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Synthesis of Antitumor Dendritic Imides

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Abstract—Dendritic imides were synthesized and evaluated as antitumor compounds. Compounds **8** and **11** showing a promising profile as inhibitors of *lck* but their antiproliferative activity against HT-29 was not so relevant.

Introduction

DNA intercalating agents are among the most common anticancer drugs used in the clinical therapy of human tumors. In our laboratory, we have discovered the antitumor potential of a series of mono and bisintercalating agents bearing naphthalimide chromophores.[1] Two compounds from this class (Fig. 1) amonafide and elinafide have been selected for phase II clinical trials.[2] On the other hand, although dendrimers with chains of amine ramification have been employed in gene therapy,[3] their chemical modification to join intercalating chromophores as antitumor compounds has never been described. Thus, the synthesis and pharmacological profile of such compounds could be of great interest. Our goal was to improve the therapeutic properties of these imides by means of increasing the binding affinity towards the DNA molecule when branching the basic chain to generating dendrite-like structures[4] (Fig. 2). Thus, the increase of potential hydrogen bonds and favorable electrostatic interactions between the protonated amino groups of the dendritic chains and the negatively charged sugar phosphate backbone should largely increase the affinity for DNA of these polymeric structures and the potency of the intercalator.[5] Although it is well known that the addition of polyamines to a solution of DNA–ethidium bromide complex displaces the intercalating agent from the double helix, it has been predicted that a multitude of anionic site charges are available for binding of additional basic functions beyond those present in the intercalator framework,[6] in a similar way like it has been proposed in our work.

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Chemistry

We have synthesized a series of imide derivatives linked to a branched basic chain. To carry out the synthesis, we first built the dendritic chain by a divergent procedure, as described by Vögtle[7] and Wörner,[8] starting from a bi-directional core. The chosen core was *N*-*tert*-butoxycarbonyl derivative, **1**. A repetition of a double

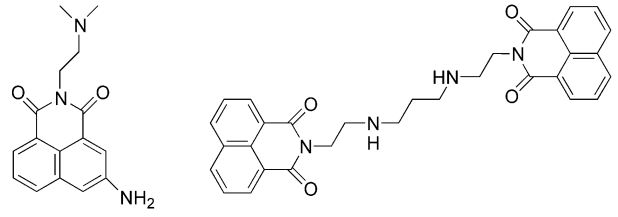

Figure 1.

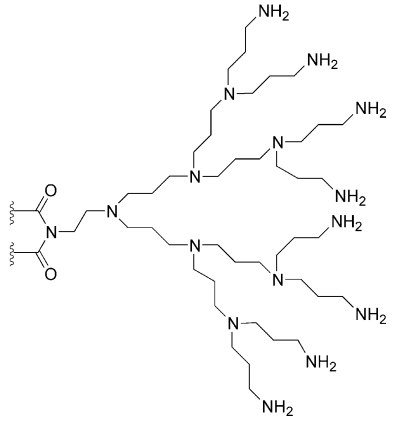

Figure 2.

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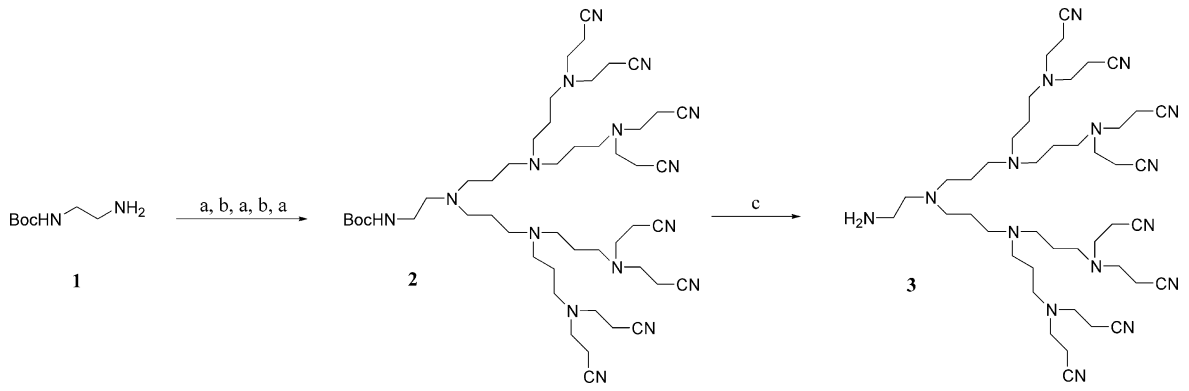
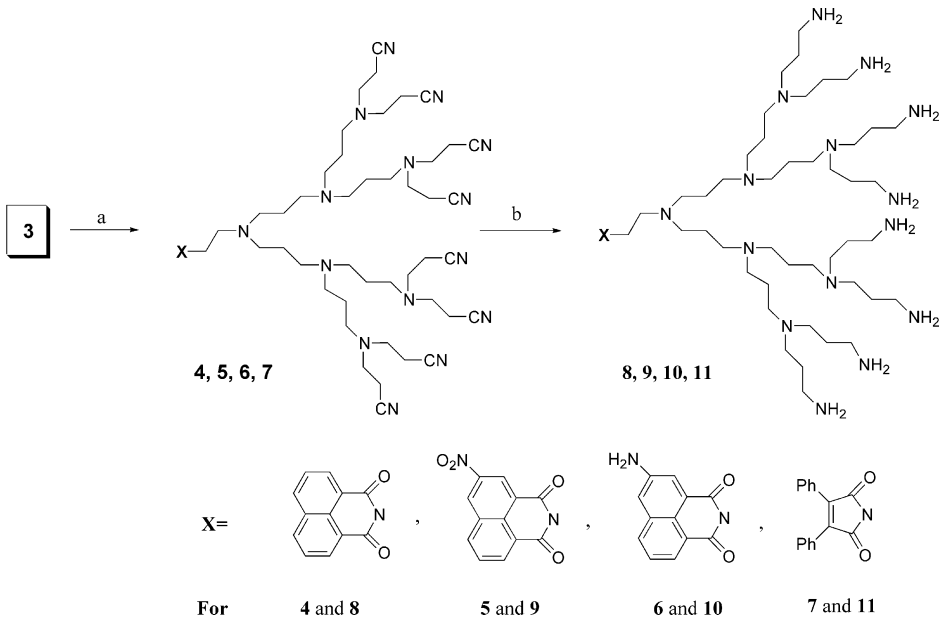


Scheme 1. (a) Acrylonitrile, MeOH; (b) H_2, Ni-Raney, 1.4 M NaOH in 95% EtOH; (c) TFA, CH_2Cl_2/anysol 1:1, 20 min, 80%.



Scheme 2. (a) Aromatic anhydride, absolute EtOH, rt; **4**: 18 h, 92%; **5**: 18 h, 53%; **6**: 48 h, 60%; **7**: 2 h, 80%; (b) H_2, Ni-Raney, 1.4 M NaOH in 95% EtOH; **8**: 48 h, 52%; **9**: 24 h, 60%; **10**: 24 h, 49%; **11**: 16 h, 77%.

Michael addition of acrylonitrile to primary amines[9] followed by hydrogenation with Ni-Raney at room temperature and at 60 psi in a solution of 1.4 M NaOH provided finally the octacyano **2**, with a good overall yield. Cleavage of the *tert*-butoxycarbonyl group with trifluoroacetic acid provided compound **3**, with the free amine moiety (Scheme 1). Imide derivatives were prepared by nucleophilic addition of the dendritic amine **3** to the appropriate 1,8-naphthalic anhydride-3-substituted to provide compounds **4**–**6**.[10] Also, we synthesized the imide derivative of 3,4-diphenyl maleic anhydride **7**, because this system exhibits relevant cytostatic activity.[11] Finally, reduction of end cyano groups of compounds **4**–**7** in described same conditions, led us to the target molecules **8**–**11**[12] (Scheme 2).

Biological Evaluation

In vitro cytotoxicity against human colon carcinoma cell line HT-29 was carried out.[13] Also, we tested some of these compounds against *lck* because this receptor has a great interest and this biological system was available at the laboratory.[14] The activity profile against HT-29 cell lines was not relevant probably due to poor permeabilities. However, compounds **8** and **11** showed high activity as inhibitors of *lck*. It appears that *lck* expression is largely confined to T cells, and its over expression may contribute to certain T-cell tumors.[15] Thus, selective inhibition of the *lck* function may be of

Table 1. Biological activities against HT-29 and *lck*

Compd	HT-29 ID_{50} (μM)	*Lck* IC_{50} (μM)
3	>100	3.8
4	>100	>50
5	>100	33.8
6	>100	5.02
7	57	>50
8	44	0.39
9	—[a]	—[a]
10	—[a]	—[a]
11	100	0.10
Amonafide	38	—[a]
Elinafide	0.014	—[a]

[a]Not determined.

interest for the treatment of several types of lymphomas such as non-Hodgkin[16] which express *lck* function. These results open up a new area of future research (Table 1). In conclusion, in this work we have synthesized new imide derivatives linked to a dendritic chain. Biological assays show no relevance for the activity of these compounds against HT-29 cell lines and values range around 10^{-5} μM. However, results obtained for the inhibition of *lck* seem promising and an excellent starting point for further development of this type of approximation in the search for new antitumor compounds.

Acknowledgements

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4. This work was presented at the 90th Annual Meeting of American Association of Cancer Research, Philadelphia, PA, April 10–14, 1999. Domínguez, G., Braña, M. F., de Pascual-Teresa, Sáez, B. *Proceedings of 90th Annual Meeting of AACR*, **1999**, *40*, 122.
5. This approach was further supported by molecular modeling studies in which has been determinated that these compounds are likely to bind a DNA octamer of a model d[GCACGTCG]$_2$ sequence through the central 5′-CpG-3′ step via either the mayor or the minor groove.[4]
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9. Typical procedure for synthesis of oligonitriles via cyanoethylation: To a cold solution of the polyamine (1.0 g, 1.9 mmol) in methanol (18 mL) were added dropwise 19 mL (288 mmol) of acrylonitrile. After stirring 1 h at 10 °C and 4 days at 60 °C, the quantitative biscyanoethylation was obtained. The solvent was evaporated under vacuo to obtain an oil which was purified by low pressure distillation.
10. Typical procedure for synthesis of imide derivatives: To a solution of **3** (0.89 g, 1.08 mmol) in 50 mL of absolute ethanol was added 0.21 g, (1.08 mmol) of 1,8-naphthalic anhydride and the mixture was stirred for 18 h at room temperature. The solvent was removed under reduced pressure to obtain 0.89 g (92%) of **4** as a light brown oil. ^{1}H NMR ($CDCl_3$): δ 1.64 (m, 12H, 6CH_2, $CH_2CH_2CH_2$), 2.50 (t, 24H, 12CH_2, CH_2CH_2N, J = 6.6 Hz), 2.59 (t, 16H, 8CH_2, NCH_2CH_2CN, J = 7.1 Hz), 2.75 (t, 2H, CH_2, NCH_2CH_2NCO, J = 7.1 Hz), 2.86 (t, 16H, 8CH_2, CH_2CN, J = 87.1 Hz), 4.26 (t, 2H, NCH_2CH_2NCO, J = 7.1 Hz), 7.78 (t, 2H, Har, J = 7.7 Hz), 8.24 (d, 2H, Har, J = 8.2 Hz), 8.5 (d, 2H, Har, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$): δ 163.8, 133.8, 131.3, 130.8, 127.7, 126.7, 122.1, 118.7, 52.3, 51.5, 51.2, 50.9, 49.1, 44.1, 37.6, 24.3, 23.9, 16.5. IR (neat): 2240, 1700, 1610 cm^{-1}.
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12. Typical procedure for synthesis of primary amines via reduction: To 25 mL of a 1.4 M solution NaOH in 95% ethanol, 1.13 g (1.12 mmol) of **4**, were added. The mixture was treated with 1.8 g of Ni-Raney and it was hydrogenated at 60 psi for 48 h at room temperature. The catalyst was filtered through Celite and washed with 95% ethanol. After diluting the filtrate with H_2O, ethanol was evaporated and the residue extracted several times with CH_2Cl_2. Thereby, the NaOH concentration in the aqueous phase was increased in every extraction step. The organic layers were dried with Na_2SO_4 and the solvent evaporated under vacuo to give 0.58 g (50%) of **8** as a light brown oil. ^{1}H NMR (D_2O): δ 1.40–1.56 (m, 28H, 14CH_2, $CH_2CH_2CH_2$), 2.32–2.43 (m, 42H, 21CH_2, CH_2N), 2.70 (m, 16H, 8CH_2, CH_2NH_2), 3.97 (bs, 2H, CH_2, CH_2NCO), 7.59 (bs, 2H, Har), 8.19 (bs, 4H, Har). ^{13}C NMR (D_2O): δ 163.3, 128.9, 127.6, 127.5, 127.4, 57.1, 50.3, 50.1, 48.0, 38.3, 27.6, 27.0, 24.8, 20.9. IR (neat): 3500, 1700, 1610 cm^{-1}.
13. The cytotoxicity of these compounds against HT-29 was measured using a standard MTT assay.[17] Human colon carcinoma cell line HT-29 was obtained from American Type Culture Collection.
14. Human recombinant *lck* catalytic domain was produced in a baculovirus expression system. Purified protein was used and IC_{50} values were determined by an enzyme linked immunosorbent (ELISA) phosphorylation assay using the universal tyrosine kinase substrate Poly(Glu,Tyr) 4:1 at 5 mM ATP.[18]
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