Table S1. Cytotoxicity and antiviral activity of phenyl-benzotriazoles against HTNV

| Series 1 compounds R | Vero E6 CC50a | HTNV EC50b | Vero-76 CC50c | MT-4 CC50d | MDBK CC50e | BHK-21 CC50f |
|----------------------|---------------|-------------|---------------|------------|------------|-------------|
| a NH2                | >30           | >30         | >100          | >100       | >100       | >100        |
| b NHCOCH3            | 20            | >20         | 30            | 35         | 43         | 53          |
| c NHCOCH2CH3         | 20            | >20         | 30            | 28         | ≥100       | 54          |
| e N(COCH2CH2CH3)2    | 25            | >25         | 20            | 35         | 14         | 16          |
| f NHCO-4-CH3-Ph      | >30           | >30         | >100          | >100       | >100       | ≥100        |
| g NHCO-4-Cl-Ph       | >30           | ≥30         | ≥100          | >100       | >100       | >100        |
| h NHCO-4-NO2-Ph      | >30           | 21          | >100          | >100       | >100       | 96          |
| i NHCO-4-OCH3-Ph     | >30           | >30         | >100          | 33         | >100       | >100        |
| j NHCO-3,4,5-OCH3-Ph | >30           | 90          | 77            | >100       | >100       | >100        |

| Series 2 compounds   |               |             |               |            |            |              |
|----------------------|---------------|-------------|---------------|------------|------------|-------------|
| a NH2                | >30           | >30         | >100          | 52         | ≥100       | >100        |
| b NHCOCH3            | >30           | >30         | >100          | >100       | >100       | >100        |
| c NHCOCH2CH3         | >30           | >30         | >100          | >100       | >100       | >100        |
| d N(COCH2CH2CH3)2    | >30           | ≥30         | ≥100          | 15         | 73         | 26          |
| e N(COCH2CH2CH3)2    | >30           | >30         | >100          | 24         | 84         | 62          |
| f NHCO-4-CH3-Ph      | >30           | 22          | >100          | >100       | >100       | >100        |
| g NHCO-4-Cl-Ph       | >30           | >30         | 95            | >100       | >100       | >100        |
| h NHCO-4-NO2-Ph      | >30           | >30         | >100          | >100       | >100       | >100        |
| j NHCO-3,4,5-OCH3-Ph | >30           | 4 (>7.5)    | 80            | 63         | >100       | 35          |
| k NHCONHC2H5CH3      | >30           | 26          | >100          | >100       | >100       | >100        |
| l NHCONH(CH2)2CH3    | 30            | 6 (>6)      | 30            | >100       | 78         | 40          |
| m NHCONH(CH2)2CH3    | >30           | >30         | 95            | >100       | >100       | >100        |
| n NHCONH-cyclohexyl  | >30           | 4 (>7.5)    | 90            | >100       | >100       | 71          |

| Series 3 compounds   |               |             |               |            |            |              |
|----------------------|---------------|-------------|---------------|------------|------------|-------------|
| f NHCO-4-CH3-Ph      | >30           | >30         | >100          | >100       | >100       | >100        |
| j NHCO-3,4,5-OCH3-Ph | >30           | >30         | >100          | >100       | >100       | >100        |
| k NHCONHC2H5CH3      | >30           | >30         | >100          | nd         | >100       | >100        |
| l NHCONH(CH2)2CH3    | >30           | >30         | >100          | >100       | >100       | >100        |
| m NHCONH(CH2)2CH3    | >30           | >30         | >100          | >100       | >100       | >100        |
| n NHCONH-cyclohexyl  | >30           | >30         | 95            | >100       | >100       | >100        |

| Series 4 compounds   |               |             |               |            |            |              |
|----------------------|---------------|-------------|---------------|------------|------------|-------------|
| f NHCO-4-CH3-Ph      | >30           | >30         | >100          | >100       | >100       | >100        |
| j NHCO-3,4,5-OCH3-Ph | >30           | >30         | >100          | nd         | >100       | >100        |
| k NHCONHC2H5CH3      | >30           | >30         | >100          | >100       | >100       | >100        |
| l NHCONH(CH2)2CH3    | >30           | >30         | >100          | >100       | >100       | >100        |
| m NHCONH(CH2)2CH3    | >30           | >30         | >100          | >100       | >100       | >100        |
| n NHCONH-cyclohexyl  | >30           | >30         | 95            | >100       | >100       | >100        |

Reference compound

Ribavirin RBV >100 37 (>2)

Data represent mean values ± SD for three independent determinations. For values where SD is not shown, variation among triplicate samples was less than 15%. Results for active compounds are in bold character.

*Compound concentration (µM) affecting the morphology of Vero-E6 monolayers, as determined by optical microscope examination.

*Compound concentration (µM) required to reduce the foci number of HTNV by 50% in Vero-E6 monolayers.

*Compound concentration (µM) required to reduce the viability of mock-infected MT-4, Vero-76, MDBK and BHK21 cells by 50%, as determined by the MTT method.

( ) Selectivity Index
Cells

Cell lines were purchased from American Type Culture Collection (ATCC): Vero E6 cells (ATCC CRL 1586) [B. Klempa, P. T. Witkowski, E. Popugaeva, B. Auste, L. Koivogui, E. Fichet-Calvet, T. Strecker, J. ter Meulen, D. H. Krüger, Sangassou Virus, the First Hantavirus Isolate from Africa, Displays Genetic and Functional Properties Distinct from Those of Other Murinae-Associated Hantaviruses. J Virol. (2012); 86(7): 3819–3827]; CD4+ human T-cells containing an integrated HTLV-1 genome (MT-4) ; Madin Darby Bovine Kidney (MDBK) [ATCC CCL 22 (NBL-1) Bos Taurus]; Baby Hamster Kidney (BHK-21) [ATCC CCL 10 (C-13) Mesocricetus auratus]; Monkey kidney (Vero-76) [ATCC CRL 1587 Cercopithecus Aethiops] [G. Sanna, S. Madeddu, G. Giliberti, S. Piras, M. Struga, M. Wrzosek, G. Kubiak-Tomaszewksa, A.E Koziol,. O. Savchenko, T. Lis, J. Stefanska, P. Tomaszewski, M. Skrzypcki, D. Szulczyk, Synthesis and Biological Evaluation of Novel Indole-Derived Thioureas. Molecules (2018), 23, 2554].

Cytotoxicity assays

Exponentially growing MT-4 cells were seeded at an initial density of 4x10^5 cells/ml in 96-well plates in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin G and 100 μg/mL streptomycin. MDBK, BHK were seeded in 96-well plates at an initial density of 6x10^5 and 1x10^6, respectively, in Minimum Essential Medium with Earle’s salts (MEM-E), L-glutamine, 1mM sodium pyruvate and 25 mg/L kanamycin, supplemented with 10% horse serum (MDBK) or 10% fetal bovine serum (FBS) (BHK-21). Vero-76 cells were seeded in 96-well plates at an initial density of 5x10^5 cells/mL, in Dulbecco’s Modified Eagle Medium (D-MEM) with L-glutamine and 25 mg/L kanamycin, supplemented with 10% FBS. Cell cultures were then incubated at 37 °C in a humidified, 5% CO₂ atmosphere, in the absence or presence of serial dilutions of test compounds. The test medium used for the cytotoxic assay as well as for antiviral assay contained 1% of the appropriate serum. Cell viability was determined after 72-120 hrs at 37 °C by MTT method [R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, D. Schols, P. Herdewijin, J. Desmyter, E. De Clercq, Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds, J. Virol. Methods 20 (1988) 309-321]. Vero E6 cells were seeded at an initial density of 4x 10^5 cells/mL in 6-well plates, in culture medium (EMEM 25mM HEPES buffer) supplemented with 1% L-glutamine, 10% fetal bovine serum (FBS), 1% sodium pyruvate (NaPy), 1% non-essential amino acids (NEAA) and 0.1% gentamycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO₂ atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 7 days at 37 °C by the Crystal violet staining method.