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NOVEL PHENYL NITROGEN MUSTARD AND HALF-MUSTARD DERIVATIVES OF DISTAMYCIN A

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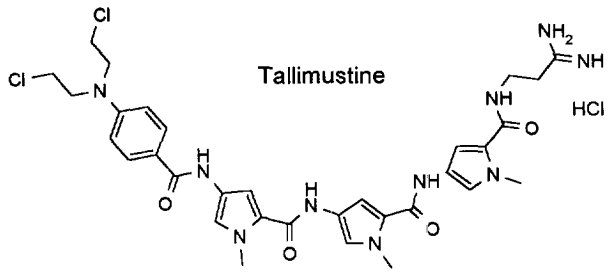
Abstract : The design, synthesis, *in vitro* and *in vivo* activities of novel benzoyl and cinnamoyl nitrogen mustard and half-mustard derivatives of distamycin A are described and structure-activity relationships are discussed. The equipotent activities of N-ethyl-N-chloroethyl half-mustards and N,N-dichloroethyl mustards and the superior activities of cinnamoyl derivatives are the most relevant features of the series. © 1997 Elsevier Science Ltd.

DNA minor groove binders represent a new class of antitumor agents whose DNA sequence specificity may lead to a high selectivity of action [1]. The main representatives of this class which have reached the clinic are the antitumor agents derived from CC-1065 [2] and the nitrogen mustard tallimustine [3]. The latter is a benzoyl nitrogen mustard derivative of non-cytotoxic distamycin A [4] which exhibits a high efficacy against a variety of murine tumours and human xenografts. Tallimustine was shown to bind to the minor groove AT-rich sequences, as distamycin does, and, in contrast with conventional nitrogen mustards, to alkylate at adenine N(3) sites with no evidence of guanine N(7) alkylation [5].

In recent years, among several papers dealing with distamycin and distamycin-like derivatives as DNA minor groove binders, great attention has been paid to the role of the oligopyrrolic frame or its isosters, the so called lexitropsins [6], and some nitrogen mustards of isosteric distamycin derivatives have been described [7]. However, less attention has been paid to the study of the reactivity of the alkylating moiety, and in particular to the role of the mustard moiety, with the notable exception of the tethering of chlorambucil to distamycin and its analogs [8].



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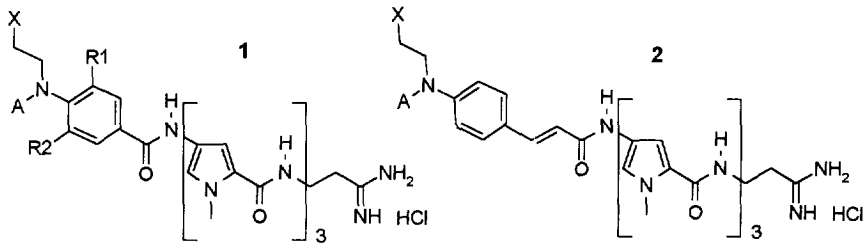
In this paper we report the synthesis, *in vitro* and *in vivo* activities of novel benzoyl and cinnamoyl nitrogen mustard and half-mustard derivatives of distamycin A, of general formula **1** and **2**, which are close analogues or vinylogues of tallimustine and we discuss their structure-activity relationships.



X = F, Cl, Br, OH ; **A** = H, CH_2-CH_3 , CH_2-CH_2-CH_3, CH_2-CH_2X ; $\mathbf{R_1}$ = H, F, CH_3 ; $\mathbf{R_2}$ = H, CH_3

The rationale that led to the synthesis of tallimustine was to tether to the distamycin frame, which plays the role of minor groove binding ligand, a potentially very mild alkylating moiety, represented by the benzoic acid *para*-nitrogen mustard (BAM). The aim was to avoid, as much as possible, aspecific alkylation of both intra and extracellular biological nucleophiles. The combination of a weak alkylating moiety and of a DNA minor groove binder may explain the very high DNA sequence specificity of tallimustine, which is able to alkylate adenine N(3) only when this is present in the sequence 5'TTTTGA[5].

The weak alkylating power of BAM is due to its very low chemical reactivity [9], caused by the poor electronic availability of the aniline-type nitrogen, which is decreased by aromatic conjugation with the *para* carbonyl group. As a matter of fact BAM is devoid of significant cytotoxicity [10].

Compounds **1** and **2** were designed with the aim of gaining more information about the not fully established mechanism of action of tallimustine, and improving the pharmacological profile of the latter. In particular the cytotoxicity / myelotoxicity ratio required improvement in view of the fact that myelotoxicity has been the dose limiting toxicity of tallimustine in the clinic [11].

The rationale followed was to obtain very close analogs of tallimustine with different chemical reactivity of the mustard moiety, and less close analogs characterised by a fine tuning of the alkylating power and by different topological and conformational features of phenyl nitrogen mustard. Except in the case of dibromo mustard analogs of tallimustine, which was designed as a possible tool for the isolation of a DNA-ligand complex, we discarded the idea of simply maximising the reactivity of the mustard. Moreover difluoro and dibromo mustard analogs and the diol derivative, were synthesised to define the role of nitrogen mustard reactivity alone. Some *ortho*-methyl and fluoro phenyl analogs of tallimustine were synthesised in view of the possible effect of this kind of substitution both on the mustard reactivity and on the DNA binding. In fact *ortho* substitution may significantly affect nitrogen mustard reactivity both because of direct inductive or mesomeric effects and because the bulky nitrogen mustard group may be twisted out of conjugation with the π electrons of the phenyl ring,

making the nitrogen a better nucleophile[12]. Additionally, the presence of a substituent on the phenyl ring may affect the conformation of this ring in the minor groove close to the putative site of alkylation.

The isomer of tallimustine bearing the mustard moiety *meta* to the carbamoyl group was synthesised both in view of the topological and conformational diversity and the foreseen increased chemical reactivity arising from the lack of electron-withdrawing mesomeric effect of the *meta* carbamoyl.

Cinnamic mustard derivatives were synthesised both to obtain some increase of the mustard reactivity, and to increase the distance between the alkylating moiety and the DNA-binding distamycin frame.

Finally some half-mustard derivatives were synthesised to evaluate the possible effect of the presence of a sole alkylating arm of the mustard. It is known that in the case of the classical nitrogen mustards, the half-mustards analogs are genotoxic but substantially non cytotoxic [13] possibly due to the impossibility of crosslinking the two DNA strands after twin N(7)-guanine alkylation [14]. In the case of tallimustine however, such crosslinking was proved not to occur,[5] so that the cytotoxicity of half-mustard analogs of tallimustine could not be excluded.

CHEMISTRY

The new compounds synthesised and tested are reported in the Table [15]. Tallimustine (**1a**) was prepared by coupling N,N-dichloroethyl benzoic mustard, with desformyl-distamycin dihydrochloride (DDD) in dioxane-H_2O *via* the acid chloride, as previously described [16]. Diol derivative **1b** was prepared by hydrolysis of the nitrogen mustard moiety by heating tallimustine in boiling water for 3 h. Difluoro mustard **1c** was prepared by coupling 1.5 equivalents of N,N-difluoroethyl-benzoic mustard with DDD *via* the imidazolide. Dibromomustard hydrobromide **1d,** was similarly prepared by coupling N,N-dibromoethyl-benzoic mustard with desformyl-distamycin dihydrobromide. All other N,N dichloroethyl benzoic mustard derivatives were prepared by coupling the corresponding BAM with DDD, *via* the acid chloride, using procedures similar to that reported for tallimustine. Half-mustards **1i**, **1j**, **1k** and **1l** were prepared by coupling the appropriate benzoic acid half-mustards with DDD in dioxane/H_2O *via* the acid chloride. Cinnamic nitrogen mustards derivatives **2a** and **2b** were prepared from the corresponding N,N dichloroethyl cinnamic mustard, or from the N-ethyl-N-chloroethyl-cinnamic mustard and DDD *via* the imidazolide. Intermediate N,N-dihaloethyl acid mustards, were prepared as described in the literature [17] while N-H,N-chloroethyl and N-alkyl-N-chloroethyl acid mustards were obtained by reductive alkylation with $ClCH_2CHO$, $NaCNBH_3$ in HCl / MeOH [18]. DDD was prepared by acidic hydrolysis of distamycin A as previously described [19].

All tested compounds were assayed *in vitro* and *in vivo* on L1210 murine leukaemia cells, evaluating cytotoxicity and antileukemic activity as previously described [20]. The chemical reactivity of the mustard moiety of some representative derivatives was evaluated determining the rate of hydrolysis [21] and alkylation of 4-(4-nitrobenzyl) pyridine (NBP) [9] following classical procedures.

Comp.[a]	X	A	R_1	R_2	in vitro IC50 ng/mL	in vivo OD mg/kg	in vivo T/C%	$k_{hydrol.}$ s^{-1}	$k_{alkyl.}$ s^{-1}
1a[b]	Cl	CH_2CH_2Cl	H	H	50.3±5.9	3.13	133	$3.8\cdot10^{-5}$	$6.2\cdot10^{-4}$
1b	OH	CH_2CH_2OH	H	H	> 10000	nd	nd	_	nd
1c	F	CH_2CH_2F	H	H	> 10000	nd	nd	nd	nd
1d	Br	CH_2CH_2Br	H	H	0.6±0.1	0.39	169	$6.4\cdot10^{-4}$	$6.2\cdot10^{-3}$
1e	Cl	CH_2CH_2Cl	F	H	222.5±17.5	nd	nd	$5.6\cdot10^{-5}$	$5.8\cdot10^{-4}$
1f	Cl	CH_2CH_2Cl	CH_3	H	11.1±1.9	3.13	175	$1.4\cdot10^{-3}$	$1.4\cdot10^{-2}$
1g	Cl	CH_2CH_2Cl	CH_3	CH_3	227.6±13.4	6.25	171	$2.8\cdot10^{-3}$	$9.7\cdot10^{-3}$
1h[c]	Cl	CH_2CH_2Cl	H	H	208.0±4.4	3.13	118	$7.4\cdot10^{-5}$	$5.5\cdot10^{-4}$
1i	Cl	H	H	H	450.0±60.0	nd	nd	nd	nd
1j	Cl	CH_2CH_3	H	H	42.0±9.0	3.13	133	$3.2\cdot10^{-4}$	$5.5\cdot10^{-4}$
1k	Cl	$CH_2CH_2CH_3$	H	H	739.1±0.2	3.13	112	nd	nd
1l	Cl	CH_2CH_3	CH_3	H	55.3±33.3	1.56	150	nd	nd
2a	Cl	CH_2CH_2Cl	_	_	7.2±2.1	6.25	267	$2.9\cdot10^{-4}$	$1.3\cdot10^{-3}$
2b	Cl	CH_2CH_3	_	_	2.9±0.2	3.13	207	nd	nd

[a] all reported compounds are hydrochloride salts except 1d which is a hydrobromide. [b] tallimustine [c] meta isomer. IC_{50} = 50% inhibitory concentration as the mean ± SE from dose-response curves of at least two experiments, drug sensitivity was 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 ; $k_{hydrol.}$: hydrolysis in acetone/water (50/50), T=66°C, pH=8. $k_{alkyl.}$: alkylation of 4-(4-nitrobenzyl)pyridine (NBP), T=66°C.

RESULTS AND DISCUSSION

The data in the Table show that the cytotoxicity depends, as expected, on the presence of a reactive mustard moiety, since diol **1b** and difluoromustard **1c** are inactive, as is distamycin itself. However the cytotoxicity does not simply depend upon the chemical reactivity of the mustard moiety, as determined from the kinetics of hydrolysis and alkylation. In fact while there is a clear relationship between the reactivity and the cytotoxicity of tallimustine **1a** and its dibromo mustard analog **1d** , this is not the case for the other derivatives as can be seen from the data for the couples tallimustine and *ortho*-fluoro derivative **1e**, or *ortho*-methyl **1f** and di-*ortho*-methyl **1g** analogs, which show significantly different cytotoxicity in spite of substantially equivalent reactivity. The high chemical reactivity of **1f** and **1g** confirms the activating role of bulky *ortho* phenyl substitution on the nitrogen mustard, while the relatively low cytotoxicity of **1g**, in spite of its reactivity, suggests that the conformation which the phenyl ring could assume in the DNA minor groove, due to *ortho* dimethyl substitution, may play a significant role in the DNA binding. Similarly the reduced cytotoxicity of *meta* isomer **1h**, despite increased chemical reactivity, underlines the role which may be played by the spatial relationship between the nitrogen mustard moiety and the distamycin frame.

Cinnamic mustard **2a** appears significantly more cytotoxic than tallimustine in accordance with its increased chemical reactivity. Somewhat unexpectedly ethyl-chloroethyl half-mustard derivatives **1j** and **2b** show cytotoxicities substantially equivalent to, or better than, two-arm benzoic and cinnamic nitrogen mustards **1a** and **2a** respectively. It must be noted that **1i** and **1k**, both benzoic half-mustards with a non-alkylating arm different

from ethyl, show reduced cytotoxicity. The different cytotoxicity of ethyl and propyl half-mustard analogs in particular, suggests that the non-alkylating arm might not play a purely electronic role, such as the + I effect for example, but may possibly sterically affect DNA binding. Moreover half-mustard **1i**, although one order of magnitude less active than tallimustine, maintains a significant cytotoxicity, at variance with what has been reported for a similar half-mustard derivative of an imidazole-containing analog of distamycin[22].

Although *in vivo* antileukemic activity, is generally poorly correlated with cytotoxicity, the very good antileukemic activity of **1f**, **1g** and in particular of two cinnamic derivatives **2a** and **2b** must be underlined. The latter compounds, which appear significantly less myelotoxic than tallimustine, have been selected for further extensive evaluation on murine solid tumours and human xenografts.

Dibromo-mustard **1d** and ortho-methyl analog **1f** have been selected as tools to investigate the interaction of tallimustine and close congeners with DNA oligonucleotides containing the $T_4G\underline{A}$ consensus sequence identified for tallimustine. These studies will be subject of a forthcoming paper.

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15 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 spectra in agreement with assigned structures. ^{1}H-NMR data of representative compounds (DMSO-d_6) are given. (Bruker AC200 spectrometer, δ in ppm, TMS as internal standard) **1d**, δ : 10.02 (s, 1H); 9.95 (s, 1H); 9.92 (s; 1H); 8.91(b.s., 2H); 8.46 (b.s., 2H); 8.20 (t, J=5.3 Hz, 1H); 7.85 (m, 2H); 7.30 (d, J_1= 1.7 Hz, 1H); 7.24 (d, J_1, 1H); 7.17 (d, J_1, 1H); 7.08 (m, 2H); 6.97 (d, J_1, 1H); 6.81 (m, 2H); 3.86 (s, 3H); 3.85 (s, 3H); 3.81 (s, 3H); 3.8-3.7 (m, 4H); 3.62 (t, J=7.0 Hz, 4H); 3.46 (t, J=6.3 Hz, 2H); 2.60 (t, J=6.3 Hz, 2H); **1f**, δ :10.19 (s, 1H); 9.97 (s, 1H); 9.91 (s, 1H); 8.7 (b.s, 4H); 8.21 (t, J=5.7 Hz, 1H); 7.74 (m, 2H); 7.29 (d, J_1=1.8 Hz, 1H); 7.28 (d, J=7.5 Hz, 1H); 7.22 (d, J_1, 1H); 7.17 (d, J_1,1H); 7.08 (d, J_1, 1H); 7.05 (d, J_1, 1H); 6.94 (d, J_1, 1H); 3.85 (s, 3H); 3.83 (s, 3H); 3.80 (s, 3H); 3.3-3.7 (m, 10H); 2.60 (t, J=6.6 Hz, 2H); 2.33 (s, 3H); **1j**, δ : 9.96 (s, 1H); 9.90 (s, 1H); 8.98 (s, 1H); 8.98 (b.s., 2H); 8.65 (b.s., 2H); 8.21 (t, J=5.9 Hz, 1H); 7.82 (m, 2H); 7.27 (d, J_1=1.7 Hz, 1H); 7.22 (d, J_1, 1H); 7.17 (d, J_1,1H); 7.07 (d, J_1, 1H); 7.05 (d, J_1, 1H); 6.94 (d, J_1, 1H); 6.75 (m, 2H); 3.85 (s, 3H); 3.83 (s, 3H); 3.80 (s, 3H) 3.72 (m, 4H); 3.47 (m, 4H); 2.32 (m, 2H); 1.10 (t, J=6.9 Hz, 3H) ; **2a** , δ :10.01 (s, 1H); 9.97 (s, 1H); 9.94 (s, 1H); 8.95 (b.s., 2H); 8.57 (b.s.,2H); 8.24 (t, J=5.6 Hz, 1H); 7.44 (m, 2H); 7.39 (d, J_1 = 15.6 Hz, 1H); 7.29 (d, J_2=1.7 Hz, 1H); 7.24 (d, J_2, 1H); 7.18 (d, J_2, 1H); 7.06 (d, J_2, 1H); 6.95 (d, J_2, 1H); 6.94 (d, J_2 1H); 6.80 (m, 2H); 6.55 (d, J_1,1H); 3.9-3.6 (m, 8H); 3.86 (s, 3H); 3.85 (s, 3H); 3.81 (s, 3H); 3.50 (m, 2H); 2.60 (m, 2H); **2b**, δ 10.00 (s, 1H); 9.94 (s, 1H); 9.91(s, 1H); 8.95 (b.s., 2H); 8.55 (b.s., 2H); 8.21 (t, J=5.8 Hz, 1H); 7.41 (m, 2H); 7.37 (d, J_1=15.6 Hz, 1H); 7.27 (d, J_2=1.7 Hz, 1H); 7.23 (d, J_2, 1H); 7.17 (d, J_2, 1H); 7.05 (d, J_2, 1H); 6.95 (d, J_2, 1H); 6.93 (d, J_2, 1H); 6.73 (m, 2H); 6.50 (d, J_1, 1H); 3..85 (s, 3H); 3.84 (s, 3H); 3.80 (s, 3H) ; 3.80-3.30 (m, 8H) 2.60 (m, 2H); 1.10 (t, J=7.0 Hz, 3H).

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