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Cytotoxic α-Bromoacrylic Derivatives of Distamycin Analogues Modified at the Amidino Moiety

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Abstract—The design, synthesis, in vitro and in vivo activities of novel α-bromoacrylic derivatives of distamycin A, modified at the amidino moiety by the replacement with basic or non-basic groups are reported. In spite of the relevance of these modifications of distamycin frame, the new derivatives are potent cytotoxics. The presence of the amidino moiety, is, therefore, not an absolute requirement for the activity. In particular due to a favorable myelotoxicity/cytotoxicity ratio, guanidino derivative PNU 166196 was selected for clinical development.

In recent years several papers dealing with distamycin A and distamycin-like derivatives as DNA minor groove binders have been published, however, although many of these papers have investigated the DNA binding-role of the oligopyrrolic frame,[1] little attention has been paid to the possible role of the strong basic amidino moiety. This moiety is typical not only of distamycin A, but also of other DNA minor groove binders, such as netropsin and synthetic diarylamidine derivatives as e.g., DAPI, berenil and pentamidine, which share with distamycin A a strong selectivity for TA-rich sequences.[2] The strong basic nature of the amidino group, which leads to its total protonation at any physiological pH, may affect both DNA binding and bioavailability.

Although distamycin or lexitropsin nitrogen mustard derivatives, in which the propionamidino moiety was replaced by aminoalkyl and anilino groups were described,[3,4] no systematic modification of the amidino moiety was undertaken until we recently reported benzoyl and cinnamoyl nitrogen mustard derivatives of distamycin A, in which the amidino moiety was modified or replaced by groups, basic and non basic, of different nature.[5]

Recently the α-bromoacrylamido derivative of four pyrrole homologue of distamycin A, PNU 151807, was reported and showed significant cytotoxicity and in vivo antileukemic activity.[6] PNU 151807, at variance with tallimustine, was found to bind but not to alkylate DNA minor groove AT-rich sequences, and it was hypothesized as the lead of a class of minor groove binders mechanistically different from tallimustine and congener mustards.[7]

This hypothesis was strengthened by the results described in a previous paper[8] in which we reported a series of halogenoacrylic derivatives of distamycin A and congeners prepared with the aim of defining the role of the halogenoacrylic moiety.

We now report the synthesis, in vitro and in vivo activities of novel α-bromoacrylic derivatives of four pyrrole units homologues of distamycin A modified at the amidino moiety (Formula I).

The modifications include the replacement of the amidino moiety of the parent PNU 151807 with basic amidino-derived analogues of different lipophilicity and bulk and with non-basic groups of different nature.

Chemistry

The novel derivatives reported in Table 1[9] were obtained by coupling α-bromoacrylamido-pyrrole-carboxylic acid, via corresponding acid chloride with desformyldistamycin derivatives appropriately modified

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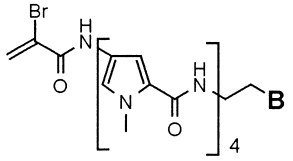


B = $C(NH)NH_2$, $C(NCH_3)NH_2$, $C(NCH_3)NHCH_3$, $C(NH)N(CH_3)_2$, C-Imidazolin-2-yl, $NHC(NH)NH_2$, $C(NOH)NH_2$, $C(NCN)NH_2$, $CONH_2$, CN

Formula 1.

Table 1.

Compound	B	In vitro[a]	In Vivo	
		IC_{50}	OD	T/C%
1[b]	$C(NH)NH_2 \cdot HCl$	6.31±1.34	1.56	200
2	$C(NCH_3)NH_2 \cdot HCl$	2.67±0.78	3.13	150
3	$C(NCH_3)NHCH_3 \cdot HCl$	1.86±0.18	1.56	258
4	$C(NH)N(CH_3)_2 \cdot HCl$	1.48±0.84	n.d.	n.d.
5	C-Imidazolin-2-yl·HCl	2.46±0.52	n.d.	n.d.
6	$NHC(NH)NH_2 \cdot HCl$	1.85±0.17	1.56	196
7	$C(NOH)NH_2$	8.58±1.55	6.25	186
8	$C(NCN)NH_2$	4.13±0.27	3.13	157
9	$CONH_2$	9.00±2.43	6.25	169
10	CN	18.1±2.27	12.5	157

[a]IC_{50} = 50% inhibitory concentration as the mean±S.E. from dose–response curves of at least two experiments, determined after 48 h of continuous exposure against L1210 cells. For in vivo studies cells were injected iv at day 0 and mice were treated iv the day after tumor injection; O.D. = optimal (non-toxic) dose < LD10. %T/C = median survival time of treated versus untreated mice×100. L1210 murine leukemia cell lines were obtained from NCI, Bethesda, USA.
[b]Compound 1 = PNU151807.

at the amidino moiety, with yields ranging from 30 to 70% (Scheme 1).

Latter intermediates in the case of amidino-derived compounds were easily obtained from distamycin A and a suitable amine derivative, in DMF at 70 °C, with yields ranging from 30 to 80% (Scheme 2).

Intermediate amide was prepared in 90% yield by alkaline hydrolysis in CH_3CN/H_2O at 70 °C, of the amidino moiety of distamycin A (Scheme 3).

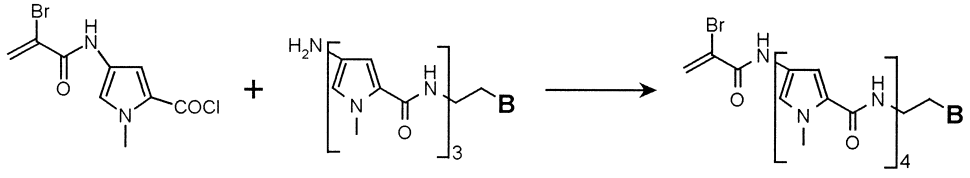
Intermediate nitrile was prepared in 75% yield, reacting distamycin A with succinic anhydride and K_2CO_3 in DMF, following an original procedure recently published by us (Scheme 4).[10]

Intermediate guanidino derivative was prepared, differently from other intermediates, by a step-by-step total synthesis from guanidinoethylamine, by iterative acylation with 4-nitro-pyrrolecarboxylic acid chloride and catalytic reduction of the nitro group, as previously reported[5] (Scheme 5).

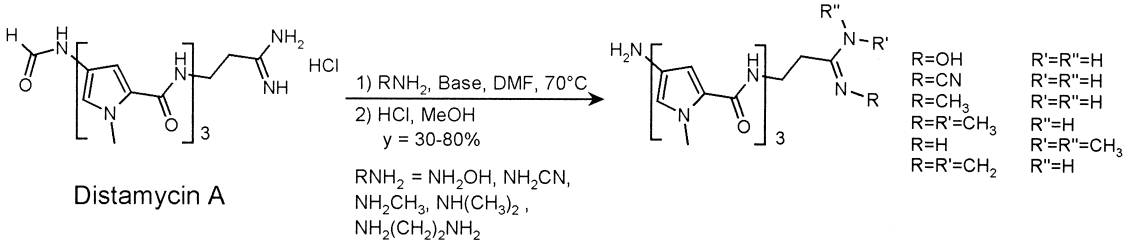
Results and Discussion

Tested compounds were assayed in vitro and in vivo on L1210 murine leukemia cells, evaluating cytotoxicity and antileukemic activity as previously described.[11]

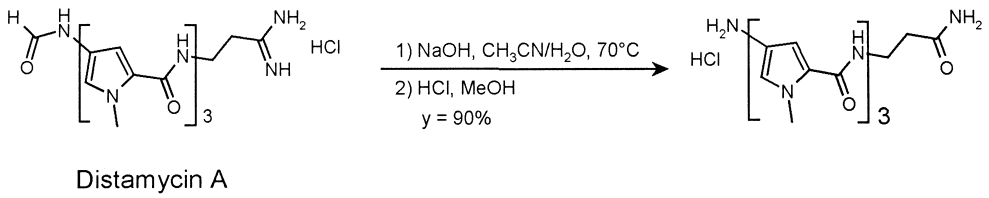
Table 1 data clearly show that the potent cytotoxicity of the parent amidino derivative PNU 151807 (**1**) is fully maintained not only by basic amidino-derived compounds of different lipophilicity and bulk, such as *N*-methylamidine (**2**), *N*,*N*′-dimethylamidine (**3**), *N*,*N*-dimethylamidine (**4**), 2-imidazoline (**5**), and by guanidine derivative (**6**), but also by non-basic amidino-derived compounds such as amidoxime (**7**) and cyanoamidine (**8**), and even by non-basic, non-amidino-derived amide **9**. Only nitrile (**10**) shows a modest decrease of activity. Alkylamidino and guanidino derivatives appear even more potent cytotoxics than the parent amidino compound. These data not only indicate that the amidino moiety is not an



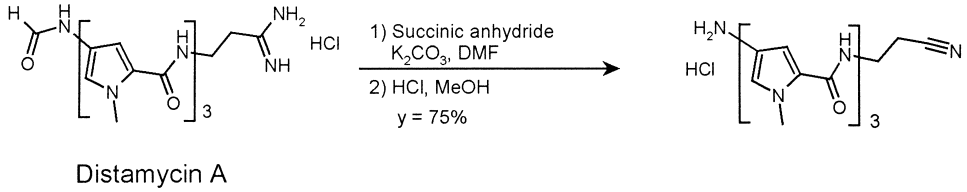
Scheme 1.



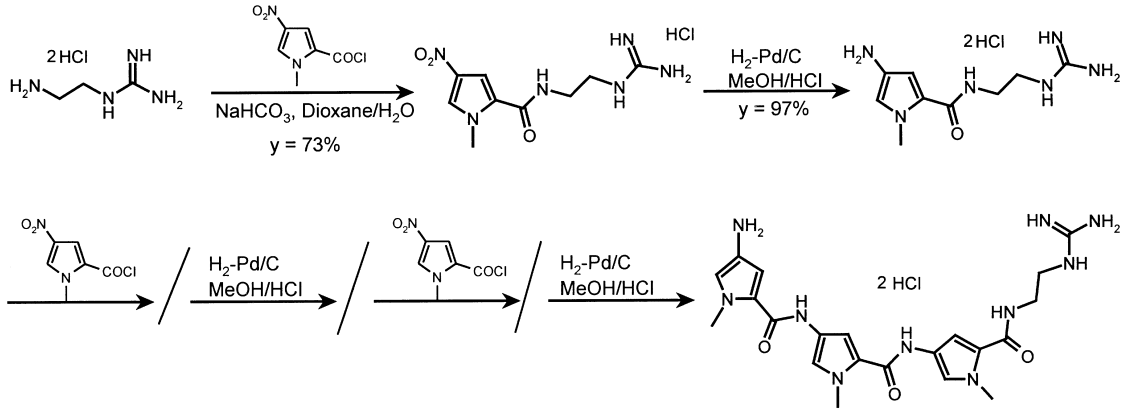
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

absolute requirement for activity, but also a lack of any correlation between the basicity of the amidine-like structure and cytotoxicity. This apparently contrasts with the common opinion that electrostatic interaction between the cationic moiety and the negatively charged DNA phosphate residues represents one of the main contributions to molecular recognition of distamycin and distamycin-like derivatives.[12]

A lack of correlation between the basicity of the amidine-replacing moiety and cytotoxicity was already demonstrated by us in the case of nitrogen mustard derivatives of distamycin A.[5] However, the high cytotoxic activity of the compounds of this series appears particularly relevant and occurs also in the case of modifications such as those represented by amidoxime (**7**) and amide (**9**), which led in the mustard series to a decrease of activity particularly relevant in the case of amidoxime.[5]

Also in vivo antileukemic activities appear equivalent to, or even better than, that of the parent PNU 151807. Some of these compounds, which appear significantly more cytotoxic than tallimustine and show a favorable myelotoxicity/cytotoxicity ratio, have been selected for further extensive evaluation in vitro and on murine solid tumors and human xenografts.

In particular the guanidino derivative **6**, PNU 166196, results 20-fold more active than tallimustine in inducing apoptosis in A2780 human ovarian carcinoma cells,[13] circumvents in vitro and in vivo resistance to alkylating agents and topo I inhibitors and it shows an outstanding myelotoxicity/cytotoxicity ratio, being its mean IC_{50} against a series of tumor cells about eighty times lower than its IC_{50} on human CFU-GM hematopoietic progenitors cells.[14]

This compounds, as the parent PNU 151807, appears unreactive in DNA alkylation assays.[14] All these facts underline the novelty of PNU 166196 for both the profile of activity and the mechanism of action. PNU 166196 is presently undergoing Phase I Clinic.

References and Notes

1. See e.g., Pindur, U.; Fischer, G. *Curr. Med. Chem.* **1996**, *3*, 379 and references therein.
2. See e.g., Zunino, F.; Animati, F.; Capranico G. *Current Pharmaceutical Design* **1995**, *1*, 83 and references therein.
3. See e.g., (a) Lee, M.; Rhodes, A.L.; Wyatt, M.; Forrow, S.; Hartley, J.A. *Anticancer Drug Design* **1993**, *8*, 173, (b) Wyatt, M.D.; Garbiras, B.J.; Haskell, M.K.; Lee, M.; Souhami, R.L.; Hartley, J.A., *Anticancer Drug Design* **1994**, *9, 511,* (c) Wyatt, M.D.; Lee, M.; Hartley, J. A. *Anticancer Drug Design* **1997**, *12*, 49.

4. Brooks, N.; Hartley, J. A.; Simpson, J. E.; Wright, S. R.; Woo, S.; Centioni, S.; Fontaine, M. D.; McIntyre, T. E.; Lee, M. *Biorg. Med. Chem.* **1997**, *5*, 1497.
5. Cozzi, P.; Beria, I.; Biasoli, G.; Caldarelli, M.; Capolongo, L.; Geroni, C.; Mongelli, N. *Biorg. Med. Chem. Lett.* **1997**, *7*, 2979.
6. D'Alessio, R.; Geroni, C.; Biasoli, G.; Pesenti, E.; Grandi, M.; Mongelli, N. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1467.
7. Marchini, V.; Cirò, M.; Gallinari, F.; Geroni, C.; Cozzi, P.; D'Incalci, M.; Broggini, M. *Br. J. Cancer* **1999**, *80*, 991.
8. Cozzi, P.; Beria, I.; Caldarelli, M.; Capolongo, L.; Geroni, C.; Mongelli, N. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1269.
9. Tested compounds were purified by silica gel column chromatography (eluant CH_2Cl_2:CH_3OH: 80:20) and gave satisfactory analytical values and ^1H NMR spectra in agreement with assigned structures. representative ^1H NMR data of the compounds (DMSO-d_6) are given. (Bruker AC200 spectrometer, δ in ppm, TMS as internal standard). (**1**): 7.94 (t, $J = 5.9$ Hz, 1H); 7.3 (b.s., 1H); 6.8 (b.s., 1H); 3.3 (m, 2H); 2.30 (t, $J = 7.2$ Hz, 2H). (**2**): 5 (b.s., 1H); 9.1 (b.s., 1H); 8.5 (b.s., 1H); 8.22 (t, $J = 5.9$ Hz, 1H); 3.48 (m, 2H); 2.79 (s, 3H); 2.62 (m, 2H). (**3**): 9.2 (b.s., 2H); 8.33 (t, $J = 6.0$ Hz, 1H); 3.44 (m, 2H); 3.00 (s, 3H); 2.79 (s, 3H); 2.73 (m, 2H). (**4**): 9.0 (b.s., 1H); 8.31 (t, $J = 5.8$ Hz, 1H); 3.46 (m, 2H) 3,22 (s, 3H); 3.03 (b.s., 3H), 2.77 (t, $J = 6.5$ Hz, 2H). (**6**): 8.10 (t, $J = 5.9$ Hz, 1H); 7.56 (t, $J = 5.9$, 1H); 7.2 (b.s., 4H); 3.30 (m, 4H). (**7**): 9.5 (b.s., 2H); 8.5 (b.s., 1H); 7.98 (t, $J = 5.9$ Hz, 1H); 3.38 (m, 2H); 2,31 (m, 2H).(**8**): 8.3 (b.s., 2H); 8.1 (b.s., 1H); 3.4 (b.s., 2H); 2,6 (b.s., 2H). (**9**): 7.94 (t, $J = 5.9$ Hz, 1H); 6.8 (b.s., 2H); 3.33 (m, 2H); 2.30 (t, $J = 7.2$ Hz, 2H). (**10**): 8.36 (t, $J = 5.9$ Hz, 1H); 3,42 (m, 2H); 2.75 (t, $J = 6.5$ Hz, 2H).
10. Caldarelli, M.; Biasoli, G.; Cozzi, P.; Mongelli, N. *Tetrahedron Lett.* **1998**, *39*, 3551.
11. Geroni, C.; Pesenti, E.; Tagliabue, G.; Ballinari, D.; Mongelli, N.; Broggini, M.; Erba, E.; D'Incalci, M. *Cancer Res.* **1993**, *53*, 308.
12. See e.g. Lown, J. W. *Chemtracts-Organic Chemistry* **1993**, *6*, 205.
13. Ciomei, M.; Pastori, W.; Cozzi, P.; Geroni, C. Proceedings of AACR, Philadelphia 1999, 40 Abst.1490, 224.
14. Geroni, C.; Pennella, G.; Capolongo, L.; Moneta, D.; Rossi, R.; Farao, M.; Marchini, S.; Cozzi, P. *Proceedings of AACR, San Francisco*, 2000, in press.