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Cytotoxic Halogenoacrylic Derivatives of Distamycin A

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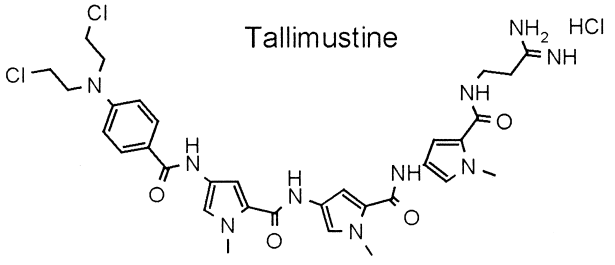
Abstract—The design, synthesis, in vitro and in vivo activities of a series of halogenoacrylic derivatives of distamycin A are described. The structure–activity relationships indicate a key role of the reactivity of α-halogenoacrylic moiety. The reactivity and the putative alkylating mechanism of these compounds are different from those of the nitrogen mustards and possibly based on a Michael type reaction. This supports the hypothesis that these compounds represent a class of minor groove binders mechanistically different from tallimustine.

DNA minor groove binders represent a class of antitumor agents whose DNA sequence specificity may lead to a high selectivity of action.[1] Representative compounds of this class, such as the antitumor agents derived from CC-1065[2] and the nitrogen mustard tallimustine,[3] have been thoroughly investigated. Tallimustine, a benzoyl nitrogen mustard derivative of non-cytotoxic distamycin A,[4] was shown to bind to the DNA minor groove AT-rich sequences and to alkylate at adenine N(3) sites.[5]

Tallimustine was the result of a drug design rationale which disclosed for the first time the possibility of obtaining antitumor agents by combining a chemically reactive moiety with a DNA binding frame, as distamycin A, which acts as a sequence-selective vector of an alkylating function.

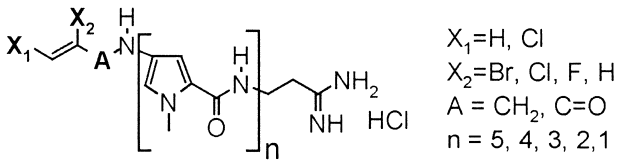


Following the same concept distamycin-derived cytotoxics showing an α-halogenoacrylamido moiety linked to distamycin or its four pyrrole homologue were reported.[6] These α-halogenoacrylic derivatives, and in particular the α-bromoacrylamido derivative of four pyrrole distamycin homologue, PNU 151807, appear endowed with significant cytotoxicity and in vivo activity against L1210 murine leukaemia. However, PNU 151807, at variance with tallimustine and congeners, was found unable to alkylate DNA minor groove AT-rich sequences, and found to inhibit kinase activity of cyclin dependent kinases.[7] This may suggest that PNU 151807 represents the lead of a new class of minor groove binders.

We now report a series of halogenoacrylic derivatives of distamycin A and congeners (Scheme 1) prepared with the aim of studying the structure–activity relationship, defining the role of the halogenoacrylic moiety and possibly giving a contribution to the definition of the mechanism of action of PNU 151807.

Chemistry

The novel[8] and tested compounds are reported in Tables 1–3, and were synthesised by coupling the appropriate acrylamido–pyrrolecarboxylic acid with *N*-desformyldistamycin dihydrochloride (DDD).



Scheme 1.

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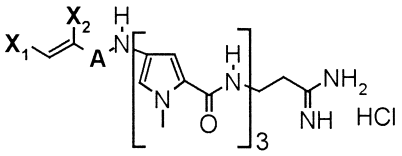


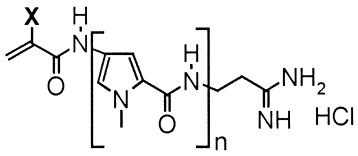


Table 1.

Comp.	A	X_2	X_2	In vitro IC_{50} nM[a,b]
1	C=O	H	Br	79.6±22.4
2	CH_2	H	Br	>1600
3	C=O	H	Cl	96.8±24.2
4	C=O	H	F	>800

[a]IC_{50} = 50% inhibitory concentration as the mean±SE from dose–response curves of at least two experiments.
[b]Drug sensitivity was determined after 48 h of continuous exposure against L1210 cells.

Table 2.

Comp.	A	X_1	X_2	In vitro IP_{ol} nM[a,c]
1	C=O	H	Br	103.5±33.0
3	C=O	H	Cl	1470.0±385.5
5[d]	C=O	Cl	H	>100,000
6[e]	C=O	Cl	H	>50,000
7	C=O	H	H	>50,000

[a]IC_{50} = 50% inhibitory concentration as the mean±SE from dose–response curves of at least two experiments.
[c]Drug sensitivity was determined after 4 h of continuous exposure against L1210 cells.
[d]*trans* isomer.
[e]*cis* isomer; L1210 murine leukaemia cell lines were obtained from NCI, Bethesda, USA.

Table 3.

Compound	X	n	In vitro[a] IC_{50} nM[b]	In vivo[c] OD mg/kg	In vivo[c] T/C%
8	Br	5	23.0[d]±5.0	0.78	167
9[e]	Br	4	6.3±1.3 13.4[d]±2.8	1.56	200
1	Br	3	98.8±24.2	12.5	100
14	Br	2	>1300	nd	nd
15	Br	1	>13,000	nd	nd
10	Cl	4	3.8±1.4	1.56	133
3	Cl	3	96.8±24.2	12.5	117
11	F	4	>700	nd	nd
4	F	3	>800	nd	nd

[a]Drug sensitivity was determined after 48 h of continuous exposure against L1210 cells.
[b]IC_{50} = 50% inhibitory concentration as the mean±SE from dose–response curves of at least two experiments.
[c]For in vivo studies cells were injected iv at day 0 and mice were treated iv the day after tumor injection; OD = optimal (non toxic) dose < LD10.
[d]Data is referred to 4 h treatment; T/C = median survival time of treated versus untreated mice×100. L1210 murine leukaemia cell lines were obtained from NCI, Bethesda, USA.
[e]Compound **9** = PNU151807.

In particular, bromoacrylamido derivative **1** was prepared coupling DDD, obtained by acid hydrolysis of distamycin A as previously described,[9] with the commercially available α-bromoacrylic acid in DMF via symmetric anhydride. The same coupling procedure was used for the synthesis of compounds **14** and **15,** using as starting material the α-bromoacrylic acid and the opportune aminopyrroloamidine derivative prepared as previously reported.[10]

Haloacrylamido derivatives **3** and **7** were prepared by coupling DDD with a slight excess of the opportune haloacrylic acid activated as acyl chloride in dioxane/H_2O mixture as previously described.[11] In a similar way, coupling the desired 4-(α-haloacrylamido)-pyrrole-2-carboxylic acid, synthesised as described in the literature,[6] with DDD or its four pyrrole analogue, compounds **9**, **10**, **11** or **8** were respectively prepared. Compound **2** was synthesised by substitution reaction of DDD with commercially available 2,3-dibromopropene in DMF.

Results and Discussion

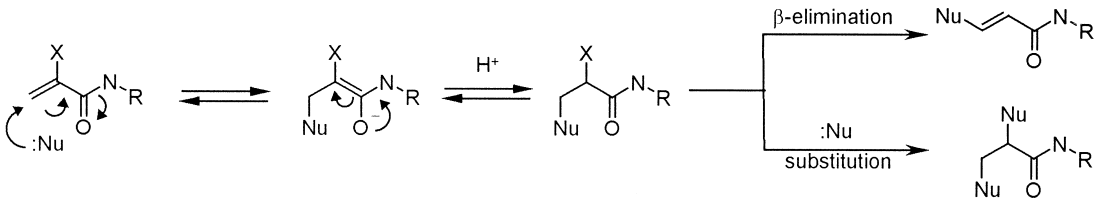
Tested compounds were assayed in vitro and in vivo on L1210 murine leukaemia cells, evaluating cytotoxicity and antileukemic activity as previously described.[12]

Table 1 shows cytotoxicity data of α-bromo/chloro/fluoro-acrylamido derivatives of distamycin A and of an α-bromovinyl analogue lacking of the carbonyl function. While bromo (**1**) and chloro (**3**) analogues show a relevant cytotoxicity, the fluoro (**4**) and α-bromovinyl (**2**) derivatives show a dramatic loss of activity.

Table 2 compares the cytotoxicities of α-bromo and α-chloroacrylamido derivatives, the two possible isomeric β-chloroacrylamido derivatives and the acrylamido derivative of distamycin A. Only α-bromo (**1**) and α-chloroacrylamido (**3**) derivatives appear cytotoxic, while β-chloroacrylamido (**5**,**6**) and acrylamido (**7**) derivatives appear devoid of significant activity.

As a whole these data suggest a key role of the reactivity of the α-halogenoacrylic moiety for cytotoxicity, in spite of the fact that PNU 151807 was found unable to alkylate DNA minor groove AT-rich sequences.

We hypothesise that the reactivity of the α-halogenacrylic moiety, due to the low reactivity of the vinylic halogen, could be based on a first-step Michael-type nucleophilic attack, possibly followed by a further reaction of the no more vinylic halogen leading to a second nuclephilic substitution or to beta elimination (Scheme 2). A mechanism of this kind implying a Michael attack followed by a classical nucleophilic displacement of bromo substituent alpha to a carbonyl was reported following the so-called Gabriel–Cromwell reaction.[13]



Scheme 2.

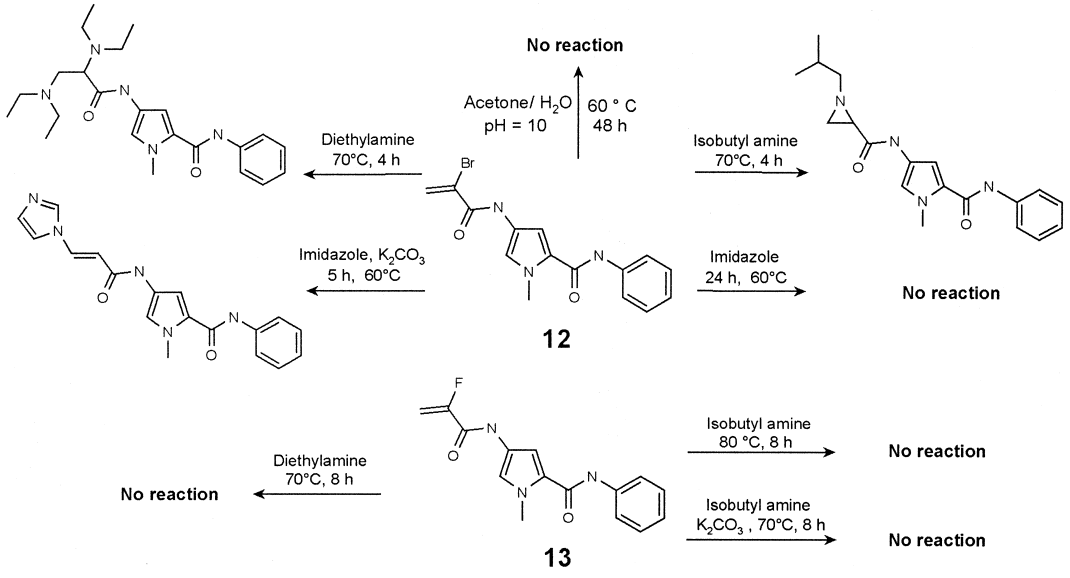
This hypothesis is supported by both the loss of activity occurring when the carbonyl function alpha to the halogen is absent (**2**), making the Michael attack impossible, and by the inactivity of acrylic (**7**), β-chloro-substituted (**5**,**6**) and of fluoroacrylic (**4**) analogues, which could be explained by the reversibility of the Michael reaction when the further reaction is impossible (**7**) or difficult, being based on the bad leaving capability of the fluoro α to the carbonyl (**4**) or on the displacement of the β halogen (**5**,**6**). Moreover we had the experimental evidence, concerning the last issue, of a significantly different reactivity toward nucleophilic attack of α-bromoacrylamido derivative **12** and of α-fluoroacrylamido derivative **13** synthesised by us as model compounds in order to evaluate the reactivity of α-halogenoacrylic moiety (Scheme 3).

Bromoacrylamido derivative **12** was stable in the aqueous alkaline conditions that, in the case of nitrogen mustards led to hydrolysis, a fact underlining a relative stability of the α-halogenoacrylic moiety. However compound **12** reacted with an excess of primary and secondary amines undergoing double nucleophilic substitution, and with imidazole ring, in the presence of K_2CO_3, undergoing nucleophilic substitution and *beta*-elimination, in accordance with the hypothesis of Scheme 2. In the same conditions fluoroacrylamido derivative **13** gave no reaction.

Data of Table 3 show that, as expected, the increase in the number of pyrrole units of the oligopeptidic frame leads to an increase of cytotoxicity. While both α-bromo and α-chloro derivatives of the same distamycin frame **9** (PNU151807) and **10**, **1** and **3**, are substantially equipotent in vitro, the four pyrrole unit derivatives **9** and **10** are about one order of magnitude more cytotoxic than three pyrrole unit congeners **1** and **3.** The same decrease of activity occurs with α-bromo derivatives with two (**14**) and one (**15**) pyrrole units, which are therefore devoid of significant activity. This is in accordance with what was previously found in the case of distamycin derived nitrogen mustards, in which the cytotoxicity increased about one order of magnitude every additional pyrrolecarbamoyl unit present in the oligopeptidic frame.[14] This possibly arises from a tighter DNA binding, depending on the increased multiplicity of interaction between the pyrrolecarbamoyl units and TA-rich sequence.[15] Apparently five pyrrole unit derivative **8** does not follow this trend of cytotoxicity. The increase in the number of pyrrole units from three to five leads also to an increase of in vivo potency, while the survival time shows a peak in the case of four pyrrole derivative **9**.

Conclusions

As a whole, for α-halogenoacrylamido derivatives of distamycin-like frame it appears that both the DNA binding capability, dependent on the multiplicity of the interaction with TA-rich sequences, and the reactivity of the α-halogenoacrylic moiety play a fundamental role for activity.



Scheme 3.

While the first feature appears in accordance with the established opinion concerning distamycin-derived minor groove binders, the latter appears particularly noteworthy due to the fact that PNU 151807 was found to bind only non covalently to DNA minor groove AT-rich sequences. This suggested to us that PNU 151807 could represent a mechanistically original minor groove binder and prompted us to further investigate this class of derivatives. A following paper[16] will report a new series of analogues whose study finally led to the identification of a new derivative for clinical development as anticancer agent.

Acknowledgements

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8. Tested compounds were purified by silica gel column chromatography (eluent 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 representative compounds (DMSO-d_6) are given. (Varian 200 and 300 MHz spectrometer, δ in ppm, TMS as internal standard): (**1**) 2.62 (t, 6.5 Hz, 2H), 3.45 (m, 2H), 3.81 (s, 3H), 3.84 (s, 3H), 3.85 (s, 3H), 6.20 (d, $J=2.8$ Hz, 1H), 6.68 (d, $J=2.8$ Hz, 1H), 6.91 (d, $J=1.7$ Hz, 1H), 7.01–7.04 (m, 2H), 7.18 (d, $J=1.7$ Hz, 1H), 7.20–7.24 (m, 2H), 8.18 (t, $J=5.7$ Hz, 1H), 8.60 (bs, 2H), 8.96 (bs, 2H), 9.88 (s, 1H), 9.93 (s, 1H), 10.29 (s, 1H); (**2**) 2.60 (t, $J=6.6$ Hz, 2H), 5.54 (d, $J=1.6$ Hz, 1H), 5.92 (d, $J=1.6$ Hz, 1H); (**3**) 5.98 (d, $J=2.1$ Hz, 1H), 6.40 (d, $J=2.1$ Hz, 1H); (**4**) 5.28 (dd, $J=15$,8, 3.5 Hz, 1H), 5.58 (dd, $J=47.8$, 3.5 Hz, 1H); (**5**) 6.59 (d, $J=13.1$ Hz, 1H), 7.29 (d, $J=13.1$ Hz, 1H); (**6**) 6.48 (d, $J=8.0$ Hz, 1H), 6.82 (d, $J=8.0$ Hz, 1H); **7** 5.67 (dd, $J=5.0$, 1.2 Hz, 1H), 6.17 (dd, $J=8.5$, 1.2 Hz, 1H), 6.40 (dd, $J=8.5$, 5.0 Hz, 1H); (**14**), 3.81 (s, 3H), 3.84 (s, 3H), 6.22 (d, $J=2.8$ Hz, 1H), 6.71 (d, $J=2.8$ Hz, 1H), 6.94 (d, $J=1.8$ Hz, 1H), 7.03 (d, $J=1.8$ Hz, 1H), 7.20 (d, $J=1.8$ Hz, 1H), 7.22 (d, $J=1.8$ Hz, 1H); (**15**) 2.59 (t, $J=6.5$ Hz, 2H), 3.40–3.55 (m, 2H), 3.79 (s, 3H), 6.21 (d, $J=2.8$ Hz, 1H), 6.64 (d, $J=2.8$ Hz, 1H), 6.90 (d, $J=1.8$ Hz, 1H), 7.08 (d, $J=1.8$ Hz, 1H), 8.22 (t, $J=5.6$ Hz 1H), 8.58 (bs, 2H), 8.90 (bs, 2H),10.22 (s, 1H).
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