Activity of bisnaphthalimidopropyl derivatives against trypanosoma brucei.

GRACA, N.A.G., GASPAR, L, COSTA, D.M., LOUREIRO, I., KONG THOO LIN, P., RAMOS, I., ROURA, M., PRUVOST, A., PEMBERTON, I.K., LOUKIL, H., MACDOUGALL, J., TAVARES, J. and CORDEIRO-DA-SILVA, A.

2016

Activity of Bisnaphthalimidopropyl Derivatives Against *Trypanosoma brucei*

Running title: BNIP derivatives against *T. brucei*

Nuno A. G. Graça[1,2,#], Luis Gaspar[1,2,#], David M. Costa[1,2,#], Inês Loureiro[1,2], Paul Kong Thoo-Lin[3], Isbaal Ramos[4], Meritxell Roura[4], Alain Pruvost[5], Ian K. Pemberton[6], Hadjer Loukil[6], Jane MacDougall[6], Joana Tavares[1,2,*] & Anabela Cordeiro-da-Silva[1,2,7,*]

[1]Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [2]IBMC-Institute for Molecular and Cell Biology, Parasite Disease Group, Porto, Portugal; [3]Institute for Health & Welfare Research, School of Pharmacy & Life Sciences, Robert Gordon University, Aberdeen, Scotland, UK; [4]Innoprot SL, Derio, Spain; [5]CEA, iBiTec-S, SPI, Laboratoire d'Etude du Métabolisme des Médicaments, Gif sur Yvette, France; [6]Photeomix, IP Research Consulting SAS, Noisy le Grand, France; [7]Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.

[#]These authors contributed equally to this work.

Corresponding author: *Anabela Cordeiro da Silva, cordeiro@ibmc.up.pt; Joana Tavares, jtavares@ibmc.up.pt

ABSTRACT

Current treatments for African trypanosomiasis are either toxic, costly, difficult to administer or prone to elicit resistance. This study evaluates the activity of bisnaphthalimidopropyl (BNIP) derivatives against *Trypanosoma brucei*. BNIPDabut, the most active compound, showed *in vitro* inhibition in the single unit nanomolar range, similar to the reference drug pentamidine, and presented low toxicity and adequate metabolic stability. Additionally, using a murine model of acute infection and live imaging, significant decrease of parasite load in BNIPDabut-treated mice was observed. However, cure was not achieved. BNIPDabut constitutes a new scaffold for antitrypanosomal drugs that deserves further consideration.

MAIN TEXT

African trypanosomiasis is an infectious disease caused by parasites of the species *Trypanosoma brucei*. The parasite is transmitted by an insect vector, the tsetse fly (*Glossina spp.*). The disease is mainly distributed in the African continent, with distinct subspecies causing different forms of human disease: *T. brucei gambiense* infection produces a chronic form that may last for years and was responsible for nearly 98% of the cases in the past decade; the acute form is caused by *T. brucei rhodesiense* and usually kills the host within weeks, accounting for the remaining 2% of the reported cases (1, 2).

Since vaccination remains elusive and vector control strategies are frequently insufficient, chemotherapy is still the most efficient option to control the disease (2-5). However, the drugs in use have many drawbacks, mostly related with cost, effectiveness, toxicity, difficult administration and the appearance of resistance (6). Therefore, the development of new drugs is urgently needed.

Bisnaphthalimidopropyl (BNIP) derivatives have previously been shown to possess both anticancer activity (7-11) and also have been shown to be antiparasitic against a related trypanosomatid *Leishmania infantum* (12-14). The potential activity of three BNIP derivatives previously synthetized (10, 11, 15, >96% pure), namely BNIPDiaminobutane (BNIPDabut), BNIPDiaminoheptane (BNIPDahep) and BNIPDiaminooctane (BNIPDaoct) (FIG 1A), against *T. brucei brucei* Lister 427 bloodstream forms (BSF) was investigated. These were selected from a series of compounds based on preliminary studies of bioavailability and *in vitro* and *in vivo* activity against both *T. brucei* and *L. infantum* (12 and unpublished data). The *in vitro* antiparasitic activity was assessed using a resazurin assay, as previously described, with minor modifications (incubation with 10^3 parasites/well, in 200 µL, 16). All three BNIPs demonstrated a potent inhibitory effect on

58 the parasites' growth, with IC_{50} within the nanomolar range (FIG 1B, TABLE 1).
59 BNIPDabut was the most active compound with an $IC_{50} \pm$ SD of 2.4 ± 1.0 nM similar to the
60 reference drug pentamidine with 2.9 ± 0.7 nM (TABLE 1). Since this class of compounds
61 has previously been described as inhibitors of the *L. infantum* Silent information regulator 2
62 related protein 1 (*Li*Sir2rp1, accession: AAN39039.1) (15), we evaluated whether
63 inhibition of the *T. brucei* orthologue, *Tb*Sir2rp1 (accession: AAX70528.1) would be a
64 possible mechanism of action. Whereas BNIPDabut was shown to inhibit the NAD^+-
65 dependent deacetylase activity of *Tb*Sir2rp1with an $IC_{50} \pm$ SD of 155 ± 42 μM, suggesting
66 that this is not the major mechanism of action (data not shown), *Li*Sir2rp1 was inhibited
67 with an $IC_{50} \pm$ SD of 35.0 ± 5.8 μM (15). The 47% identity between *Li*Sir2rp1 and
68 *Tb*Sir2rp1 obtained by protein sequence alignment (Clone Manager 9, BLOSUM 62
69 scoring matrix) might explain the differences observed (17). Moreover, no correlation
70 between the enzymatic inhibition and activity towards *T. brucei* parasites was observed. To
71 evaluate *in vitro* toxicity towards mammalian cells, all the compounds were studied with
72 the MTT assay (18) in THP1-derived macrophages and two primary cell cultures: rat
73 cortical neurons and mouse hepatocytes (TABLE 2). The CC_{50} values for these molecules
74 translate into selectivity indexes ($SI=CC_{50}/IC_{50}$) higher than 100. All BNIP derivatives
75 exhibited high SIs, with BNIPDabut in particular being at least 800 times more selective
76 towards *T. brucei* parasites. All BNIPs had potency and selectivity that warranted
77 additional characterization (TABLE 2). To further evaluate the potential toxic effects of
78 BNIPs in host cells, a set of *in vitro* assays was performed in hepatocytes and neuronal
79 primary cells. These assays evaluated different possible mechanisms of toxicity based on:
80 a) reactive oxygen species determination (CM-H2DCFDA probe, by High Content

Analysis – HCA); b) mitochondrial dysfunction (TMRM probe, by HCA); c) membrane integrity (lactate dehydrogenase quantification); d) apoptosis (caspase 3/7 activation); e) either DNA damage for hepatocytes (H2AX antibody, by HCA) or neurite outgrowth for neurons (anti-tubulin III antibody, by HCA); f) cell viability as measured by WST-8 probe and g) Hoechst staining for nuclear detection. Nimesulide (400 μM) was included as a positive control and the vehicle as a neutral control (19-21). The relative percentage of deviation from the neutral control was quantified and assigned with a number from 0 to 5 according to the following criteria: 0 (0-20% deviation), 1 (20-40%), 2 (40-60%), 3 (60-100%), 4 (100-1000%), or 5 (>1000% deviation). The sum of these values was posteriorly ranked to create a combined injury criteria that varied from no injury (0), low injury (1 to <5), moderate injury (≥5-to <12) to high injury (≥ 12). All BNIPs showed a dose-dependent injury score close to pentamidine in both cell types (FIG 2A-B). BNIPDabut had a toxicity profile indistinguishable from the reference drug pentamidine.

To infer metabolic stability, mouse microsomes were incubated over 45 minutes with 5 μM of each compound and the drug was quantified by LC-MS/MS. Similarly to pentamidine, BNIPDabut was more stable than both BNIPDahep and BNIPDaoct, with 95 to 100% of the drug not being metabolized (TABLE 3). This high metabolic stability is an indicator that the molecule is not easily subjected to common inactivation or loss of potency by reactions catalized by liver enzymes, and is kept intact in circulation for longer periods.

To determine the pharmacokinetics of BNIPDabut, a 10 mg/kg dose was administered to BALB/c mice by intravenous injection. Five minutes after, a concentration of 58 nM was achieved and during the following 24 hours remained higher than 41 nM (data not shown), thus approximately 8 times higher than the calculated IC_{90}.

105 Taking in consideration the previous results, BNIPDabut was chosen for *in vivo* efficacy
106 studies. All the experiments involving animals were carried out in accordance with the
107 IBMC Animal Ethics Committees and the Portuguese and European Authorities for Animal
108 Health guidelines. *T. b. brucei* Lister 427 parasites were transfected with a construct kindly
109 provided by M. Taylor, in which the red-shifted luciferase gene (PpyRE9H) is flanked by
110 5´VSG/3´tubulin (22). Upon transfection, clones were screened for bioluminescent signal
111 and the ones expressing the highest levels were selected. Their *in vitro* growth was
112 compared to wild type parasites and found to be similar (data not shown). *In vitro* detection
113 limits were also analyzed for BSF in a 96-well plate and determined to be about 2500 cells
114 (data not shown). BALB/c female mice were inoculated intraperitoneally with 10^4 BSF.
115 Three days post-infection, five groups of mice (n=4) were treated intravenously with:
116 saline, pentamidine at 2.5 mg/kg/day, DMSO at 16.7%, or BNIPDabut both at a 10
117 mg/kg/day and a 20 mg/kg/day dose. Pentamidine was administered for 4 days, while
118 BNIPDabut and the respective vehicle (DMSO 16.7%) were administered for 6 days (FIG
119 3A). No adverse effects were observable following any administration regimen. Treatment
120 efficacy was followed through whole animal live imaging using an IVIS Lumina LT
121 (Perkin Elmer). Parasitaemias were also assessed, and animals were euthanized after
122 reaching a parasitaemia of 10^8 parasites/mL. Similarly to pentamidine, two administrations
123 of BNIPDabut both at 10 and 20 mg/kg efficiently reduced parasitaemia below detection
124 limit ($5x10^4$/mL) (FIG 3B). However, whole mice imaging reveals that reduction of
125 bioluminescent signal to the background level (obtained with non-infected mice) is only
126 achieved in mice treated with 20 mg/kg of BNIPDabut during at least 5 consecutive days as
127 opposed to 2 days treatment for the pentamidine group. Although treatment with 20mg/kg
128 BNIPDabut apparently cleared the infection, cure was not achieved as the parasite burden

relapsed when treatment was stopped (FIG 3B). Indeed, BNIPDabut (10 and 20 mg/kg) treatment increases mice survival, but in contrast with pentamidine, animals were not cured (FIG 3C). A hypothesis is that BNIPDabut, although highly trypanocidal cannot reach and clear all parasites, either due to potency, distribution, or both. A frequent observation was that recurrence of parasitaemia was preceded by imaging of parasite loads in the peritoneal zone, where animals were originally injected with the parasites. It has been demonstrated that trypanosomes invade extravascular tissues as a defense mechanism against host immunity, and that this process may be related with relapses after treatment interruption (23, 24). Indeed, the presence of parasites in the extravascular tissues might explain the discrepancy of radiance values between days 4 and 12 while average parasitaemia remain similar in mice treated with BNIPDabut (10 and 20 mg/kg) (FIG 3C). Nonetheless, it remains to be elucidated whether BNIPDabut is active on a mouse model of the late stage of the disease as this is the central objective in drug discovery against human African trypanosomiasis. Additional chemical modifications to BNIPDabut may improve potency and/or distribution of the drug, while maintaining or improving the toxicity and metabolism profile.

In conclusion, this work demonstrates that BNIPDabut has potent *in vitro* and *in vivo* antitrypanosomal activity with acceptable toxicity and high metabolic stability. However, chemical modifications are needed in order to improve its pharmacodynamic and/or pharmacokinetic properties.

FUNDING

The research leading to these results has received funding from: the European Community's Seventh Framework Programme under grant agreement No.602773 (Project

KINDReD) and Fundação para a Ciência e Tecnologia (FCT)/Ministério da Educação e Ciência (MEC) cofunded by FEDER, partnership agreement PT2020, through the Research Unit No.4293.

L.G. was supported by the Fundação para a Ciência e Tecnologia through grant SFRH/BD/81604/2011. N.A.G.G. was supported by NMTrypI– PR300103-BD scholarship. D.M.C. was supported by KINDReD-PR301404-BD III scholarship.

J.T. is an Investigator FCT funded by National funds through FCT and co-funded through European Social Fund within the Human Potential Operating Programme.

ACKNOWLEDGEMENTS

Luciferase-expressing plasmid was kindly provided by Martin Taylor, who constructed it with the firefly luciferase gene variant *PpyRE9h* from Bruce Branchini Lab.

REFERENCES

1. **Franco JR, Simarro PP, Diarra A, Jannin JG.** 2014. Epidemiology of human African trypanosomiasis. Clin Epidemiol **6:**257-275.
2. **Franco JR, Simarro PP, Diarra A, Ruiz-Postigo JA, Jannin JG.** 2014. The journey towards elimination of gambiense human African trypanosomiasis: not far, nor easy. Parasitology **141**(6)**:**748-760.
3. **Horn D.** 2014. Antigenic variation in African trypanosomes. Mol Biochem Parasitol **195**(2)**:**123-129.

4. **Radwanska M, Guirnalda P, De Trez C, Ryffel B, Black S, Magez S.** 2008. Trypanosomiasis-induced B cell apoptosis results in loss of protective anti-parasite antibody responses and abolishment of vaccine-induced memory responses. PLoS Pathog **4**(5)**:**e1000078. doi:10.1371/journal.ppat.1000078.

5. **Schofield CJ, Kabayo JP.** 2008. Trypanosomiasis vector control in Africa and Latin America. Parasit Vectors **1**(1)**:**24.

6. **Brun R, Blum J, Chappuis F, Burri C.** 2010. Human African trypanosomiasis. Lancet **375**(9709)**:**148-159.

7. **Ralton LD, Bestwick CS, Milne L, Duthie S, Kong Thoo Lin P.** 2009. Bisnaphthalimidopropyl spermidine induces apoptosis within colon carcinoma cells. Chem Biol Interact **177**(1)**:**1-6.

8. **Barron GA, Bermano G, Gordon A, Kong Thoo Lin P.** 2010. Synthesis, cytotoxicity and DNA-binding of novel bisnaphthalimidopropyl derivatives in breast cancer MDA-MB-231 cells. Eur J Med Chem **45**(4)**:**1430-1437.

9. **Bestwick CS, Ralton LD, Milne L, Kong Thoo Lin P, Duthie SJ.** 2011. The influence of bisnaphthalimidopropyl polyamines on DNA instability and repair in Caco-2 colon epithelial cells. Cell Biol Toxicol **27**(6)**:**455-463.

10. **Oliveira J, Ralton L, Tavares J, Codeiro-da-Silva A, Bestwick CS, McPherson A, Thoo Lin PK.** 2007. The synthesis and the in vitro cytotoxicity studies of bisnaphthalimidopropyl polyamine derivatives against colon cancer cells and parasite Leishmania infantum. Bioorg Med Chem **15**(1)**:**541-545.

11. **Dance AM, Ralton L, Fuller Z, Milne L, Duthie S, Bestwick CS, Lin PK.** 2005. Synthesis and biological activities of bisnaphthalimido polyamines derivatives:

cytotoxicity, DNA binding, DNA damage and drug localization in breast cancer MCF 7 cells. Biochem Pharmacol **69**(1)**:**19-27.

12. **Tavares J, Ouaissi A, Silva AM, Lin PK, Roy N, Cordeiro-da-Silva A.** 2012. Anti-leishmanial activity of the bisnaphthalimidopropyl derivatives. Parasitol Int **61**(2)**:**360-363.

13. **Tavares J, Ouaissi A, Lin PK, Tomas A, Cordeiro-da-Silva A.** 2005. Differential effects of polyamine derivative compounds against Leishmania infantum promastigotes and axenic amastigotes. Int J Parasitol **35**(6)**:**637-646.

14. **Costa Lima S, Rodrigues V, Garrido J, Borges F, Kong Thoo Lin P, Cordeiro da Silva A.** 2012. In vitro evaluation of bisnaphthalimidopropyl derivatives loaded into pegylated nanoparticles against Leishmania infantum protozoa. Int J Antimicrob Agents **39**(5)**:**424-430.

15. **Tavares J, Ouaissi A, Kong Thoo Lin P, Loureiro I, Kaur S, Roy N, Cordeiro-da-Silva A.** 2010. Bisnaphthalimidopropyl derivatives as inhibitors of Leishmania SIR2 related protein 1. ChemMedChem **5**(1)**:**140-147.

16. **Bowling T, Mercer L, Don R, Jacobs R, Nare B.** 2012. Application of a resazurin-based high-throughput screening assay for the identification and progression of new treatments for human African trypanosomiasis. Int J Parasitol Drugs Drug Resist **2:**262-270.

17. **Eddy SR.** 2004. Where did the BLOSUM62 alignment score matrix come from? Nat Biotech **22**(8)**:**1035-1036.

18. **van Meerloo J, Kaspers GL, Cloos J.** 2011. Cell Sensitivity Assays: The MTT Assay, p 237-245. *In* Cree IA (ed), Cancer Cell Culture, vol 731. Humana Press.

19. **Mingatto FE, Rodrigues T, Pigoso AA, Uyemura SA, Curti C, Santos AC.** 2002. The critical role of mitochondrial energetic impairment in the toxicity of nimesulide to hepatocytes. J Pharmacol Exp Ther **303**(2)**:**601-607.

20. **Tripathi R, Tripathi P, Pancholi SS, Patel CN.** 2014. The genotoxic and cytotoxic effects of nimesulide in the mouse bone marrow. Drug Chem Toxicol **37**(3)**:**255-260.

21. **Borkotoky D, Panda SK, Sahoo GR, Parija SC.** 2014. Genotoxicity of nimesulide in Wistar rats. Drug Chem Toxicol **37**(2)**:**178-183.

22. **McLatchie AP, Burrell-Saward H, Myburgh E, Lewis MD, Ward TH, Mottram JC, Croft SL, Kelly JM, Taylor MC.** 2013. Highly sensitive in vivo imaging of Trypanosoma brucei expressing "red-shifted" luciferase. PLoS Negl Trop Dis **7**(11)**:**e2571. doi:10.1371/journal.pntd.0002571.

23. **Whitelaw DD, Gardiner PR, Murray M.** 1988. Extravascular foci of Trypanosoma vivax in goats: the central nervous system and aqueous humor of the eye as potential sources of relapse infections after chemotherapy. Parasitology **97**(01)**:**51-61.

24. **Claes F, Vodnala SK, van Reet N, Boucher N, Lunden-Miguel H, Baltz T, Goddeeris BM, Buscher P, Rottenberg ME.** 2009. Bioluminescent imaging of Trypanosoma brucei shows preferential testis dissemination which may hamper drug efficacy in sleeping sickness. PLoS Negl Trop Dis **3**(7)**:**e486.

FIGURE LEGENDS

FIG 1 *In vitro* antitrypanosomal activity of BNIP compounds.

A) Chemical structures of pentamidine and BNIP derivatives BNIPDabut, BNIPDahep, BNIPDaoct. B) Growth inhibition curves of *Trypanosoma brucei brucei* BSF incubated *in vitro* with the indicated concentrations of pentamidine, BNIPDabut, BNIPDahep or BNIPDaoct for 72h. Parasite density was evaluated using resazurin. Dots and error bars represent the mean + standard deviation of antiparasitic activity. Data of 3 independent experiments.

FIG 2 *In vitro* toxicity of BNIP derivatives.

A) Hepatotoxicity injury score. The score was calculated as the sum of individual scores obtained from a panel of *in vitro* cytotoxicity assays that include: measurement of reactive oxygen species using CM-H2DCFDA and cells imaging by high content analysis; assessment of mitochondrial dysfunction measured by TMRM probe dynamics in cells and image by high content analysis; membrane integrity assayed by lactate dehydrogenase quantification; DNA damage by imaging with H2AX antibody and high content analysis; and apoptosis by caspase 3/7 activation; Hoechst staining for nuclear detection; and cell viability by WST-8 probe. Nimesulide (400 μM), an approved drug with a mild toxicological profile, was included as a toxicity control. Individual scores are calculated based on the relative percentage of deviation from the negative control quantified and assigned with a number from 0 to 5 according to the following criteria: 0 (0-20% deviation), 1 (20-40%), 2 (40-60%), 3 (60-100%), 4 (100-1000%), or 5 (>1000% deviation). The data represent the mean sum of these values + standard deviation B) Neurotoxicity Injury Score. The score was calculated similarly to the hepatotoxicity score but instead of DNA damage by H2AX antibody, an assay to test neurite outgrowth as

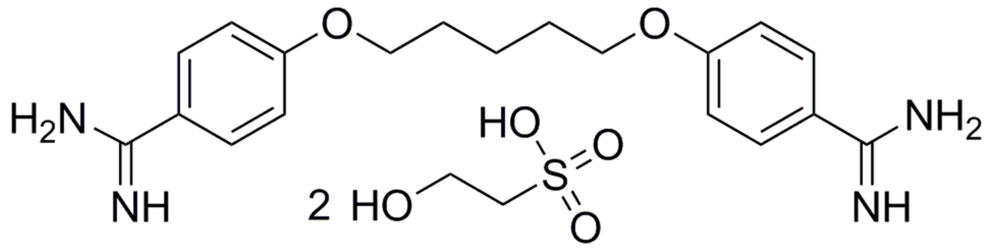
imaged with an anti-tubulin III antibody and high content analysis was performed. The data represent the mean sum of these values + standard deviation. Data of 2 independent experiments.

FIG 3 BNIPDabut *in vivo* efficacy against *T. b. brucei*.

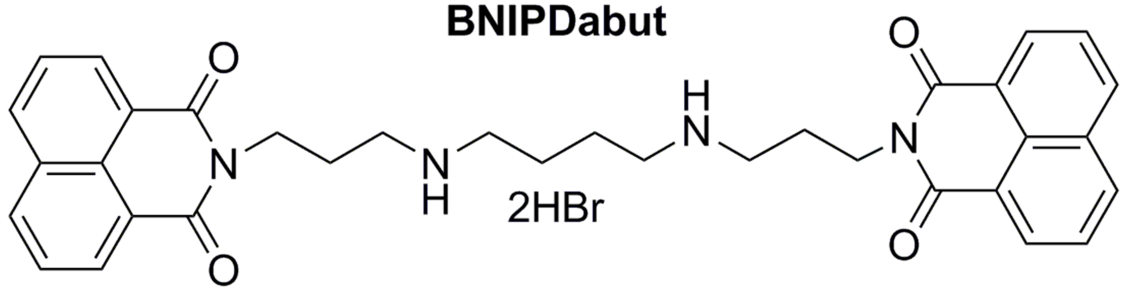
A) Schematic of the experimental design to evaluate the *in vivo* efficacy of BNIPDabut. B) Mice were infected with 10^4 LUC^+ BSF by intraperitoneal injection and initiated the different treatments 3 days post-infection. Whole mice bioluminescence imaging was done at days 3, 4, 9 and 12 using an IVIS LUMINA LT and upon injection of 2.1mg luciferin. Bioluminescence average radiance (p/sec/cm^2/sr) of whole mice was quantified and the mean + standard deviation (n=4) is shown in bars. Parasitemia was determined using a haemocytometer and the mean + standard deviation is represented by red dots. Red crosses represent the parasitaemia of the only animal where parasites could be detected and quantified. Parasitaemia detection limit is $5x10^4$ parasites/mL. C) Kaplan-Meyer survival curves of the infected mice treated with controls and experimental doses of BNIPDabut. B-C) The data is representative of 3 independent experiments.

A

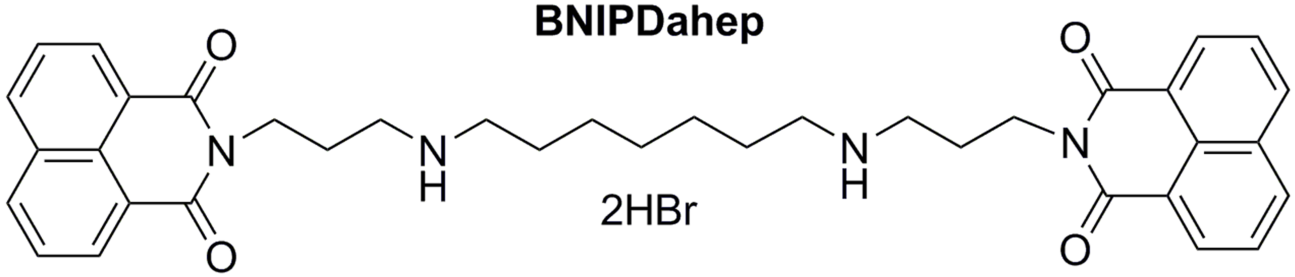
Pentamidine



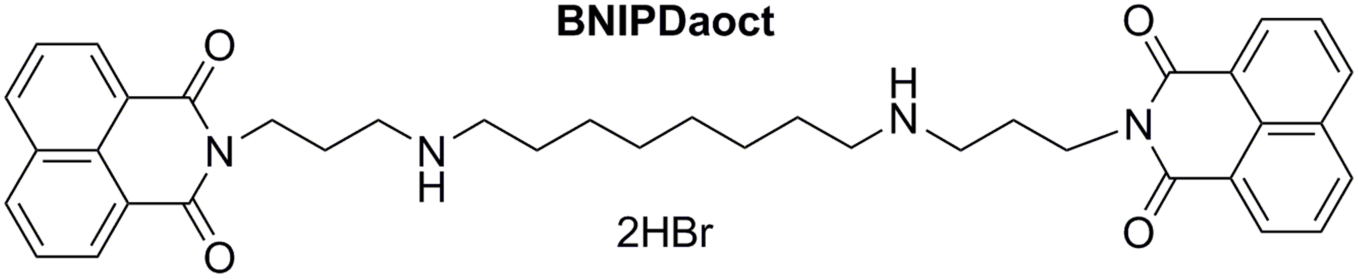
BNIPDabut



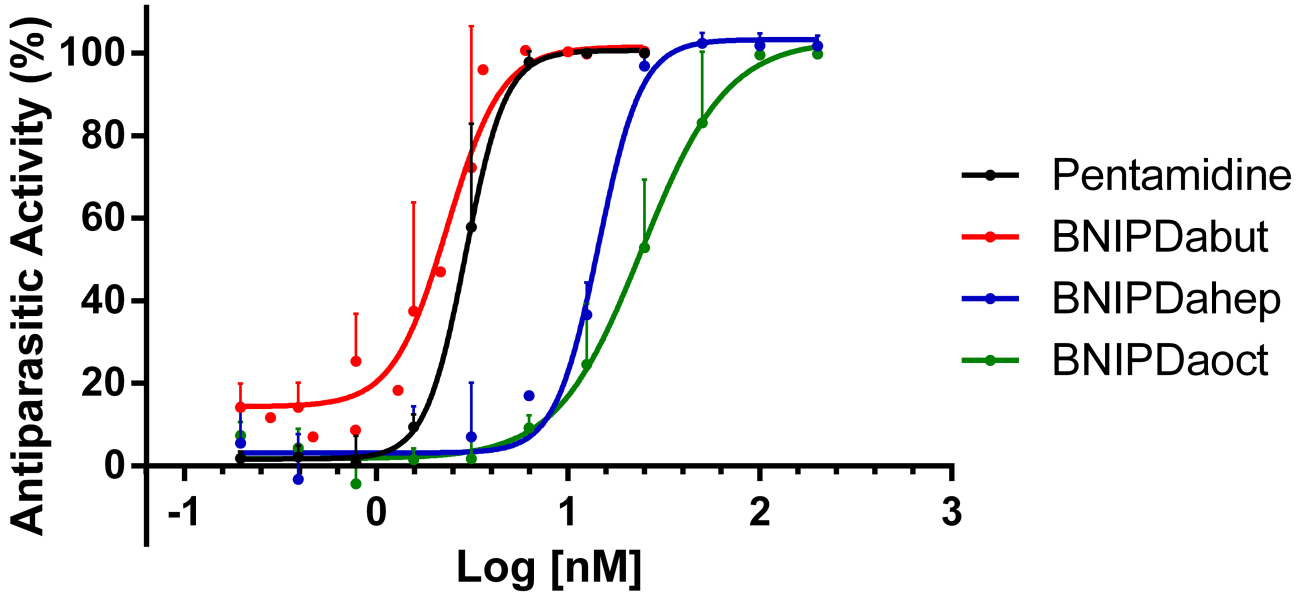
BNIPDahep

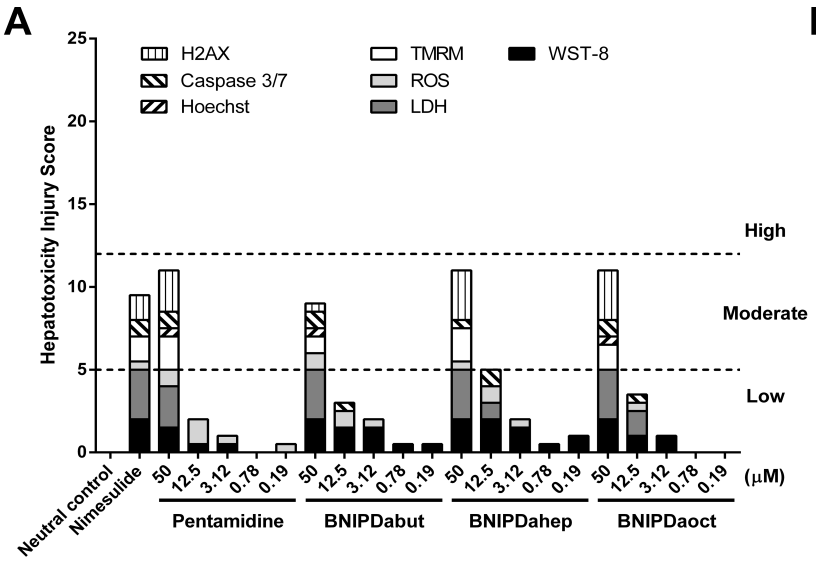


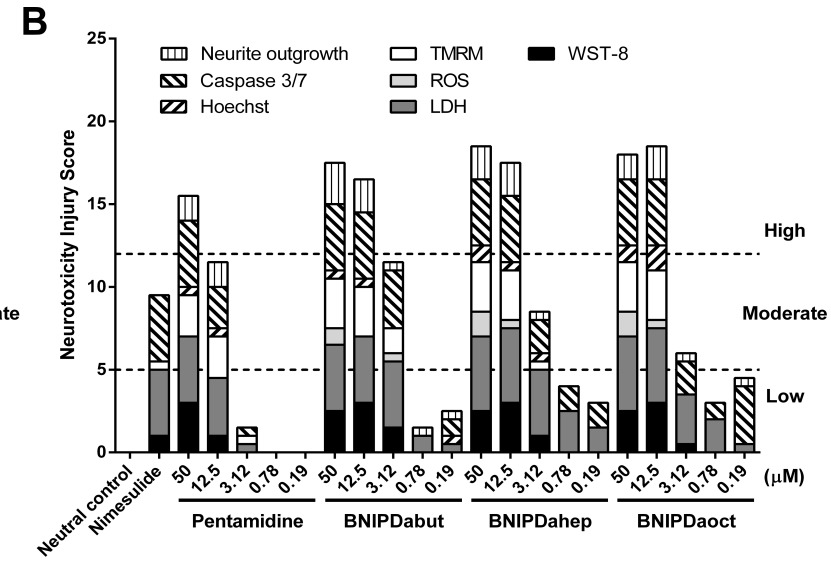
BNIPDaoct



B




A
H2AX
Caspase 3/7
Hoechst
TMRM
ROS
LDH
WST-8
Hepatotoxicity Injury Score
25
20
15
10
5
0
High
Moderate
Low
Neutral control
Nimesulide
50
12.5
3.12
0.78
0.19
(μM)
Pentamidine
BNIPDabut
BNIPDahep
BNIPDaoct


B
Neurite outgrowth
Caspase 3/7
Hoechst
TMRM
ROS
LDH
WST-8
Neurotoxicity Injury Score
25
20
15
10
5
0
High
Moderate
Low
Neutral control
Nimesulide
50
12.5
3.12
0.78
0.19
(μM)
Pentamidine
BNIPDabut
BNIPDahep
BNIPDaoct

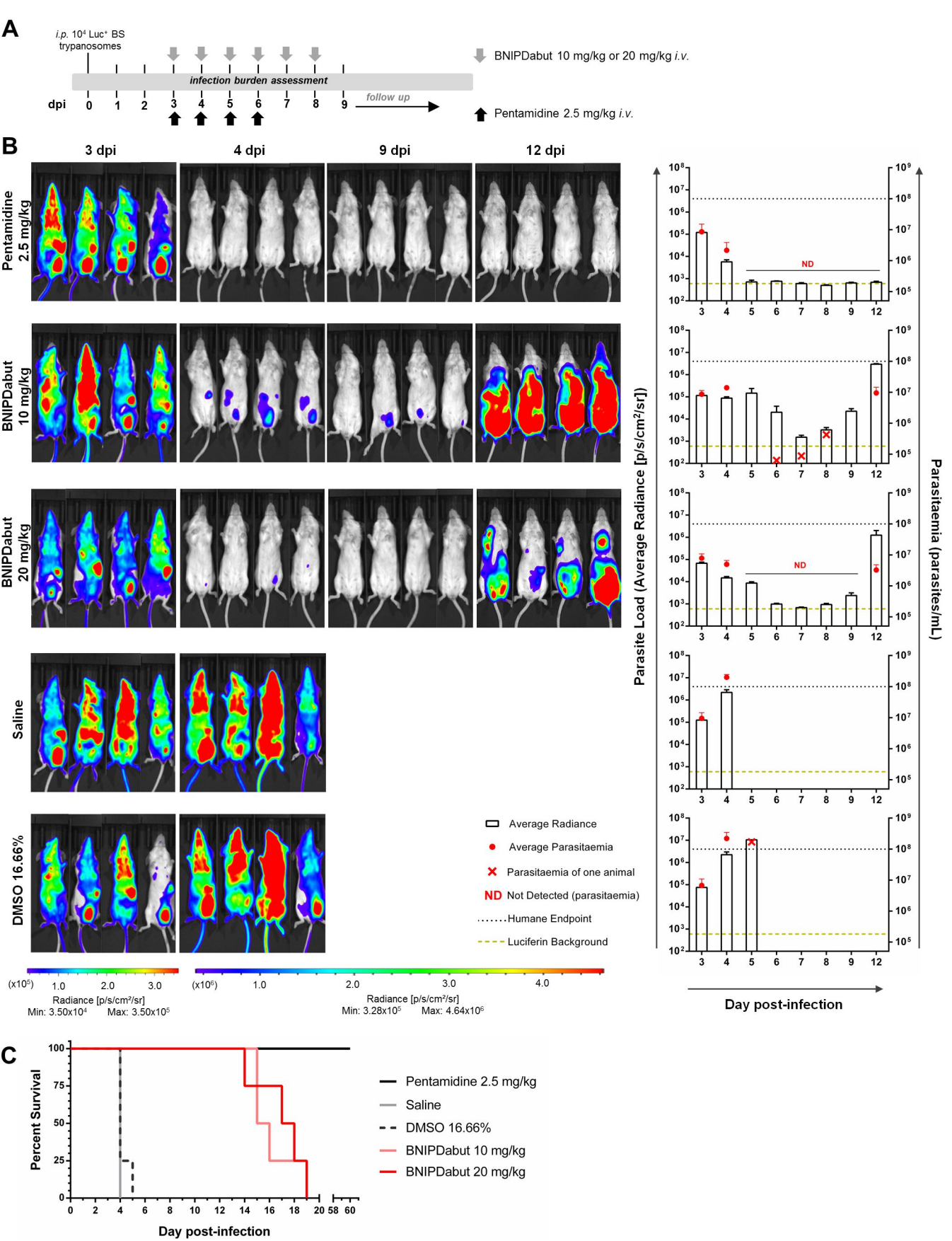

A
i.p. 10⁴ Luc⁺ BS trypanosomes
infection burden assessment
dpi 0 1 2 3 4 5 6 7 8 9
follow up
BNIPDabut 10 mg/kg or 20 mg/kg i.v.
Pentamidine 2.5 mg/kg i.v.
B
3 dpi
4 dpi
9 dpi
12 dpi
Pentamidine 2.5 mg/kg
BNIPDabut 10 mg/kg
BNIPDabut 20 mg/kg
Saline
DMSO 16.66%
(x10⁵) 1.0 2.0 3.0
Radiance [p/s/cm²/sr]
Min: 3.50x10⁴ Max: 3.50x10⁵
(x10⁶) 1.0 2.0 3.0 4.0
Radiance [p/s/cm²/sr]
Min: 3.28x10⁵ Max: 4.64x10⁶
Average Radiance
Average Parasitaemia
Parasitaemia of one animal
ND Not Detected (parasitaemia)
Humane Endpoint
Luciferin Background
Parasite Load (Average Radiance [p/s/cm²/sr])
Parasitaemia (parasites/mL)
ND
Day post-infection
C
Percent Survival
Day post-infection
Pentamidine 2.5 mg/kg
Saline
DMSO 16.66%
BNIPDabut 10 mg/kg
BNIPDabut 20 mg/kg

TABLE 1 *In vitro* activity of BNIP derivatives against *Trypanosoma brucei* L427 bloodstream forms

	IC_{50} ± SD (nM)	IC_{90} ± SD (nM)
Pentamidine	2.94 ± 0.74	5.26 ± 0.58
BNIPDabut	2.35 ± 0.99	3.83 ± 1.40
BNIPDahep	14.32 ± 1.21	23.07 ± 1.01
BNIPDaoct	26.15 ± 10.43	63.36 ± 21.19

TABLE 2 *In vitro* cytotoxity of BNIP derivatives in different cell types

	CC_{50} ± SD (μM)			Selectivity Index*		
	THP1	Hepatocytes	Neurons	THP1	Hepatocytes	Neurons
Pentamidine	47.73 ± 3.32	18.21 ± 0.66	8.23 ± 0.88	16259	6203	2803
BNIPDabut	5.90 ± 0.40	9.19 ± 0.06	2.06 ± 1.69	2514	3916	878
BNIPDahep	3.34 ± 0.11	4.23 ± 0.48	2.31 ± 1.65	233	295	161
BNIPDaoct	3.88 ± 0.59	18.35 ± 4.58	3.97 ± 1.30	148	702	152

*Selectivity Index = CC_{50} cell line/IC_{50} *T. brucei*

TABLE 3 Mouse microsomal stability

	Metabolic stability (%)	*In vitro* Intrinsic Clearance Cl_{int} (µL/min/mg protein)	Degradation non NADPH-dependent (%)
Pentamidine	95 - 100	-	< 5
BNIPDabut	95 - 100	-	< 5
BNIPDahep	85	7	< 5
BNIPDaoct	64	20	9