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Antitrypanosomal activity of quaternary naphthalimide derivatives

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Abstract—Sleeping sickness caused by *Trypanosoma brucei gambiense* and *rhodesiense* is fatal if left untreated. Due to the toxicity of drugs currently used and the emerging resistance against these drugs new lead compounds are urgently needed. Within the frame of a broad screening program for drugs with antitrypanosomal activity, some highly potent tertiary and quaternary mono- and bis-naphthalimides being active in the lower micromolar and nanomolar range of concentration have been identified. These compounds are easily available via a two- or three-step microwave-driven synthesis with high yield.
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Naphthalimides are a class of compounds known to have high antitumoral activity against both murine and human tumor cells.[1] The planar aromatic moiety of the N,N-dimethylaminoethyl-1,8-naphthalimides intercalates within the DNA via the major groove.[2–4] Mitonafide and amonafide (see Fig. 1), characterized by a nitro- and an amino group in 3-position of the naphthalimide, respectively, did not show sufficient activity when initially tested in clinical trials.[5] However, the in vitro antitumoral activity increased by forming corresponding bisintercalating agents, that is, bisnafide and elinafide (Fig. 1). In fact, both compounds exhibited very high in vivo and in vitro activity, and elinafide was transferred to clinical trials against solid tumors.[1]

High antitumoral activity relates to the presence of two large planar chromophores, typically π-deficient aromatic moieties and a flexible basic linker consisting of C2-N_{sec}-C2,3-N_{sec}-C2 chain.[6–11] Bisquaternary mono- and bisphthalimide and naphthalimide derivatives,[12,13] which were originally developed as allosteric modulators of muscarinic receptors, share the aforementioned features. Initial screening of derivatives, characterized by

a C3-N_{quart}-C6-N_{quart}-C3 middle chain, revealed no cytotoxic activity against different cell lines. Following the intercalation model developed by Cushman's group[14,15] this result can be explained by the middle chain being too long (at least three more C atoms). Additionally, the two permanent positive charges of the bisquaternary bisnaphthalimides may possibly prevent a perfect intercalation by interaction with the negatively charged backbone of the DNA.
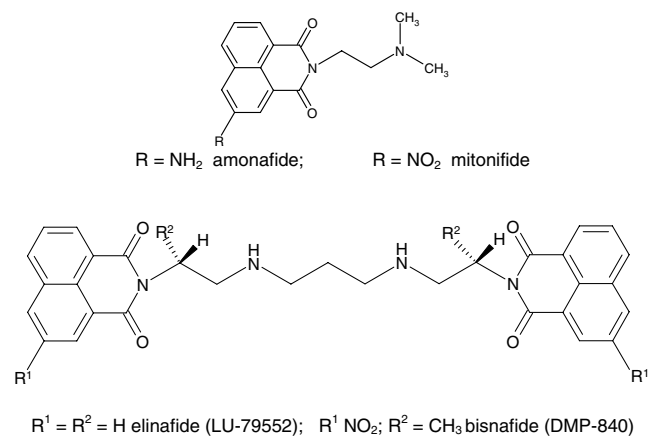

R = NH$_2$ amonafide;   R = NO$_2$ mitonifide

R^1 = R^2 = H elinafide (LU-79552);   R^1 NO$_2$; R^2 = CH$_3$ bisnafide (DMP-840)

Figure 1. Structural formulae of representative naphthalimides with antitumoral activity.

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Within the frame of a broad screening program for drugs with antitrypanosomal activity a series of systematically varied phthalimide and naphthalimide compounds (synthesis, see Scheme 1, structural formulae, see Table 1), whose syntheses were recently described,[12,13,16] was forwarded to a broad library screening for activity against *Trypanosoma brucei brucei*, one of the causant of African trypanosomiasis being the sleeping sickness in humans. Additionally, a parallel cytotoxicity assay was performed in the macrophage cell line J774.1. The antitrypanosomal activities determined in *T. brucei brucei* according to Räz et al.[17] Huber and Koella,[18] and Vicik et al.[19] and the cytotoxicities in macrophages measured according to Ahmed et al.[20] are summarized in Table 1.

Previous screening of a dozen of bisphthalimide compounds with various substituents on the aromatic moiety and the alkyl residues on the propyl chains revealed no antitrypanosomal activity for either compound (compounds and biological data not shown). In contrast, the mononaphthalimide **1**, which represents a half of the 'bis'-molecules, exhibited an activity against trypanosomes in the lower micromolar range of concentration. The activity was increased by elongation of the alkyl chain attached to

the quaternary nitrogen (**2**). No cytotoxicity in macrophages was observed for both compounds.

Adding a 5-methylphthalimidopropylammonium moiety to **1** revealed a kind of hybrid compound (**4**) of similar antitrypanosomal activity. Interestingly compound **3** having no methyl groups in the propyl chain attached to the naphthalimide was 5 times less active than **4**. If the compound's antitrypanosomal activity is caused by intercalation as reported for the antitumoral activity, the two methyl groups in the lateral propyl chain (**4**) should have hindered the interaction with the DNA. Correspondingly, N-methylation was reported to be disadvantageous for antitumoral activity.[21] Thus, the higher antitrypanosomal activity of **4** in comparison to **3** may be an indication for different mode of actions for antitumoral and antitrypanosomal properties.

Comparison of the compounds **4–8** having a different substitution at the aromatic part of the naphthalimide revealed highest antitrypanosomal activity for the non-substituted compound (**4**). Especially the substitution in 3-position is disadvantageous (cf. **5** and **7**). These results are in line with the antitumoral acting
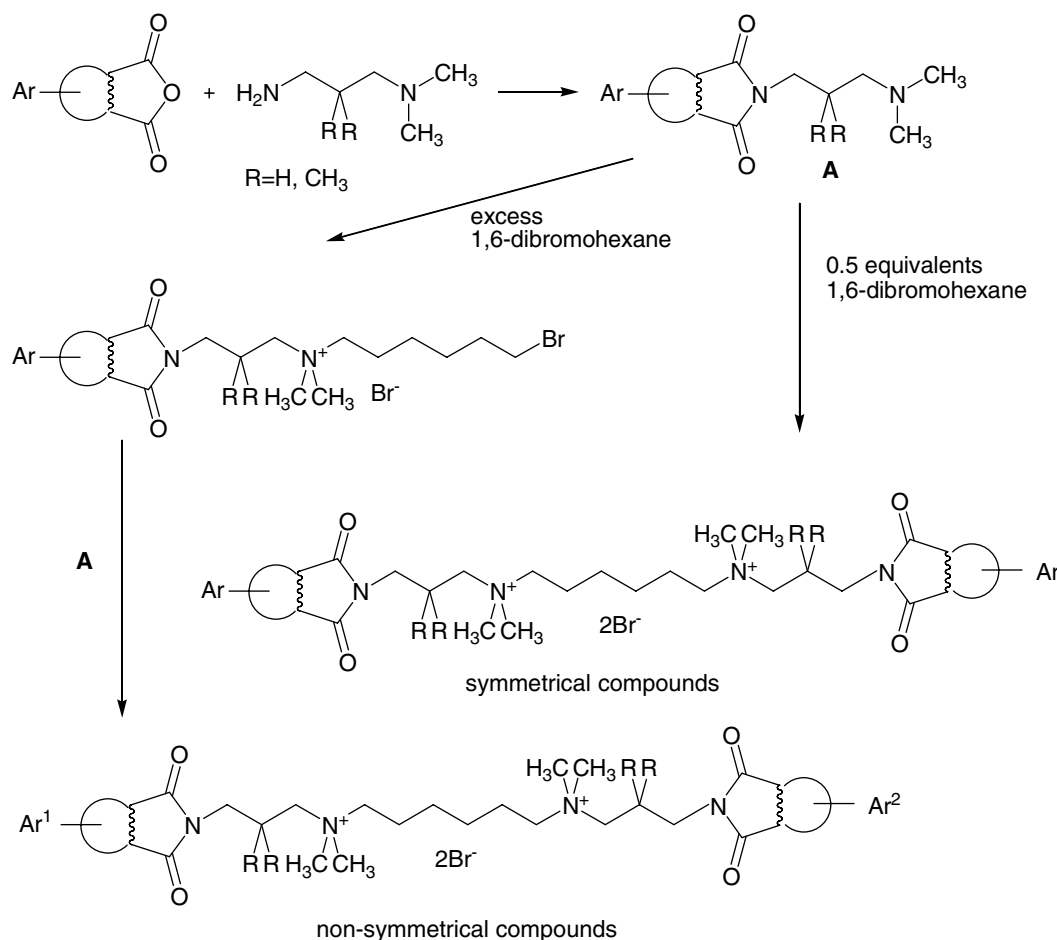
Scheme 1. General synthetic pathway for symmetrical and non-symmetrical compounds. Ar-imide = phthalimide, methyl-phthalimide, and naphthalimide; they are identical or different (see Table 1). The propyl linker can be either non-substituted or methylated. Synthetic details of the pathway are reported in Ref. 12,13.
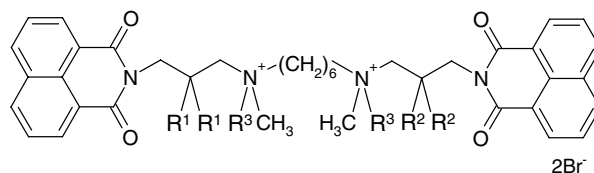
Table 1. Antitrypanosomal activity and cytotoxicity of the quaternary naphthalimide compounds **1–11**[a] (number of experiments = 3)
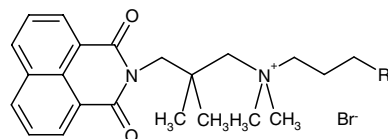
| 1 | R = H |
| 2 | R = $(CH_2)_3Br$ |
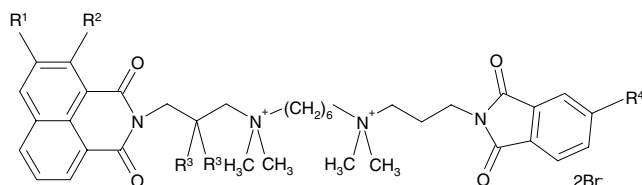
3 - 8

Compound	R^1	R^2	R^3	R^4	*Trypanosoma brucei brucei* IC$_{50}$ (μM) after 48 h	Cytotoxicity IC$_{50}$ (μM) after 48 h
1					1.20 ± 0.29	>100
2					0.69 ± 0.32	>100
3	H	H	H	CH_3	27.93 ± 2.83	>100
4	H	H	CH_3	CH_3	4.62 ± 1.00	>100
5	NH_2	H	CH_3	H	24.25 ± 7.26	>100
6	H	NH_2	CH_3	H	5.95 ± 1.23	>100
7	NO_2	H	CH_3	H	22.89 ± 2.80	>100
8	H	NO_2	CH_3	H	8.78 ± 1.24	>100

9 -11

Compound	R^1	R^2	R^3	*Trypanosoma brucei brucei* IC$_{50}$ (μM) after 48 h	Cytotoxicity IC$_{50}$ (μM) after 48 h
9	H	H	H	0.03 ± 0.01	3.24 ± 0.10
10	CH_3	H	CH_3	4.27 ± 1.29	>100
11	CH_3	CH_3	CH_3	1.17 ± 0.28	>100

[a] For comparison: *Trypanosoma brucei brucei* IC$_{50}$ (μM) after 48 h for pentamidine–diisethionate 0.0027 μM, eflornithin–HCl 22.9 μM, suramine–Na 0.3 μM.

compounds in the literature, that is, the unsubstituted bisnaphthalimide compound elinafide exhibited the highest antitumoral activity. However, the highly potent mononaphthalimides mitonafide and amonafide have the same substitution pattern as compounds **5** and **7** which showed the lowest antitrypanosomal activity in the entire series of compounds tested. These contradictory findings may again indicate a different mode of action of naphthalimides in trypanosomes and in tumor cell lines.

Replacement of the phthalimide moiety with a second naphthalimide residue resulted in compounds of higher trypanocidal activity, especially if methyl groups are present in the propyl chain (cf. **10** vs **11**). The activity of the bisquaternary compounds **10** and **11** was surpassed by the activity of the bistertiary compound **9**

being active in the nanomolar range of concentration. Interestingly compound **9** does not have any methyl groups in the propyl chain.

In contrast to all other compounds whose IC$_{50}$ values for cytotoxicity in macrophages were found to be higher than 100 μM, **9** showed an IC$_{50}$ value of 3.24 ± 0.10 μM. Thus, going from a quaternary to a tertiary compound increases the antitrypanosomal activity but also induces toxicity. Although the factor is 100 between antitrypanosomal activities and cytotoxicity this result has to be taken into account for further developments.

Taken together, the mono- and bistertiary and -quaternary bisnaphthalimides tested in this study are promising new lead structures for the development of new antitrypanosomal compounds. Some of them are active

against *T. brucei brucei* in the submicromolar range of concentration and are almost not cytotoxic. Due to the limited number of compounds in the series tested it is difficult to decide whether mono- or bisnaphthalimides are the better leads.

Such a drug discovery approach was recently called 'piggy-back' strategy[22] which is useful when a molecular target in parasites is being pursued for other indications as it facilitates the identification of chemical starting points. It has to be noted that structure–activity relationships emerging from the parasite assays are unlikely to be the same as observed for the original indications, that is, antitumoral activity[1] and allosteric modulation of antagonist binding to the muscarinic receptors[13] herein. However, besides increasing the antitrypanosomal activity, the mechanism of action has to be elucidated in future studies.

New antitrypanosomal compounds are urgently needed because the chemotherapy currently depends on very 'old' drugs that lack adequate efficacy and cause serious side effects. Besides being highly potent trypanocidal compounds, an additional advantage of the naphthalimide compounds presented herein is the fact that they can be easily synthesized in two or three steps with high yields (see Scheme 1), especially if performed in the microwave.[23] Thus, the production costs will be low which is of high importance for drugs against tropical diseases.

Acknowledgments

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.12.088.

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