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Cytotoxic Alpha-Bromoacrylic Derivatives of Low Molecular Weight

Italo Beria,[a,*] Marina Caldarelli,[a] Cristina Geroni,[b] Nicola Mongelli,[a] Benedetta Reinach,[c] Luisella Vignati[c] and Paolo Cozzi[a]

[a]*Chemistry Department, Pharmacia Discovery Research Oncology, Viale Pasteur 10, 20014 Nerviano, Milan, Italy*
[b]*External Research Department, Pharmacia Discovery Research Oncology, Viale Pasteur 10, 20014 Nerviano, Milan, Italy*
[c]*Global Drug Metabolism Department, Pharmacia Discovery Research Oncology, Viale Pasteur 10, 20014 Nerviano, Milan, Italy*

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Abstract—In vitro and in vivo activities of a small series of α-bromoacrylic derivatives of low molecular weight (MW) are described and compared with those of α-bromoacrylic derivatives of distamycin-like frames. Low MW compounds, when lacking of a strong basic moiety, are potent cytotoxics, while analogues bearing a strong basic moiety are not. This suggests the existence of an active transport mechanism for distamycin-derived cytotoxics characterized by strong basic amidino or guanidino moieties. Low MW compounds are inactive in vivo, possibly because of the metabolic lability of α-bromoacrylic moiety. The same moiety is however present in a series of potent anticancer distamycin-like minor groove binders, for example, PNU-166196 (brostallicin), a fact that underlines the features of the latter.

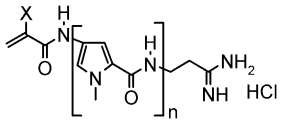
Introduction

We have recently reported a series of potent cytotoxic antitumor agents showing an α halogenoacrylamido moiety linked to a distamycin-like frame, some of which modified also at the amidine terminus typical of distamycin A. The SAR of these minor groove binders was discussed, particularly as far as the role of the length of the polypyrrolic frame and the α halogenoacrylamido moiety are concerned.[1,2]

While both α-bromo and α-chloro derivatives of the same four or three pyrrole units frame are substantially equipotent in vitro, the three pyrrole unit derivatives, both bromo and chloro, are about one order of magnitude less cytotoxic than the corresponding four pyrrole congeners.

The same decrease of activity occurs with α-bromo derivatives with two and one pyrrole units, which are therefore devoid of significant activity (Table 1).

Table 1. In vitro and in vivo activity of α-halogenoacrylamido distamycin derivatives **1**–**8** against L1210 murine leukemia



Compd	X	*n*	In vitro[a] IC_{50}, nM	In vivo[b]	
				OD mg/kg	T/C%
1	Br	4	6.3 (±1.3)	1.56	200
2	Br	3	79.6 (±22.4)	12.5[c]	175
3	Br	2	1300	nd	nd
4	Br	1	>2500	nd	nd
5	Cl	4	3.8 (±1.4)	1.56	133
6	Cl	3	96.8 (±24.2)	12.5	117
7	F	4	>800	nd	nd
8	H	4	2000	nd	nd

IC_{50} = 50% inhibitory concentration as the mean ± SE from dose–response curves of at least two experiments.
[a]Drug sensitivity was determined after 48 h of continuous exposure against L1210 cells.
[b]For in vivo studies cells were injected iv at day 0 and mice were treated iv the day after tumor injection.
[c]Cells were injected ip at day 0 and mice were treated ip 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 leukaemia cell lines were obtained from NCI, Bethesda, USA.

*Corresponding author. Fax: +39-02-48303833; e-mail: italo.beria@pharmacia.com

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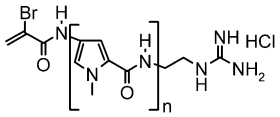
Table 2. In vitro cytotoxicity of α-bromoacrylic guanidino derivatives **9–11**



Compd	*n*	In vitro[a] IC_{50} (nM)
9	4	1.8 (±0.1)
10	3	27.0 (±3.1)
11	2	>500

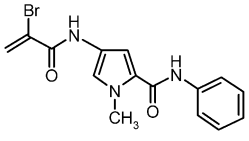
[a]See footnote a in Table 1.

This feature is in accordance with what was previously found in the case of distamycin derived nitrogen mustards[3] and was considered to arise from a tighter DNA binding depending on the increased multiplicity of interaction between the pyrrolecarboxamide units and minor groove TA-rich sequences.[4]

An identical trend of cytotoxicity (Table 2) has been more recently found for α-bromoacrylic derivatives of four, three and two pyrrole units distamycin like frames ending with a guanidino moiety, that is, PNU-166196 (brostallicin) **9**, a potent antitumor agent undergoing Phase II clinical trials.[5]

As far as the role of the α halogenoacrylamido moiety is concerned, the cytotoxicity data clearly indicated a key role for the reactivity of the α-halogenoacrylic moiety, that we hypothesized to be based on a first-step Michael-type nucleophilic attack, followed by further substitution reaction of the no more vinylic halogen.

In fact while α-bromo and chloro derivatives **1**, **5** show a relevant cytotoxicity, the fluoro and acrylamido derivatives **7**, **8** appear devoid of significant activity. Our hypothesis was supported by the study of the reactivity toward nucleophilic attack of α-bromoacrylamido derivative **12** synthesized as model compound.[1]



12

Unexpectedly compound **12**, tested in our routine in vitro activity test on L1210 murine leukemia, proved to be endowed with a significant cytotoxic activity, higher than α-bromoacrylic derivative of distamycin **2**, at variance with monopyrrole analogue **4** ending with the amidinoethyl terminus typical of distamycin. This prompted us to synthesize and test some low MW non-polypyrrolic α-bromoacrylic derivatives.

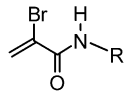
Chemistry

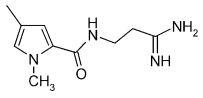
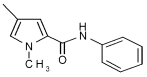
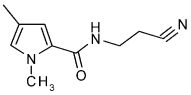
The novel compounds[6] reported in Table 4, except compounds **13**, **15**, **17** and **18** were synthesized by coupling the commercially available α bromoacrylic acid, activate as symmetric anhydride, with the suitable amines and in presence of a base.

In particular the reaction to obtain compound **16**, was carried out in acetonitrile/H_2O mixture using $NaHCO_3$ as base, while reactions to yield compounds **12** and **14** were carried out in acetonitrile using triethylamine as base.

Compound **13** was synthesized by coupling 4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxylic acid chloride[1] with 3-aminopropanenitrile fumarate. Compound **15** and **17** were synthesized from α-bromoacryloylchloride and the commercially available 4-fluoroaniline or 4-amino-benzamidine dihydrochloride, respectively. The reactions for the preparation of compounds **13** and **15** were carried out in methylene chloride and acetonitile respectively, in presence of triethylamine as base, while for the compound **17**, a mixture of dioxane/H_2O in presence of $NaHCO_3$ as a base was used. Compound **18** was prepared by coupling the α-fluoroacrylic acid, activated as acyl chloride with 4-amino-1-methyl-*N*-phenyl-1H-pyrrole-2-carboxamide hydrochloride in acetonitrile and triethylamine as base.

Table 3. In vitro cytotoxicity against L1210 murine leukemia cells for some low MW compounds



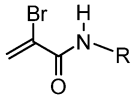
Compd	R	IC_{50} nM[a]
4		>2500
12		43.0
13		51.9
14		36.8

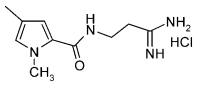
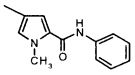
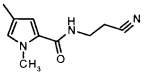
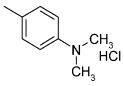
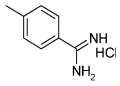
[a]See footnote a in Table 1.

Results and Discussion

The key structural feature that characterized compound **12**, in comparison to inactive compound **4**, was the lack of the basic amidino moiety. Therefore, we synthesized and tested a close analogue of **4** in which the amidino moiety was replaced by the non basic cyano group (**13**) and the simple α-bromoacrylic anilide (**14**) in which any residue of the distamycin frame was absent. Both compounds showed a significant cytotoxicity, closely comparable with that of **12** (Table 3). It must be underlined that α-bromoacrylic acid per se is not cytotoxic (L1210: IC_{50} >120 μM).[7]

Table 4. In vitro and in vivo activity of low MW derivatives



Compd	R	IC_{50} (nM)[a]	Calcd pK_a	OD iv[b] mg/kg	%T/C
4		>2500	11.7	nd	nd
12		43.0	ns	25.0	100
13		51.9	ns	40	100
14		36.8	ns	12.5	117
15		58.6	ns	>50	100
16		54.0	5.0	25.0	100
17		>5000	8.2	nd	nd

[a]See footnote a in Table 1.
[b]See footnote b in Table 1.

These data suggest that α-bromoacrylic derivatives of low MW are potent cytotoxics only when lacking of a strong basic moiety. The extreme structural simplicity of compound **14** prompted us to synthesize and test some close analogues substituted on the phenyl ring by simple groups able to modulate the chemico-physical features of the molecule and the electronic effect on the α-bromoacrylic moiety, apparently determinant for the activity. Table 4 reports also the calculated values of pK_a,[8] for basic compounds. Few of these derivatives were also tested for antileukemic activity in vivo.

The data of Table 4 show that in vitro activities of different α-bromoacrylic anilides are, in molar terms, substantially the same, in spite of different electronic effects of *para* substituents, and equivalent to those of neutral α-bromoacrylic pyrrole derivatives **12** and **13**, with the relevant exception of basic amidino derivative **17**, which shows the same inactivity of basic amidino derivative **4**.

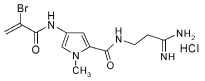
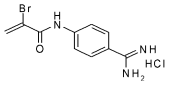
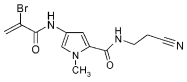
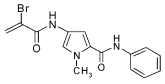
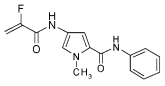
We hypothesize that the inactivity of compounds **4** and **17** could be explained by the lack of cellular membrane permeability depending upon the ionization of the molecules due to the presence of strongly basic amidino moiety. The mild basicity of dimethylanilino derivative **16** should avoid full ionization of the molecule at pH 7.4, thus allowing cellular membrane permeability and explaining in vitro activity.

Table 4 shows, moreover, unexpected lack of antileukemic activity in vivo for compounds **12**, **14**, **15** and **16**, accompanied also by a substantial lack of significant toxicity, particularly evident for compound **15**, in spite of their relevant cytotoxicity in vitro. A possible explanation for in vivo inactivity could be a massive metabolic degradation, which may depend upon the presence of the same α-bromoacrylic moiety which is responsible for the cytotoxicity and which is the sole reactive moiety of α-bromoacrylic anilides. The lack of in vivo activity for compound **15** should exclude a metabolic hydroxylation on the phenyl ring as a possible explanation of the inactivity of **14**.

Again there is an apparent contradiction between the maintenance of a potent in vivo activity for distamycin like polypyrrolic frames with three or more pyrrole units and the inactivity of compounds **12**, **14**, **15** and **16**, in spite of common presence of the α-bromoacrylic moiety, which is apparently determinant for both cytotoxicity and metabolic degradation in the case of low MW derivatives.

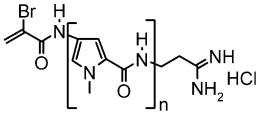
A preliminary attempt to evaluate the cell permeability of low MW compounds by the Caco-2 cells assay, did not give conclusive results because of the very low mass balance shown by many compounds, with the notable exception of amidino compounds **4** and **17**, whose very low permeability, made reliable in view of its high mass

Table 5. Caco-2 cell permeability of low MW derivatives

Compd		P_{app}[a] $\times 10^{-6}$ cm/s	Mass balance[b]	IC_{50} (nM)
4		0.7	83.6	> 2500
17		3.9	62.0	> 5000
13		uc	26.5	51.9
12		1.2	1.7	43.0
18		39.6	88.0	> 5000

All the compounds were incubated at a concentration of 10 μM in presence of 0.1% of dimethyl sulfoxide.
[a]Apparent permeability coefficient determined after 120 min of incubation according to the method reported by Artusson.[9]
[b]Total recovery of the compound calculated at the end of the experiment. UC = unable to calculate. Caco-2 cells were grown on permeable filters of 12 polycarbonate transwell for 21 days.

Table 6. Caco-2 cell permeability for a series of α-bromoacrylamidino derivatives



Compd	*n*	P_{app}[a] $\times 10^{-6}$ cm/s[a]	Mass balance[b]	IC_{50} (nM)
1	4	38.20	117	6.3
2	3	15.21	75	98.8
3	2	5.58	87	1300
4	1	0.69	84	> 2500

[a]See footnote a in Table 5.
[b]See footnote b in Table 5.

balance, is an accordance with their lack of significant cytotoxicity (Table 5).

The low mass balance of the low MW compounds **13** and **12** should arise from the chemical reactivity of α-bromoacrylic moiety, as confirmed by the high mass balance shown by non-cytotoxic, unreactive[1] α-fluoroacrylic analogue **18**.

The apparent contradiction with the relevant in vitro activity of distamycin-like polypyrrolic frames with three or more pyrrole units, for example, compounds **1** and **2**, which share with compounds **4** and **17** the strong basic amidino moiety, could be explained by the presence of an active transport mechanism, that may be justified by the biotic nature of distamycin, but hardly conceivable for low MW xenobiotics, for which a passive transport should be expected.

Table 6 show preliminary permeability data, performed using Caco-2 cells line on a series of polypyrrole amidino derivatives, wherein there is a progressive increasing of permeability from one to four pyrrole units apparently in accordance with their increased cytotoxicity.

Conclusion

The reported data show that low MW α-bromoacrylic derivatives, even simple α-bromoacrylic anilides, are endowed with relevant cytotoxic activity in vitro, provided that they are lacking of strong basic moieties. However these compounds are inactive in vivo, a fact apparently depending on a possible metabolic lability of α-bromoacrylic moiety.

These features underline the peculiarity of potent in vitro and in vivo activities shown by α-bromoacrylic distamycin-like derivatives characterized by a polypyrrole frame ending with strong basic amidino or guanidino moieties. For these compounds an active transport mechanism should be hypothesized, in addition to minor grove binding capability, to explain potent cytotoxicity.

The reason why in the caco-2 cell assay, good mass balance values were found only for α-bromoacrylic derivatives ending with an amidino moiety is open to speculation.

Studies aimed to clarify these issues are foreseen also in order to better define the mechanistic profile of PNU

166196 (brostallicin). The results could also give useful information concerning the hypothesized therapeutic role of polyazolecarboxamides investigated by different groups for chemical gene regulation because of their DNA high affinity and binding specificity.[10]

References and Notes

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5. Cozzi, P. *Idrugs* **2001**, *4*, 573.
6. Tested compounds were purified by silica gel column chromatography giving satisfactory analytical values and ^{1}H NMR spectra in agreement with assigned structures. ^{1}H NMR data of representative novel compounds (DMSO-d_6 or *$CDCl_3$) are given. (Varian 200 and 300 MHz spectrometer, δ in ppm, TMS as internal standard): (**10**) 10.28 (s, 1H), 9.93 (s, 1H), 9.89 (s, 1H), 8.09 (t, $J=5.3$ Hz, 1H), 7.54 (m, 1H), 7.40–6.90 (bs, 4H), 7.22 (d, $J=1.8$ Hz, 1H), 7.21 (d, $J=1.8$ Hz, 1H), 7.18 (d, $J=1.8$ Hz, 1H), 7.04 (d, $J=1.8$ Hz, 1H), 7.02 (d, $J=1.8$ Hz, 1H), 6.94 (d, $J=1.8$ Hz, 1H), 6.65 (d, $J=2.9$ Hz, 1H), 6.20 (d, $J=2.9$ Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.40–3.20 (m, 2H); (**11**) 10.29 (s, 1H), 9.92 (s, 1H), 8.10 (t, $J=5.3$ Hz, 1H), 7.59 (m, 1H), 7.40–6.90 (bs, 4H), 7.19 (d, $J=1.8$ Hz, 1H), 7.17 (d, $J=1.8$ Hz, 1H), 7.02 (d, $J=1.8$ Hz, 1H), 6.92 (d, $J=1.8$ Hz, 1H), 6.67 (d, $J=2.9$ Hz, 1H), 6.20 (d, $J=2.9$ Hz, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.40–3.20 (m, 2H); (**12**) 10.27 (s, 1H), 9.83 (s, 1H), 7.69 (m, 2H), 7.29 (m, 2H), 7.26 (d, $J=1.8$ Hz, 1H), 7.10 (d, $J=1.8$ Hz, 1H), 7.02 (m, 1H), 6.67 (d, $J=2.9$ Hz, 1H), 6.21 (d, $J=2.9$ Hz, 1H), 3.83 (s, 3H); (**13**) 10.29 (s, 1H), 8.39 (t, $J=6.4$ Hz 1H), 7.22 (d, $J=1.8$ Hz, 1H), 6.91 (d, $J=1.8$ Hz, 1H), 6.68 (d, $J=3.0$ Hz, 1H), 6.23 (d, $J=3.0$ Hz, 1H), 3.83 (s, 3H), 3.40 (m, 2H), 2.74 (t, $J=6.4$ Hz, 2H); (**14***) 8.34 (bs, 1H), 7.58 (m, 2H), 7.37 (m, 2H), 7.22 (m, 1H), 7.13 (d, $J=1.7$ Hz, 1H), 6.14 (d, $J=1.7$ Hz, 1H); (**15***) 8.33 (bs, 1H), 7.56 (m, 2H), 7.13 (d, $J=1.7$ Hz, 1H), 7.10 (m, 2H), 6.14 (d, $J=1.7$ Hz, 1H); (**16**) 10.45 (s, 1H), 7.72 (m, 2H), 7.53 (bs, 2H), 6.79 (d, $J=2.9$ Hz, 1H), 6.30 (d, $J=3.3$ Hz, 1H), 3.04 (s, 6H); (**17**) 10.67 (s, 1H), 9.22 (bs, 2H), 8.83 (bs, 2H), 7.78–7.89 (m, 4H), 6.82 (d, $J=3.1$ Hz, 1H), 6.39 (d, $J=3.1$ Hz, 1H).
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