56 (4H, d, J = 4.1 Hz, η^5-C_5H_4), 4.50 (2H, s, —NCH_2CO—), 4.21 (5H, s, η^5-C_5H_5), 3.57–3.53 (2H, m, CH_2), 3.42 (2H, br s, CH_2), 1.66 (4H, br s, CH_2), 1.57 (2H, s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 168.2, 165.7, 162.9, 136.7, 117.4, 76.6, 72.2 (2C), 70.7 (2C), 70.2 (5C), 45.9, 43.5, 42.4, 26.3, 25.4, 24.4. HRMS: m/z (ESI) found 439.0779 $[M + H]^+$, calcd for $C_{21}H_{23}FeN_2O_3S$, 438.3230. Anal. Calcd for $C_{21}H_{22}FeN_2O_3S$ (%): C, 57.54; H, 5.06; N, 6.39; S, 7.31. Found (%): C, 57.34; H, 5.76; N, 6.09; S, 6.69.

2.4 | X-ray Crystallographic Analysis

A single crystal was covered in a small amount of paratone N oil and mounted on a MiTeGen microloop.

X-ray intensity data were collected at 100 K using a Bruker SMART APEX CCD with graphite monochromated Mo radiation ($\lambda = 0.71073$ Å). The detector to crystal distance was 60 mm. Data were collected using phi and omega scans and were scaled and reduced using the APEXII software unit (Bruker SAINT). Unit cell dimensions were refined on all the data and the space group was assigned on the basis of systematic absences and intensity statistics. The structure was solved using SHELXS-97 (2008) and refined using SHELXL-2016/6.[25] Hydrogen atoms were placed in calculated positions and included in the model during later stages of the refinement. The program X-SEED,[26] an interface to SHELX, was used during the structure solution and refinements.

CCDC 1588274 contains the supplementary crystallographic data for compound **5c**. The data including copies of this information can be obtained free of charge from the Cambridge Crystallographic Data Centre at http://www.ccdc.cam.ac.uk/conts/retrieving.html or The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk.

2.5 | Growth Inhibition Assays

2.5.1 | *In vitro* antitrypanosomal assay

Trypanosoma brucei brucei 427 trypomastigotes were cultured in Iscove's modified Dulbecco's medium (Lonza) supplemented with 10% foetal calf serum, HMI-9 supplement,[27] hypoxanthine and penicillin/streptomycin at 37 °C in a 5% CO_2 incubator. Serial dilutions of test compounds were incubated with the parasites in 96-well plates for 24 h and residual parasite viability in the wells determined by adding 20 μl of 0.54 mM resazurin in phosphate buffered saline (PBS) and incubating for an additional 2–4 h. Reduction of resazurin to resorufin by viable parasites was assessed by fluorescence readings (excitation 560 nm, emission 590 nm) with a Spectramax M3 plate reader. Fluorescence readings were converted to percentage parasite viability relative to the average readings obtained from untreated control wells. IC_{50} values were determined by plotting viability versus log[compound] and performing nonlinear regression using GraphPad Prism (v. 5.02) software.

2.5.2 | *In vitro* cytotoxicity assay

HeLa cells (Cellonex) were cultured in Dulbecco's modified Eagle medium (Lonza) supplemented with 10% foetal calf serum and antibiotics (penicillin/streptomycin/amphotericin B) at 37 °C in a 5% CO_2 incubator. Cells were plated in 96-well plates at a cell density of 2×10^4 cells per well and grown overnight. Serial dilutions of test compounds were incubated with the cells for an additional 24 h, and cell viability in the wells assessed by adding 20 μl of 0.54 mM resazurin in PBS for an additional 2–4 h. Fluorescence readings (excitation 560 nm, emission 590 nm) obtained for the individual wells were converted to percentage cell viability relative the average readings obtained from untreated control wells. Plots of cell viability versus log[compound] were used to determine IC_{50} values by nonlinear regression using GraphPad Prism (v. 5.02).

2.5.3 | *In vitro* antiplasmodial assay

Activity was determined against chloroquine-resistant isolate of human malaria (*P. falciparum* Dd2). Parasites were maintained in continuous culture using a modified method of Trager and Jensen.[28] Growth medium was supplemented with Albumax II (Gibco), a bovine serum albumin preparation, instead of human serum. Cultures did not exceed 4% haematocrit and parasitemia was diluted to 1% when the cultures were in the trophozoite stage. The compounds were tested in triplicate on at least three occasions *in vitro* against the human malaria parasite. Compounds were prepared to 0.02 g ml^{-1} stock solutions in dimethyl sulfoxide and sonicated for 10 min to enhance solubility. Compounds that did not dissolve completely were tested as a suspension. Stock solutions were stored at −20°C. Dilutions to the desired starting concentration of each compound were prepared in complete medium immediately prior to use on each occasion. Dose–response experiments were carried out in order to determine the IC_{50} value of each compound. The experiment was conducted using 2% parasitemia and 1% haematocrit in the plate. Compounds were prepared to double the desired highest starting concentration in a 96-well plate and then serially diluted twofold in complete medium to produce a wide range of different concentrations, to which an equivalent volume of prepared parasite stock was added, yielding the desired concentration of each compound. An erythrocyte control and a drug-free parasite control were included for each row, representing 0 and 100% parasite survival, respectively. Plates were housed in airtight chambers containing 4% CO_2 and 3% O_2 in nitrogen and left for 48 h at 37°C. Quantitative assessment of antimalarial activity was determined from the dose–response experiments using the parasite lactate dehydrogenase assay described by Makler *et al.*[29] The IC_{50} values were obtained using a nonlinear dose–response curve fitting analysis via GraphPad Prism v.4.0 software.

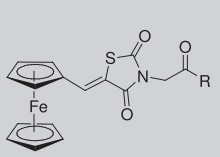
3 | RESULTS AND DISCUSSION

3.1 | Synthesis and Characterisation of Desired Compounds

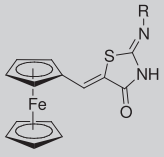
The series of ferrocene-containing thiazolidine-2,4-dione derivatives are summarised in Table 1, and the synthetic procedures followed for preparation of key intermediates and target compounds **5a–d**, **7**, **8a**, **8b** and **11** are outlined in Schemes 1 and 2. The synthesis commenced with a reaction of appropriate commercially accessible amines **1a–d**, with chloroacetyl chloride for 1 h to give rise to the respective starting chloroacetyl amides **2a–d** in yields ranging between 50 and 56% (Scheme 1).

Treating these starting amides **2a–d** with thiazolidine-2,4-dione **3**, in acetone under basic conditions, successfully yielded the desired key intermediates **4a–d** in moderate to excellent yields. Knoevenagel condensation of **4a–d** with ferrocenecarboxaldehyde resulted in a series of C-3-substituted ferrocene-based thiazolidine-2,4-dione derivatives **5a–d** in yields of 52–95%. Attempts to synthesise chloroacetyl amide of 1-methylpiperazine using the same synthetic procedure adopted in Scheme 1 (step i) proved unrewarding. To solve this, the synthetic protocol employing methylbromoacetate as shown in Scheme 1 (steps iv–vii) afforded the 1-methylpiperazine (**8a**) and piperidine (**9b**) derivatives through the key acid intermediate **7** in moderate yields of 44 and 61%, respectively. To assess the effect of changing the thiazolidine-2,4-dione

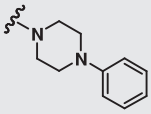
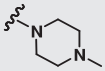
TABLE 1 *In vitro* bioassays data for synthesised compounds[a]



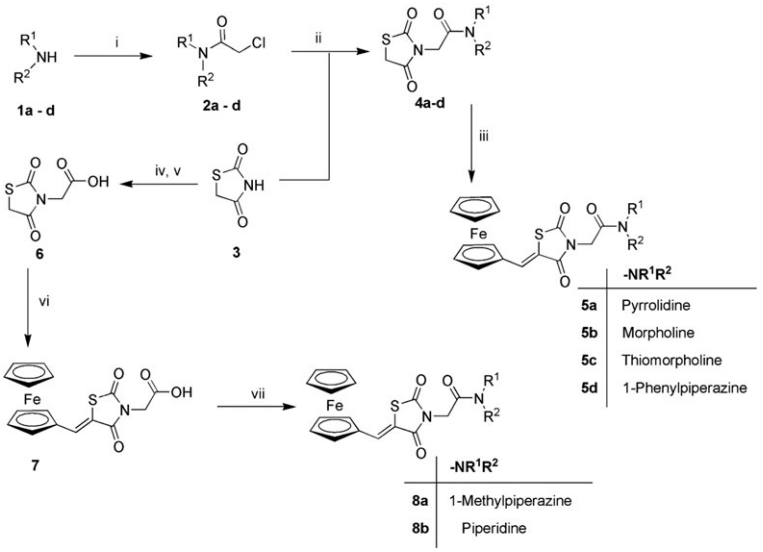
5a - d, 7, 8a-b



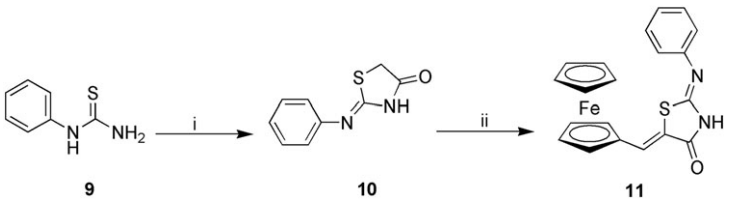
11

Compound	R	IC_{50} (μM) *T. b. brucei*	IC_{50} (μM) Dd2	Parasite viability (%) *T. b. brucei*	HeLa viability (%)
5a		12.4	>20	4.66	108.3
5b		ND	19.2	57.2	96.0
5c		ND	19.6	69.2	109.2
5d		ND	18.8	102.3	100.5
7		5.14	>20	1.91	94.9
8a		1.94	>20	1.33	108.1
8b		3.31	18.6	1.48	108.0
11		ND	14.6	104.3	104.2
TZD-4		7.51	4.45	16.7	109.9
PMD		0.0014	—	0.00088	—
CQ		—	0.28	—	—
EMT		—	—	—	0.084

[a]Antitrypanosomal activity (as IC_{50} using *T. b. brucei* 427 and parasite viability at 20 μM), antiplasmodial activity (as IC_{50} using a *P. falciparum* Dd2 assay) and cytotoxicity (viability of HeLa cells at 20 μM). The values for the PMD, CQ and EMT standards are the IC_{50} obtained in the respective assays. ND, not determined when a compound did not reduce parasite viability to <25%.



SCHEME 1 Reagents and conditions: (i) chloroacetyl chloride, triethylamine, 0 °C, 1 h, 52–56%; (ii) K_2CO_3, acetone, 24 h, 53–60%; (iii) ferrocenecarboxaldehyde, EtOH, piperidine, 60 °C, 6–24 h, 52–95%; (iv) methyl bromoacetate, K_2CO_3, acetone, 60 °C, 24 h, 48%; (v) 2 N HCl, AcOH, 100 °C, 8 h, 57%; (vi) ferrocenecarboxaldehyde, EtOH, piperidine, 60 °C, 8 h, 83%; (vii) DMF, piperazine, EDC·HCl, HOBt·H_2O, DIPEA, 0–25 °C, 24 h, 44–61%



SCHEME 2 Reagents and conditions: (i) chloroacetyl chloride, AcONa, 60 °C, 4 h; (ii) ferrocenecarboxaldehyde, EtOH, piperidine, 60 °C, 8 h, 86%

moiety with 2-phenyliminothiazolidin moiety, compound **11** was synthesised from commercially available phenylthiourea **9** in excellent yield of 86% as shown in Scheme 2.

All the synthesised compounds were fully characterised using common analytical techniques: IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques, HRMS and elemental analysis. From the ^{1}H NMR spectra, the products could be easily deduced with a clear disappearance of the aldehyde —CH proton of ferrocenecarboxaldehyde at 10.13 ppm and appearance of a sharp singlet signal of newly generated vinyl —CH double bond between 7.78 and 7.77 ppm. The protons of the η^5-C_5H_4 ring appeared as a singlet (sometimes a doublet) in the region 4.58 to 4.54 ppm. Similarly, the protons of the η^5-C_5H_5 ring appeared as a singlet in the region 4.22 to 4.23 ppm. The ^{13}C NMR spectra of these compounds showed between 13 and 16 carbon peaks with a diagnostic methine carbon often in the region 137.1 ppm, and four characteristic carbon signals in the region 76.9 to 69.8 ppm, an indication of a monosubstituted ferrocenyl subunit. The η^5-C_5H_5 ring carbons appear as a single high-intensity signal in the region 70.2 to 69.8 ppm.

The configuration of the target compounds was also confirmed using ^{1}H NMR and single-crystal X-ray diffraction analysis (Figure 2, showing only compound **5c**). ^{1}H NMR spectra for all the target compounds suggested a *Z*

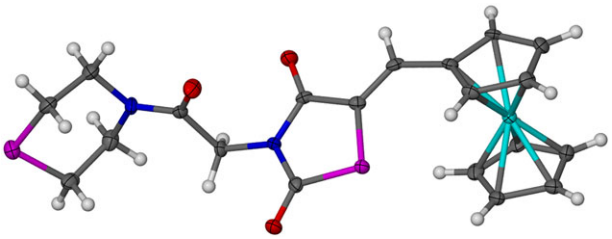


FIGURE 2 Molecular structure of ferrocenyl-containing thiazolidine-2,4-dione hybrid **5c** determined using single-crystal X-ray diffraction

configuration for the C═C bond, the diagnostic signals of the vinyl —CH protons resonating at higher chemical shift (i.e. 7.78–7.77 ppm) as singlets, an observation which is consistent with a *Z* configuration,[30,31] since the *E* diastereomer would have resonated at a value lower than 6.64 ppm (i.e. upfield).[32]

3.2 | Single-Crystal X-ray Diffraction Analysis

In order to confirm the conformations of the new thiazolidine-2,4-dione derivatives based on the organometallic ferrocenyl unit, single-crystal X-ray diffraction was utilised to determine the molecular structure of a representative thiazolidine-2,4-dione derivative, compound **5c** (Figure 2; crystallographic data are provided in Table S1 in the supporting information). Dark red crystals

of compound **5c** suitable for X-ray diffraction were obtained by vapour diffusion of *n*-hexane into dichloromethane solution containing **5c**. Compound **5c** crystallises in the centrosymmetric space group *P*-1 (triclinic crystal system) with two molecules in the unit cell, $Z = 2$. The solid-state structure confirms that the configuration around the double bond (C11–C12) is *Z*. The packing arrangement is characterised by several intermolecular C—H···O hydrogen bonds that span the crystal structure (pertinent hydrogen bond lengths and angles are reported in Table S2 in the supporting information).

3.3 | *In Vitro* Biological Evaluation

All synthesised compounds were evaluated *in vitro* for their antiplasmodial activity against the chloroquine-resistant (CQ^R) Dd2 strain of *P. falciparum* malaria parasite, antitrypanosomal activity against *T. b. brucei* 427 trypomastigotes, and cytotoxicity against the human cervix adenocarcinoma (HeLa) cell line. Chloroquine (CQ), pentamidine (PMD) and emetine (EMT) were included as reference compounds. Initially, the assays were performed in triplicate using 20 μM solutions of the synthesised compounds, and the results are displayed in Table 1. At this concentration, compounds **5a**, **7**, **8a**, **8b** and TZD-4 resulted in below 20% viability of the trypanosomal parasites species, while the other compounds were inactive at the same concentration. Thus, *T. b. brucei* IC_{50} values were determined for these five compounds. The acid intermediate **7** (IC_{50} = 5.14 μM) and its two derivatives, **8a** (IC_{50} = 1.94 μM) and **8b** (IC_{50} = 3.31 μM), showed improved antitrypanosomal activity compared to the original hit compound TZD-4 (IC_{50} = 7.51 μM), with compound **8a** being the most active. Since the only major differences between compounds **5a**, **5b**, **5c**, **8a** and **8b** are the size of secondary cyclic amino side groups and the presence of electronegative atoms other than nitrogen (oxygen and sulfur), the observed enhanced activity appeared to suggest that hydrophobicity is an important parameter. However, the introduction of bulkier amide group as exemplified by **5d** leads to marked loss of activity, and these results mirror a similar trend observed by Rahmani *et al.*[33]

Against the CQ^R Dd2 *P. falciparum* strain none of tested target compounds showed any appreciable activity compared to the hit compound TZD-4. Based on the presented data, it is quite clear that the synthesised target compounds showed selectivity towards *T. b. brucei* parasite species compared to the *P. falciparum* parasite. To determine whether the observed activities of target compounds were independent of any eukaryotic toxicity, the compounds were evaluated against HeLa cell line at 20 μM. All tested compounds showed minimal growth inhibition of HeLa cell line at that concentration, resulting in >90% HeLa cell viability in all cases, thus confirming that the observed activities are specific to the protozoan parasites.

4 | CONCLUSIONS

In summary, this study describes the design, synthesis and biological evaluation of new ferrocenyl analogues incorporating the TZD framework. The activities of these rationally designed and synthesised compounds were then compared to those of the TZD-4 hit compound. A number of these compounds were more active against *T. b. brucei* compared to TZD-4, while for antiplasmodial activity TZD-4 was superior compared to all compounds from the series which showed moderate activity with IC_{50} (Dd2) > 10 μM. The observed selective activities of compounds **8a** and **b** against *T. b. brucei* have necessitated further structure–activity relationship studies that are currently underway in our laboratories.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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