**A new approach to chemotherapy: drug-induced differentiation kills African trypanosomes**

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## Supplementary information tables

**Table S1. 212 hits and the data from the GUS differentiation and Alamar blue viability assays.** GUS activity S/B = GUS signal of drug treated samples to signal of background samples, optimal concentration for GUS activation, $IC_{50}$: 50% inhibitory concentration. NA: not applicable (there was no GUS signal). All assays were performed with a 40 h exposure time on *T. b. brucei.* GUSone. The initial parasite density was $7\times10^5$/ml in the GUS assay and $2.5\times10^5$/ml in the Alamar blue assay.

| PubChem ID | GUS activity [S/B] | optimal conc. for GUS [µg/ml] | Viability $IC_{50}$ [µg/ml] |
|---|---|---|---|
| 405_5923_0447 | 1.7 | 25 | 34.4 |
| 582_5690_8152 | 2.9 | 12.5 | 14.2 |
| 628_8463_9573 | 5.3 | 12.5 | 18.8 |
| 5105854 | 1.3 | 25 | 27.2 |
| 5259437 | 3.2 | 25 | 22.6 |
| 5352515 | 4.6 | 6.25 | 8.0 |
| 5426064 | 1.7 | 12.5 | 25.0 |
| 5527585 | 2.5 | 25 | >50 |
| 5729045 | 2.9 | 12.5 | 19.5 |
| 5569698 | 3.8 | 12.5 | 18.4 |
| 5734991 | 3.8 | 25 | 21.0 |
| 5740240 | 5.8 | 6.25 | 8.6 |
| 5742422 | 4.6 | 25 | 23.6 |
| 5742784 | 3.8 | 12.5 | 18.1 |
| 6367347 | 1.9 | 25 | 25.8 |
| 5879554 | 3.1 | 25 | 32.8 |
| 7493764 | 2.5 | 6.25 | 15.7 |
| 95703437 | 2.9 | 12.5 | 13.0 |
| BTB 14532 | 4.0 | 25 | 39.6 |
| CD 05692 | 1.2 | 12.5 | 26.0 |
| 8L-729 | 1.2 | 12.5 | 27.8 |
| 10L-630S | 1.1 | NA | >50 |
| 2K-091 | 1.7 | 12.5 | 22.0 |
| STOCK3S-02777 | 3.4 | 25 | 28.9 |
| STOCK2S-80114 | 1.8 | 25 | 25.3 |
| STOCK2S-82575 | 2.9 | 6.25 | 13.3 |
| STOCK2S-88445 | 1.9 | 25 | 12.8 |
| STOCK2S-89152 | 1.8 | 25 | 26.9 |
| STOCK2S-96769 | 1.0 | NA | 31.5 |
| STOCK2S-08783 | 2.6 | 6.25 | 12.6 |
| STOCK1S-31097 | 2.7 | 12.5 | 20.9 |

| | | | |
|---|---|---|---|
| STOCK1S-83980 | 3.4 | 12.5 | 20.8 |
| STOCK2S-40414 | 3.0 | 25 | 18.3 |
| STOCK2S-02216 | 1.3 | 6.25 | 7.5 |
| STOCK1S-06041 | 2.4 | 25 | 24.4 |
| STOCK3S-27791 | 2.8 | 12.5 | 23.5 |
| STOCK3S-28703 | 2.2 | 25 | 32.6 |
| STOCK1S-09923 | 3.8 | 3.125 | 1.9 |
| STOCK2S-13266 | 1.5 | 12.5 | 18.9 |
| STOCK1S-23169 | 1.2 | 12.5 | 18.9 |
| STOCK3S-17959 | 1.9 | 6.25 | 24.5 |
| STOCK3S-36150 | 4.4 | 12.5 | 10.4 |
| STOCK3S-44534 | 4.5 | 12.5 | 10.9 |
| STOCK3S-81258 | 2.2 | 6.25 | 13.6 |
| STOCK4S-16503 | 1.7 | 12.5 | 11.4 |
| STOCK1S-93356 | 1.6 | 25 | 28.4 |
| STOCK2S-38696 | 1.2 | 3.125 | 4.6 |
| STOCK2S-14011 | 2.2 | 6.25 | 10.1 |
| STOCK5S-44811 | 2.6 | 12.5 | 25.6 |
| STOCK5S-48033 | 2.0 | 25 | 23.7 |
| STOCK5S-48081 | 1.5 | 25 | >50 |
| STOCK5S-44766 | 3.0 | 12.5 | 25.6 |
| STOCK5S-54405 | 2.9 | 25 | 20.0 |
| STOCK5S-55771 | 3.4 | 12.5 | 10.4 |
| STOCK5S-77305 | 1.4 | 12.5 | 18.0 |
| STOCK5S-76783 | 2.2 | 6.25 | 16.3 |
| STOCK5S-72614 | 1.4 | 6.25 | 12.9 |
| STOCK5S-91387 | 5.2 | 12.5 | 10.3 |
| STOCK5S-68323 | 4.6 | 12.5 | 12.3 |
| STOCK5S-80549 | 3.2 | 12.5 | 14.3 |
| STOCK5S-83458 | 4.2 | 12.5 | 11.6 |
| STOCK5S-89031 | 2.4 | 12.5 | 19.2 |
| STOCK5S-96369 | 2.1 | 6.25 | 9.8 |
| STOCK6S-01740 | 6.1 | 25 | 32.6 |
| STOCK5S-50422 | 2.5 | 12.5 | 16.9 |
| STOCK5S-57687 | 1.7 | 25 | 25.8 |
| STOCK5S-89689 | 3.6 | 6.25 | 6.1 |
| STOCK5S-92524 | 2.3 | 12.5 | 18.2 |
| STOCK5S-90569 | 2.7 | 12.5 | 12.4 |
| STOCK5S-79972 | 1.6 | 25 | 26.4 |
| STOCK6S-14101 | 1.3 | 25 | >50 |
| STOCK6S-12050 | 1.4 | 25 | >50 |
| STOCK6S-10803 | 4.8 | 12.5 | 23.4 |
| STOCK6S-14165 | 5.6 | 6.25 | 14.6 |
| STOCK6S-12339 | 3.4 | 6.25 | 15.6 |
| STOCK6S-10284 | 4.1 | 6.25 | 17.1 |
| STOCK6S-06338 | 3.5 | 6.25 | 15.3 |
| STOCK6S-09080 | 2.2 | 12.5 | 25.4 |
| STOCK6S-18317 | 3.0 | 12.5 | 14.3 |
| STOCK6S-22702 | 2.2 | 25 | 48.8 |
| STOCK6S-27511 | 3.5 | 25 | 41.0 |
| STOCK6S-40884 | 0.9 | NA | 50.9 |
| STOCK6S-34290 | 3.1 | 6.25 | 7.7 |
| STOCK1N-21603 | 2.3 | 6.25 | 7.3 |
| AE-641/30156002 | 3.9 | 6.25 | 7.1 |
| AH-034/08306008 | 1.2 | 25 | 45.2 |
| AG-690/36516036 | 1.0 | NA | >50 |
| AG-690/36644020 | 1.0 | NA | >50 |
| AK-249/36810004 | 1.2 | 25 | >50 |

| | | | |
|---|---|---|---|
| AC-907/34131044 | 0.9 | NA | >50 |
| AE-842/34026055 | 1.2 | 25 | 30.4 |
| AE-842/34646010 | 0.9 | NA | 40.8 |
| STOCK4S-90544 | 3.4 | 3.125 | 8.1 |
| STOCK4S-57232 | 3.7 | 12.5 | 13.6 |
| STOCK4S-54253 | 4.1 | 12.5 | 10.8 |
| STOCK4S-82648 | 1.3 | 25 | 26.1 |
| STOCK5S-20823 | 1.7 | 25 | 19.6 |
| STOCK5S-06163 | 2.9 | 6.25 | 7.3 |
| STOCK3S-37136 | 2.9 | 6.25 | 7.0 |
| STOCK5S-49612 | 1.4 | 3.125 | 4.4 |
| STOCK4S-66388 | 0.9 | NA | 26.4 |
| STOCK5S-13066 | 2.8 | 25 | 33.1 |
| STOCK5S-18906 | 2.2 | 12.5 | 28.9 |
| STOCK5S-25534 | 1.4 | 12.5 | 31.0 |
| STOCK5S-18704 | 1.6 | 25 | 26.0 |
| STOCK5S-38263 | 3.2 | 6.25 | 12.4 |
| STOCK5S-28510 | 1.1 | NA | 44.4 |
| BRZ127:5:E7 | 2.2 | 6.25 | 17.5 |
| CAK201:43:E1 | 1.0 | NA | >50 |
| T5473027 | 1.5 | 6.25 | 8.8 |
| T5817819 | 2.2 | 6.25 | 15.3 |
| T5314847 | 4.8 | 12.5 | 19.4 |
| T0520-7534 | 1.3 | 25 | >50 |
| T5990785 | 1.0 | NA | 42.9 |
| T0509-0656 | 1.1 | NA | >50 |
| T5407434 | 1.4 | 6.25 | 12.5 |
| T5845297 | 3.2 | 6.25 | 12.3 |
| T0505-5074 | 3.4 | 12.5 | 10.2 |
| T5227647 | 5.4 | 6.25 | 6.5 |
| T5263000 | 3.7 | 6.25 | 8.4 |
| T5544185 | 1.3 | 25 | 39.2 |
| T5410513 | 1.1 | NA | 42.5 |
| T5547254 | 1.1 | NA | 53.7 |
| T5548412 | 1.4 | 25 | 46.6 |
| T5621982 | 3.9 | 3.125 | 4.5 |
| T5703243 | 1.1 | NA | >50 |
| T5801187 | 1.2 | 25 | 47.8 |
| T5848463 | 1.6 | 25 | 46.1 |
| T5929635 | 1.7 | 25 | 25.7 |
| T6048356 | 1.4 | 25 | 26.4 |
| T6092106 | 1.2 | 25 | 43.4 |
| T6224950 | 1.0 | NA | >50 |
| T6225499 | 1.0 | NA | >50 |
| T6240036 | 2.4 | 12.5 | 23.7 |
| T0505-3851 | 2.8 | 25 | 38.8 |
| T0515-2003 | 4.3 | 25 | 22.6 |
| T5226301 | 1.0 | NA | 22.1 |
| T5383989 | 1.2 | 25 | >50 |
| T5383642 | 1.2 | 12.5 | 26.2 |
| T5540719 | 1.3 | 25 | 30.2 |
| T5541143 | 1.0 | NA | 26.1 |
| T5985287 | 1.3 | 12.5 | 23.4 |
| T6066027 | 1.5 | 12.5 | 27.1 |
| T5650357 | 3.0 | 25 | 34.3 |
| T5700438 | 3.1 | 6.25 | 8.3 |
| T5731482 | 3.0 | 25 | 42.0 |
| T5734439 | 1.6 | 25 | 22.4 |

| | | | |
|---|---|---|---|
| T5872380 | 3.0 | 25 | 26.4 |
| T5872712 | 1.6 | 25 | 42.5 |
| T5872760 | 1.5 | 12.5 | 30.9 |
| T6004974 | 1.2 | 25 | 37.0 |
| T6117781 | 3.7 | 25 | 31.8 |
| T6214849 | 4.9 | 12.5 | 15.3 |
| T6248417 | 1.2 | 25 | 41.6 |
| T6238737 | 1.2 | 25 | 39.7 |
| 5144511 | 1.0 | NA | >5 |
| 5421628 | 1.0 | NA | >5 |
| 5546507 | 1.0 | NA | >5 |
| 5571547 | 1.0 | NA | >5 |
| 5574341 | 1.0 | NA | >5 |
| 5618307 | 1.0 | NA | >5 |
| 6049586 | 1.0 | NA | >5 |
| 6155812 | 1.0 | NA | >5 |
| 7821616 | 1.0 | NA | >5 |
| 7978712 | 0.9 | NA | >5 |
| CGX-02167237 | 1.0 | NA | >5 |
| CGX-03309303 | 1.0 | NA | >5 |
| 033_0221_0172 | 1.0 | NA | >5 |
| 11G-031 | 1.0 | NA | >5 |
| STOCK3S-28947 | 1.0 | NA | 2.7 |
| STOCK3S-00823 | 1.1 | NA | >5 |
| STOCK1S-65123 | 1.0 | NA | >5 |
| STOCK1S-64351 | 1.1 | NA | >5 |
| STOCK3S-11937 | 2.9 | 2.5 | 2.1 |
| STOCK3S-76410 | 2.3 | 2.5 | 3.0 |
| STOCK1S-93075 | 1.1 | NA | >5 |
| STOCK1S-54857 | 0.9 | 2.5 | >5 |
| STOCK4S-38774 | 1.0 | NA | >5 |
| STOCK4S-62985 | 1.4 | 2.5 | 4.0 |
| STOCK6S-15817 | 4.4 | 1.25 | 1.5 |
| STOCK4S-60288 | 1.0 | NA | 3.2 |
| STOCK5S-42531 | 3.3 | 0.625 | 1.2 |
| STOCK5S-82907 | 4.3 | 1.25 | 1.6 |
| STOCK5S-69744 | 1.0 | NA | >5 |
| STOCK5S-85745 | 1.0 | NA | >5 |
| STOCK6S-01108 | 1.0 | NA | >5 |
| STOCK6S-03252 | 4.2 | 1.25 | >5 |
| STOCK6S-41308 | 1.0 | NA | >5 |
| STOCK6S-32250 | 2.8 | 0.313 | 0.3 |
| STOCK6S-37013 | 4.1 | 1.25 | 1.6 |
| STOCK6S-42437 | 4.3 | 1.25 | 1.5 |
| STOCK6S-38758 | 2.9 | 0.313 | 0.6 |
| STOCK6S-35180 | 3.4 | 0.625 | 1.6 |
| STOCK6S-41114 | 1.1 | NA | >5 |
| AF-399/09020026 | 0.9 | NA | >5 |
| STOCK1S-31919 | 4.2 | 0.313 | 0.8 |
| STOCK1S-42137 | 5.7 | 0.156 | 0.2 |
| STOCK1S-13735 | 12.4 | 12.5 | 13.6 |
| STOCK1S-04771 | 7.0 | 12.5 | 11.6 |
| STOCK3S-56977 | 0.9 | NA | 18.1 |
| STOCK3S-92457 | 2.3 | 6.25 | 13.0 |
| STOCK3S-87227 | 1.4 | 12.5 | 17.9 |
| STOCK4S-04391 | 8.5 | 12.5 | >25 |
| STOCK1S-13266 | 1.5 | 12.5 | >25 |
| 5464668 | 3.9 | 5 | 5.0 |

| | | | |
|---|---|---|---|
| 5558382 | 5.2 | 1.25 | 1.3 |
| 6374290 | 2.0 | 5 | 5.7 |
| STOCK4S-44678 | 0.6 | NA | 2.3 |
| STOCK6S-03600 | 1.5 | 10 | 8.8 |
| STOCK3S-38373 | 1.5 | 5 | 7.3 |
| STOCK4S-34434 | 2.3 | 0.625 | 5.7 |
| T5496829 | 1.5 | 2.5 | 2.3 |

**Table S2.** Viability of *T. b. gambiense* STIB930, *T. b. brucei* GUSone and *T. b. rhodesiense* STIB900 after 40 hours drug exposure. The initial parasite density was $3.5\times10^5$/ml in the Alamar blue assay.

| Compound | STIB930 $IC_{50}$ [µg/ml] | STIB900 $IC_{50}$ [µg/ml] | GUSone $IC_{50}$ [µg/ml] |
|---|---|---|---|
| DIP-01 | 25.5 | 26.9 | 27.1 |
| DIP-02 | 0.42 | 0.66 | 0.97 |
| DIP-03 | 0.04 | 0.11 | 0.15 |
| DIP-04 | 28.3 | 37.6 | 36.1 |
| DIP-05 | 3.1 | 2.9 | 8.9 |
| DIP-06 | 0.24 | 0.56 | 2.94 |
| DIP-07 | 0.25 | 0.40 | 0.95 |
| DIP-08 | 2.7 | 2.3 | 7.5 |
| DIP-09 | 3.6 | 8.3 | 19.2 |
| DIP-10 | 0.89 | 0.96 | 2.11 |
| DIP-11 | 2.0 | 1.1 | 5.4 |
| DIP-12 | 3.6 | 4.1 | 10.0 |
| DIP-13 | 24.5 | 4.6 | 5.4 |
| DIP-14 | 9.9 | 8.7 | 26.2 |
| DIP-15 | 3.6 | 4.6 | 9.2 |
| DIP-16 | 1.7 | 3.8 | 13.1 |
| DIP-17 | 3.3 | 3.9 | 8.2 |
| DIP-18 | 4.8 | 3.5 | 7.1 |
| DIP-19 | 0.22 | 0.24 | 0.61 |
| DIP-20 | 2.4 | 3.2 | 8.5 |
| DIP-21 | 0.83 | 1.37 | 3.98 |
| DIP-22 | 1.1 | 1.5 | 3.9 |
| DIP-23 | 2.1 | 2.9 | 9.3 |
| DIP-24 | 1.4 | 1.2 | 2.3 |
| DIP-25 | 3.0 | 2.6 | 4.9 |
| DIP-26 | 1.7 | 2.0 | 3.6 |
| DIP-27 | 2.4 | 4.3 | 6.7 |
| DIP-28 | 1.7 | 2.2 | 4.7 |
| Melarsoprol | 0.006 | 0.008 | 0.009 |
| Pentamidine | 0.003 | 0.005 | 0.006 |

**Table S3. VSG loss triggered by *cis*-aconitate (CA).** Dose response of CA (5 – 20 mM). Triplicate samples were analysed by flow cytometry. The median fluorescence intensity (MFI) was calculated for all datasets and is given in the table together with the corresponding means and standard deviations. The ratio of the mean of CA treated cells to untreated cells is given as T/B. StDev: standard deviation.

| | | 18 hours | | | | | | 24 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. mM | Triplicates of MFI | | | Mean | StDev | T/B | Triplicates of MFI | | | Mean | StDev | T/B |
| untreated | | 61.0 | 61.4 | 56.9 | 59.8 | 2.5 | B | 38.0 | 47.2 | 48.3 | 44.5 | 5.7 | B |
| CA | 5 | 156 | 144 | 138 | 146.0 | 9.2 | 2.4 | 137 | 143 | 128 | 136.0 | 7.5 | 3.1 |
| | 10 | 228 | 233 | 235 | 232.0 | 3.6 | 3.9 | 310 | 355 | 306 | 323.7 | 27.2 | 7.3 |
| | 20 | 311 | 341 | 313 | 321.7 | 16.8 | 5.4 | 441 | 472 | 396 | 436.3 | 38.2 | 9.8 |

**Table S4. VSG loss for DIP compounds.** For all compounds and concentrations indicated triplicate samples were analysed by flow cytometry. The median fluorescence intensity (MFI) was calculated for all datasets and is given in the table together with the corresponding means and standard deviations. The ratio of the mean of treated cells to untreated cells is given as T/B. *CA was tested at 20 mM. StDev: standard deviation.

| | | 18 hours | | | | | | 24 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. µg/ml | Triplicates of MFI | | | Mean | StDev | T/B | Triplicates of MFI | | | Mean | StDev | T/B |
| untreated | | 77.4 | 93.3 | 88.4 | 86.4 | 8.1 | B | 18.7 | 33.8 | 78.4 | 43.6 | 31.0 | B |
| CA | 20* | 357 | 259 | 431 | 349.0 | 86.3 | 4.0 | 598 | 698 | 779 | 691.7 | 90.7 | 15.9 |
| DIP-01 | 5 | 135 | 165 | 229 | 176.3 | 48.0 | 2.0 | 131 | 173 | 189 | 164.3 | 30.0 | 3.8 |
| | 10 | 196 | 235 | 247 | 226.0 | 26.7 | 2.6 | 161 | 259 | 242 | 220.7 | 52.4 | 5.1 |
| | 20 | 393 | 393 | 415 | 400.3 | 12.7 | 4.6 | 469 | 482 | 495 | 482.0 | 13.0 | 11.1 |
| DIP-02 | 0.156 | 124 | 116 | 93.5 | 111.2 | 15.8 | 1.3 | 121 | 111 | 77.9 | 103.3 | 22.6 | 2.4 |
| | 0.323 | 150 | 120 | 112 | 127.3 | 20.0 | 1.5 | 118 | 124 | 99.1 | 113.7 | 13.0 | 2.6 |
| | 0.625 | 384 | 393 | 391 | 389.3 | 4.7 | 4.5 | 359 | 523 | 482 | 454.7 | 85.3 | 10.4 |
| DIP-03 | 0.039 | 71.2 | 83.9 | 85.2 | 80.1 | 7.7 | 0.9 | 20.3 | 40.3 | 74.3 | 45.0 | 27.3 | 1.0 |
| | 0.078 | 120 | 108 | 94.1 | 107.4 | 13.0 | 1.2 | 24.5 | 47.5 | 69.9 | 47.3 | 22.7 | 1.1 |
| | 0.156 | 477 | 528 | 485 | 496.7 | 27.4 | 5.7 | 292 | 352 | 343 | 329.0 | 32.4 | 7.5 |
| DIP-07 | 0.625 | 104 | 114 | 119 | 112.3 | 7.6 | 1.3 | 65.1 | 27.7 | 24.7 | 39.2 | 22.5 | 0.9 |
| | 1.25 | 172 | 138 | 145 | 151.7 | 18.0 | 1.8 | 116 | 75.3 | 39.4 | 76.9 | 38.3 | 1.8 |
| | 2.5 | 615 | 614 | 649 | 626.0 | 19.9 | 7.2 | 588 | 530 | 552 | 556.7 | 29.3 | 12.8 |
| DIP-19 | 0.323 | 112 | 76.9 | 91.2 | 93.4 | 17.7 | 1.1 | 147 | 139 | 135 | 140.3 | 6.1 | 3.2 |
| | 0.625 | 114 | 116 | 68.8 | 99.6 | 26.7 | 1.2 | 161 | 166 | 151 | 159.3 | 7.6 | 3.7 |
| | 1.25 | 200 | 185 | 232 | 205.7 | 24.0 | 2.4 | 390 | 254 | 267 | 303.7 | 75.0 | 7.0 |

**Table S5. VSG loss of standard drugs.** For all compounds and concentrations indicated triplicate samples were analysed by flow cytometry. The median fluorescence intensity (MFI) was calculated for all datasets and is given in the table together with the corresponding means and standard deviations. The ratio of the mean of treated cells to untreated cells is given as T/B. *CA was tested at 20 mM. StDev: standard deviation.

| | | 24 hours | | | | | |
|---|---|---|---|---|---|---|---|
| | Conc. ng/ml | Triplicates of MFI | | | Mean | StDev | T/B |
| untreated | | 13.9 | 17.8 | 16.5 | 16.1 | 2.0 | B |
| CA | 20* | 109 | 170 | 106 | 128.3 | 36.1 | 8.0 |
| Pentamidine | 1.25 | 16.7 | 15.5 | 14.4 | 15.5 | 1.2 | 1.0 |
| | 2.5 | 18.9 | 19.1 | 22.1 | 20.0 | 1.8 | 1.2 |
| | 5 | 73.7 | 70.4 | 63.9 | 69.3 | 5.0 | 4.3 |
| Melarsoprol | 5 | 7.14 | 10.1 | 8.88 | 8.7 | 1.5 | 0.5 |
| | 10 | 26.9 | 25.3 | 16.9 | 23.0 | 5.4 | 1.4 |
| | 20 | 168 | 174 | 149 | 163.7 | 13.1 | 10.2 |
| Nifurtimox | 2,500 | 11.9 | 11.9 | 10.9 | 11.6 | 0.6 | 0.7 |
| | 5,000 | 49.5 | 41.9 | 40.6 | 44.0 | 4.8 | 2.7 |
| | 10,000 | 225 | 245 | 222 | 230.7 | 12.5 | 14.3 |

**Table S6. Primers used in quantitative reverse transcription PCR.**

| **Primer name** | **Sequence** | **G**ene(s) amplified |
|---|---|---|
| **C1_fwd_qPCR** | ttgtgacgacgagagcaaac | Tb927.10.12970 |
| **C1_rev_qPCR** | agtggttgaacgccaaatgc | ditto |
| **Proc_f_qPCR** | tggcacctcgttccctttatc | Tb927.6.450, Tb927.6.480, Tb927.6.510, Tb927.6.520, Tb927.10.10250, Tb927.10.10260 |
| **Proc_r_qPCR** | aaatcccacgccagagaagag | ditto |

## Supplementary information figures

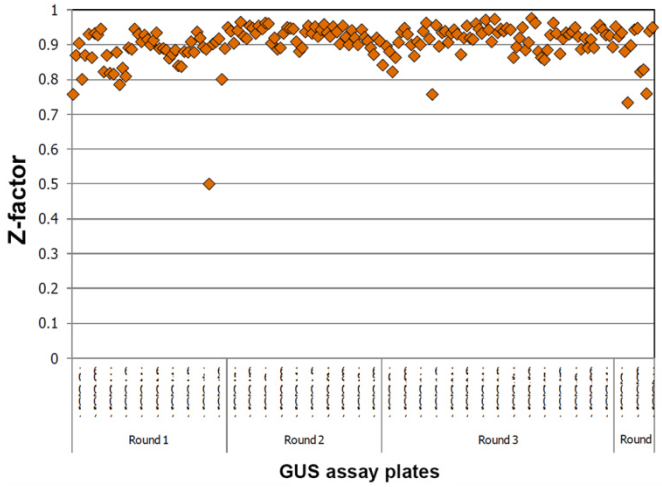



**Figure S1. Z'-factor of each plate in the MTS.** The high Z'-factor of 0.9 in average indicates a highly robust assay. Controls: trypanosomes with *cis*-aconitate (CA) served as the positive control and trypanosomes without any drug served as the negative control.

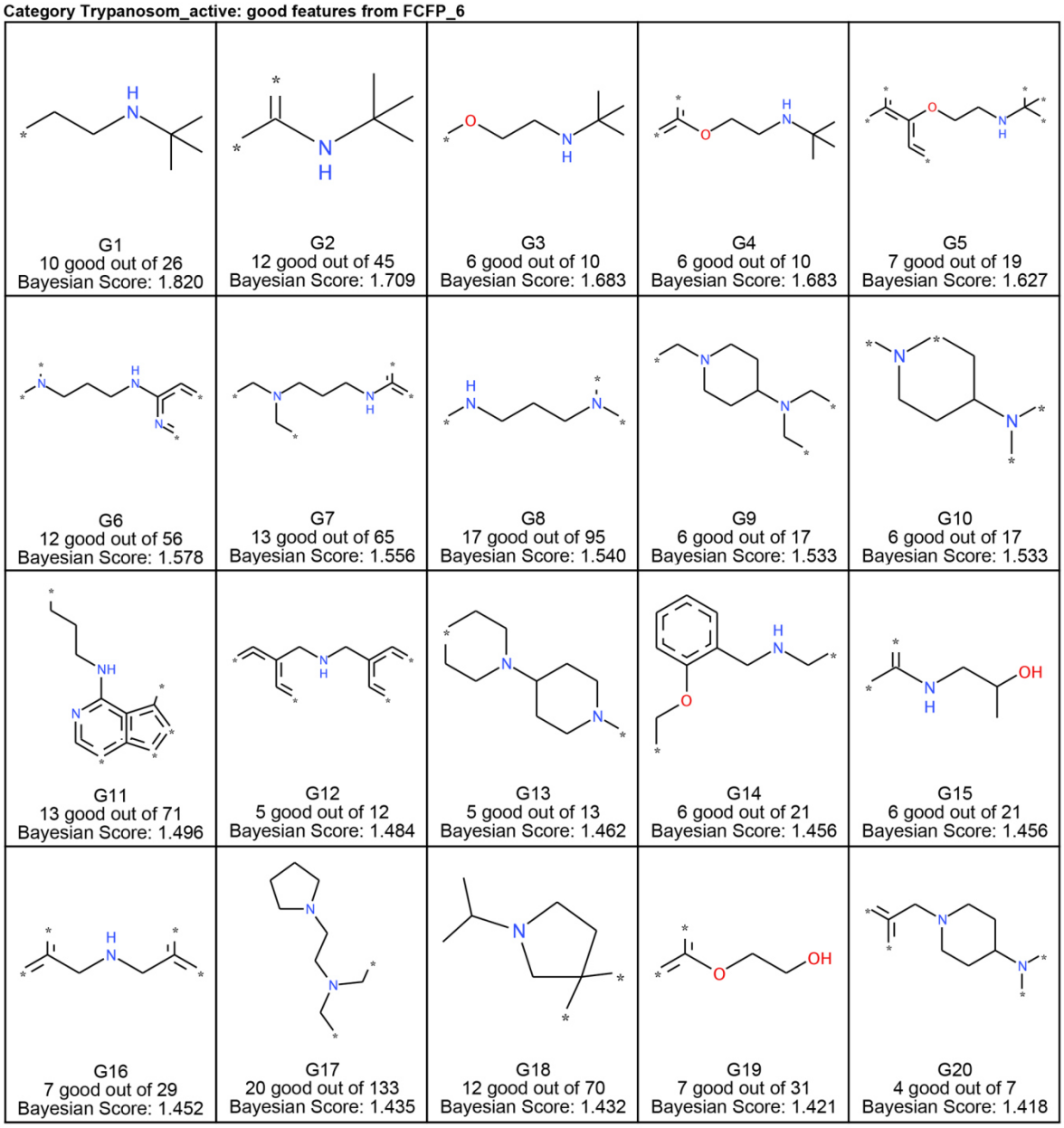
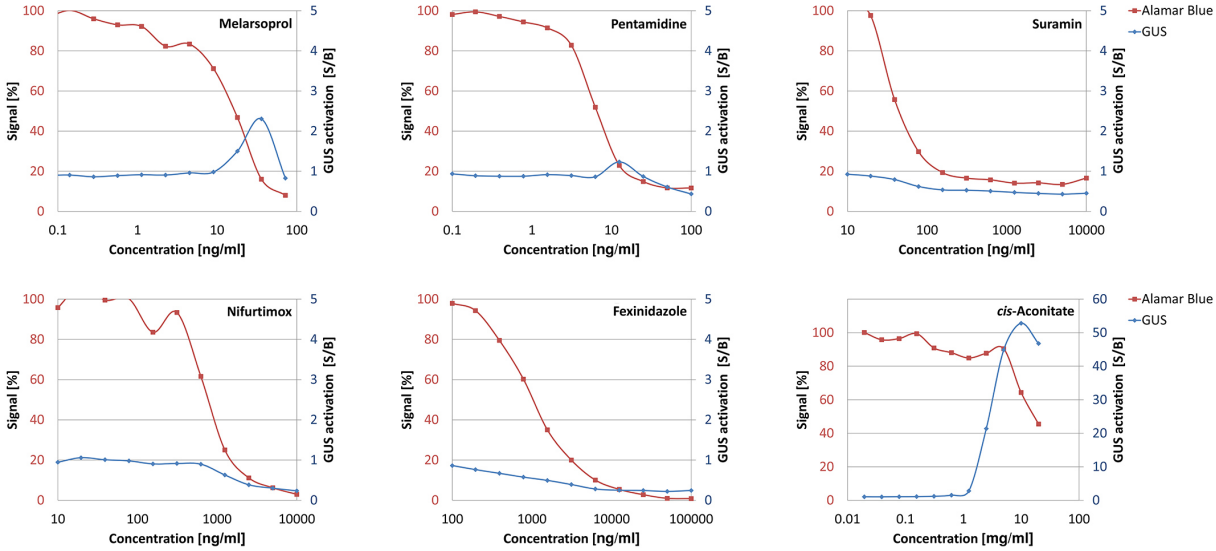



**Figure S2. Substructure features significantly enriched in compounds activating GUS compared to the total test set of 7495 compounds.** The 212 active compounds and all other compounds screened were designated as active and inactive, respectively. Default descriptors including FCFP_6 fingerprints were used as molecular descriptors to identify features enriched in the active compounds, using the Pipeline Pilot implementation of a two-class Bayesian categorization model (Biovia, San Diego, USA).

**Figure S3: Anti-trypanosomal control drugs tested in the GUS reporter assay and the viability assay.** The standard drugs melarsoprol, pentamidine, suramin, nifurtimox and fexinidazole were tested for viability (Alamar Blue) and for GUS activation with a 40 hour drug exposure time and initial parasite density of $5\times10^5$ GUSone trypanosomes /ml. All compounds were tested over a ≥1,000-fold concentration range with 2x dilution steps. The dose response plots are from a single experiment. Among the standard trypanocidal drugs tested, only melarsoprol gave a GUS signal (S/B>2). No GUS activation was observed following exposure to pentamidine, suramin, nifurtimox and fexinidazole, including non-toxic concentrations. At highly trypanocidal concentrations the GUS signal is below the background signal (trypanosomes without drug) resulting in S/B ratio <1.



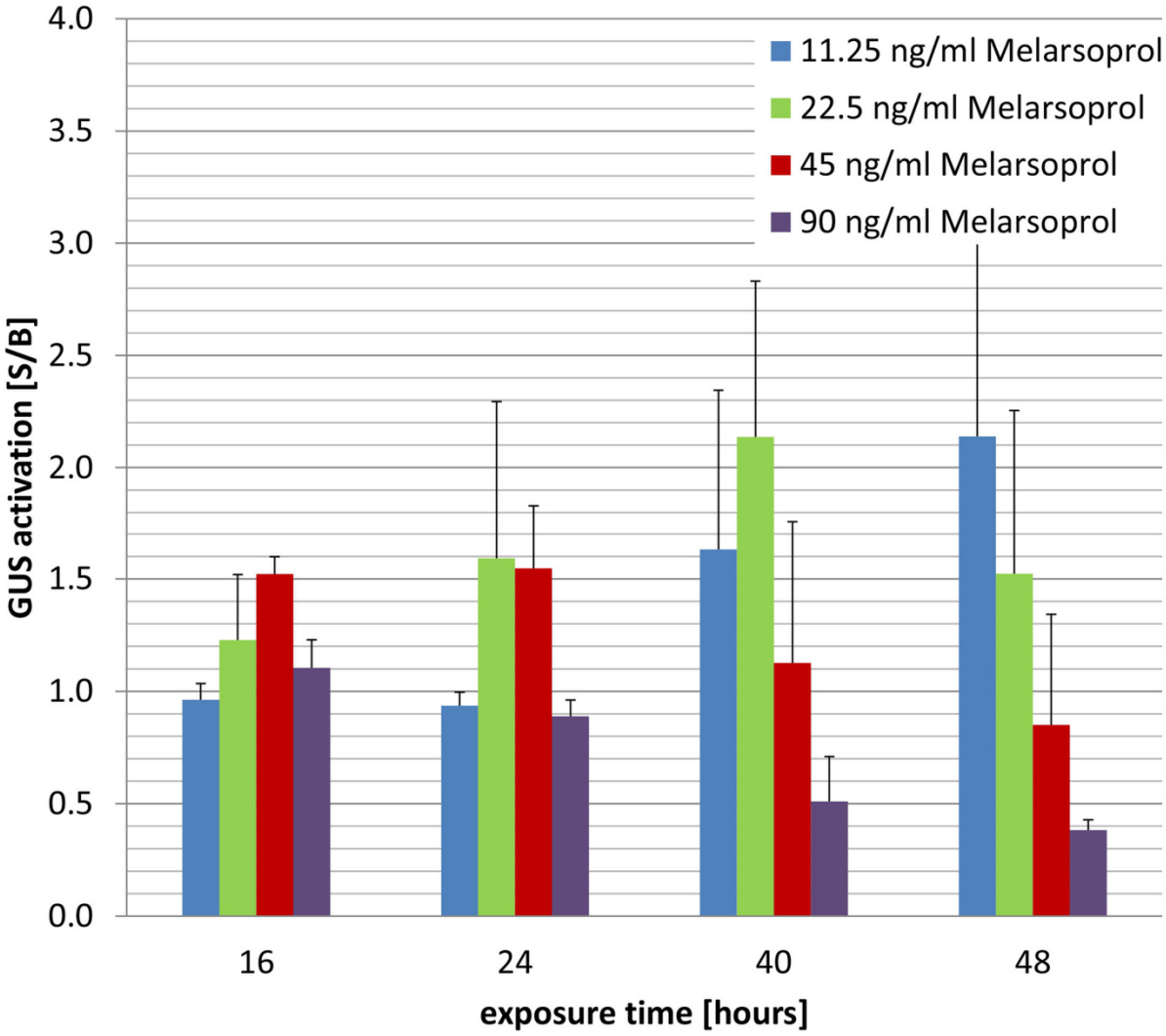



**Figure S4. Melarsoprol induces GUS expression up to 2x over background.** The starting trypanosome density was $8\times10^5$ parasites/ml for 16 h and 24 h, $5\times10^5$ parasites/ml for 40 h and $2.5\times10^5$ parasites/ml for 48 h exposure. The S/B was 2 fold at 22.5 ng/ml after 40 h exposure or at 11.25 ng/ml after 48 h. S/B: Ratio of GUS signal of melarsoprol-treated trypanosomes to GUS signal of untreated trypanosomes. Bars indicate standard deviations of S/B from at least three independent assays.

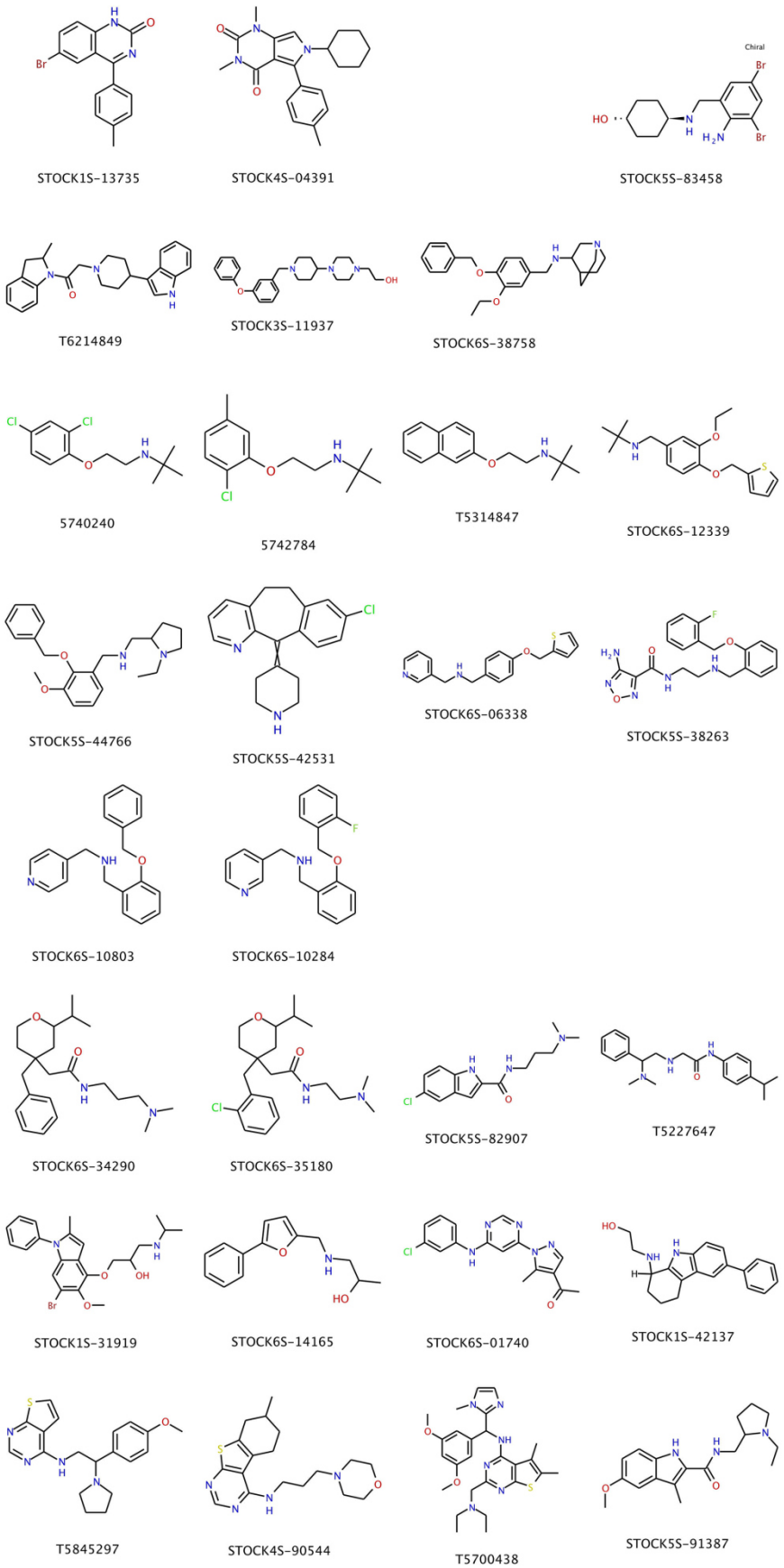



**Figure S5. Chemical structures of 28 DIP-compounds with PubChem ID.** Structures were grouped by similar features or certain sub-sets.

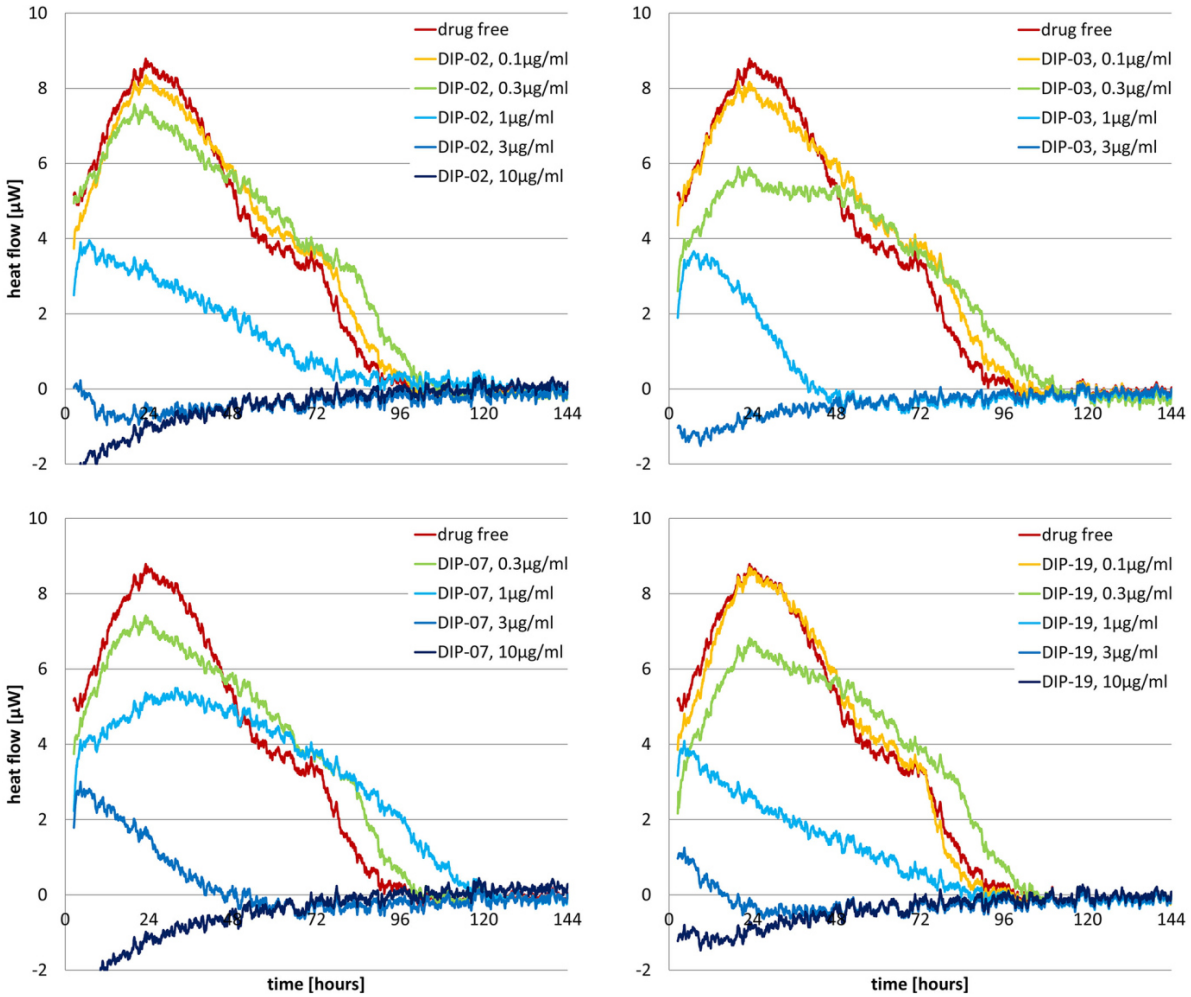



**Figure S6. Microcalorimetric analysis of 4 DIP compounds at various concentrations.** Details of the assay are provided in the methods section. The time to kill was short: at the highest concentration the DIP compounds killed over 99% of the cells before the equilibration time (i.e. ≤4 h). At the second highest concentration the parasites were killed within 48 h. DIP-01 is insoluble at the high concentrations required for fast killing and was therefore omitted from the experiment.

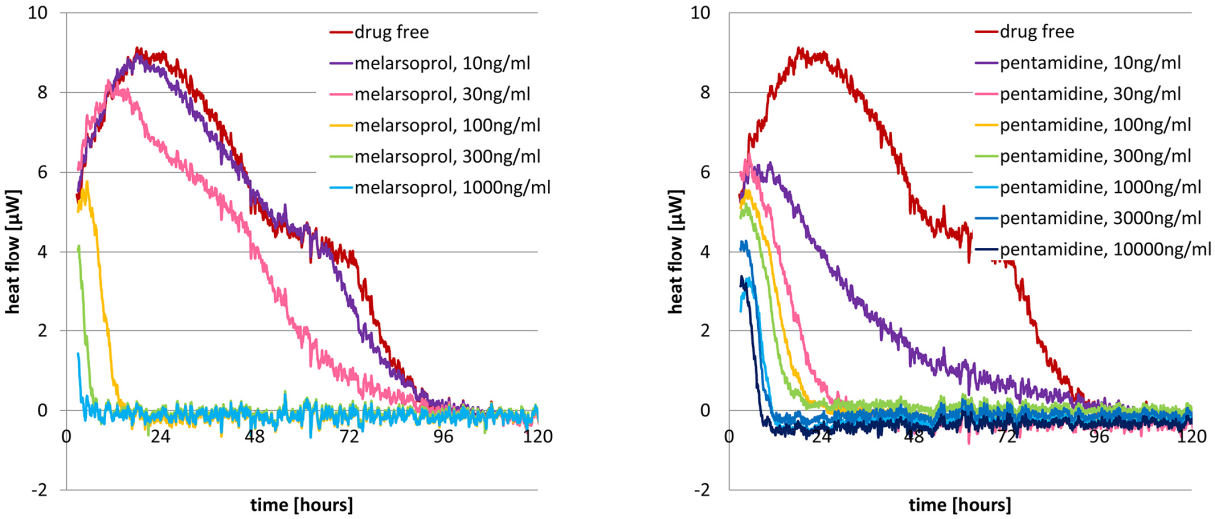



**Figure S7. Microcalorimetric analysis of trypanocidal control compounds at various concentrations.** Dose dependency and time of drug action are different for melarsoprol and pentamidine. Pentamidine is slower acting at high drug concentrations (>10-fold $IC_{50}$) and a wider concentration range is required to cover low to strong inhibition than for melarsoprol and the 4 DIP compounds (Figure S6).

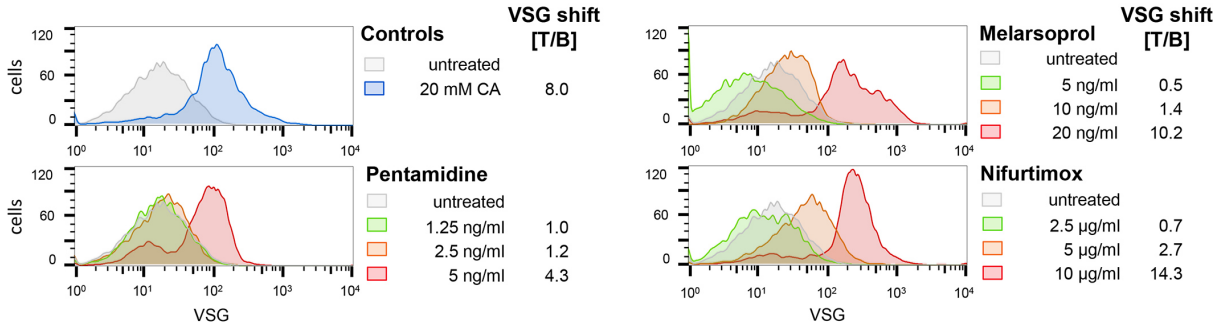



**Figure S8. Loss of VSG of standard drugs at 37°C.** The density of the VSG coat was monitored by staining with anti-VSG221 antibodies and flow cytometry. Assays were performed in triplicate with a 24 h drug exposure time. To display the dose-dependent VSG shifts, representative curves of different drug concentrations were plotted together with the corresponding untreated control. MFI: median fluorescence intensity. T/B: VSG changes are expressed as a ratio of the MFI after treatment to the MFI of the untreated control. Full flow cytometry data are available in Table S5.