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Phenyl Sulfur Mustard Derivatives of Distamycin A

Paolo Cozzi,[a,*] Italo Beria,[a] Marina Caldarelli,[a] Laura Capolongo,[b] Cristina Geroni,[b] Stefania Mazzini[c] and Enzio Ragg[c]

[a]*Department of Chemistry, Pharmacia & Upjohn, Discovery Research Oncology, Viale Pasteur 10, 20014 Nerviano, Milan, Italy*
[b]*Department of Pharmacology, Pharmacia & Upjohn, Discovery Research Oncology, Viale Pasteur 10, 20014 Nerviano, Milan, Italy*
[c]*Department of Molecular Sciences, Faculty of Agriculture, University of Milan Milan, Italy*

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Abstract—The design, synthesis, and cytotoxic activity of novel benzoyl and cinnamoyl sulfur mustard derivatives of distamycin A are described and structure–activity relationships are discussed. These sulfur mustards are more potent cytotoxics than corresponding nitrogen mustards in spite of the lower alkylating power, while their sulfoxide analogues are substantially inactive. Cinnamoyl sulfur mustard derivative (**7**) proved to be one of the most active distamycin-derived cytotoxics, about 1000 times more potent than melphalan. © 2000 Elsevier Science Ltd. All rights reserved.

DNA minor groove binders represent a class of antitumor agents whose DNA sequence specificity may lead to a high selectivity of action.[1] The main representatives of this class which reached the clinic are the antitumor agents derived from CC-1065, i.e., adozelesin, carzelesin and bizelesin,[2] and tallimustine (**1**).[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 tumors and human xenografts. Tallimustine was shown to bind to the minor groove AT-rich sequences, and in contrast with conventional nitrogen mustards, to alkylate at adenine N(3) sites with no evidence of guanine N(7) alkylation.[5]

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, unspecific alkylation of both intra and extracellular biological nucleophiles. The weak alkylating power of BAM,[6] is caused by the poor electronic availability of the aniline-type nitrogen, further decreased by aromatic conjugation with the *para* carbonyl group.

Recently we synthesised the cinnamic nitrogen mustard derivative of distamycin (**2**), i.e., a vinylogue of tallimustine, which proved to be significantly more cytotoxic than tallimustine.[7] We synthesised also the ethyl-chloroethyl half-mustard analogue (**3**) of tallimustine and its half-mustard cinnamic congener (**4**), which showed cytotoxicities substantially equivalent to, or better than those of the corresponding two arm nitrogen mustard derivatives **1** and **2**.[7] This underlines the mechanistic diversity of these compounds from classical nitrogen half-mustards which are substantially non cytotoxic,[8] possibly due to the impossibility of crosslinking the two DNA strands.[9]

The activity of distamycin half-mustards derivatives prompted us to the synthesis of sulfur mustard analogues of tallimustine and congeners, as one-arm alkylating derivatives of minor groove binder distamycin A.

A further motivation for the synthesis of phenyl sulfur mustard derivatives of distamycin was represented by the foreseen low reactivity of the thiiranium (sulfonium) cation, which should be the alkylating intermediate of sulfur mustards.[10] The low reactivities is caused by the aromatic conjugation with the electron withdrawing carbonyl or vinylcarbonyl function. These sulfur mustards should fulfil the rationale already followed in the case of tallimustine, with the aim to avoid aspecific reactivity as much as possible.

In spite of the fact that the traditional definition of mustard, applied to cytotoxic *N,N*-bis-haloethyl derivatives, arises from the nickname of mustard gas given to bis-dichloroethyl-sulfide *S*-$(CH_2CH_2Cl)_2$, a chemical weapon used in the course of first world war, the sulfur mustards

*Corresponding author. Fax: +39-02-4838-5351; e-mail: paolo.cozzi@eu.pnu.com

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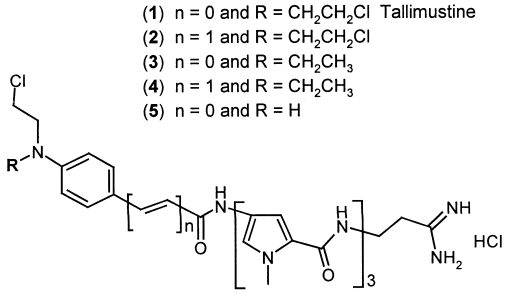
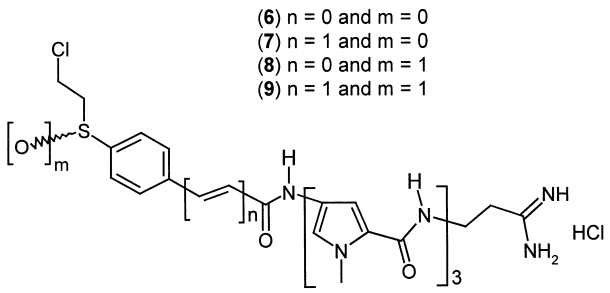
have found only a limited attention as therapeutic cytotoxics.[11] This was due to the fact that the tethering of a haloethyl sulfur moiety to an organic template gives rise to a one-arm alkylating compound with the same limits cited for nitrogen half-mustards, i.e., the impossibility of crosslinking the two DNA strands. Bifunctionality of sulfur mustards is apparently required for cytotoxicity, and sulfur mustards in which the two 2-chloroethyl substituents are on different sulfur atoms were investigated to meet this requirement.[12]



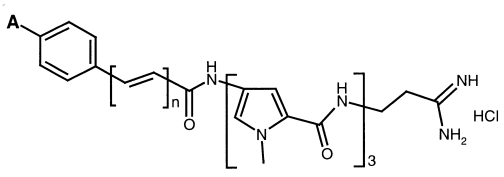
In this paper we report the synthesis, in vitro activities of benzoyl and cinnamoyl sulfur mustard derivatives of distamycin A, **6** and **7**, their sulfoxides **8** and **9**, and we discuss their structure–activity relationships also in comparison with their nitrogen mustard counterparts.

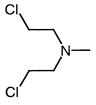
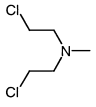
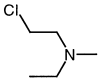
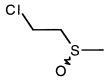
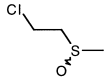
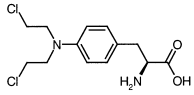


Chemistry

The sulfur mustard derivatives of distamycin A and reference nitrogen mustard derivatives are reported in the Table 1. Nitrogen mustard derivatives **1**–**5** were synthesised as previously reported[7] by coupling the corresponding benzoic or cinnamic acid mustards with desformyldistamycin. Novel sulfur mustards distamycin derivatives and their sulfoxides[13] were obtained analogously from corresponding benzoic or cinnamic acid sulfur mustards and their sulfoxides and desformyldistamycin, by coupling with DCC and HBT in DMF (yield = 45–70%). Benzoic and cinnamic sulfur mustard intermediates for **6** and **7**, were prepared by the reaction of corresponding 4-mercaptobenzoic and mercaptocinnamic

Table 1.



Compound[a]	A	*n*	In vitro IC_{50} (nM)	K^{alkyl} s^{-1} M^{-1}	Compound[a]	A	*n*	In vitro IC_{50} (nM)	K^{alkyl} s^{-1} M^{-1}
1[b]		0	68.5±8.0	6.2×10^{-4}	**6**		0	20.6±0.1	1.4×10^{-4}
2		1	9.5±2.8	1.3×10^{-3}	**7**		1	0.9±0.1	5.6×10^{-4}
3		0	60.0±1.3	5.5×10^{-4}	**8**		0	>600	5.0×10^{-5}
4		1	2.9±0.9	1.9×10^{-3}	**9**		1	>600	6.2×10^{-5}
5		0	>600	1.0×10^{-4}	**10**[c]			980.5±642.4	2.0×10^{-3}

[a]All reported compounds are hydrochloride salts.
[b]Tallimustine.
[c]Melphalan. 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. L1210 murine leukaemia cell lines were obtained from NCI, Bethesda, USA. k^{alkyl} = alkylation of 4-(4-nitrobenzyl)pyridine $T = 66\,^{\circ}C$. Nitrogen mustard derivatives **1**–**5** were described in ref 7.

acid respectively, with 2-chloroethanol and NaOH at 25 °C (yield = 90%), followed by chlorination with thionyl chloride in toluene (yield = 70%) in the case of the benzoic derivative, or with mesyl chloride in pyridine (yield = 60%) in the case of cinnamic compound. Benzoic and cinnamic sulfoxide mustard intermediates for **8** and **9** were obtained by oxidation of corresponding 2-chloroethyl sulfur mustards of benzoic and cinnamic acid with $NaIO_4$ in $MeOH/H_2O$ (yield = 70%).

Results and Discussion

All tested compounds were assayed in vitro on L1210 murine leukaemia cells, evaluating cytotoxicity as previously described.[14] The chemical reactivity of the mustard moieties were evaluated by determining the rate of alkylation of 4-(4-nitrobenzyl) pyridine (NBP) following classical procedures.[15]

These sulfur mustard derivatives show a cytotoxicity/reactivity ratio more favourable than the corresponding two and one-arm nitrogen mustards (**6** versus **1**, **3**, **5** and **7** versus **2**, **4**). The NBP alkylation rate of benzoyl sulfur mustard **6** is about four times lower than that of corresponding nitrogen mustards **1** and **3** and only little greater than that of nitrogen half-mustard **5**, however its cytotoxicity is about three times greater than that of **1** and **3** and about 30 times greater than that of **5**. Cinnamoyl sulfur mustard **7**, PNU 193821, which shows a chemical reactivity equivalent to that of tallimustine and its half-mustard **3**, is more than 60 times more potent than the latter. Compound **7** is about three times less reactive, but about 1000 times more cytotoxic, against L1210 leukaemia cells, than the classical nitrogen mustard melphalan (**10**).

Noteworthy, the corresponding sulfoxide derivatives **8** and **9** of sulfur mustards **6** and **7** show a poor alkylating power versus NBP, about one order of magnitude lower than that shown by sulfur mustards and tallimustine. This suggests that the formation of a thiiranium cation, which is no more possible in the case of sulfoxides, may play a key role in the alkylation mechanism of sulfur mustards towards NBP. More relevant is the substantial lack of cytotoxicity of sulfoxide derivatives **8** and **9**, which suggests that the formation of a thiiranium cation is possibly also the basis of the mechanism leading to cytotoxicity.

Conclusions

Benzoyl and cinnamoyl sulfur mustard derivatives of distamycin A described here represent a structural novelty among reported mustards because bifunctionality of sulfur mustards is apparently required for cytotoxicity. These compounds confirm the possibility, first demonstrated by nitrogen half-mustard derivatives of distamycin,[7] of achieving potent cytotoxic activity by means of one-arm alkylating derivatives of the latter, i.e., when the reactive one-arm mustard moiety is tethered to a sequence selective minor groove binding ligand.

Moreover these novel sulfur mustards, compared with their nitrogen counterparts, show a greater cytotoxicity and a very low chemical reactivity. While their low reactivity was hypothesised as a part of the design rationale, their cytotoxic activity is beyond what could be foreseen. Cinnamoyl sulfur mustard PNU 193821 proved to be the most potent distamycin-derived cytotoxic found in our laboratories ($IC_{50} = 0.9$nM against L1210 leukaemia cells, to be compared with $IC_{50} = 68.5$nM for tallimustine and $IC_{50} = 980.5$nM for melphalan).

Studies aimed to investigate the possible interaction of these derivatives with DNA oligonucleotides containing the $T_4G\underline{A}$ consensus sequence identified for tallimustine have been planned, in order to verify possible mechanistic analogies or differences with the latter, and will be the subject of a future paper.

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13. 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. ^1H NMR data of compounds **6**–**9** (DMSO-d_6) are given. (Bruker AC200 spectrometer, δ in ppm, TMS as internal standard): (**6**) 10.34 (s, 1H), 9.98 (s, 1H), 9.92 (s, 1H), 8.9 (b.s., 2H), 8.6 (bs, 2H), 8.21 (t, $J = 5.6$ Hz, 1H), 7.91 (m, 2H), 7.47 (m, 2H), 7.32 (d, $J = 1.7$ Hz, 1H), 7.24 (d, $J = 1.7$ Hz, 1H), 7.18 (d, $J = 1.7$ Hz, 1H), 7.10 (d, $J = 1.7$ Hz, 1H), 7.06 (d, $J = 1.7$ Hz, 1H), 6.95 (d, $J = 1.7$ Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.78 (t, $J = 7.3$ Hz, 2H), 3.48 (m, 4H), 2.60 (t, $J = 6.5$ Hz, 2H); (**7**): 10.23 (s, 1H), 9.96 (s, 1H), 9.91 (s, 1H), 8.9 (b.s., 2H), 8.6 (bs, 2H), 8.21 (t, $J = 5.6$ Hz, 1H), 7.55 (m, 2H), 7.46 (d, $J = 15.8$ Hz, 1H), 7.41 (m, 2H), 7.30 (d, $J = 1.7$ Hz, 1H), 7.23 (d, $J = 1.7$ Hz, 1H), 7.17 (d, $J = 1.7$ Hz, 1H), 7.01 (d, $J = 1.7$ Hz, 1H), 6.96 (d, $J = 1.7$ Hz, 1H), 6.95 (d, $J = 1.7$ Hz, 1H), 6.77 (d, $J = 15.8$ Hz, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.76 (t, $J = 7.3$ Hz, 2H), 3.49 (m, 2H), 3.40 (t, $J = 7.3$ Hz, 2H), 2.60 (t, $J = 6.4$ Hz, 2H); (**8**): 10.56 (s, 1H), 10.00 (s, 1H), 9.92 (s, 1H), 9.0 (bs, 2H), 8.6

(bs, 2H), 8.21 (t, $J=5.6$ Hz, 1H), 8.14 (m, 2H), 7.83 (m, 2H), 7.35 (d, $J=1.7$ Hz, 1H), 7.24 (d, $J=1.7$ Hz, 1H), 7.18 (d, $J=1.7$ Hz, 1H), 7.12 (d, $J=1.7$ Hz, 1H), 7.06 (d, $J=1.7$ Hz, 1H), 6.94 (d, $J=1.7$ Hz, 1H), 4.0-3.8 (m, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.49 (m, 2H), 3.46 (m, 1H), 3.26 (m, 1H), 2.61 (t, $J=6.6$ Hz, 2H); (**9**): 10.38 (s, 1H), 9.98 (s, 1H), 9.92 (s, 1H), 8.80 (bs, 4H), 8.22 (t, $J=6$ Hz, 1H), 7.80 (m, 2H), 7.74 (m, 2H), 7.55 (d, $J=15.6$ Hz, 1H), 7.32 (d, $J=1.7$ Hz, 1H), 7.24 (d, $J=1.7$ Hz, 1H), 7.18 (d, $J=1.7$ Hz, 1H), 7.06 (d, $J=1.7$ Hz, 1H), 6.98 (d, $J=1.7$ Hz, 1H), 6.94 (d, $J=1.7$ Hz, 1H), 6.93 (d, $J=15.6$ Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.96–3.76 (m, 2H), 3.48 (m, 2H), 3.45–3.20 (m, 2H), 2.61 (t, $J=6.5$ Hz, 2H).

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