Synthesis, in vitro Cytotoxicity and Trypanocidal Evaluation of Novel 1,3,6-Substituted Non-fluoroquinolones

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ABSTRACT
Sleeping sickness (trypanosomiasis) is a neglected tropical disease that affects mostly the poorest communities in sub-Saharan Africa. Toxic side effects associated with the use of current anti-trypanosomal drugs, which in some cases kill faster than the disease itself, necessitate the search for new drugs with better safety margins. To this effect, a small library bearing different substituents at position -1, -3, and -6 of the quinolone nucleus were synthesized and evaluated in vitro against HeLa cell lines and Trypanosoma brucei brucei for cytotoxicity and trypanocidal potentials, respectively. While most of these compounds showed no cytotoxic effect, they exhibited moderate to weak anti-trypanosomal activities. The SAR studies of this series provide new information worth considering in future exploration of the quinolone scaffold in search of more potent and safe trypanocidal agents.

KEYWORDS
Sleeping sickness, trypanosomiasis, quinolones, non-fluoroquinolones.

1. Introduction
Human African trypanosomiasis (HAT), also referred to as sleeping sickness, is among the WHO’s list of neglected tropical diseases (NTDs). It mostly affects people living in rural areas of sub-Saharan Africa, where medical facilities are scarce and drug purchasing power of inhabitants is very low. At least 2184 new cases of the disease were reported in 2016, and approximately 60 million people living in 36 different countries are presently at risk of contracting the disease.

HAT takes two forms caused by two different subspecies of Trypanosoma brucei. T. b. gambiense is the pathogenic subspecies causing the form of HAT commonly found in central and western Africa, whereas T. b. rhodesiense is the subspecies responsible for the form of HAT prevalent in eastern and southern Africa. It has been noted that the two sub-pathogenic species coexist in Uganda. These pathogens are transmitted between humans following the bite of an infected tsetse fly. The disease exists in two stages: haemolymphatic stage – wherein the parasites are localized in blood and lymphatic systems, and an encephalitic stage – wherein the parasites have invaded the central nervous system.

Current treatment options are limited to just four drugs. In any case of HAT, the drug to be used is dictated by the form and stage of the disease. Pentamidine is the drug of choice for treating the haemolymphatic stage of HAT caused by T. b. gambiense, while a combination of nifurtimox and eflornithine is used to treat the encephalitic stage. In cases of T. b. rhodesiense, suramin is the recommended drug for treating the haemolymphatic stage, while malarsoprol is used to treat the encephalitic stage. Besides limited treatment options, the foregoing drugs are far from ideal. They all have poor oral bioavailability (and hence are administered intravenously), and considerable toxic side effects. For example, pentamidine causes hyper or hypoglycaemia and hypotension, suramin causes renal failure, eflornithine causes alopecia and seizures, while at least 5.9 % of patients on malarsoprol die from its toxicity, creating a scenario wherein patients either die from an acute illness or die faster from a pill.

The overwhelming life-threatening side effects of existing drugs used to treat sleeping sickness create a dire need for new compounds with better drug properties such as high oral bioavailability, and a high safety margin. The therapeutic potentials of quinolones cannot be over emphasized. Compounds containing this scaffold are currently in use as drugs to treat bacterial and viral infections as well as other conditions such as cancers. Fluoroquinolones (Fig. 1a) in clinical use have been extensively screened against trypanosomes and their activity profiles established as moderate to poor. A hit optimization study by Hiltsensperger and co-workers generated a potent library of fluoroquinolones characterized by benzylamides, (acyclic amines, and aliphatic chains at positions -3, -7, and -1 of the quinolone nucleus. However, the lead compound (Fig. 1b) in this series suffers from poor solubility, necessitating further work on this class of compounds. Unlike fluoroquinolones, the anti-trypanosomal potentials of non-fluorinated quinolones have not been extensively investigated, with just one study on non-fluorinated quinolones bearing substituents at position -1 and -2, respectively, being reported (Fig. 1c). To further expand the SAR around the non-fluoroquinolone scaffold, we conceptualized and synthesized a library of 18 non-fluorinated quinolones bearing unique concurrent substituents at position -1, -3, and -6. Target compounds were subjected to in vitro cytotoxicity and anti-trypanosomal evaluation. At 20 µM concentration, most of the compounds exhibited less than 50 % parasite viability while having little effect on the viability of HeLa cell lines (see Supplementary information). This suggests that the quinolone scaffold...
can be tailored via chemical synthesis to generate safe and potent drug substrates to treat trypanosomiasis.

2. Results and Discussion

We conceptualized and synthesized a set of 18 novel compounds that bear different substituents at positions -1, -3 and -6 of the quinolone scaffold. Conceptualized compounds were synthesized as depicted in Scheme 1. Briefly, 4'-nitroacetophenone was reduced to 4'-aminoacetophenone using reduced iron powder and acetic acid. 4'-Aminoacetophenone and 4-chloroaniline were each treated with diethyl ethoxymethylene malonate in refluxing acetonitrile to form condensed methylenemalonate esters, which underwent cyclization in boiling diphenylether at 245–250 °C for 5 min to form compound 2. Deprotonation of 2 using K₂CO₃, followed by N-alkylation with alkylhalides afforded target compounds 3a–h in yields ranging between 40 and 70%. Ester functionality in compounds 3d–h underwent selective aminolysis in the presence of a ketone to afford target compounds 4a–j in 30–50% yields. This transformation was realized using DBU as a base. All targetted compounds were characterized using proton and carbon NMR, HRMS and IR. The carbon NMR spectra of all compounds show a peak at c. 174 ppm, which is indicative of an oxo carbon within the quinolone nucleus (C-4) and the peak at c. 164 ppm is assigned to ester or amide carbonyl carbon (C-3a). With the exception of compounds 3a–c, the proton NMR spectra of all compounds show a singlet signal at ~2.3–2.6 ppm, which is assigned to a methyl attached to a carbonyl carbon (CH₃C=O). The carbonyl carbon is also evident in the respective carbon spectra at ~197 ppm. The presence of a triplet peak (J = 5.9 Hz) at c. 9.9 ppm in the proton NMR spectra of compounds 4a–j, which is absent in compounds 3d–h, suggests the successful conver-

Figure 1 Representative structures of fluoroquinolones (a and b) and non-fluoroquinolones (c) investigated for anti-trypanosomal activities.

Scheme 1
Synthesis of target compounds
Reagents and conditions: (a) Acetonitrile, diethyl ethoxymethylene malonate, reflux 12 h, (b) Diphenyl ether, 250 °C, 5 min, (c) K₂CO₃, DMF, alkylhalide (1.2 eqv), 12 h, (d) Amine (5 eqv), DBU (1.2 eqv), CHCl₃, reflux 12 h.
sion of ester to amide. It is also worth noting that the acquisition of NMR spectroscopic data was at times hindered by compounds crystallizing out of the solutions. This sometimes necessitated the use of hot solvents to encourage compounds to remain in solution long enough to obtain 1H and 13C NMR spectroscopic data (see Supplementary information). The absorption band at 1654/cm on the IR spectrum further confirms the presence of amide.

This focused library was screened in vitro against human cervix adenocarcinoma (HeLa) cell lines to investigate potential cytotoxicity effects. The compounds were incubated at 20 µM in 96-well plates containing HeLa cells for 48 h. The numbers of cells surviving upon drug exposure were determined using resazurin reduction to resorufin by live cells and reading resorufin fluorescence in a multiwell plate reader. Compounds were tested in duplicate, and a standard deviation (S.D.) calculated. Results are expressed as % viability based on fluorescence readings in treated wells versus untreated control wells. Emetine (which induces cell apoptosis) was used as positive control. With the exception of compound 4f (% viability, –19 %), and 4j (% viability, –13 %), which strongly inhibited HeLa cell lines, the rest of the series had little to no effect on HeLa cell viability. This observation suggests that this series with necessary optimization could serve as templates for the development of non-toxic anti-trypanosomal agents.

The compounds were further evaluated in vitro for anti-trypanosomal activities by screening against the 427 strain of T. b. brucei. Compounds were added to in vitro cultures of T. b. brucei in 96-well plates at a 20 µM concentration. After an incubation period of 48 h, the numbers of parasites surviving drug exposure were determined by adding resazurin. As in HeLa cells, resazurin is reduced to resorufin by living parasites. Resorufin is a fluorophore (Exc560/Em590) and can thus be quantified in a multiwell fluorescence plate reader. Compounds were tested in duplicate wells, and a standard deviation (S.D.) calculated. Results are expressed as % viability – the resorufin fluorescence in compound-treated wells relative to untreated controls. Pentamidine (an existing drug for treatment of trypanosomiasis) was used as a positive control. At 20 µM concentration, 11 compounds inhibited parasite growth below 50 % (Table 1); however, only compounds inhibiting parasite viability below 25 % with little or no effect on HeLa cell line were considered for IC50 determination. The IC50 values for the selected compounds are summarized in Fig. 2.

Structure–activity relationship analysis across this series suggests that modifications at position -1, -3, and -6 of the quinolone scaffold influence anti-trypanosomal activities. For example, comparing the activity profiles of compounds 3b (IC50 19 µM) and 3g (IC50 24 µM), all having the same substituents at position -1 and -3 and differing in substitution pattern only at position -6 suggests that a chloride substituent at this position leads to increase anti-trypanosomal activity than a ketone. Also, comparing compounds 3d (IC50 32 µM) bearing an alkyl chain at position -1 and 3g (IC50 24 µM) bearing a substituted-benzyl moiety at position -1, indicates that the presence of a substituent on the aromatic ring at position -1, is important for anti-trypanosomal activity.

Table 1 In vitro cytotoxicities and anti-trypanosomal activities of target compounds expressed as percentage viabilities.

| Comp. | % Viability ± S.D. |
|-------|--------------------|
|       | T. b. brucei | HeLa cells |
| 3a    | 21.2 ± 4.0 | 97.6 ± 5.6 |
| 3b    | 4.0 ± 0.7  | 100.2 ± 6.2 |
| 3c    | 97.3±3.3  | 102.7 ± 7.6 |
| 3d    | 21.0 ± 3.7 | 77.0 ± 0.7 |
| 3e    | 43.8 ± 3.3 | 96.7 ± 3.7 |
| 3f    | 87.3 ± 7.0 | 107.2 ± 1.6 |
| 3g    | 5.5 ± 5.2  | 103.6 ± 3.7 |
| 3h    | 42.3 ± 8.4 | 108.6 ± 5.3 |
| 4a    | 95.4 ± 10  | 92.6 ± 3.6 |
| 4b    | 4.8 ± 0.1  | 86.1 ± 2.4 |
| 4c    | 105.8 ± 1.6| 110.7 ± 6.7 |
| 4d    | 31.2 ± 12.2 | 88.9 ± 5.8 |
| 4e    | 100.0 ± 2.7 | 102.6 ± 5.7 |
| 4f    | -1.3 ± 0.3 | -19.2 ± 0.04|
| 4g    | 24.7 ± 7.6 | 102.1 ± 5.8 |
| 4i    | 64.5 ± 7.8 | 91.0 ± 4.9 |
| 4j    | 50.3 ± 4.4 | -14.0 ± 0.6 |
| PE    | 0.0        | N.D           |
| EMT   | N.D*       | 0.0           |

*PE = pentamidine, *EMT = emetine, *N.D = not determined.

Figure 2 Plot of percentage viability against log concentration for compound 3a, 3b, 3d, 3g, 4b and 4g as well as the standard (PE = pentamidine) and their corresponding IC50 values.
tuted-benzyl moiety at position -1 seems to promote anti-trypanosomal activities over alkyl chains. We also observed that the substituent on the benzyl moiety at position -1 also influences anti-trypanosomal activities. This is evident when comparing the effects of compounds 3a, 3b and 3c on parasite viability at a concentration of 20 µM. Compounds 3a and 3b bearing -NO₂ and -Br, respectively, exhibited more than 75% parasite growth inhibition, while compound 3c having -CF₃, substituent exhibited less than 5% parasite growth inhibition. These results suggest that electron-withdrawing units promote activity while electron-donating unit leads to poor activity. The substitution pattern at position -3 also seems to greatly influence activity while electron-donating unit leads to poor activity. The substituent exhibited less than 5% parasite growth inhibition.

3. Conclusion

We have synthesized a series of novel quinolones with varied substituents at position -1, -3 and -6 of the quinolone scaffold. While most compounds in this series showed no promising cytotoxicity potentials, compounds 4g (IC₅₀ 24 µM) and 4f (IC₅₀ 7 µM), both of which differ only in the substituent at position -3, suggests that an amide moiety at position -3 seems to enhance activity over ethyl ester.

4. Experimental

4.1. General Method

All the chemicals and solvents used were purchased from various chemical suppliers and were used without further purification. Melting points were determined using a Reichert hot stage microscope and are uncorrected. The progress of the reactions was monitored by thin layer chromatography (TLC) using Merck Kieselgel 60 Å: 70–230 (0.068–0.2 mm) silica gel mesh. ¹H and ¹³C NMR spectra were recorded on Bruker Biospin 300 MHz, or 400 MHz spectrometers, and the chemical shifts are given in δ values referenced to solvents and are reported in parts per million (ppm). The high-resolution mass spectrometric data of final compounds was recorded on a Waters Synapt G2 quadrupole time-of-flight (QTOF) mass spectrometer operated with an electrospray ionization probe in the positive mode (University of Stellenbosch). The instrument was operated with an electrospray ionization probe in the positive mode. The starting quinolones were obtained in 40–70% yield following this procedure.

4.2. Synthesis of Compounds

4.2.1. General Method for the Preparation of N-alkylated Compounds 3a–h

A mixture of 2 (3.86 mmol, 1 g, 1 eq), K₂CO₃ (5.00 mmol, 0.53 g), alkyl halide (5 eq) in acetone (30 mL) was refluxed for 15 h. Upon reaction completion as indicated by TLC, the mixture was filtered, and the filtrate evaporated to dryness to obtain a crude N-alkylated product which was purified through silica gel column chromatography using CH₂Cl₂/MeOH (10:1) as the mobile phase. Compounds 3a–h were obtained in 40–70% yield following this procedure.
Ethyl 6-acetyl-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylate, 3d

Red powder, 0.65 g (70 %), \(R_f = 0.71\) (DCM/MeOH 1:1). \(R_f = 0.84\) (d, \(J = 2.1\) Hz, 1H, H-5), 2.22 (dd, \(J = 8.9, 2.1\) Hz, 1H, H-7), 7.46 (d, \(J = 9.0\) Hz, 1H, H-8), 4.39–4.31 (m, 2H, H-1a), 4.23 (q, \(J = 7.2\) Hz, 2H, H-3c), 2.63 (s, 3H, H-6b), 1.50 (t, \(J = 7.3\) Hz, 3H, H-3d), 1.37–1.20 (m, 3H, H-1b). \(1^\text{C}\) NMR (75 MHz, CDCl₃) \(\delta\) 196.9 (6a), 173.9 (4), 165.4 (3a), 149.1 (2), 141.6 (3), 133.3 (6), 131.4 (5), 129.5 (7), 128.7 (4a), 116.2 (8), 112.4 (8a), 61.1 (3c), 49.1 (1a), 26.6 (6b), 14.5 (1b), 14.4 (3d). IR (neat, cm⁻¹): 3053, 2982, 2921, 1712, 1685, ESI-HRMS \([M+H]^+\) calcd for \(\mathrm{C}_{21}\mathrm{H}_{16}\mathrm{O}_4\), found 350.1391.

Methyl 6-acetyl-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylate, 3e

Brown powder, 0.680 g (68 %), \(R_f = 0.81\) (DCM/MeOH 1:1). \(R_f = 0.84\) (d, \(J = 3.4\) Hz, 1H, H-5), 8.15 (dd, \(J = 9.1, 3.4\) Hz, 1H, H-7), 7.73 (d, \(J = 8.0\) Hz, 2H, H-1c), 7.60 (d, \(J = 7.1\) Hz, 2H, H-1d), 7.43 (d, \(J = 9.1\) Hz, 1H, H-8), 5.82 (s, 2H, H-1a), 3.89 (s, 3H, H-3c), 2.49 (s, 3H, H-6b). \(1^\text{C}\) NMR (75 MHz, DMSO) \(\delta\) 197.1 (C-6a), 173.9 (4), 164.4 (3a), 151.4 (C-2), 142.2 (C-3), 140.2 (C-1b), 135.7 (C-1d), 133.3 (C-6), 132.3 (C-1c), 132.0 (C-5), 129.7 (C-1e), 127.6 (C-4a), 121.5 (C-8), 118.8 (C-8a), 111.6 (C-1f), 55.3 (C-3c), 53.7 (C-1a), 26.6 (6b). IR (neat, cm⁻¹): 3100, 2970, 2921, 1700, 1680. ESI-HRMS \([M+H]^+\) calcd for \(\mathrm{C}_{21}\mathrm{H}_{16}\mathrm{F}_{3}\mathrm{O}_4\), found 414.0403.

Ethyl 6-acetyl-1-benzyl-4-oxo-1,4-dihydroquinoline-3-carboxylate, 3f

White powder, 0.80 g (58 %), \(R_f = 0.81\) (DCM/MeOH 1:1). \(R_f = 0.84\) (d, \(J = 3.4\) Hz, 1H, H-5), 8.15 (dd, \(J = 9.1, 3.4\) Hz, 1H, H-7), 7.73 (d, \(J = 8.0\) Hz, 2H, H-1c), 7.60 (d, \(J = 7.1\) Hz, 2H, H-1d), 7.43 (d, \(J = 9.1\) Hz, 1H, H-8), 5.82 (s, 2H, H-1a), 3.89 (s, 3H, H-3c), 2.49 (s, 3H, H-6b), 1.50 (t, \(J = 7.3\) Hz, 3H, H-3d). \(1^\text{C}\) NMR (75 MHz, DMSO) \(\delta\) 197.1 (C-6a), 173.9 (4), 164.4 (3a), 151.4 (C-2), 142.2 (C-3), 140.2 (C-1b), 135.7 (C-1d), 133.3 (C-6), 132.3 (C-1c), 132.0 (C-5), 129.7 (C-1e), 127.6 (C-4a), 121.5 (C-8), 118.8 (C-8a), 111.6 (C-1f), 55.3 (C-3c), 53.7 (C-1a), 26.6 (6b). IR (neat, cm⁻¹): 3100, 2970, 2921, 1700, 1680. ESI-HRMS \([M+H]^+\) calcd for \(\mathrm{C}_{21}\mathrm{H}_{18}\mathrm{F}_{3}\mathrm{O}_4\), found 350.1391.

Ethyl 6-acetyl-1-ethylaryl-4-oxo-1,4-dihydroquinoline-3-carboxylate, 3g

Brown powder, 0.58 g (62 %), \(R_f = 0.81\) (DCM/MeOH 1:1), \(R_f = 0.84\) (d, \(J = 8.6\) Hz, 1H, H-7), 7.81–7.52 (m, 3H, H-1c, H-8), 7.19 (d, \(J = 7.6\) Hz, 2H, H-1d), 5.72 (s, 2H, H-1a), 3.79 (s, 3H, H-3c), 2.50 (s, 3H, H-6b). \(1^\text{C}\) NMR (75 MHz, DMSO) \(\delta\) 197.1 (C-6a), 173.1 (C-4), 164.4 (3a), 151.4 (C-2), 142.2 (C-3), 135.7 (C-1d), 133.3 (C-6), 132.3 (C-1c), 129.2 (C-5), 127.8 (C-1e), 127.7 (C-4a), 127.5 (C-8), 119.8 (C-8a), 111.6 (C-1f), 55.3 (C-3c), 53.7 (C-1a), 26.6 (6b). IR (neat, cm⁻¹): 3048, 2972, 2931, 1702, 1682. ESI-HRMS \([M+H]^+\) calcd for \(\mathrm{C}_{21}\mathrm{H}_{20}\mathrm{O}_4\), found 414.0335.

4.2.2. General Method for the Preparation of Amides 4a–j

A mixture of 3 (1 g, 1 eq.), DBU (320 µL, 0.33 g, 2.1 mmol), an appropriate amine (5 eq.), and chloroform (15 mL) in a 100 mL round-bottom flask was stirred under reflux for 24–30 h. Upon completion, the mixture was evaporated to dryness and resultant crude subjected to silica gel column chromatography eluting with CHCl₃/MeOH (10:1). Fractions containing the desired product were combined, evaporated to dryness and recrystallized from ethanol. Compounds 4a–j were obtained in 30–50 % yield following this procedure. 6-Acetyl-1-ethyl-N-(2-methoxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 4a
Orange powder, 0.028 g (32 %), \( R_f = 0.48 \) (DCM/MeOH 1:1), m.p. 163–165 °C; \( ^1{H} NMR \) (300 MHz, DMSO) \( \delta \) 9.93 (s, 1H, NH), 8.88 (s, 1H, H-2), 8.83 (d, \( J \) = 1.5 Hz, 1H, H-5), 8.32–8.22 (m, 1H, H-7), 7.97 (d, \( J \) = 9.0 Hz, 1H, H-8), 4.55 (q, \( J \) = 7.0 Hz, 2H, H-1a), 3.49 (s, 3H, H-3O), 3.42–3.04 (m, 4H, H-3c–H-3d), 2.68 (s, 3H, H-6b), 1.39 (t, \( J \) = 7.0 Hz, 3H, H-7b). \( ^1{C} NMR \) (75 MHz, DMSO) \( \delta \) 197.6 (C-6a), 175.9 (C-4), 164.2 (C-3a), 148.8 (C-2), 142.1 (C-3), 132.9 (C-6), 132.1 (C-5), 127.9 (C-7), 127.1 (C-4a), 118.4 (C-8) 112.6 (C-8a), 71.3 (C-3d), 58.3 (C-3f), 49.1 (C-1a), 38.8 (C-3c), 27.5 (C-6b), 14.9 (C-1b). IR (neat, cm\(^{-1}\)): 3393, 3041, 2970, 2929, 1682, 1656. ESI-HRMS \( m/z \) [M+H]\(^+\) calc for C\(_{17}\)H\(_{14}\)N\(_{2}\)O\(_{3}\), 317.1496, found 317.1497.

6-Acetyl-1-ethyl-N-(2-(2-hydroxyethoxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 4b

White powder, 0.235 g (33 %), \( R_f = 0.39 \) (DCM/MeOH 1:1), m.p. 127–129 °C; \( ^1{H} NMR \) (400 MHz, CDCl\(_3\)) \( \delta \) 10.12 (s, 1H, NH), 8.97 (s, 1H, H-1), 8.75 (s, 1H, H-5), 8.27 (d, \( J \) = 7.9 Hz, 1H, H-7), 7.54 (d, \( J \) = 7.9 Hz, 1H, H-8), 4.31 (q, \( J \) = 7.1 Hz, 2H, H-1a), 3.74–3.61 (m, 8H, H-3c–H-3d, H-3f–H-3g), 2.67 (s, 3H, H-6b), 1.53 (t, \( J \) = 7.2 Hz, 3H, H-1b). \( ^1{C} NMR \) (101 MHz, CDCl\(_3\)) \( \delta \) 196.8 (C-6a), 176.7 (C-4), 164.7 (C-3a), 147.9 (C-2), 141.5 (C-3), 133.6 (C-6), 131.9 (C-5), 129.2 (C-7), 127.9 (C-4a), 116.2 (C-8), 113.5 (C-8a), 72.7 (C-3g), 69.7 (C-3f), 61.7 (C-3d), 49.3 (C-1a), 39.0 (C-3c), 26.6 (C-6b), 14.6 (C-1b). IR (neat, cm\(^{-1}\)): 3333, 3252, 3001, 2970, 2929, 1682, 1654. ESI-HRMS \( m/z \) [M+H]\(^+\) calc for C\(_{17}\)H\(_{14}\)N\(_{2}\)O\(_{3}\), 347.1601, found 347.1604.

6-Acetyl-1-ethyl-N-(2-(2-hydroxyethyl)amino)ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, 4c

White powder, 0.475 g (48 %), \( R_f = 0.11 \) (DCM/MeOH 1:1), m.p. 157–159 °C; \( ^1{H} NMR \) (300 MHz, DMSO) \( \delta \) 9.97 (t, \( J \) = 5.9 Hz, 1H, NH-3b), 8.91 (s, 1H, H-2), 8.84 (d, \( J \) = 2.2 Hz, 1H, H-5), 8.30 (dd, \( J \) = 9.0, 2.2 Hz, 1H, H-7), 8.01 (d, \( J \) = 9.0 Hz, 1H, H-8), 5.27 (s, 1H, H-3h), 4.56 (q, \( J \) = 7.1 Hz, 2H, H-1a), 3.67 (t, \( J \) = 6.3 Hz, 4H, H-1c–H-3c), 3.15 (t, \( J \) = 8.8 Hz, 2H, H-3g), 3.03 (t, \( J \) = 5.4 Hz, 2H, H-3c), 2.68 (s, 3H, H-6b), 1.39 (t, \( J \) = 7.1 Hz, 3H, H-1b). \( ^1{C} NMR \) (75 MHz, DMSO) \( \delta \) 197.2 (C-6a), 175.8 (C-4), 165.1 (C-3a), 149.2 (C-2), 141.8 (C-13), 133.2 (C-6c), 132.2 (C-7), 127.7 (C-4a), 118.5 (C-8), 112.3 (C-8a), 56.9 (C-3g), 49.7 (C-1a), 49.0 (C-3c), 47.1 (C-3f), 35.3 (C-3d), 27.2 (C-6b), 14.9 (C-1b). IR (neat, cm\(^{-1}\)): 3313, 3243, 3081, 2879, 2819, 1687, 1656. ESI-HRMS \( m/z \) [M+H]\(^+\) calc for C\(_{17}\)H\(_{22}\)N\(_{2}\)O\(_{3}\), 446.1762, found 446.1762.

N-(3-(1H-imidazol-1-yl)propyl)-6-acetyl-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxamide, 4d

White powder, 0.020 g (48 %), \( R_f = 0.01 \) (DCM/MeOH 1:1), m.p. 178–180 °C; \( ^1{H} NMR \) (300 MHz, DMSO) \( \delta \) 9.93 (t, \( J \) = 4.8 Hz, 1H, NH), 9.10 (s, 1H, H-2), 8.85 (d, \( J \) = 1.7 Hz, 1H, H-5), 8.19 (dd, \( J \) = 8.9, 1.7 Hz, 1H, H-7), 7.79 (d, \( J \) = 9.0 Hz, 1H, H-8), 7.54 (d, \( J \) = 8.3 Hz, 2H, H-1c), 7.20 (d, \( J \) = 8.3 Hz, 2H, H-1d), 5.79 (s, 2H, H-1a), 4.62 (t, \( J \) = 5.2 Hz, 2H, H-3c), 3.60–3.49 (m, 6H, H-3d–H-3g), 2.43 (s, 3H, H-6b). \( ^1{C} NMR \) (75 MHz, DMSO) \( \delta \) 197.1 (C-6a), 176.2 (C-4), 164.1 (C-3a), 150.2 (C-2), 142.2 (C-3), 135.7 (C-1d), 133.4 (C-6c), 132.2 (C-1c), 132.0 (C-5), 129.3 (C-7), 127.4 (C-1b), 121.6 (C-4a), 118.9 (C-8), 112.7 (C-8a), 111.9 (C-1e), 58.7 (C-3g), 55.8 (C-1a), 49.4 (C-3c), 48.9 (C-3d), 36.9 (C-3c), 27.2 (C-6b). IR (neat, cm\(^{-1}\)): 3320, 3020, 3000, 2970, 2929, 1682, 1657. ESI-HRMS \( m/z \) [M+H]\(^+\) calc for C\(_{22}\)H\(_{24}\)N\(_{2}\)O\(_{3}\), 486.1023, found 486.1021.
**Anti-trypanosomal Assay**

*Trypanosoma brucei brucei* (ATCC® 302605) was grown in Iscove's Modified Dulbecco's Medium (IMDM; Lonza) supplemented with 10 % fetal calf serum and antibiotics (penicillin/streptomycin/amphotericin B) at 37 °C in a 5% CO2 incubator. Cells were plated in 96-well plates at a cell density of 2×104 cells per well and grown overnight. 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 with the cells for an additional 24 h, and cell viability in the wells assessed by adding 20 µL 0.54 mM resazurin in 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) in a Spectramax M3 plate reader. Fluorescence readings were converted to % parasite viability relative to the average readings obtained from untreated control wells. IC50 values were determined by plotting % viability vs. log[compound] and performing non-linear regression using GraphPad Prism (v. 5.02) software.

**Cytotoxicity Assay**

HeLa cells (Cellonex) were cultured in Dulbecco's modified Eagle's medium (DMEM; Lonza) supplemented with 10 % fetal calf serum and antibiotics (penicillin/streptomycin/amphotericin B) at 37 °C in a 5 % CO2 incubator. Cells were plated in 96-well plates at a cell density of 2×104 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 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 % cell viability relative to the average readings obtained from the individual wells assessed by adding 20 µL 0.54 mM resazurin in PBS for an additional 24 h, and cell viability in the samples treated with the compounds was assessed by fluorescence readings (excitation 560 nm, emission 590 nm) in a Spectramax M3 plate reader. Fluorescence readings were converted to % cell viability relative to the average readings obtained from untreated control wells. IC50 values were determined by plotting % viability vs. log[compound] and performing non-linear regression using GraphPad Prism (v. 5.02) software.

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**6-Acetyl-1-(4-bromobenzyl)-N-[2-(2-hydroxyethoxy)ethyl]-4-oxo-1,4-dihydroquinoline-3-carboxamide, 4i**

Orange powder, 0.367 g (44 %), Rf = 0.11 (DCM/MeOH 10:1), m.p. 193–195 °C; 1H NMR (300 MHz, DMSO) δ 9.03 (t, J = 5.9 Hz, 1H, NH), 9.22 (s, 1H, H-2), 8.92 (dd, J = 5.9, 2.1 Hz, 1H, H-5), 8.28 (dd, J = 8.9, 2.2 Hz, 1H, H-7), 8.19 (d, J = 9.0 Hz, 1H, H-8), 8.14 (dd, J = 8.9, 2.2 Hz, 2H, H-1d), 6.03 (s, 2H, H-6b). 13C NMR (75 MHz, DMSO) δ 197.1 (C-6a), 176.1 (C-4), 165.0 (C-3a), 150.4 (C-2), 142.2 (C-3), 137.1 (C-1d), 132.6 (C-1c), 126.3 (C-1b), 126.3 (C-1a), 118.9 (C-8), 112.7 (C-8a), 111.9 (C-1e), 127.4 (C-1g), 69.7 (C-3f), 60.7 (C-3g), 55.0 (C-3a), 53.5 (C-1f), 39.7 (C-3c), 27.2 (C-6f). IR (neat, cm−1): 3298, 3071, 2971, 2929, 1682, 1655. ESI-HRMS m/z [M+H]+ calcd for C22H21Cl2N2O4 447.0878, found 447.0879.

**6-Acetyl-N-[2-(2-hydroxyethy)amino)ethyl]-4-oxo-1-(4-trifluoro methyl)benzyl)-1,4-dihydroquinoline-3-carboxamide, 4j**

Orange powder, 0.367 g (44 %), Rf = 0.11 (DCM/MeOH 10:1), m.p. 193–195 °C; 1H NMR (300 MHz, DMSO) δ 10.03 (t, J = 5.9 Hz, 1H, NH), 9.22 (s, 1H, H-2), 8.92 (dd, J = 5.9, 2.1 Hz, 1H, H-5), 8.28 (dd, J = 8.9, 2.2 Hz, 1H, H-7), 8.19 (d, J = 9.0 Hz, 1H, H-8), 8.14 (dd, J = 8.9, 2.2 Hz, 2H, H-1d), 6.03 (s, 2H, H-6b). 13C NMR (75 MHz, DMSO) δ 197.1 (C-6a), 176.1 (C-4), 165.0 (C-3a), 150.4 (C-2), 142.2 (C-3), 137.1 (C-1d), 132.6 (C-1c), 126.3 (C-1b), 126.3 (C-1a), 118.9 (C-8), 112.6 (C-8a), 56.9 (C-3f), 55.9 (C-1a), 49.6 (C-3e), 47.0 (C-3d), 35.9 (C-3c), 27.2 (C-6b). IR (neat, cm−1): 3298, 3252, 3081, 2974, 2926, 1682, 1654. ESI-HRMS m/z [M+H]+ calcd for C19H16F2N4O4 476.1972, found 476.1979.
untreated control wells. Plots of % cell viability vs. log[compound] were used to determine IC_{50} values by non-linear regression using GraphPad Prism (v. 5.02).

Supplementary Material
Supplementary information is provided in the online supplement.

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References
1. R. Brun, R. Schumacher, C. Schmid, C. Kunz and C. Burri, The phenomenon of treatment failures in human African trypanosomiasis, 
*Trav. Med. Int. Health*, 2001, 6, 906–914.
2. WHO. Neglected tropical diseases http://www.who.int/neglected_diseases/diseases/en/ accessed on 14 August 2017.
3. M. Kaiser, M. Bray, M. Cal, B. Trunz, E. Torreele and R. Brun, Anti-trypanosomal activity of fexinidazole, a new oral nitrimidazole drug candidate for treatment of sleeping sickness, *Antimicrob. Agents Chemother.*, 2011, 55, 5602–5608.
4. M. Berninger, I. Schmidt, A. Ponte-Sucrub and U. Holzgrabe, Novel lead compounds in preclinical development against African sleeping sickness, *Med. Chem. Commun.*, 2017, 8, 1872–1890.
5. WHO. Trypanosomiasis, human African (Sleeping sickness), http://www.who.int/mediacentre/factsheets/fs259/en/ accessed on 14 August 2017.
6. H.-H. Tran, Z. Zheng, X. Wen, S. Manivannan, A. Pastor, M. Kaiser, R. Brun, R. Snyder and T. Back, Synthesis and activity of nucleoside-based antiprotozoan compounds, *Bioorg. Med. Chem.*, 2017, 25, 2091–2104.
7. X. Wang, D. Inaoka, T. Shiba, E. Balogun, S. Allmann, Y. Watanabe, M. Banerjee, D. Paraia, P. Dhar, M. Roy, R. Barik, S. Chattopadhyay and J. Morris and D. Whitehead, Evaluation of substituted ebselen derivatives as potential trypanocidal agents, *J. Chem. Med. Biol.*, 2015, 3, 138.
8. H. Hirumi and K. Hirumi, Continuous cultivation of *Trypanosoma brucei*, 2008, 3. 186–194.
9. N. Tiberti, A. Hainard and J.-C. Sanchez, Translation of human African trypanosomiasis biomarkers towards field application, *Transl. Prot.*, 2013, 12, 12–24.
10. L. MacLean, H. Reiber, P. Kennedy and J. Sternberg, Stage progression and neurological symptoms in *Trypanosoma brucei rhodesiense* sleeping sickness: role of the CNS inflammatory response, *PLOS Negl. Trop. Dis.*, 2012, 6, e1857.
11. P. Kennedy, The continuing problem of human African trypanosomiasis (sleeping sickness), *Ann. Neurol.*, 2008, 64, 116–126.
12. R. Brun, M. Rothert, B. Henke, L. Jeacock, D. Horn and E. Beitz, Pentamidine is not a perfumet but a nanomolar inhibitor of the *Trypanosoma brucei* aquaglyceroporin-2, *PLOS Pathog.*, 2016, 12, e1005436.
13. M. Banerjee, D. Paraia, P. Dhar, M. Roy, R. Barik, S. Chattopadhyay and J. Morris and D. Whitehead, Evaluation of substituted ebselen derivatives as potential trypanocidal agents, *J. Chem. Med. Biol.*, 2015, 3, 138.
14. E. Alford, D. Schump, J. Amici Heradi, A. Riedel, C. de Patoul, M. Quere and F. Chappuis, Nifurtimox-eflornithine combination therapy for second-stage gambiense human African trypanosomiasis: Médecins Sans Frontières experience in the Democratic Republic of Congo, *Clin. Infect. Dis.*, 2013, 56, 195–203.
15. R. Jacobs, B. Nare and M. Phillips, State of the art in African trypanosomiasis drug discovery, *Curr. Top. Med. Chem.*, 2011, 13, 1255–1274.
16. M. Barrett, D. Boykin, R. Brun and R. Tidwe, Human African trypanosomiasis: pharmacological re-engagement with a neglected disease, *Br. J. Pharmac.*, 2007, 152, 1155–1171.
17. D. Malvy and F. Chappuis, Sleeping sickness, *Clin. Microbiol. Infect.*, 2011, 17, 986–995.
18. G. Pohlig, S.C. Bernhard, J. Blum, C. Burri, A. Mponya, J-P. Fina Lubaki, A. Mpoto, B. Munungu, M. Bilenge, V. Miseu, J. Franco, N. Dituvanga, R. Tidweil and C. Olson, Efficacy and safety of pentamidine versus pentamidine maleate for treatment of first stage sleeping sickness in a randomized, comparator-controlled, international phase 3 clinical trial, *PLOS Negl. Trop. Dis.*, 2016, 10, e0004363.
19. P. Kennedy, Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness), *Lancet Neurol.*, 2013, 12, 186–194.
20. C. Burri, Chemotherapy against human African trypanosomiasis: Is there a road to success?, *Parasitol.*, 2010, 137, 1987–1994.
21. R. Betek, F. Smitt, R. Haynes and D. N’Da, Recent progress in the development of anti-malarial quinolones, *Mal. J.*, 2014, 13, 339.
22. E. Neenert, C. Burri and T. Shapiro, Antitrypanosomal activity of fluoroquinolones, *Antimicrob. Agents Chemother.*, 1999, 43, 2066–2068.
23. J. Keiser and C. Burri, Antitrypanosomal activities of fluoroquinolones with pyrrolidinyl substitutions, *Antimicrob. Agents Chemother.*, 2017, 61, e0003773.
24. E. Nenortas, C. Burri, T. Kulikowicz and T. Shapiro, Antitrypanosomal activities of fluoroquinolones with pyrrolidinyl substitutions, *Antimicrob. Agents Chemother.*, 2003, 47, 3015–3017.
25. G. Hilensperger, N. Jones, S. Niedermeier, A. Stich, M. Kaiser, J. Jung, S. Puhl, A. Damme, H. Braunschweig, L. Meinel, M. Engstler and U. Holzgrabe, Synthesis and structure-activity relationships of new quinolone-type molecules against *Trypanosoma brucei*, *J. Med. Chem.*, 2012, 55, 2538–2548.
26. A. Wube, A. Hufner, W. Seebacher, M. Kaiser, R. Brun, R. Bauer and F. Bucar, 1,2-Substituted 4-(1H)-quinolones: synthesis, antimarial and antitrypanosomal activities in vitro, *Molecules*, 2014, 19, 14204–14202.
27. R. Betek, D. Coertzen, F.J. Smit, L.-M. Birkholtz, R.K. Haynes and D.D. N’Da, Straightforward conversion of dequino- into inexpensive tractable new derivatives with significant anti-malarial activities, *Bioorg. Med. Chem. Lett.*, 2016, 26, 3006–3009.
28. H. Hirumi and K. Hirumi, Continuous cultivation of *Trypanosoma brucei* blood stream forms in a medium containing a low concentration of serum protein without feeder cell layers, *J. Parasitol.*, 1989, 75, 983–989.
Supplementary material to:

R.M. Beteck, M. Isaacs, H.C. Hoppe and S.D. Khanye,

Synthesis, \textit{in vitro} Cytotoxicity and Trypanocidal Evaluation of Novel 1,3,6-Substituted Non-fluoroquinolones

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Synthesis, in vitro cytotoxicity and trypanocidal evaluation of novel 1,3,6-substituted non-fluoroquinolones.

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NMR ($^1$H, $^{13}$C, DEPT135) AND MS SPECTRA AND BIOLOGICAL DATA OF COMPOUNDS

Compound 3a
Compound 3b
Compound 3c
Compound 3d
Compound 3e
Compound 3f
Compound 3g
Compound 3h
Compound 4a
COMPOUND 4c
COMPOUND 4d
Compound 4e

\[ \text{Diagram of Compound 4e} \]
Compound 4f
Compound 4g, HIT Compound
Compound 4h
Compound 4i
Figure S11a: Compounds inhibitory potential against *T. b. brucei* parasites at 20µM.

Compounds were added to *in vitro* cultures of *T. b. brucei* in 96-well plates at a fixed concentration of 20 µM. After an incubation period of 48 hours, the numbers of parasites surviving drug exposure were determined by adding resazurin. Reduction of resazurin to resorufin by living cells was quantified in a multiwell fluorescence plate reader (Exc560/Em590). The results are expressed as parasite % viability relative to untreated controls.
Compounds were tested in duplicate wells, and a standard deviation (SD) calculated. Only compounds exhibiting less than 20% parasite viability were considered for IC$_{50}$ determination.

Figure S11b: Compounds inhibitory potential against *T. b. brucei* parasites at 20µM.

Compounds were added to *in vitro* cultures of *T. b. brucei* in 96-well plates at a fixed concentration of 20 µM. After an incubation period of 48 hours, the numbers of parasites surviving drug exposure were determined by adding resazurin. Reduction of resazurin to resorufin by living cells was quantified in a multiwell fluorescence plate reader (Exc$_{560}$/Em$_{590}$). The results are expressed as parasite % viability relative to untreated controls. Compounds were tested in duplicate wells, and a standard deviation (SD) calculated. Only compounds exhibiting less than 20% parasite viability were considered for IC$_{50}$ determination.
Figure SI2a: Compound cytotoxicity against HeLa cells at 20µM.

Compounds were added to in vitro cultures of HeLa (human cervix adenocarcinoma) cells in 96-well plates at a fixed concentration of 20 µM. After an incubation period of 48 hours, the numbers of cells surviving drug exposure are determined by adding resazurin, which was reduced to resorufin by living cells. Resorufin was quantified in a multiwell fluorescence plate reader (Exc560/Em590). The results are express as cell % viability. Compounds were tested in duplicate wells, and a standard deviation (SD) calculated. With the exception of compound RB009, this series shows no extensive cytotoxicity against HeLa cells.
Figure SI2b: Compound cytotoxicity against HeLa cells at 20µM.

Compounds were added to in vitro cultures of HeLa (human cervix adenocarcinoma) cells in 96-well plates at a fixed concentration of 20 µM. After an incubation period of 48 hours, the numbers of cells surviving drug exposure are determined by adding resazurin which was reduced to resorufin by living cells. Resorufin is was quantified in a multiwell fluorescence plate reader (Exc560/Em590). The results are express as cell % viability. Compounds were tested in duplicate wells, and a standard deviation (SD) calculated. With the exception of compound RB025, this series shows no cytotoxicity against HeLa cells.