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# SYNTHESIS AND ANTITUMOR ACTIVITY OF NOVEL DISTAMYCIN DERIVATIVES

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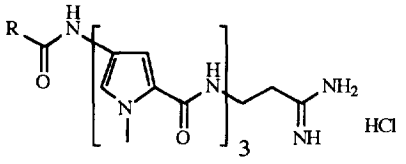
**Abstract:** Several distamycin derivatives were synthesized from deformyl distamycin by coupling with different azole carboxylic acids bearing an alkylating moiety. Some of them showed good activities *in vitro* and *in vivo* against L 1210 murine leukemia. 

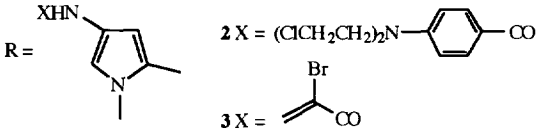
## Introduction

Distamycin A **1** is an antiviral antibiotic, metabolite of *Streptomyces distallicus* [1,2] isolated in 1962 in Farmitalia laboratories and subsequently obtained by total synthesis[3-7], which binds to the minor groove of double-helical DNA at sites of 4 or 5 successive A,T base pairs. This compound is not active as an antitumor agent.

Recently, Mongelli and co-workers[8] synthesized distamycin analogs **2** and **3** where the formyl group was replaced with a pyrrole 2-carbonyl moiety bearing two different alkylating groups, an α-bromo acryloyl or a benzoyl nitrogen mustard . These compounds showed relevant cytotoxicity on several tumor cell lines and *in vivo* antitumor activity. These interesting properties prompted us to synthesize potential new minor groove binders as novel carriers to deliver DNA-reacting functional groups (nitrogen mustard or α-bromo-acryloyl moieties) into the DNA minor groove.

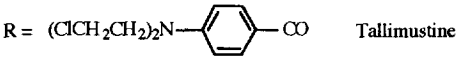


**1** R = H Distamycin A



**2** X = $(ClCH_2CH_2)_2N$—C6H4—CO

**3** X = $CH_2=C(Br)-CO$



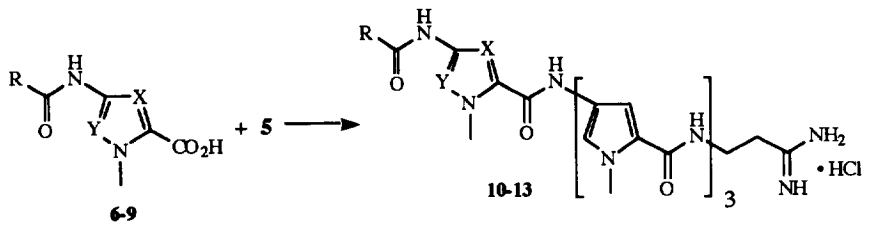
**4** R = $(ClCH_2CH_2)_2N$—C6H4—CO Tallimustine

We wish to report here the synthesis and the preliminary biological evaluation of distamycin derivatives, in which the pyrrole ring bearing the alkylating moiety of compounds of formulae **2** and **3**, have been replaced by isosteric imidazole or pyrazole rings.

## Chemistry

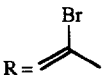
In the synthesis of new distamycin derivatives **10-13**, the key step was the coupling between several heterocyclic carboxylic acids bearing the alkylating moieties and the N-deformyl distamycin **5**, obtained from distamycin A according to a reported procedure[9] (Scheme 1).

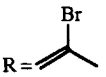
**Scheme 1**




**6** R = $(ClCH_2CH_2)_2N$–C6H4– Y=N, X=CH **10**[16]

**7** R = $(ClCH_2CH_2)_2N$–C6H4– Y=CH, X=N **11**[16]

**8** R =  Y=CH, X=N **12**[17]

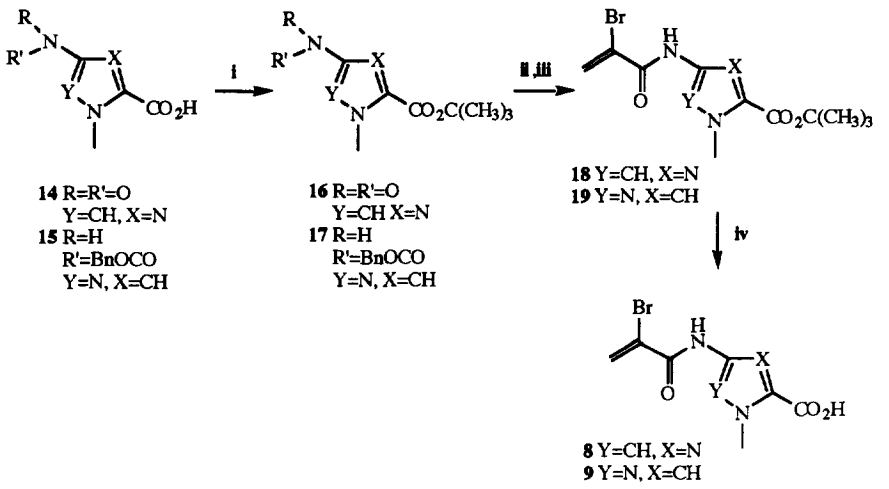
**9** R =  Y=N, X=CH **13**[17]

The condensation of acids **6-9** with deformyl distamycin **5** was performed using an excess (1.5 equivalent) of 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride (EDC) as the coupling agent, in DMF as a solvent, in the presence of Hunig's base, at room temperature and with identical reaction times (16 h). The corresponding tetraoligopeptides **10-13** were obtained in acceptable yields (32-43%), after purification by silica gel flash-chromatography.

The synthesis of heterocyclic acids **6** and **7**, bearing as alkylating moiety the benzoyl N-mustard, was performed by coupling ethyl 1-methyl-4-aminoimidazole-2-carboxylate[10,11] or methyl 1-methyl-3-aminopyrazole-5-carboxylate[12] respectively, with p[bis (2-chloroethyl)amino]-benzoyl chloride[13,14], followed by alkaline hydrolysis of the ester group.

The synthetic approach employed for the preparation of compounds **8** and **9** was carried out by esterification of 4-nitro-1-methylimidazole-2-carboxylic acid[10,11] **14** and 3-benzyloxycarbonylamino-1-methylpyrazole-5-carboxylic acid[12] **15** in the presence of t-butylbromide[15] to give **16** and **17**, respectively (Scheme 2).

**Scheme 2**




**Reagents**: **i**) $(CH_3)_3CBr$, BTEAC, $K_2CO_3$, DMF; **ii**) $H_2$, C/Pd; **iii**) α-bromo acrylic acid, EDC, DMF; **iv**) $CF_3COOH$

Catalytic reduction of the nitro group of **16** or removal of the protecting group of **17**, gave the corresponding amine. Subsequent acylation with α-bromo acrylic acid in DMF in the presence of EDC afforded **18** and **19** respectively, that in turn were trasformed into the corresponding carboxylic acids **8** and **9** by removal of the *t*-butyl group with trifluoroacetic acid.

**Results and Discussion**

The activity of synthesized compounds was tested *in vitro* and *in vivo* on L1210 murine leukemia (obtained from NCI, Bethesda, USA). The cytotoxicity and the antileukemic activity was evaluated as previously described.[18]

As shown in Table 1, the cytotoxicity of the alkylating tetrapeptides, with the notable exception of imidazolic benzoyl nitrogen mustard **11**, was much greater than that of the parent distamycin A. In addition, we were interested to compare the cytotoxic activity of our compounds with that of tallimustine **4**,[19-20] a distamycin-derived nitrogen mustard, endowed with potent anticancer activity and presently undergoing Phase II clinical trials.

In contrast with the poor activity shown by **11**, the pyrazole analog **10** showed significant cytotoxicity, comparable with that of tallimustine. The poor activity of **11** may be related to a different binding pattern to DNA recognition sequence, due to a possible role of the N(3) lone pair of the imidazole ring[21].

**Table 1**

| Compound | *in vitro* [22] $IC_{50}$ (ng/mL) | *in vivo* [23] O.D. (mg/Kg) | *in vivo* [23] % T/C |
|---|---|---|---|
| **Distamycin A** | 5216.0 | 200 | 113 |
| **Tallimustine** | 50.3 | 3.13 | 175 |
| **2**[8] | 16.3 | 0.39 | 138 |
| **3**[8] | 4.7 | 3.125 | 206 |
| **10** | 29.1 | 3.13 | 144 |
| **11** | 2000.0 | 6.25 | 125 |
| **12** | 35.0 | 6.25 | 163 |
| **13** | 9.9 | 6.25 | 200 |

$IC_{50}$ = 50% inhibitory concentration represents the mean from dose-response curves of at least three experiments.

O.D= optimal dose; optimal non toxic dose < LD10.

%T/C= median survival time of treated vs. untreated mice x 100.

The type of alkylating group had a significant effect on the cytoxicity of compounds having the same oligopeptide frame. Compound **12** bearing a α-bromoacryloyl moiety was at least 60-fold more potent that the benzoyl mustard counterpart.

In summary, we synthesized a novel class of distamycin derivatives endowed with good cytoxicity and antileukemic activity *in vivo*. The compounds bearing an α-bromo-acryloyl moiety, in particular, appeared of relevant interest.

The activity of this class of derivatives apparently depends both on the nature of the alkylating moiety and the heterocyclic units of the oligopeptidic frame, which may determine a specific DNA recognition possibly by hydrogen bonding.

We are currently undertaking an extensive biophysical and biochemical evaluation of this group of molecules in order to gain information about the sequence selectivity of DNA alkylation.

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## References and notes

1. Di Marco, A.; Gaetani, M.; Orezzi, P.; Scotti, T.; Arcamone, F. *Cancer Chemother. Rep.*, **1962**, *18*, 5.
2. Arcamone, F.; Orezzi, P.G.; Barbieri, W.; Nicolella, V.; Penco, S. *Gazz. Chim. Ital.*, **1967**, *97*, 1097.
3. Arcamone, F.; Penco, S.; Orezzi, P.; Nicolella, V.; Pirelli, A. *Nature*, **1964**, *203*, 1064.
4. Bialer, M.; Yagen, B.; Mechoulam, R.; Becker, Y. *J. of Pharm. Sc.*, **1980**, *69*, 1334.
5. Ragnarsson, U.; Grehen, L. *J. Org. Chem.*, **1981**, *46*, 3492.
6. Bialer, M.; Yagen, B.; Mechoulam, R. *Tetrahedron*, **1978**, *34*, 2389.
7. Lown, J.W.; Krowichi, K. *J. Org. Chem.*, **1985**, *50*, 3774.
8. D'Alessio, R.; Geroni, C.; Biasoli, G.; Pesenti, E.; Grandi, M.; Mongelli, N.; *Bioorg.& Med. Chem. Lett.* **1994**, *4*, 1467-1472.
9. Arcamone, F.; Nicolella, V.; Penco, S.; Redaelli, S. *Gazzetta Chim. Ital.*, **1969**, *99*, 632.
10. Dwyer, T.J.; Geierstaner, B.H.; Bathini, Y.; Lown, J.W.; Wemmer, D.E. *J. Am. Chem. Soc.*, **1992**, *114*, 5911-5919.
11. Lown, J.W.; Krowichi, K. *J. Org. Chem.*, **1987**, *52*, 3493.
12. Lee, Ho H.; Cain, B.F.; Penny, W.A.; Buckleton, J.S.; Clark, G.R. *J. Org. Chem.*, **1989**, *54*, 428-431.
13. Lee, M.; Rhodes, A.L.; Wyatt, D.M.; D'Incalci, M.; Forrow, S.; Hartely, J.A. *J. Med. Chem.*, **1993**, *36*, 863-870.
14. Eiderfield, R.C.; Liao, T.K.; *J. Org. Chem.*, **1961**, *26*, 4996-4997.
15. Chevallet, P.; Garouste, P.; Malawskw, B.; Martinez, J. *Tetrahedron Lett.*, **1993**, *34*, 7409-7412.

16. Characterization of compounds **10** and **11**. **10**: white solid, 263° (MeOH-ethyl ether); 200 MHz $^1$H-NMR (d$_6$-DMSO) δ 10.65 (1H, bs), 10.47 (1H, bs), 10.05 (1H, s), 9.97 (1H, s), 8.9 (3H, bs), 8.25 (1H, bs), 7.94 (2H, d, J = 9 Hz), 7.47 (1H, s), 7.32 (1H, d, J = 1.5 Hz), 7.26 (1H, d, J = 1.5 Hz), 7.20 (1H, d, J = 1.5 Hz), 7.12 (1H, d, J =1.5 Hz), 7.07 (1H, d, J = 1.5 Hz), 6.95 (1H, d, J = 1.5 Hz), 6.82 (2H, d, J = 9 Hz), 4.06 (3H, s), 3.88 (3H, s), 3.64 (3H, s), 3.61 (3H, s), 3.75 (8H, m), 3.5 (2H, t, J = 6 Hz), 2.50 (2H, t, J = 6 Hz); **11**: yellow amorphous solid, 223° (MeOH-ethyl ether); 200 MHz $^1$H-NMR (d$_6$-DMSO) δ 10.44 (1H, s), 10.03 (1H, s), 10.00 (1H, s), 9.94 (1H, s), 9.1-8.6 (4H, m), 8.24 (1H, m), 7.94-7.90 (2H, d J = 7.2 Hz), 7.60 (1H, s), 7.28 (1H, s), 7.25 (2H, s), 7.18 (1H, s), 7.08 (1H, s), 6.96 (1H, s), 6.84-6.79 (2H, d J = 7.1 Hz), 3.86 (3H, s), 3.85 (3H, s), 3.81 (3H, s), 3.79 (3H, s), 3.53-3.40 (8H, m), 2.6-2.3 (4H, m).

17. Characterization of compounds **12** and **13**. **12**: white amorphous solid, 295° (MeOH-ethyl ether); 200 MHz $^1$H-NMR (d$_6$-DMSO) δ 10.58 (1H, s), 10.14 (1H, s), 10.00 (1H, s), 9.94 (1H, s), 8.97 (2H, s), 8.61 (2H, s), 8.24 (1H, m), 7.54 (1H, s), 7.28 (1H, s), 7.24 (1H, s), 7.18 (2H, s), 7.08 (1H, s), 6.96 (1H, s), 6.82-6.80 (1H, d J = 3.2 Hz), 6.32-6.31 (1H, d J = 3.2 Hz), 4.11 (3H, s), 3.99 (3H, s), 3.86 (3H, s), 3.85 (3H, s), 3.81 (3H, s), 2.6-2.4 (4H, m); **13**: white solid, 271° (MeOH-ethyl ether); 200 MHz $^1$H-NMR (d$_6$-DMSO) δ 11.05 (1H, s), 10.51 (1H, s), 10.03 (1H, s), 9.95 (1H, s), 8.99 (2H,s), 8.63 (1H,s), 8.25 (1H, bs), 7.36 (1H, s), 7.32 (1H, s), 7.25 (1H, s), 7.2 (1H, s), 7.1 (1H, s), 7.07 (1H, s), 6.6 (1H, d, J = 1.7 Hz), 6.32 (1H, d, J = 1.7 Hz), 4.05 (3H, s), 3.87 (3H, s), 3.84 (3H, s), 3.81 (3H, s), 3.48 (2H, t, J = 6 Hz), 2.58 (2H, t, J = 6 Hz).

18. Geroni, C.; Pesenti, E.; Tagliabue, G.; Ballinari, D.; Mongelli, N.; Broggini, M.; Erba, E.; D'Incalci, M.; Spreafico, F.; Grandi, M.; *Int. J. Canc.* **1993**, *68*, 916.

19. Broggini, M.; Ballinari, D.; Spinelli, L; Geroni, C.; Spreafico, F.; D'Incalci, M. *Proc. Am. Cancer Soc.*, **1990**, *31*, 348.

20. Broggini, M.; D'Incalci, M. *Anti-Cancer Drug Design*, **1994**, *9*, 373.

21. Xie, G.; Gupta, R.; Lown, J.W.; *Anti-Cancer Drug Design*, **1995**, *10*, 389.

22. Drug sensivity was determined by counting surviving cells after 48h of continuous exposure to at least 4 concentrations of each drug.

23. CD2F1 mice were given an injection of $10^5$ cells i.p. and treated on day 1.

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