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NOVEL PHENYL NITROGEN MUSTARD AND HALF-MUSTARD DERIVATIVES OF AMIDINO-MODIFIED DISTAMYCIN

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Abstract : The design, synthesis, and *in vitro* and *in vivo* activities of novel benzoyl and cinnamoyl nitrogen mustard and half-mustard derivatives of distamycin A, in which the amidino moiety has been replaced by moieties of different physico-chemical features, are described and structure-activity relationships are discussed. Some amidino-modified derivatives show significant cytotoxicity and antileukemic activity.
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Tallimustine, a benzoic acid N-mustard derivative of distamycin A, is a potent cytotoxic and antitumor agent[1] which, like distamycin A itself, has been shown to bind to the DNA minor groove AT-rich sequences[2].

In recent years several papers dealing with distamycin 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 distamycin frame[3], little attention has been paid to the possible role of the strongly basic amidino moiety which is typical not only of distamycin A, but also of other DNA minor groove binders, such as netropsin, DAPI and berenil [4]. This role , due to the strongly basic nature of the amidino group which leads to its total protonation at any physiological pH, may affect both the DNA binding and bioavailability.

Although distamycin or lexitropsin derivatives in which the propionamidino moiety has been replaced by a dimethylaminoethyl / propyl group[5] have previously been described, no systematic modification of the amidino moiety has been undertaken until now.

In a previous paper [6] we described the synthesis and the biological activities of novel benzoyl and cinnamoyl nitrogen mustard and half-mustard derivatives of distamycin A. Here we report the synthesis, and *in vitro* and *in vivo* activities of novel benzoyl and cinnamoyl nitrogen mustard and half-mustard derivatives of distamycin, the compounds of general formula **1** and **2.** In these compounds the amidino moiety has been modified or replaced by various groups, leading to electronic, lipophilic and steric modifications of the DNA binding frame and to different physico-chemical features of the whole distamycin-derived molecule.

As a whole the amidino moiety was modified to include ionizable, acidic or basic, and non-ionizable groups, wherein the basic groups are of different strength (estimated pKa ranging approximately from 8 to 12), and wherein the nitrogen atoms, present in most compounds, are either sp^2 or sp^3 hybridised.

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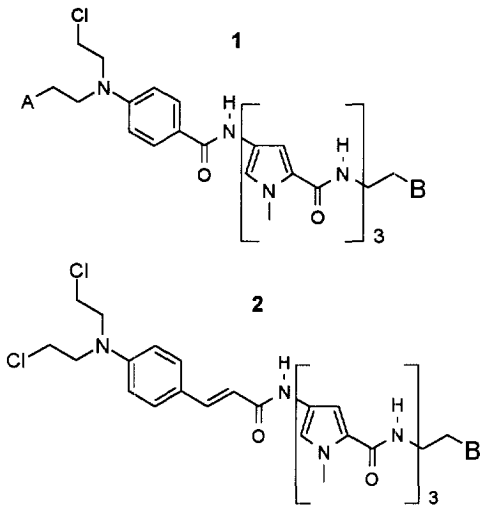
However the amidino moiety was in most cases replaced by moieties that maintained the amidine structure, notwithstanding significant modification of the physico-chemical features.



A = Cl, H ; B = $C(NH)NH_2$, $CH_2N(CH_3)_2$, , $CONH_2$, CN, COOH, CH_2OH, $C(NOH)NH_2$, $C(NNH_2)NH_2$, $C(NCN)NH_2$, $C(NCH_3)NH_2$, $C(NCH_3)NHCH_3$ $NHC(NH)NH_2$, imidazolin-2-yl, imidazol-2-yl

CHEMISTRY

New compounds[7], most of which are benzoyl nitrogen mustard derivatives, while only few are half-mustard benzoyl and cinnamic mustard derivatives, are reported in the Table, together with tallimustine **1a**, its ethyl half-mustard **1o** and cinnamic mustard analog **2a** as reference compounds. The mustard and half-mustard derivatives containing the modified amidino moieties amidoxime (**1b,2b**), amidrazone (**1c**), cyanamidine (**1d,1p.2c**) mono methylamidine (**1e,1q,2d**), dimethylamidine (**1f,2e**), imidazoline (**1g**) and imidazole (**1h**), a formal amidine, were prepared by the direct reaction of the parent amidine with the appropriate amine derivative. Although the reaction of the amidino moiety with different amine derivatives has been reported in the literature [8], the occurrence of this kind of nucleophilic attack in our case appears noteworthy due to the potential electrophilic reactivity of the nitrogen mustard moieties. Under our conditions, no significant degradation of the nitrogen mustard moiety occurred and the reactions proceeded with satisfactory yields, ranging, non optimised, from 30 to 80 %.

In detail amidoxime **1b** was prepared from tallimustine and 3 equivalents of H_2NOH free base, obtained *in situ* from $H_2NOH.HCl$ and triethylamine, in DMF at 70°C; amidrazone **1c** was prepared under the same conditions with NH_2NH_2. HCl; cyanamidine **1d** was prepared analogously with 3 equivalents of H_2NCN sodium salt,

obtained *in situ* with NaH in DMF; N-methylamidine **1e** and N,N-dimethylamidine **1f** were prepared by reacting tallimustine, dissolved in DMF, with 3 equivalents of aqueous CH_3NH_2 at 25° C or 6 equivalents of aqueous CH_3NH_2 at 80°C respectively; imidazolin-2-yl derivative **1g** was prepared from tallimustine and 3 equivalents of ethylendiamine in CH_3CN/H_2O (2 / 1) at 25°C and analogously imidazol-2-yl derivative **1h** was prepared with 3-aminopropionaldheyde dimethylacetale at 70°C, followed by hydrolysis with 2N HCl .

Guanidino derivative **1i**, at variance with true amidino derivatives, was prepared by total synthesis. Thus 2-guanidinoethylamine[9] was reacted with 4-nitro-1-methyl-pyrrole-2-carboxylic acid chloride and then the oligopyrrolic frame of distamycin was built, step by step, by iterative reduction of nitro group and subsequent coupling with 4-nitro-1-methyl-pyrrole-2-carboxylic acid chloride, as reported for the synthesis of distamycin [10]. Final coupling with benzoic acid nitrogen mustard, *via* chloride, gave **1i**.

Analogously, dimethylamino derivative **1j** was prepared by coupling benzoic acid nitrogen mustard, *via* chloride, with the oligopyrrolic frame built up step by step as described [11].

Amide derivative **1k** was prepared in 85 % yield by the hydrolysis of the amidino moiety of tallimustine with NaOH in refluxing CH_3CN/H_2O for 1 h. The fact that no hydrolysis of the mustard moiety occurred under these conditions confirms the stability of the nitrogen benzoyl mustard to nucleophilic attack.

Nitrile derivative **1l** was prepared in 65 % yield, reacting tallimustine with succinic anhydride and K_2CO_3 in DMF at 60°C. This unusual and interesting conversion of an amidine group into the corresponding nitrile will be the subject of a future paper.

Carboxylic acid derivative **1m** was prepared by coupling N,N-dichloroethyl benzoic mustard in dioxane-H_2O *via* acid chloride, with the aminoacid derivative obtained, in 85 % yield, from the hydrolysis of distamycin A with NaOH in refluxing MeOH/H2O for 22 h. Carbinol derivative **1n** was prepared, in 40 % yield, by reduction of acid **1m** with B_2H_6 in THF at 25°C.

Half mustard derivatives **1p**, **1q** and **1r**, were prepared with the same procedures and conditions described for **1d**, **1e**, **1i** from the corresponding half mustard **1o**. Cinnamic derivatives **2b-2f** were prepared, with the same procedures and conditions described for their benzoic mustard counterparts.

Tallimustine **1a** and the other intermediate distamycin-derived half-mustard **1o** and mustard **2a** were prepared, as described[6], by coupling the corresponding phenyl nitrogen mustard acid, with desformyl-distamycin dihydrochloride. All tested compounds were assayed *in vitro* and *in vivo* on L1210 murine leukaemia cell line evaluating cytotoxicity and antileukemic activity, reported in the Table, as previously described [12].

RESULTS AND DISCUSSION

Five benzoyl nitrogen mustard derivatives, cyanoamidine **1d**, N-methylamidine and N,N-dimethylamidine **1e** and **1f**, imidazol-1-yl derivative **1h** and guanidino derivative **1i**, maintain a cytotoxicity substantially equivalent to

that of tallimustine. While **1e,1f,** and **1i** are strongly basic amidine-like derivatives, cyanoamidine **1d** is not basic at all.

Comp.	A	B	in vitro IC50 ng/mL	in vivo OD mg/kg	in vivo T/C%
1a[a]	Cl	$C(NH)NH_2.HCl$	50.3±5.9	3.13	133
1b	Cl	$C(NOH)NH_2$	1476.7±163.1	12.5	129
1c	Cl	$C(NNH_2)NH_2$	738.1±36.2	12.5	136
1d	Cl	$C(NCN)NH_2$	37.9±5.6	6.25	129
1e	Cl	$C(NCH_3)NH_2.HCl$	38.5±11.3	3.13	150
1f	Cl	$C(NCH_3)NHCH_3.HCl$	36.5±11.3	3.13	100
1g	Cl	C-Imidazolin-2-yl.HCl	142.5±17.5	n.d.	n.d.
1h	Cl	C-Imidazol-2-yl.HCl	69.0±16.0	6.25	117
1i	Cl	$NHC(NH)NH_2.HCl$	64.4±3.6	12.5	114
1j	Cl	$CH_2N(CH_3)_2.HCl$	1004.0±113.0	25	100
1k	Cl	$CONH_2$	130.0±33.0	n.d.	n.d.
1l	Cl	CN	94.0±6.0	25	133
1m	Cl	COOH	959.7±212.0	50	100
1n	Cl	CH_2OH	>2000	n.d.	n.d.
1o	H	$C(NH)NH_2.HCl$	42.0±9.1	3.13	133
1p	H	$C(NCN)NH_2$	362.8±171.1	6.25	267
1q	H	$C(NCH_3)NH_2.HCl$	21.2±6.7	3.13	129
1r	H	$NHC(NH)NH_2.HCl$	62.2±14.4	6.25	158
2a	-	$C(NH)NH_2.HCl$	7.2±2.1	6.25	267
2b	-	$C(NOH)NH_2$	42.4±21.3	6.25	150
2c	-	$C(NCN)NH_2$	5.4±1.4	12.5	183
2d	-	$C(NCH_3)NH_2.HCl$	3.9±1.6	3.13	192
2e	-	$C(NCH_3)NHCH_3.HCl$	6.4±2.5	1.56	100
2f	-	$NHC(NH)NH_2.HCl$	7.4±2.0	3.13	200

[a] tallimustine ; IC_{50} = 50% inhibitory concentration as the mean ± SE 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 i.v. at day 0 and mice were treated i.v. the day after tumor injection; O.D.= optimal (non-toxic) dose <LD10 : %T/C = median survival time of treated vs. untreated mice x 100. L1210 murine leukaemia cell lines were obtained from NCI, Bethesda, USA .

Moreover, the weakly basic imidazolyl compound **1h**, which only formally can be considered an amidine derivative, shows a cytotoxicity better than that of imidazoline **1g**, a strongly basic cyclic amidine. These data indicate a lack of correlation between the basicity of the amidine-like structure and cytotoxicity. On the other hand, the low cytotoxicity of amidoxime **1b** and amidrazone **1c** indicate that the presence of an amidino-like structure does not guarantee significant activity. Among non-amidine-like derivatives the low cytotoxicity of dimethylamino derivative **1j** must be underlined. Under our conditions this compound appears markedly less active than tallimustine, in contrast to reports by other authors[5b] . On the contrary, the significant cytotoxicities of non-basic carbamoyl and cyano derivatives **1k** and **1l** are notable if compared with the low or very low activities of carboxylic and carbinol derivatives **1m** and **1n** respectively. Carbamoyl and cyano derivatives **1k** and **1l** present a pattern of sequence specificity of DNA alkylation different from that of tallimustine [13].

The positive role played by some modifications of the amidino moiety in the benzoyl mustard series, is confirmed also in the case of half-mustard derivatives, as shown by methylamidine **1q** and guanidine **1r**, and in the case of cinnamic mustards derivatives, as shown by methylamidine **2d**, dimethylamidine **2e**, cyanoamidine **2c**, and guanidine **2f**. These compounds maintain or even improve the cytotoxicity of the amidine parent compounds **1o** and **2a** respectively. On the other hand the amidoxime derivative **2b** of the cinnamic mustard shows a decreased activity in comparison to the parent compound, as does the corresponding amidoxime **1b** of the benzoyl series.

The *in vivo* antileukemic activity is apparently poorly correlated both with cytotoxicity and the basicity of the modified amidino moiety. The activity of methylamidino derivatives appears good and is roughly comparable to that of the parent amidino derivatives (e.g. **1e** vs. **1a** ; **1q** vs. **1o** : **2d** vs. **2a**), while on the contrary, dimethylamidino derivatives such as **1f** and **2e**, appear inactive despite significant cytotoxicity. On the whole cinnamic mustards appear to be more active *in vivo* than their benzoic counterparts, as occurs for the parent amidino derivative **2a** in comparison with tallimustine **1a**, with the aforementioned exception of dimethylamidino derivative **2e**. Cinnamic mustards **2d** and **2f**, endowed with significant *in vitro* and *in vivo* antileukemic activity, have been considered for further extensive pharmacological evaluation.

In conclusion, while no clear-cut structure-activity relationship can be drawn and it is difficult to find common physico-chemical features both among active and inactive compounds, it appears that the presence of the amidino moiety, or even of a basic moiety, is not an absolute requirement for *in vitro* and *in vivo* activity of distamycin mustards derivatives.

This apparently contrasts with the established 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[14].

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6 Previous paper in this issue

7 Tested compounds were purified by silica gel column chromatography (eluant CH_2Cl_2 / CH_3OH : 80 / 20) and gave satisfactory analytical values and ^{1}H-NMR data, in agreement with assigned structures. ^{1}H-NMR data concerning the amidino-modified moiety of representative compounds (DMSO-d_6) are given. (Bruker AC 200 spectrometer, δ in ppm, TMS as internal standard). **1b**: 8.82 (s,1H); 7.87 (t, J=5.7Hz, 1H); 5.4 (b.s., 2H); 3.32 (m, 2H); 2.20 (m, 2H). **1c**: 8.7 (b.s., 2H); 8.19 (t, J=5.7Hz, 1H); 5.0 (b.s., 2H); 3.44 (m, 2H); 2.57 (t, J=6.5Hz, 2H). **1d**: 8.0 (b.s., 1H); 8.1 (b.s., 1H); 8.84 (m, 1H); 3.48 (q, J=6.7Hz, 2H); 2.61 (m, 2H). **1e**: 9.55 (b.s., 1H); 9.1 (b.s., 1H); 8.6 (b.s., 1H), 8.26 (t, J=5.8Hz, 1H), 3.50 (m, 2H); 2.79 (s, 3H), 2.57 (m, 2H). **1g**: 10.0 (b.s., 2H); 8.29(t, J=5.7Hz, 1H); 3.6-3.9 (m 4H); 3.48 (m, 2H); 2.68 (t, J=6.7Hz, 2H). **1h**: 11.80 (b.s., 1H); 8.12 (t, J=5.8Hz, 1H); 6.87 (s, 2H); 3.42 (m, 2H); 2.81 (m, 2H). **1i**: 8.12 (b.s., 1H); 7.65 (b.s., 1H); 7.2 (b.s., 4H); 3.1-3.4 (m, 4H). **1j**: 8.12 (t, J=5.8Hz, 1H), 4.09 (t, 2H); 2.90 (t, 2H); 2.65 (s, 6H); 1.98 (m, 2H). **1k**: 7.96 (t, J=5.9Hz, 1H); 7.34 (b.s., 2H); 3.33 (m, 2H); 2.30 (t, J=7.2Hz, 2H). **1l**: 8.34 (t, J=6.0Hz, 1H); 3.40 (m, 2H); 2.72 (t, J=6.4Hz, 2H).**1m**: 8.08 (t, J=5.8Hz, 1H); 3.30 (m, 8H).

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