Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 459–461

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Preliminary Cytotoxicity of Nitrogen Mustard Derivatives of Distamycin A

Yuqiang Wang,* Susan C. Wright and James W. Larrick

Panorama Research, Inc., 2462 Wyandotte Street, Mountain View, CA 94043, USA

Received 31 January 2002; accepted 24 October 2002

Abstract—Distamycin and nitrogen mustard conjugates, in which the nitrogen mustard unit was coupled to the C-terminus of the pyrrole, were synthesized. The switching of the nitrogen mustard unit from the N-terminus to the C-terminus did not compromise the compound's cytotoxicity. Compound **3**, bearing three pyrrole units, was highly toxic to human K562 leukemia cells in vitro with an IC_{50} value of 0.03 μM. Addition of a *trans* double bond to the molecule had little effects on cytotoxicity.

Toxic side effects associated with chemotherapy are a major problem in the treatment of neoplastic diseases. Therefore, a great deal of research has been focused on the development of more specific therapeutic strategies to reduce toxicity to normal cells. Nitrogen mustards like cyclophosphamide, chlorambucil, and melphalan are widely used antitumor drugs. These drugs mainly alkylate guanine-N7 in the major groove of DNA and cause interstrand DNA cross-link, which is believed to be responsible for the antitumor activity.[1] However, these drugs also alkylate other bases of DNA and cause DNA single strand breaks and DNA–protein cross-linking.[2]

DNA minor groove binding compounds are a new class of anticancer agents, which binds to DNA with a high sequence-specificity.[3,4] This high sequence-selective binding of DNA in the minor groove has been exploited to develop drugs to increase their therapeutic efficacy.[3,4] Distamycin A is a natural antibiotic, which binds to AT-rich regions of B-DNA with high affinity.[5] Conjugates of distamycin and nitrogen mustard have been studied extensively recently, and some compounds of this class have significant antitumor activity in experimental animals.[3,4,6,7] For example, tallimustine, a benzoic acid nitrogen mustard derivative of distamycin, had been in Phase II clinical trials.[8] PUN 166196, a bromoacrylic derivative of distamycin, is currently under clinical investigations (Fig. 1).[7]

It is reasoned that attaching a chemical reactive group such as a nitrogen mustard to a DNA sequence-specific binder such as distamycin will result in a molecule that can alkylate DNA in a sequence-specific manner, and thus reduce undesired side effects.[8]

In all distamycin nitrogen mustard derivatives reported, the nitrogen mustard moiety has been conjugated through the 4-amino of the pyrrole, leaving the molecule with a positively charged amidino, guanidino or a ternary amino group at the C-terminus. We now want to determine if placing a nitrogen mustard moiety at the C-terminus of the pyrrole will affect the antitumor activity. Thus, a series of novel distamycin nitrogen mustard derivatives was designed (Fig. 2).

In these new molecules, a nitrogen mustard is placed at the C-terminus of the pyrrole and an *n*-butyramido group was at the N-terminus. Previously, Wang et al. have found that CC-1065 analogues bearing an *n*-butyramido substituent at C4-position of the DNA-binding pyrrole unit increased antitumor activity in vitro.[9,10] For this reason, all targeted compounds are incorporated with a C4-butyramido substituted pyrrole.

CC-1065 analogues bearing a CPI (1,2,8,8a-tetrahydro-7-methyl-cyclopropa[*c*]pyrrolo[3,2-*e*]indol-4-(5*H*)-one) subunit with a *trans* double bond have been found to have greatly increased antitumor activity in vitro compared to those without a *trans* double bond.[9,10] Distamycin derivatives with a cinnamic nitrogen mustard appear to be more potent than those with a benzoic

*Corresponding author. Fax: +1-650-694-7717; e-mail: yuqiangwang@worldnet.att.net

0960-894X/03/$ - see front matter
PII: S0960-894X(02)00986-1

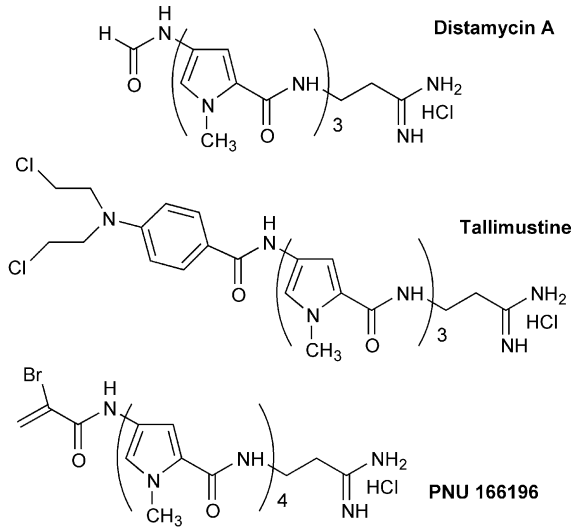


Figure 1. Structures of distamycin A, tallimustine, and PNU 166196.

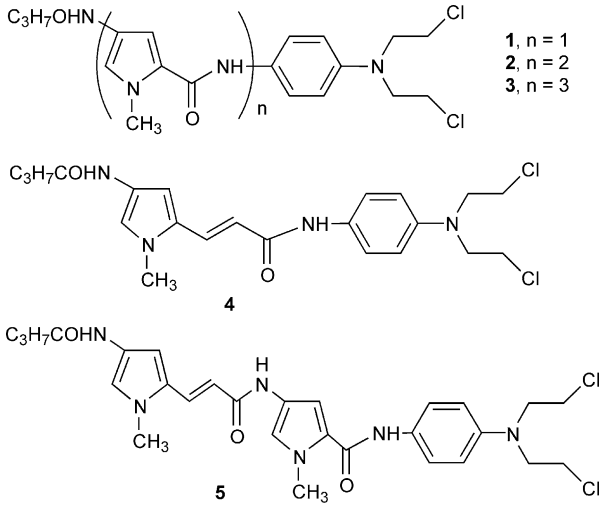
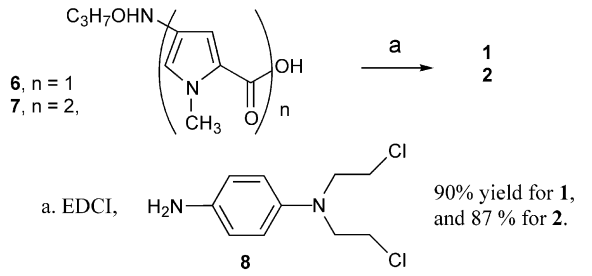


Figure 2. Structures of new distamycin nitrogen mustards.

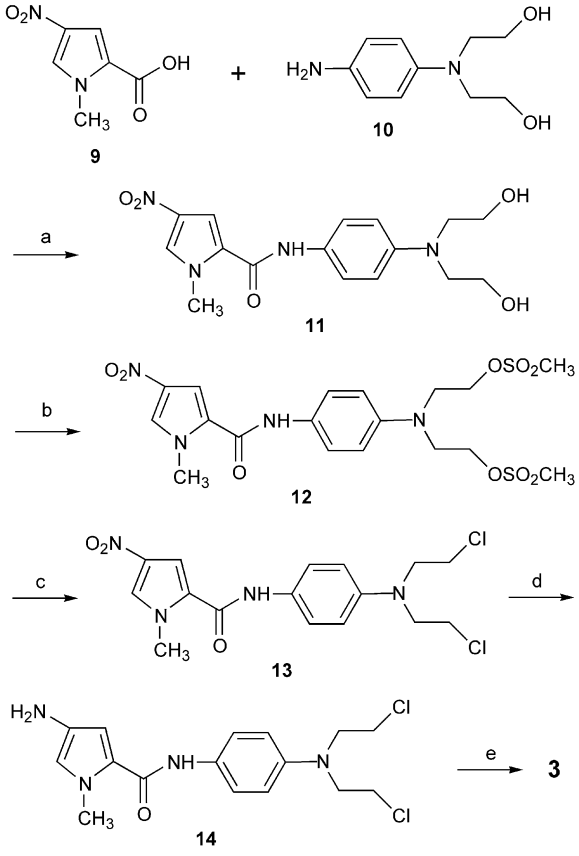
nitrogen mustard.[7] For these reasons, we choose to incorporate a *trans* double bond in some of the new compounds (**4** and **5**) to increase their potency. It has been shown that an increase in the number of pyrrole units results in an increase of DNA binding, which in turn leads to an increase in antitumor activity.[3,4,7] Therefore, the antitumor activity of the new compounds is expected to be **3** > **2** > **1**. We herein report the synthesis and cytotoxicity of the new distamycin nitrogen derivatives.

Chemistry[11]

Compounds **6** and **7** were synthesized as described previously,[9] and were coupled to 4-amino-*N, N*-bis(2-chloroethyl)aniline (**8**)[12] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) to afford **1** and **2**, respectively (Scheme 1). When the same strategy was used to synthesize **3**, the yield was very low (<20%). Thus, alternatively, **3** was also synthesized as shown in Scheme 2.

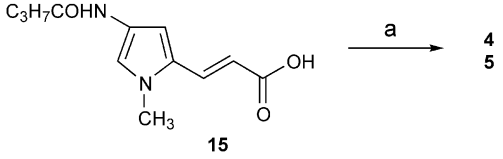


Scheme 1.



Scheme 2. (a) EDCI; (b) CH_3SO_2Cl, $(C_2H_5)_3N$; (c) LiCl, 31% yield from **9** to **13**; (d) H_2/Pd/C, 100% yield; (e) **7**, EDCI, 55% yield.

Acid **9**[9] was coupled to amino **10**[13] in the presence of EDCI to produce **11**. The latter was treated with methanesulfonyl chloride to generate the mesyl derivative **12**, which was converted to **13** by treatment with lithium chloride. The nitro group of **13** was reduced by hydrogenation affording **14**. The latter was then coupled to **7** in the presence of EDCI to generate target **3**. Compounds **4** and **5** were synthesized by coupling acid **15** with amines **8** and **14**, respectively, in the presence of EDCI (Scheme 3).



Scheme 3. (a) **8** or **14**, EDCI, 48% yield for **4**, and 37% for **5**.

Table 1. Cytotoxicity against K562 leukemia cells in vitro[a]

Compd	IC_{50} (μM)[b]
1	0.95
2	0.25
3	0.03
4	1.2
5	0.21
Chlorambucil	35

[a]The assay was set up in triplicate in 96-well flat-bottom microtiter plates. All cells were seeded at 5000 cells/well in RPMI-1640 plus 10% FCS. Drugs were added, and the total volume was adjusted to 0.2 mL/well. Total incubation time was 48 h with the addition of ^{3}H-thymidine for the last 24 h of incubation. The assay was harvested and radioactivity was counted.
[b]IC_{50} values are defined as the minimal drug concentration necessary to inhibit incorporation of [^{3}H] thymidine by 50%, and are the averages of three experiments.

Results and Discussion

The antitumor activity of the new compounds was determined against human chronic leukemia K562 cells in vitro, and the results were shown in Table 1. As expected, compound **3** (IC_{50}: 0.03 μM), bearing three pyrroles, is more potent than **2** (IC_{50}: 0.25 μM), bearing two pyrroles, which is more potent than **1** (IC_{50}: 0.95 μM), bearing one pyrrole. Generally, for distamycin nitrogen mustards, the potency of the compound will increase approximately 10-fold with the addition of one pyrrole unit up to a total of four pyrroles. Interestingly, it appears that switching the nitrogen mustard unit from the N-terminus to the C-terminus of the pyrrole did not compromise the compound's cytotoxicity. For example, the cytotoxicity of most distamycin nitrogen mustard conjugates is in the range of high μM to middle nM.[7]

In contrast to the finding with distamycin cinnamic nitrogen mustard derivatives, in which, a *trans* double bond appears to increase potency,[7] the addition of a *trans* double bond in the new molecules, **4** and **5**, had little effect on cytotoxicity. For example, the cytotoxicity of **1** and **4** is almost the same (IC_{50} value of 0.95 and 1.2 μM). There is little difference between the potency of **2** and **5** (IC_{50} value of 0.25 and 0.21 μM).

Conclusion

For the first time, distamycin and nitrogen mustard conjugates, in which the nitrogen mustard unit was coupled to the C-terminus of the pyrrole, were synthesized. The switching of the nitrogen mustard unit from the N-terminus to the C-terminus did not compromise the compound's cytotoxicity. Compound **3**, bearing three pyrrole units, was highly toxic to human K562 leukemia cells in vitro. Addition of a *trans* double bond to the molecule had little effect on cytotoxicity.

References and Notes

1. Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723.
2. Farmer, P. B. *Pharmacol. Ther.* **1987**, *35*, 301.
3. Reddy, B. S.; Sharma, S. K.; Lown, J. W. *Curr. Med. Chem.* **2001**, *8*, 475 and references therein.
4. Denny, W. A. *Curr. Med. Chem.* **2001**, *8*, 533 and references therein.
5. Dervan, P. B. *Science* **1986**, *232*, 464.
6. D'Incalci, M.; Sessa, C. *Exp. Opin. Invest. Drugs* **1997**, *6*, 875 and references therein.
7. Cozzi, P. *Il Farmaco* **2000**, *55*, 168 and references therein.
8. Arcamone, F. M.; Animati, F.; Barbieri, B.; Configliacchi, E.; D'Alessio, R.; Giuliani, F. C.; Lazzari, E.; Menozzi, M.; Mongelli, N.; Penco, S.; Verini, M. A. *J. Med. Chem.* **1989**, *32*, 774.
9. Wang, Y.; Gupta, R.; Huang, L.; Luo, W.; Lown, J. W. *Anti-Cancer Drug Des.* **1996**, *11*, 15.
10. Fregeau, N. L.; Wang, Y.; Pon, R. T.; Wylie, W. A.; Lown, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 8917.
11. The tested compounds were purified by silica gel column chromatography, eluting with a mixture of ethyl acetate and hexane. The compounds were characterized by elemental analysis with satisfactory results. Their ^{1}H NMR and MS spectra were in agreement with the assigned structures. ^{1}NMR (DMSO-d_6) data of **1**–**5** and MS spectra are given. (**1**) 9.69 (s, 1H, NH), 9.54 (s, 1H, NH), 7.51 (d, 2H, C_6H_4, J = 9.2 Hz), 7.15 (d, 1H, Py–H, J = 1.8 Hz), 6.88 (d, 1H, Py–H, J = 1.8 Hz), 6.72 (d, 2H, C_6H_4, J = 9.2 Hz), 3.80 (s, 3H, NCH_3), 3.70 (brs, 8H, CH_2CH_2Cl). 2.20 (t, 2H, $CH_2CH_2CH_3$, J = 7.4 Hz), 1.62–1.56 (m, 2H, $CH_2CH_2CH_3$), 0.95 (t, 3H, $CH_2CH_2CH_3$, J = 7.3 Hz). EIHRMS calcd for $C_{20}H_{26}Cl_2N_4O_2$ 424.1433, found 424.1432. (**2**) 9.86 (s, 1H, NH), 9.72 (s, 1H, NH), 9.61 (s, 1H, NH), 7.53 (d, 2H, C_6H_4, J = 9.2 Hz), 7.25 (d, 1H, Py–H, J = 1.4 Hz), 7.15 (d, 1H, Py–H, J = 1.9 Hz), 7.05 (d, 1H, Py-H, J = 1.8 Hz), 6.88 (d, 1H, Py–H, J = 1.8 Hz), 6.72 (d, 2H, C_6H_4, J = 9.1 Hz), 3.83 (s, 3H, NCH_3), 3.71 (brs, 8H, CH_2CH_2Cl). 2.21 (t, 2H, $CH_2CH_2CH_3$, J = 7.4 Hz), 1.62–1.56 (m, 2H, $CH_2CH_2CH_3$), 0.90 (t, 3H, $CH_2CH_2CH_3$, J = 7.3 Hz). FABHRMS calcd for $C_{26}H_{32}Cl_2N_6O_3$ 546.1913, found 546.1903. (**3**) 9.86 (s, 1H, NH), 9.82 (s, 1H, NH), 9.67 (s, 1H, NH), 9.58 (s, 1H, NH), 7.53 (d, 2H, C_6H_4, J = 9.2 Hz), 7.24 (s, 1H, Py–H), 7.22 (s, 1H, Py–H), 7.14 (s, 1H, Py–H), 7.05 (m, 2H, Py–H), 6.88 (s, 1H, Py–H), 6.73 (d, 2H, C_6H_4, J = 9.2 Hz), 3.86–3.83 (m, 9H, NCH_3), 3.71 (brs, 8H, CH_2CH_2Cl). 2.20 (t, 2H, $CH_2CH_2CH_3$, J = 7.8 Hz), 1.60–1.58 (m, 2H, $CH_2CH_2CH_3$), 0.90 (t, 3H, $CH_2CH_2CH_3$, J = 7.3 Hz). FABHRMS calcd for $C_{32}H_{38}Cl_2N_8O_4$ 668.2393, found 668.2360. (**4**) 9.69 (s, 1H, NH), 9.66 (s, 1H, NH), 7.54 (d, 2H, C_6H_4, J = 8.4 Hz), 7.38 (d, 1H, CH=CH, J = 15.5 Hz), 6.72 (d, 2H, C_6H_4, J = 8.9 Hz), 6.46 (s, 1H, Py–H), 6.38 (d, 1H, Py–H, J = 15.5 Hz), 3.70 (brs, 8H, CH_2CH_2Cl). 3.64 (s, 3H, NCH_3), 2.19 (t, 2H, $CH_2CH_2CH_3$, J = 7.4 Hz), 1.62–1.55 (m, 2H, $CH_2CH_2CH_3$), 0.87 (t, 3H, $CH_2CH_2CH_3$, J = 7.3 Hz). FABHRMS calcd for $C_{22}H_{29}Cl_2N_4O_2$ 451.1668, found 451.1675. (**5**) 9.88 (s, 1H, NH), 9.67 (s, 1H, NH), 9.58 (s, 1H, NH), 7.54 (d, 2H, C_6H_4, J = 8.8 Hz), 7.36 (d, 1H, CH=CH, J = 15.5 Hz), 7.28 (s, 1H, Py–H), 7.17 (d, 1H, Py–H, J = 1.5 Hz), 6.91 (d, 1H, Py–H, J = 1.8 Hz), 6.73 (d, 2H, C_6H_4, J = 9.2 Hz), 6.47 (s, 1H, Py–H), 6.37 (d, 1H, Py–H, J = 15.5 Hz), 3.83 (s, 3H, NCH_3), 3.71 (brs, 8H, CH_2CH_2Cl). 3.64 (s, 3H, NCH_3), 2.19 (t, 2H, $CH_2CH_2CH_3$, J = 7.4 Hz), 1.59–1.55 (m, 2H, $CH_2CH_2CH_3$), 0.89 (t, 3H, $CH_2CH_2CH_3$, J = 7.3 Hz). FABHRMS calcd for $C_{28}H_{34}Cl_2N_6O_3$ 572.2069, found 572.2069.
12. Palmer, B. D.; Wilson, W. R.; Pullen, S. M.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 112.
13. Gourdie, T. A.; Valu, K. K.; Gravatt, G. L.; Boritzki, T. J.; Baguley, B. C.; Wakelin, L. P. G.; Wilson, W. R.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 1177.