Supplementary material to:

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

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

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

Compound 3a
Compound 3c
Compound 3d
Compound 3e
Compound 3f
Compound 3g
Compound 3h
Compound 4a
Compound 4b
COMPOUND 4c
Compound 4e
Compound 4g, HIT Compound
Compound 4i
Compound 4J
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\textsubscript{50} determination.

![Trypanosome assay](image)

**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\textsubscript{560}/Em\textsubscript{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\textsubscript{50} determination.
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.