Pergamon

*Bioorganic & Medicinal Chemistry Letters*, Vol. 6, No. 11, pp. 1247-1252, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00205-3

# STRUCTURE-ACTIVITY RELATIONSHIP OF NOVEL TALLIMUSTINE DERIVATIVES: SYNTHESIS AND ANTITUMOR ACTIVITY

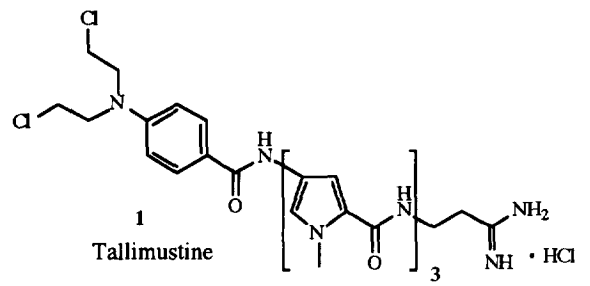
Pier Giovanni Baraldi*[1], Italo Beria[2], Barbara Cacciari[1], Laura Capolongo[2], Paolo Cozzi[2], Nicola Mongelli*[2], Romeo Romagnoli[1], Giampiero Spalluto[1].

[1] *Dipartimento di Scienze Farmaceutiche, Università di Ferrara, 44100 Ferrara, Italy*
[2] *Pharmacia & Upjohn, Oncology, Preclinical Research, Milano, Italy*

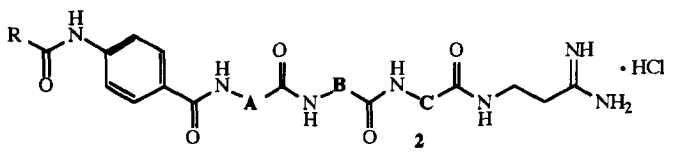
**Abstract:** Oligopeptide-like derivatives structurally related to the antitumor agent tallimustine, where one or two pyrrole rings were replaced by pyrazole or thiazole rings and bearing benzoyl nitrogen mustard or bromoacryloyl moieties were synthesized and evaluated *in vitro* and *in vivo* against L1210 murine leukemia. Compounds **9** and **12** showed antitumor activity higher than or comparable with that of tallimustine. 

## Introduction

Distamycin A derivatives bearing alkylating moieties show significant cytotoxicity and antitumor activity, in particular tallimustine **1** (FCE 24517) shows a broad spectrum of antitumor activity in a series of experimental tumor models[1] and is currently undergoing Phase II clinical trials[2]. Its mode of action is still under investigation. However it has been hypothesized that its activity may be related to its ability to alkylate adenine N(3) in the DNA minor groove with high sequence specificity[3-6].

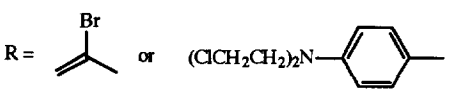


**1** Tallimustine



**2**

**A, B, C**= different heterocycles



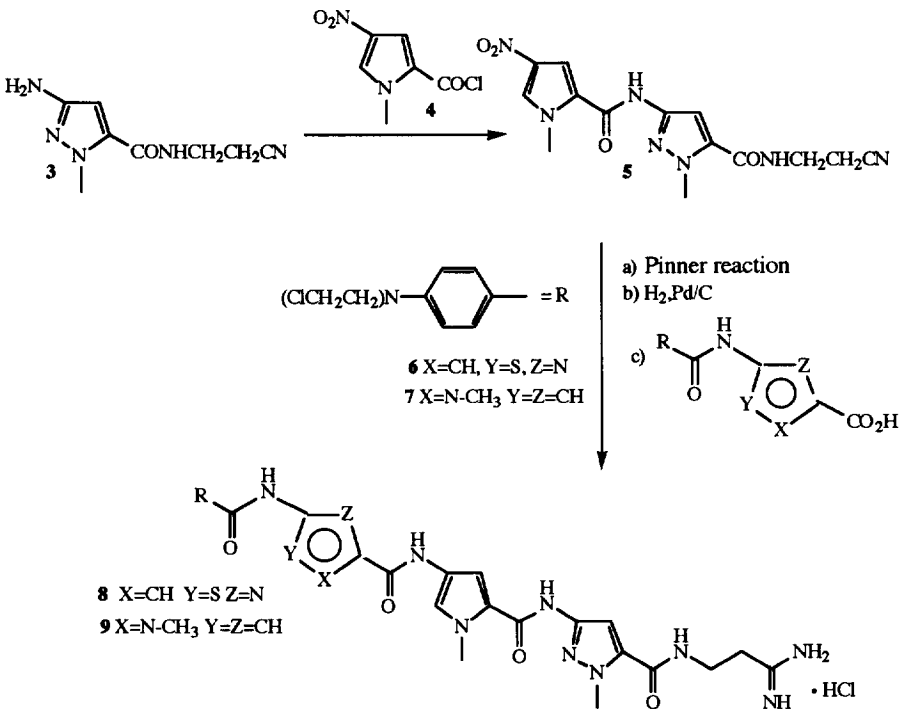
With the aim of identifying novel compounds as potential anticancer agents, we synthesized a new series of tallimustine analogs of general formula **2**, where one or more pyrrole moieties were replaced by

heterocycles such as pyrazole and thiazole. The replacement of the pyrrole ring with various kinds of heterocyclic moieties, namely imidazole and thiazole, capable of specific DNA recognition by hydrogen bond formation, was reported to lead to oligopeptide derivatives showing strong binding ability to double-stranded DNA at specific GC rich regions[7].

### Chemistry

The synthesis of the tallimustine analogs **8** and **9** was carried out following a convergent approach (Scheme 1).

**Scheme 1**

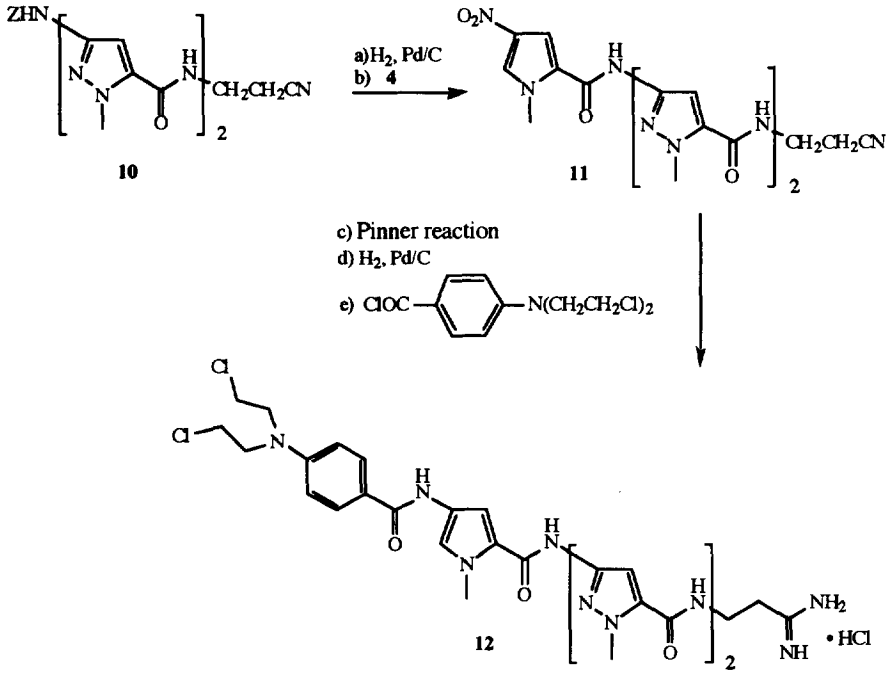



The condensation of acyl chloride[8] **4** with amine[9] **3** afforded **5** in 83% yield. Treatment of **5** under Pinner reaction condition[10] led to the conversion of the nitrile group to the amidinium hydrochloride. Catalytic hydrogenation of the nitro group and condensation with the thiazole or pyrazole derivatives **6** and **7** bearing a benzoyl mustard moiety, in the presence of EDC as coupling agent led to the formation of the oligopeptides **8** and **9**[11] respectively.

The synthesis of intermediates **6** and **7** was acheived by condensation between ethyl 2-aminothiazole-4-carboxylate[12] and methyl 4-amino-1-methylpyrrole-2-carboxylate[13] respectively, with 4-[bis(2-chloroethyl)amino] benzoyl chloride[14] followed by alkaline hydrolysis of the ester group.

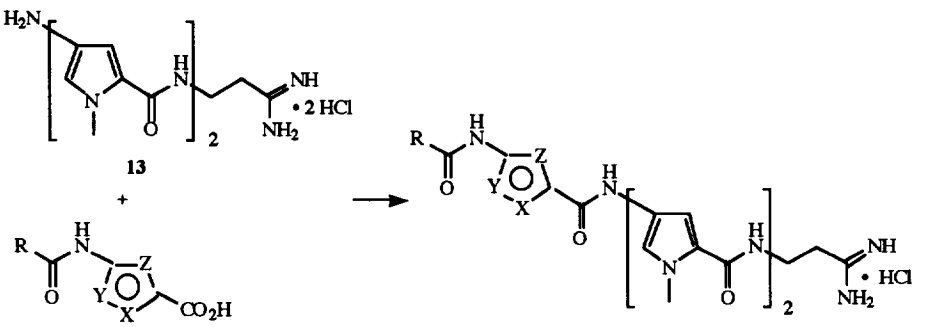
Compound **12** was prepared with a convergent approach, in which the benzoyl mustard moiety was introduced at the last stage as described in Scheme 2. Removal of the protecting group from known compound[9] **10** by catalytic hydrogenation, followed by condensation with **4** gave **11**. Treatment of **11** under Pinner reaction conditions gave the corresponding nitro-amidine, which after a reduction-condensation process in the presence of EDC led to the oligopeptide **12** in moderate yield (30%).

**Scheme 2**




The synthesis of compounds **17-20** is outlined in Scheme 3 and was carried out in analogy to the procedure reported before (see Scheme 1). The required amino-amidine **13** was synthesized starting from 1-methyl-4-nitropyrrole-2-carboxylic acid according to a published procedures[8,13].

**Scheme 3**



**13**

**6** X=CH Y=S Z= N

R= $(ClCH_2CH_2)_2N$—C$_6$H$_4$—

**14** X= N-$CH_3$ Y= N Z= CH

R=$(ClCH_2CH_2)_2N$—C$_6$H$_4$—

**15**[14] X=CH Y=S Z= N

R= $CH_2=C(Br)-$

**16**[14] X= N-$CH_3$ Y= N Z= CH

R= $CH_2=C(Br)-$

**17**

**18**

**19**

**20**

## Results and Discussion

All the compounds synthesized (**8,9,12,17-20**) were assayed *in vitro* and *in vivo* on L1210 murine leukemia cell lines (obtained from NCI, Bethesda, USA), (Table 1). The cytotoxicity and the antileukemic activity were evaluated as previously described.[18] The effects of pyrrole replacement in terms of cytotoxicity and antileukemic activity and the influence of the type of alkylating moiety were analyzed. Some of the compounds (**9,12,20**) showed cytotoxicity higher than or comparable with that of tallimustine.

In particular, compound **9**, in which the terminal N-methyl pyrrole ring of tallimustine **1** was replaced by N-methyl pyrazole, proved as active as tallimustine. Otherwise, the replacement of two pyrrole units by two pyrazoles, as in compound **12**, led to a 5 fold decrease of activity. The same observation can be made for compounds **18** and **20**, where the N-methyl pyrazole is linked to the alkylating moiety.

Derivatives bearing a thiazole nucleus directly linked to the alkylating group (**8, 17** and **19**), showed a relevant decrease of the activity in comparison with pyrrole or pyrazole analogs (**9, 18** and **20**).

As reported elsewhere[15], the hypothesis that, for the same oligopeptide chain, the α-bromoacryloyl moiety may give better results in terms of cytotoxicity and antileukemic activity in comparison to the benzoyl mustard (compound **20** vs. **18**) was confirmed.

**Table 1**

| Compound | *in vitro*[16] $IC_{50}$ (μg/mL) | *in vivo*[17] O.D (mg/Kg) | *in vivo*[17] %T/C |
|---|---|---|---|
| **Tallimustine** | 0.05 | 3.13 | 175 |
| **8** | 7.20 | n.d | n.d |
| **9** | 0.03 | 6.25 | 213 |
| **12** | 0.11 | 25 | 259 |
| **17** | 32.46 | n.d | n.d |
| **18** | 1.39 | 12.5 | 118 |
| **19** | 23.82 | n.d | n.d |
| **20** | 0.15 | 30 | 181 |

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

O.D= optimal dose; optimal non toxic dose<$LD_{10}$.

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

nd = not determined

The substitution of the last pyrrole ring with a pyrazole is not worthy and has led to the discovery of a new lead **9**, which showed the same cytotoxicity of tallimustine **1** and was less toxic in vivo (O.D.= 6.25 mg/Kg vs. 3.13 mg/Kg) with an increased survival time (% T/C). Although we did not perform studies to evaluate the binding of these compounds to DNA, these results might suggest that their activity is affected both by the nature of the alkylating group and by the oligopeptide sequence.

Studies that may prove the specific binding of these compounds to AT-rich regions of DNA minor groove are in progress, as is the synthetic work aimed to find an optimal combination between oligopeptide-sequence and alkylating moiety.

**Acknowledgment:** We wish to thank Pharmacia & Upjohn and Ministero Università e Ricerca Scientifica (MURST) (40 and 60%) for generous financial support of this work.

**References and notes**


1. Di Marco, A.; Gaetani, M.; Orezzi, P.; Scotti, T.; Arcamone, F. *Cancer Chemother. Rep.*, **1962**, *18*, 15.
2. Arcamone, F.; Penco, S.; Orezzi, P.; Nicolella, V.; Pirelli, A. *Nature*, **1964**, *203*, 1064.
3. Broggini, M.; Erba, E.; Ponti, M.; Ballinari, D.; Geroni, C.; Spreafico, F.; D'Incalci, M. *Cancer Res.*, **1991**, *51*, 199.
4. Broggini, M.; D'Incalci, M. *Anti-Cancer Drug Design*, **1994**, *9*, 373.
5. Pezzoni, G.; Grandi, M.; Basoli, G.; Capolongo, L.; Ballinari, D.; Giuliani, F.C.; Barbieri, B.; Pastori, A; Pesenti, E.; Mongelli, N. *British Journal of Cancer*, *64*, 1047.
6. Fontana, M.; Letsingi, M.; Mondello, C.; Braghetti, A.; Montecucco, A.; Ciarrocchi, G. *Anti-Cancer Drug Design*, **1994**, 7, 131.
7. Geierstanger, B.H.; Dwyer, T.J.; Bathini, Y.; Lown, J.W.; Wemmer, D.E. *J. Am. Chem. Soc.*, **1993**, *115*, 4474.
8. Lown, J.W.; Krowicki, K.J.; *J. Org. Chem.*, **1985**, *50*, 3774.
9. Lee, H.H.; Boyd, M.; Gravatt, G.L.; Denny, W.A. *Anti-Cancer Drug Des.*, **1991**, *6*, 501-517.
10. Pinner, A.; Klein, F. *Chem. Ber.*, **1877**, *10*, 1889.
11. Characterization of **9**: white amorphous solid, 206-210 °C (MeOH-ethyl ether); 200 MHz $^1$H-NMR (d6-DMSO) δ 10.7 (1H, s), 10.6 (1H, s), 10.5 (1H, s), 10.0 (1H, s), 9.00 (2H, m); 8.92-8.70(1H, m), 8.64 (2H, s), 7.97-7.93 (2H, d J = 7.2 Hz), 7.47 (1H, s), 7.37 (1H, s), 7.33 (1H, s); 7.28 (2H, s), 7.14 (1H, s), 6.84-6.80 (2H, d J = 7.1 Hz), 4.06 (3H, s), 4.00 (3H, s), 3.81 (3H, s), 3.62-3.39 (8H, m), 2.65-2.58 (4H, m).
12. Sprangue, J.M.; Lincoln, R.M.; Ziegler, C *J. Am. Chem. Soc.*, **1946**, *68*, 266.
13. Bailly, C.; Pommery, N.; Houssin, R.; Henichart, J.; *J. Pharm. Sc.*, **1989**, *78*, 910.
14. Elderfield, R.C.; Liao, T.K. *J. Org. Chem.*, **1961**, *26*, 4996.
15. Baraldi, P.G.; Beria, I.; Cacciari, B.; Capolongo, L.; Cozzi, Mongelli, N.; Romagnoli, R.; Spalluto, G. *Bioorg. Med. Chem. Lett.*, submitted for publication .
16. Drug sensivity was determined by counting surviving cells after 48 h of continuous exposure to at least 4 concentrations of each drug.
17. CDF21 mice were given an injection of $10^5$ cells i.p. and treated on day 1.
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.


(*Received in Belgium* 4 *January* 1996; *accepted* 29 *April* 1996)