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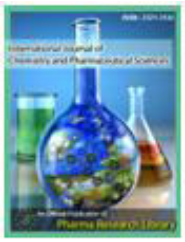

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Exploiting the Antiparasitic Activity of Naphthalimides Derivatives

Gabriela Cristo[1,2],Luís Gaspar[1,2], Jennifer Noro[3], Catarina Baptista[1,2], Paul Kong Thoo-Lin[4], Maria José Alves[3] and Anabela Cordeiro-da-Silva[1,2,5,*]

[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]*Department of Chemistry, Campus de Gualtar, University of Minho, Braga, Portugal;*
[4]*School of Pharmacy and Life Sciences, Robert Gordon University, Riverside East, Garthdee Road, Aberdeen AB101GJ, Scotland*
[5]*Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto. Portugal*

A B S T R A C T

A set of 1,8-naphtalimides derivatives were synthesized and tested against three protozoans that cause important human diseases: *Leishmania infantum*, *Trypanosomabrucei* and *Trypanosoma cruzi*. Additionally, toxicity was determined by growth inhibition of THP-1 derived macrophages. The results suggest that chemical modifications in the carbon chain linking the naphthalimide and the substituting groups have different effects in the parasites. This work should provide new insights for the design and optimization of more potent and directed naphthalimide derivatives against these organisms.

Keywords: *Leishmania infantum, Trypanosoma brucei, Trypanosoma cruzi*

A R T I C L E I N F O

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***Corresponding Author**
Anabela Cordeiro-da-Silva
Instituto de Investigação e Inovação em Saúde,
Universidade do Porto, Porto, Portugal,
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1. Introduction

Parasitic diseases caused by trypanosomatids are still an important health problem, mainly in tropical and subtropical areas. In addition, temperate regions of our globe, including North America and the Asia-Pacific region

are also affected by disease-causing protozoans like *Leishmania spp., Trypanosoma cruzi and Trypanosoma brucei* These are the species of trypanosomatids most associated with human health, being responsible for high mortality and morbidity[1]. There are still no vaccines and the actual chemotherapies are far from satisfactory, owing to the emergence of resistances, serious side effects, and its limited efficacy. Therefore, it is imperative to continue the discovery of new drugs to treat these diseases[2].

2. Experimemtal

Naphthalimides and bis-naphthalimides are classes of compounds bearing aromatic groups that have generated intense interest by scientists around the world because of their many reported biological activities. Special attention has been devoted to the high anticancer activity of naphthalimides, which is due to their interactions with DNA by a mechanism of intercalation[3–5]. In addition, recent studies showed that 1,8-naphthalimide derivatives also demonstrated other biological activities, such as antitrypanosomal [6]. In this context, this work proposes to study the relationship between the structure and the activity of a set of 1,8-naphtalimide derivatives with 2, 3 or 4 carbons linking the naphthalimide moiety to different functional groups (amine, imine, guanidine, urea, amide, and 1,2,3-triazole) against three trypanosomatids: *Leishmania infantum*, *Trypanosoma brucei* and *Trypanosomacruzi*. These 1,8-naphtalimide derivatives compounds had been recently synthesized and include naphthalimidoalkyl amines 1a, b and its heterocyclic imine derivatives 2a-l, heterocyclic amine derivatives 3f,g, guanidine derivatives 4a-c, urea derivatives 5a-l, amide derivatives 6a-l and triazol derivatives 7d-f[7,8].

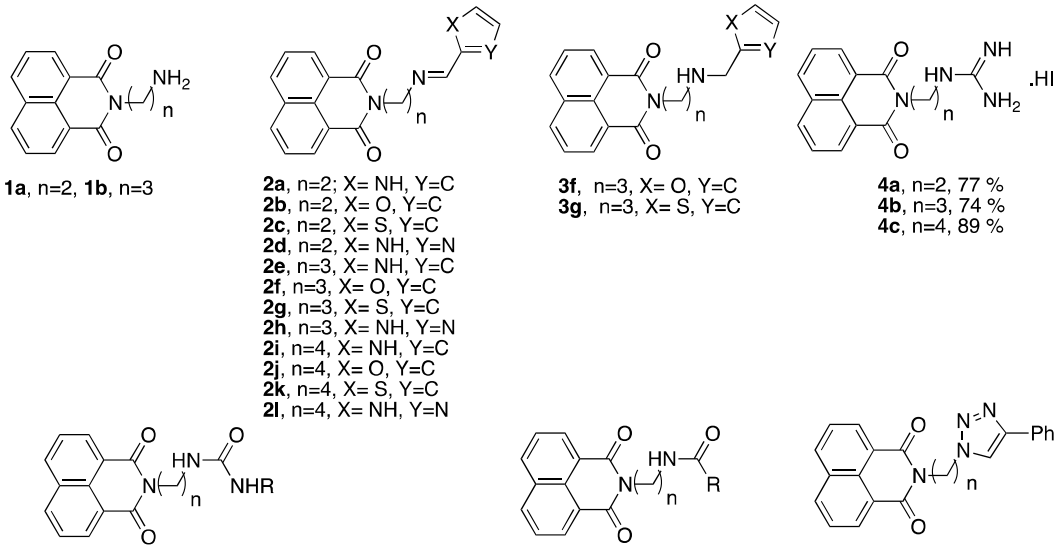

Figure 1: Naphthamides compounds 1a,b, 2a-l, 3f,g, 4a-c, 5a-l, 6a-l, 7d-f used in trypanosomatids tests: *Leishmania infantum, Trypanosoma brucei* and *Trypanosoma cruzi*

3. Results and Discussion

All the naphthalimides compounds stock solutions were prepared in DMSO in a concentration of 10 mm and stored at -20 °C. Anti *T. cruzi* (Y strain) activity was performed by high content screening (adapted from [9]); for *T. brucei* (L427 Wild Type) blood stream forms and axenic amastigote of *L. infantum* (clone MHOM/MA671TMA-P263) activity was determined using the resazurin-based assay[10,11]; intracellular amastigote activity of *L. infantum* was performed by luciferase assay (adapted from[12]). The cellular toxicity of the compounds was evaluated on THP-1 differentiated macrophages using the MTT assay[13]. The table summarizes the results obtained for the compounds with relevant anti-parasitic activity at 10 μM concentration. Overall, our results showthat the best activity was against *T.brucei*. The group of naphthalimidoalkyl amines represented by compound1a and1b show strong inhibition at concentration 10μM (101±1% inhibition); however compound 1apresentshigher toxicity with NOAEL (no observed adverse effect level)> 25μ M in comparison with 1b NOAEL > 100μM, suggesting that shorter a carbon chain (n =2) increases the cytotoxicity. The group of heterocyclic imines compounds 2a-l generally reveals good activity against *T.brucei*. Within the imino furan subgroup, compounds 2b, 2f and 2j, only 2f with 3 carbon atoms chain shows to be active (101 ± 1% inhibition), and in the subgroup incorporating imidazole (2d, 2h and 2l) compound 2l, with 4 carbon atoms chain, is the least active. The toxicity for heterocyclic imines is lower with 3 and 4 carbon chains. The compound with the best balance activity-toxicity belongs to

compound 2e, with a three-carbon atom chain and a pyrrole unit. Heterocyclic amines compounds are also active against *T. brucei*, our results showing almost complete inhibition with compound 3f (99 ± 1% inhibition) and 3g (96 ± 3% inhibition). However, compound 3g shows lower cytotoxicity than 3f, with NOAEL > 50μM and > 25μM, respectively. Guanidine substituted compounds 4a-calso reveals some anti-*T.brucei* activity, with a clear tendency for toxicity to decrease with the increase of the carbon chain length. Within the urea-substituted compounds 5a-l the most active compounds against *T.brucei*, at the concentrations tested, is 5fand 5l both with 4 carbon atom chains. Within this group, when the carbon chain increases up to four carbon atoms, so does the activity increase. Also the nature of the terminal groups in compounds 5a-lis also found to be important for activity. Comparing the cytotoxicity of the four carbon chain members in this group (5c, 5f, 5i and 5l), only compound 5cwas found non toxic, suggesting that un-substituted ureas are less toxic than substituted ureas against THP-1 cell line. In relation to the triazole group, only compound 7f shows activity against *T. brucei* (90 ± 3% inhibition), with toxicity increasing when the triazole group is linked by a 3 carbon atoms chain. Regarding *T. cruzi*, amine 1aand guanidine 4ashows modest anti-parasitic activity (34 ± 9 % inhibition and 45 ± 9% inhibition, respectively). Urea 5l bearing a 4 carbon atoms chain and a *n*-butyl group as substituent shows to be the most active compound of all tested against *T. cruzi*, (81 ± 8 % inhibition). However, all compounds that show some activity against *T. cruzi* are accompanied by an increase in cytotoxicity, suggesting a nonspecific mechanism. Previous studies conducted in our laboratory have shown that bis-naphthalimidopropyl (BNIP) derivatives compounds exert significant effects against *L.infantum* [14]. Surprisingly, no significant anti-leishmanial activity was found for all the groups studied; either against *L.infantum*intracellular and axenic amastigotes. A hypothesis is that the activity is dependent on a second naphthilimide group in the molecule. Overall, the toxicity of the synthesized compounds with a 2 carbon atoms linker presents the higher toxicity. An exception was observed with the substituted urea-derived group, where the 4 carbon atoms chain compounds are the most toxic, with the exception urea 5c. The group of amides 6a-l compounds does not present relevant anti-parasitic activity (data not shown). In conclusion, naphthalimides bearing primary alkylamines and un-substituted ureas are the hits for further research in *Trypanosome brucei*, in combination with longer linkers, and *O*-, *N*-functionalized linkers. No interesting results have been found to the other two trypanosomatids studied.

Table 1: Anti-parasitic activity against *Leishmania infantum* intracellular amastigotes and axenic amastigotes, *Trypanosoma brucei*, *Trypanosoma cruzi* and cytotoxicity in THP1-derived macrophages for the compounds tested.

Functional groups: naphthalimidoalkylamines		**10μM single dose testing (%activity ± SD)**				
Compound	**Radical / Number of carbons**	***Leishmania infantum* intracellular amastigotes**	***Leishmania infantum* axenic amastigotes**	***Trypanosoma brucei***	***Trypanosoma cruzi***	**Toxicity NOAEL (μM)**
1a	R=NH_2 / n=2	23 ± 17	5 ± 5	101 ± 1	34 ± 9	> 25
1b	R=NH_2 / n=3	N. A.	N. A.	101 ± 1	N. A.	> 100
Functional group: Heterocyclic imine						
2a	X=NH/Y=C/ n=2	N. A.	9 ± 7	101 ± 2	22 ± 13	> 25
2b	X=O/ Y=C/ n=2	N. A.	5 ± 0	17 ± 12	N. A.	> 25
2c	X=S/Y=C/ n=2	N. A.	1 ± 3	8 ± 5	N. A.	> 25
2d	X=NH/Y=N/ n=2	N. A.	2 ± 1	101 ± 1	25 ± 4	> 25
2e	X=NH/Y=C/ n=3	N. A.	N. A.	101 ± 1	N. A.	> 100
2f	X=O/Y=C/ n=3	N. A.	20 ± 21	101 ± 1	N. A.	> 50
2g	X=S/Y=C/ n=3	N. A.	N. A.	101 ± 1	N. A.	> 50
2h	X=NH/Y=N/n=3	6 ± 13	23 ± 16	99 ± 2	N. A.	> 50
2i	X=NH/Y=C/ n=4	4 ± 12	1 ± 12	53 ± 5	N. A.	> 50
2j	X=O/Y=C/ n=4	N. A.	19 ± 4	26 ± 10	N. A.	> 50
2k	X=S/Y=C/ n=4	N. A.	27 ± 3	94 ± 2	N. A.	> 50
2l	X=NH/Y=N/ n=4	N. A.	24 ± 12	24 ± 3	N. A.	> 100
Functional group: Heterocyclic amine						
3f	X=O/Y=C/N=3	N. A.	13 ± 1	99 ± 1	17 ± 15	> 25
3g	X=S/ Y=C/N=3	N. A.	9 ± 4	96 ± 3	25 ± 10	> 50
Functional group: Guanidine						
4a	R=H/ n=2	N. A.	4 ± 10	100 ± 1	45 ± 9	> 25
4b	R=H/ n=3	N. A.	22 ± 8	101 ± 1	N. A.	> 50
4c	R=H/ n=4	N. A.	10 ± 2	N. A.	N. A.	> 100

Functional group: Ureas						
5a	R=H/ n=2	N. A.	14 ±1 5	N. A.	N. A.	> 25
5b	R=H/ n=3	N. A.	27 ±1 2	15 ±16	3 ± 8	> 100
5c	R=H/ n=4	N. A.	16 ± 5	75 ± 12	N. A.	> 100
5d	R=4-$NO_2C_6H_4$/ n=2	N. A.	. 11 ± 17	37 ± 8	N. A.	> 25
5e	R=4-$NO_2C_6H_4$/ n=3	N. A.	N. A.	38 ± 6	26 ± 1	> 25
5f	R=4-$NO_2C_6H_4$/ n=4	N. A.	10 ± 10	94 ± 4	N. A.	> 10
5g	R=CH_2Ph/ n=2	N. A.	30 ± 6	5 ± 4	N. A.	> 25
5h	R=CH_2Ph/ n=3	N. A.	16 ± 0	26 ± 6	21 ± 17	> 10
5i	R=CH_2Ph/ n=4	N. A.	12 ± 12	74 ± 2	5 ± 8	> 10
5j	R=$(CH_2)_3CH_3$/ n=2	N. A.	14 ± 1	18 ± 10	N. A.	> 50
5k	R=$(CH_2)_3CH_3$/ n=3	N. A.	17 ± 14	18 ± 3	20 ± 12	> 25
5l	R=$(CH_2)_3CH_3$/ n=4	N. A.	12 ± 12	96 ± 2	81 ± 8	> 10
Functional group: 1,2,3–Triazole						
7e	n=2	6 ±16	13 ± 2	12 ± 4	6 ±12	> 100
7d	n=3	N. A.	11 ± 4	16 ± 6	N. A.	> 50
7f	n=4	17 ± 2	N. A.	90 ± 3	22 ± 5	> 100

Results show means activities of at least three independent assays.
NOAEL= No-Observed Adverse Effect Level (MTT assay in PMA-differentiated THP-1 cells);
N. A. = no activity.

4. Acknowledgements

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[8] Compound **2h**: white solid (84.7 %); m.p.: 212-213 °C; δ_H(400 MHz, DMSO) 1.97-2.04 (m, 2H), 3.65 (t, *J*= 6.8 Hz, 2H), 4.11 (t, *J*= 7.6 Hz, 2H), 7.03 (s, 1H), 7.14 (s, 1H), 7.83 (t, *J*= 7.6 Hz, 2H), 8.19 (s, 1H), 8.41 (d, *J*= 8.4 Hz, 2H), 8.45 (d, *J*= 7.2 Hz, 2H), 12.4 (br s, 1H) ppm. Compound **2j**: brown solid (45.4 %); m.p.: 110-111°C; δ_H(400 MHz, $CDCl_3$) 1.82-1.87 (m, 4H), 3.68 (t, *J*= 6.8 Hz, 2H), 4.25 (t, *J*= 7.2 Hz, 2H), 6.47 (dd, *J*= 3.2, 1.6 Hz, 1H), 6.79 (br s, 1H), 7.51 (s, 1H), 7.76 (dd, *J*= 8.4, 7.2 Hz, 2H), 8.12 (s, 1H), 8.21 (dd, *J*= 8.4, 1.2 Hz, 1H), 8.60 (dd, *J*= 7.2, 1.2 Hz, 2H) ppm. Compound **2l**: white solid (92.3 %); m.p.: 225-226 °C; δ_H(400 MHz, DMSO) 1.68-1.69 (m, 4H), 3.59 (t, *J*= 4.8 Hz, 2H), 4.08 (t, *J*= 6.4 Hz, 2H), 7.02 (s, 1H), 7.16 (s, 1H), 7.84 (dd, *J*= 8.0, 7.2 Hz, 2H), 8.15 (s, 1H), 8.42 (dd, *J*= 8.4, 0.8 Hz, 2H), 8.46 (dd, *J*= 7.2, 1.2 Hz, 2H), 12.4 (br s, 1H) ppm.

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