Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 12, pp 1467-1472, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00172-3

STRUCTURE-ACTIVITY RELATIONSHIP OF NOVEL DISTAMYCIN A DERIVATIVES : SYNTHESIS AND ANTITUMOR ACTIVITY

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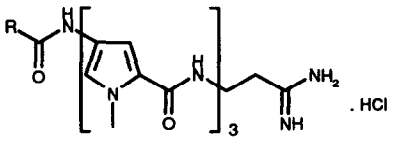
Abstract : Synthesis and biological evaluation of a group of distamycin A derivatives bearing new alkylating moieties is presented.

Distamycin A (**1**) is an antibiotic originally isolated from *Streptomyces Distallicus* [1] and subsequently obtained by total synthesis [2] (fig.1). It forms a strong reversible complex with double helical B-DNA with high selectivity for adenine-thymine rich sequences [3]. Distamycin A possesses antiviral activity [4] but is not active as an antitumor agent.

Distamycin A derivatives with alkylating moieties, active *in vitro* on several tumor cell lines and possessing antitumor activity *in vivo*, have been synthesized [5,6]. Some of them have been shown to be highly cytotoxic. Particularly, FCE 24517 (Tallimustine, **7a**) exhibited a broad spectrum antitumor activity in a series of experimental tumor models [7] and is currently in phase II clinical trials [8]. Its mode of action is under investigation: it has been hypothesised that its greater activity is due to its ability to alkylate N-3 of adenines in DNA with a high sequence specificity [9]. The mechanism of resistance selected appears to be specific for compounds of the same chemical class [10,11,12].



1 : R = H

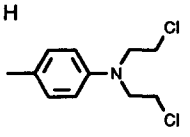
7a : R = 

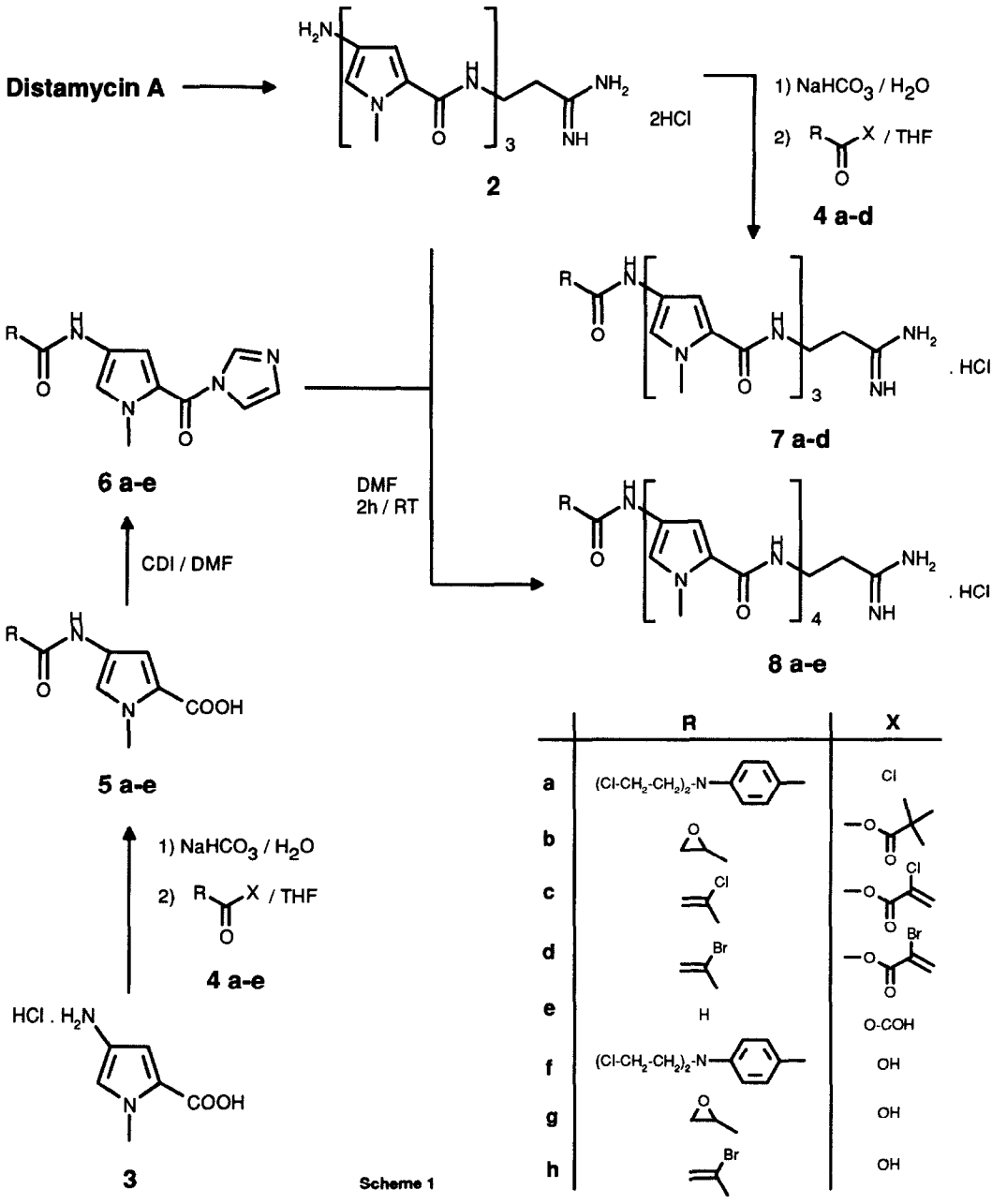
Figure 1

With the objective to identify novel promising candidates we have synthesized a series of distamycin analogs bearing different alkylating moieties with three or four pyrrole rings. The compounds have been assayed *in vitro* and *in vivo* on L1210 murine leukemia cells and *in vitro* on two sublines resistant to FCE 24517 and doxorubicin (DX).

Synthesis and structure-activity relationship of these compounds are described.

Synthesis

The starting material utilized for the synthesis of the cited compounds is N-deformyldistamycin **2** obtained from distamycin A **1** according to already reported procedures [13] (scheme 1).



Scheme 1

Synthesis of compounds **7 a-d**[14] was performed by reacting the intermediate **2** with an excess of a suitable acylating agent (**4 a-d**). As shown in scheme 1, optimization of yields could be obtained using a particular leaving group X for each alkylating group R considered.

Synthesis of four pyrrole ring derivatives **8 a-e** was accomplished first by introducing the alkylating moiety on a single pyrrole unit and then connecting the monomers obtained to the Distamycin skeleton. Thus N-methyl-4-amino-2-carboxylic acid **3** was reacted with the appropriate acylating agent **4 a-e** to obtain the intermediates **5 a-e**. Activation of **5 a-e** with N,N'-carbonyldiimidazole afforded the imidazolyl derivatives **6 a-e** as crystalline stable compounds. Finally coupling of **6 a-e** with N-deformyldistamycin **2** in DMF at 40°C for 2 hours gave the desired derivatives **8 a-e**[14] in good yields.

Biological evaluation

The activity of all synthesized compounds has been tested *in vitro* and *in vivo* on L1210 murine leukemia and *in vitro* on L1210 cells resistant to doxorubicin (L1210/DX) and to FCE 24517 (L1210/24517). The cytotoxicity and the antitumor activity on L1210 leukemia (obtained from NCI, Bethesda, USA), have been evaluated as previously described [10] . As shown in Table 1, distamycin A, its four ring homolog **8 e** and the alkylating moieties **4 f**, **4 g** and **4 h** present very low cytotoxic activity. Moreover, distamycin A is inactive *in vivo*.

Conversely, all derivatives bearing an alkylating moiety are more cytotoxic than the parent compound and are active *in vivo* (%T/C ranging from 171 to 206). Increasing the number of pyrrole rings, in each class of derivatives, we found an increase in cytotoxicity and *in vivo* potency without improving the antitumor activity.

Table 2 presents the *in vitro* activity of distamycin A analogues on resistant leukemias; results obtained show that, as far as multi drug resistance (MDR) is concerned, distamycin A as well as **8e**, **7a**, **8a**, **7c** and **8c** are inactive (resistance index (R.I.) from 175.4 to 28), whereas the epoxy (**7b**, **8b**) and bromoacrylic derivatives (**7d**, **8d**) present low R.I. . All compounds, bearing distamycin A framework however substituted, present high levels of cross-resistance on L1210/24517 cells (R.I. from 169.9 to 11.4), whereas the phenyl mustard alkylating moiety (**4f**) does not (R.I. 1.1).

Conclusion

As previously reported for FCE 24517 [6,7], the insertion of new alkylating moieties such as epoxycarbonyl or halogenoacriloyl on the N-terminal position of distamycin A, confers to this molecule a potent antiproliferative activity. It appears that the bioactivity of this class of derivatives, is due to both the alkylating group and to the DNA-recognising oligo(N-methylpyrrole carboxyamide) moiety. Although a better antitumor activity couldn't

be obtained increasing the number of pyrrole rings of distamycin A framework , a higher potency was acquired. All distamycin A derivatives are inactive on cells resistant to FCE 24517 showing that the alkylating moiety is not involved in the selection of this resistance mechanism.

Table 1. CYTOTOXICITY AND ANTITUMOR ACTIVITY ON L 1210 MURINE LEUKEMIA

COMPOUND	*in vitro*[1]	*in vivo*[3]	
	IC_{50} (ng/ml)[2]	O.D (mg/kg)[4]	% T/C[5]
Distamycin A	5216 ± 853	200	113
8e	285 ± 75	n.d.	n.d.
FCE 24517 (7a)	24.4 ± 3.3	3.125	175
8a	16.3 ± 0.6	0.39	138
7b	229.2 ± 53	50	188
8b	38.9 ± 7	3.125	188
7c	56 ± 14	10	184
8c	2.7 ± 1	3.125	171
7d	49.6 ± 14	12.5	175
8d	4.7 ± 1	3.125	206
4f	17039 ± 3589	n.d.	n.d.
4g	131397±2540	n.d.	n.d.
4h	18802±1250	n.d.	n.d.

1. Drug sensitivity was determined counting surviving cells after 48 h. of continuous exposure to at least 4 concentrations of each drug.
2. 50% inhibitory concentration (IC_{50}) represents the mean ± SE from dose-response curves of at least three experiments.
3. CD2F1 mice were given an injection of 10^5 cells i.p. and treated on day 1.
4. O.D. : optimal dose
5. % T/C : Median survival time of treated mice / Median survival time of controls x 100.

Table 2. RESISTANCE OF L1210/DX AND L1210/24517 CELLS TO DISTAMYCIN DERIVATIVES.

COMPOUND	L1210/DX R.I.[1]	L1210/24517 R.I.[1]
Distamycin A	45.6	14.1
8e	175.4	169.9
FCE 24517 (7a)	28	21.7
8a	n.d.	65.4
7b	7.1	44.4
8b	1.7	24.1
7c	40.3	24.8
8c	59.9	69.6
7d	8.8	11.4
8d	3.8	27
4f	1.4	1.1
4g	1.0	0.5
4h	1.1	0.5

1. Drug sensitivity was determined counting surviving cells after 48h. of continuous exposure. R.I. (resistance index): ratio between IC_{50} values on resistant cells and sensitive cells.

On the other hand , R.I. of compounds **8b** and **8d** on L1210/DX cells show that MDR resistance could be overcome using alkylating moieties more reactive than phenyl mustard.

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14. ^{1}H-NMR data of all new compounds are given below (Bruker WP 80 SY spectrofotometer, δ in ppm, TMS as internal standard, DMSO-d_6).

7b : δ 2.64(2H, m), 2.89(2H, m), 3.50(2H, m), 3.55(1H, dd), 3.81(3H,s), 3.84(6H, s), 6.9-7.3(6H, m), 8.22(1H, bt), 8.4-9.4(4H, br), 9.9(3H, br) ppm ; **7c** : δ 2.65(2H, t), 3.50(2H, m), 3.80(3H, s), 3.83(3H, s), 3.84(3H, s), 5.98(1H, d), 6.40(1H, d), 6.9-7.3(6H, m), 8.20(1H, t), 8.75(2H, bs), 9.04(2H, bs), 9.89(1H, s), 9.95(1H, s), 10.32(1H, s) ppm ; **7d** : δ 2.62(2H, t), 3.45(2H, m), 3.81(3H, s), 3.85(6H, s), 6.20(1H, d), 6.70(1H, d), 6.9-7.3(6H, m), 8.18(1H, t), 8.60(2H, bs), 8.96(2H, bs), 9.88(1H, s), 9.93(1H, s), 10.29(1H, s) ppm ; **8a** : δ 2.63(2H, t), 3.20-3.90(10H, m), 3.80-3.85(12H, s), 6.83(2H, d, J=8.85), 6.92-7.32(8H, m), 7.87(2H, d, J=8.85), 8.20(1H, bt), 8.95(4H, br), 9.90(3H, br), 10.001H, br) ppm ; **8b** : δ 2.63(2H, m), 2.89(2H, m), 3.50(2H, m), 3.57(1H, dd), 3.78-3.85(12H, s), 6.92-7.23(8H, m), 8.22(1H, bt), 8.65(4H, br), 9.92(4H, br) ppm ; **8c** : δ 2.62(2H,t), 3.2-4.00(14H, m), 5.99(1H, d), 6.39(1H, d), 6.90-7.30(8H, m), 8.20(1H, t), 8.80(2H, bs), 9.00(2H,bs), 9.90(2H, s), 9.93(1H, s), 10.30(1H, s) ppm ; **8d** : δ 2.63(2H, t), 3.50(2H, t), 3.80(3H, s), 3.84(3H, s), 3.85(6H, s), 6.19(1H, d), 6.69(1H, d), 6.90-7.25(8H, m), 8.12(1H, t), 8.63(2H, bs), 8.89(2H, bs), 9.80(1H, s), 9.83(1H, s), 9.86(1H, s), 10.30(1H, s) ppm

(*Received in USA* 16 *February* 1994; *accepted* 3 *May* 1994)