Pure Appl. Chem., Vol. 75, Nos. 2–3, pp. 187–194, 2003.

DNA minor-groove binders. Design, synthesis, and biological evaluation of ligands structurally related to CC-1065, distamycin, and anthramycin*

Pier Giovanni Baraldi‡, Mojgan Aghazadeh Tabrizi, Delia Preti, Francesca Fruttarolo, Barbara Avitabile, Andrea Bovero, Giovanna Pavani, Maria del Carmen Nunez Carretero, and Romeo Romagnoli

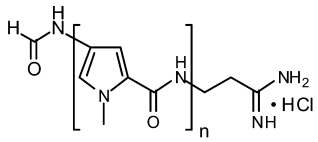
University of Ferrara, Dipartimento di Scienze Farmaceutiche, Via Fossato di Mortara 17/19, 44100 Ferrara, Italy

Abstract: Many natural and synthetic anticancer agents with the ability to interact with DNA have been discovered, but most have little sequence-specificity and often exhibit severe toxicity to normal tissues. Thus, there has been considerable interest in molecular biology and human medicine to find small molecules that can alkylate the DNA in a sequence-specific manner and modify the function of nucleic acids irreversibly. Analogs of naturally occurring antitumor agents, such as distamycin A, which bind in the minor groove of DNA, represent a new class of anticancer compounds currently under investigation. Distamycin A has driven researchers' attention not only for its biological activity, but also for its nonintercalative binding to the minor groove of double-stranded B-DNA, where it forms a strong reversible complex preferentially at the nucleotide sequences consisting of 4–5 adjacent adenine-thymine (AT) base pairs. The pyrrole-amide skeleton of distamycin A has been also used as DNA sequence-selective vehicles for the delivery of alkylating functions to DNA targets, leading to a sharp increase of its cytotoxicity, in comparison to that, very weak, of distamycin itself. In the last few years, several hybrid compounds, in which derivatives of naturally occurring antitumor agents, such as anthramycin or the alkylating unit of the antibiotic CC-1065, have been tethered to distamycin frames. The DNA alkylating and cytotoxic activities against several tumor cell lines are reported and discussed in terms of their structural differences in relation to both the number of *N*-methyl pyrrole rings and the type of alkylating unit tethered to the oligopeptidic frame.

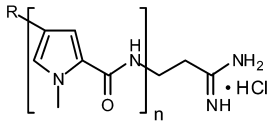
There is currently interest in the study and development of low-molecular-weight sequence-selective agents interacting with double-stranded DNA. These molecules are frequently based on natural products and have been investigated for their ability to interact selectively with the minor groove of DNA [1]. One of the most studied minor-groove binders is distamycin A.

The tripyrrole peptide distamycin A (**1**) is a naturally occurring antibiotic agent isolated in 1962 from the cultures of *Streptomyces distallicus* [2] with interesting antibacterial and antiviral activities (but inactive as an antitumor agent).

Pure Appl. Chem. **75**, 141–419 (2003). An issue of reviews and research papers based on lectures presented at the 23rd IUPAC International Symposium on the Chemistry of Natural Products, Florence, Italy, 28 July–2 August 2002 on the theme of natural products.
‡Corresponding author



1 (Distamycin A), n=3
2, n=4



3, n=3 or 4
R= 4-[(ClCH$_2$CH$_2$)$_2$N]C$_6$H$_4$CONH, CH$_2$(O)CHCONH
4-[(ClCH$_2$CH$_2$)$_2$N]C$_6$H$_4$(CH$_2$)$_3$CONH, (ClCH$_2$CH$_2$)$_2$N
CH$_2$=CBrCONH, CH$_2$=CClCONH, .

Fig. 1 Chemical structures of distamycin A (**1**), its homolog (**2**), and their derivatives with an alkylating moiety (**3**).

As reported in Fig. 1, distamycin A (**1**) is characterized by the presence of an oligopeptidic pyrrolecarbamoyl frame ending with an amidino moiety, which reversibly binds to the minor groove of DNA by hydrogen bonds, van der Waals contacts, and electrostatic interactions with a strong preference for adenine-thymine (AT)-rich sequences containing at least four AT base pairs [3].

The tetrapyrrole distamycin A homolog (**2**), although exerting very low cytotoxic activity on the L1210 leukemia cell line, is always almost 20-fold more active than the distamycin A, and increasing the number of pyrrole units of the oligopeptidic frame increases the sequence-specificity for longer tracts of AT-rich DNA, as a result of the greater availability of hydrogen bonding and van der Waals surface.

The synthesis of different analogs of distamycin A allows the establishment of some important molecular requirements for bioactivity. With the objective to identify novel promising candidates, distamycin A (**1**) and its four pyrroles homolog (**2**) have been used as DNA sequence-selective carriers of alkylating functions, in which the formyl group has been substituted by benzoyl nitrogen mustard (BAM) [10], chlorambucil (CHL), halogenoacryloyl [4], and epoxycarbonyl moieties, leading to compounds (with general formula **3**) that are substantially more cytotoxic than distamycin and **2** themselves, respectively [4].

In the last few years, several hybrid compounds, in which known antitumor compounds or simple active moieties of known antitumor agents have been tethered to distamycin and netropsin frames, have been designed, synthesized, and tested [5]. The pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) group [6], which includes the natural compounds anthramycin (**4**) and DC-81 (**5**), owes its DNA-interactive ability and resultant biological effects to an N10-C11 carbinolamine/imine moiety in the central B-ring, which is capable of covalently binding to the C2-NH_2 of guanine residues in the minor groove of DNA. X-ray and foot-printing studies on covalent DNA-PBD adduct have demonstrated a high sequence-specificity for GC-rich DNA regions, in particular for X-G-X triplets (X = purine) [7].

Recently, both our [8,9] and Lown's group [10] have reported the synthesis, biological activity, and DNA binding properties of novel hybrids (**6**–**9**), consisting respectively of one, two, three, or four pyrrole amide units linked to a PBD (**10**), through a spacer arm, in order to study the structure–activity relationship between the length of the oligopyrrolic frame, antiproliferative activity, and sequence-specificity (Fig. 2). The rationale that led to the synthesis of this series of PBD-lexitropsin conjugates was to tether the distamycin A frame, which plays the role of pure minor-groove binder, to the minor-groove alkylating moiety represented by the PBD (**10**), with the aim to obtain new derivatives that could result in more cytotoxic than the parent compounds. In these new hybrids (**6**–**9**), we conjugated the

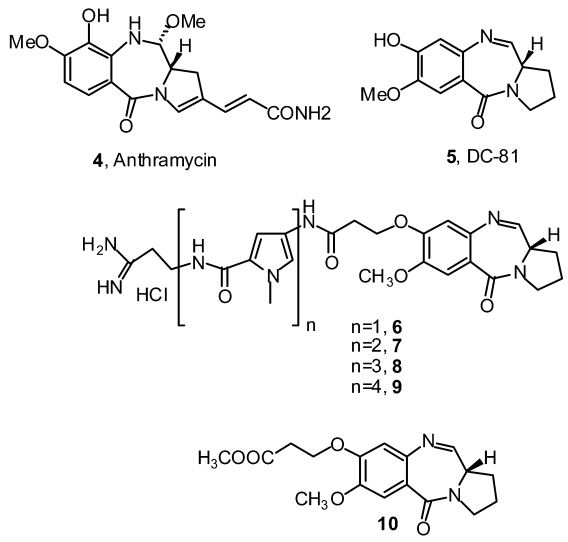


Fig. 2 Chemical structures of natural compounds anthramycin (**4**) and DC-81 (**5**), PDB-lexitropsin conjugates (**6**–**9**) and PBD derivative (**10**).

capability of the PBD (**10**) to covalently bind to GC-rich sequences, with that of distamycin's different recognition pattern.

The antiproliferative activity of the hybrids has been evaluated in vitro by using both the human chronic myeloid leukemia K562 and the T-lymphoblastoid Jurkat cell lines. The results obtained are summarized in Table 1 and compared to antiproliferative effects of the natural product distamycin A and the PBD methyl ester (**10**). The results obtained demonstrate that the hybrids **6**–**9** exhibit different DNA-binding activity with respect to both distamycin A (**1**) and PBD (**10**). In addition, a direct relationship was found between the number of pyrrole rings present in the hybrids (**6**–**9**) and the stability of drugs/DNA complexes. With respect to antiproliferative effects, it was found that the increase in the length of the polypyrrole backbone led to an increase of in vitro antiproliferative effects, i.e., the hybrid **9** containing the four pyrroles distamycin analog, was more active than **8**, both against K562 (IC_{50} μM, 0.07 vs. 0.8, respectively) and Jurkat (IC_{50} μM, 0.04 vs. 0.7, respectively) cell lines. Regarding the above-mentioned derivatives **8** and **9**, it can be observed that they are much more active than distamycin

Table 1 In vitro biological effects of distamycin A (**1**), DC-81 (**5**), PBD methyl ester (**10**) and PBD-polypyrrolic hybrids (**6**–**9**) on K562 and Jurkat cell lines.

Compound	IC_{50} (μM)	
	Jurkat	K562
Distamycin A (**1**)	20	12
DC-81 (**5**)	2.2	1
10	3	1.5
6	80	>100
7	50	6
8	0.8	0.7
9	0.07	0.04

[a]50 % inhibitory concentration represents the mean from dose–response curves of at least three experiments.

A (**1**). Only derivatives **8** and **9** retain a higher antiproliferative activity when compared to PBD (**10**) alone. In fact, compounds **6**, containing only one *N*-methylpyrrole unit, showed negligible inhibitory activity or no activity at all on K562 and Jurkat cell proliferation, with IC_{50} values of 80 and >100 μM, respectively. On the other hand, **7**, containing two pyrrole moieties, exhibited to some extent antiproliferative activity on the Jurkat cell line (IC_{50} 6 μM), being scarcely active on K562 cells (IC_{50} 50 μM). DC-81 was found to retain antiproliferative activities similar to those exhibited by compound **10**.

Taken together, these findings are consistent with the hypothesis that a tighter DNA binding, depending on the multiplicity of interactions between the pyrrole carbamoyl units and AT-rich sequences of DNA, is crucial for the antiproliferative effects of PBD-distamycin related compounds. Accordingly, the tri- and tetrapyrrole hybrids **8** and **9** are the most potent antiproliferative compounds of this series, exhibiting probably higher binding affinities with respect to the mono- and dipyrrole conjugates **6** and **7**, due to additional amido hydrogen bonds and van der Waals interactions.

These data suggest that the higher antiproliferative activity of hybrid molecules containing PBD and minor-groove oligopyrrole carriers containing three and four pyrrole moieties is due to the recognition of additional binding sites other than distamycin, as well as to an increase in the stability of drugs/DNA complexes. This is a reasonable hypothesis, since a tighter DNA binding could depend on the increased multiplicity of interactions between the increased number of pyrrolecarboxyl units and target DNA sequences.

The PBD-distamycin hybrid **8** is also able to inhibit the DNA binding of the transcription factor Sp1 [11]. The results obtained demonstrate that treatment of Sp1 target DNA with PBD-distamycin hybrid **8** renders the site unrecognizable by nuclear proteins. These data are, in our opinion, of interest, because transcription factors belonging to the Sp1 superfamily are very important for the control of transcription of cellular and viral genes, including the oncogenes Ha-ras and c-myc, the collagen-α1 (I) gene and the human immunodeficiency type 1 virus (HIV-1) [12–14].

Recently, our group has evaluated the synthesis of a series of hybrids that represent a molecular combination of polypyrrole minor-groove binders structurally related to the natural antitumor agent distamycin A, and two pyrazole analogs of the left-hand segment called cyclopropylpyrroloindole (CPI) of the potent antitumor antibiotic (+)-CC-1065 [15].

(+)-CC-1065 (**11**) (Fig. 3) is a member of the class of cyclopropylindole antitumor antibiotics first isolated from *Streptomyces zelensis* by scientists at the Upjohn Company [16], and it is a highly potent natural agent with activity both in vitro and in experimental animals [17,18]. Studies on the mechanism of cytotoxic action have shown that CC-1065 affords its biological activity through binding to double-stranded B-DNA within the minor groove at AT-rich sequences and selectively alkylating at the N_3 position of the 3′-adenine by its CPI subunit **12** [19]. Despite its high potency and broad spectrum of anti-

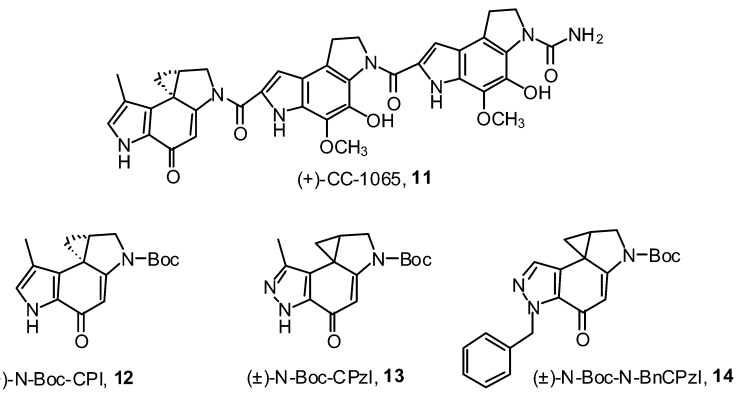


Fig. 3 Chemical structures of natural compounds (+)-CC-1065 (**11**), (+)-*N*-Boc-CPI (**12**), (±)-*N*-Boc-CPzI (**13**), and (±)-*N*-Boc-N-BnCPzI (**14**).

tumor activity, CC-1065 cannot be used in humans because it causes delayed death in experimental models [20].

Several years ago, we synthesized two CPI pyrazole analogs named (±)-*N*-Boc-CPzI **13** [21,22] and (±)-*N*-Boc-N-BnCPzI **14** [23], which demonstrate a cytotoxicity against L1210 leukemia cells that was comparable to or 10-fold lower than, respectively, that of the reference compound *N*-Boc-CPI **12** [IC_{50} = 330 nM for (+)-*N*-Boc-CPI vs. IC_{50} = 370 nM for (±)-**13** and IC_{50} = 3064 nM for (±)-**14**]. Because of their limited sequence-specificity, low affinity for DNA, and poor water solubility, it was reasoned that it may be beneficial to tether these alkylating compounds to a DNA-binding vector, such as polypyrrole pseudopeptides, which can permeate cell membranes and has the potential to control specific gene expression. The vector could, therefore, deliver the reactive group more efficiently and in a sequence-specific manner to the DNA. Moreover, water solubility made these hybrid compounds attractive to overcome the administration problem of CC-1065 derivatives.

In synthesizing these novel water-soluble hybrids, we wanted to increase the potency of pyrazole CPI analogs **13** and **14** by increasing their affinity for DNA and to determine the structure–activity relationship between the length of the oligopyrrolic frame, antitumor activity, and sequence-specificity.

These hybrid compounds (**15**–**20**) have obtained coupling of the two *N*-Boc deprotected CPI pyrazole analogs **13** and **14** with three mixed pyrazole–pyrrole compounds called lexitropsins (or information-reading oligopeptides), consisting of a varying number of pyrrole amide units (from one to three) tethered on the *N*-terminus to a 3,5-pyrazole dicarboxylic acid moiety, and structurally related to the DNA minor-groove binder distamycin A (Fig. 4).

As evident from Table 2, it was found that tethering the pyrazole CPI analogs **13** and **14** to the DNA-binding lexitropsins afforded, with few exceptions, conjugate molecules that showed enhanced cytotoxic activity against five different cancer cell lines in vitro.

The results show that the hybrids **15**–**17** were about 8- to 70-fold more potent than the alkylating unit **13**. Among these, the hybrid **17** demonstrated the highest potency across the panel of tumor cell lines, especially against T- and B-lymphoblast cells, with IC_{50} values between 7.4 and 71 nM. Against L1210 cells, the tripyrrole analog **17** was 2- to 8-fold more active than the bis and monopyrrole counterparts (compounds **16** and **15**, respectively). This is presumably due to the increased DNA binding of the "longer" compounds. The IC_{50} for **16** ranged between 19 and 45 nM with respect to the tumor cell lines L1210, Molt4, CEM, and Daudi, but the same compound was somewhat less active against FM3A (IC_{50} = 190 nM). Compound **17** was more active against L1210 cells than against the other tumor cell lines. For this series of hybrids, it is possible to correlate structure with biological activity, increasing the number of pyrrole rings from one to three results in increased cytotoxic activity.

The hybrids **18**–**20** demonstrated potent cytotoxic activity against Daudi cells (IC_{50} values ranging from 11 to 100 nM). While being somewhat less toxic to the other tumor cells (IC_{50} values ranging between 70 and 19 300 nM), they are always more cytotoxic than the alkylating unit **14** alone (with only

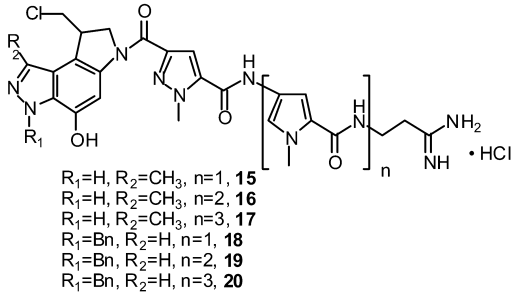


Fig. 4 Chemical structures of hybrid compounds **15**–**20** comprising either two CPI pyrazole analogs (**13** and **14**) and three mixed pyrazole–pyrrole lexitropsins structurally related to distamycin A.

Table 2 In vitro activity of alkylating units **13** and **14** and hybrids **15–20** against the proliferation of five different cancer cell lines.

Compound	IC_{50} (nM ± S.E.)				
	L1210	FM3A	Molt/4	CEM	Daudi
13	520 ± 6.6	1400 ± 40	1740 ± 50	1260 ± 30	680 ± 150
14	2710 ± 490	18 300 ± 200	8550 ± 280	6720 ± 1040	7520 ± 30
15	58 + 17	1600 ± 50	340 ± 20	230 ± 10	150 ± 40
16	19 ± 2	190 ± 6	45 ± 1	39 ± 1	22 ± 10
17	7.4 ± 0.4	31 ± 11	17 ± 4	71 ± 9	8.8 ± 0.1
18	240 ± 30	4000 ± 1000	130 ± 20	70 ± 21	11 ± 6.0
19	600 ± 90	5600 ± 1400	160 ± 60	210 ± 110	38 ± 7.0
20	400 ± 16	19 300 ± 3400	310 ± 70	400 ± 50	100 ± 10

IC_{50} = compound concentration required to inhibit tumor cell proliferation by 50 %.
Data are expressed as the mean ±SE from dose–response curves of at least three independent experiments.

few exceptions). The FM3A cell line was substantially less sensitive to hybrids **18–20**, which exhibited cytotoxic activities comparable or 3-fold higher (IC_{50} values ranging from 4 to 19 μM) than that of the alkylating unit **14** alone. In the CEM cell line, compounds **18–20** demonstrated IC_{50} values between 70–400 nM, which was 30- to 100-fold higher than that reported for **14**, with compound **18** exhibiting potent cytotoxicity (IC_{50} of 70 nM).

A fairly marked dependence on the number of pyrrolic rings for the antiproliferative activity has been observed in the **15–17** series with compound **17** comprising three pyrrolic rings proving to be the most active. The relationship between the number of pyrrole groups in the **18–20** series and their corresponding cytotoxicity did not seem to follow this pattern. In fact, the cytotoxicity was higher for compound **18**, which possesses only one pyrrole ring. It is interesting to note that the L1210 cell line was 50-fold more susceptible to the cytotoxic action of compound **20** than FM3A cells. For all cell lines, taken together, compounds possessing the same number of pyrrole rings and the alkylating unit **13** appeared to be more cytotoxic than those containing **14** as the alkylating agent.

In addition, high-resolution denaturating gel electrophoresis experiments have been performed on hybrid compounds **15–20**. When compounds **18–20** were incubated at different concentrations (ranging from 0.1 to 100 μM), no significant cleavage was observed by thermally induced strand cleavage of DNA fragment. For these derivatives, the presence of a benzyl group at the azaindole moiety significantly disrupted the alkylation reaction, and the effect of this substitution has presumably altered the positioning of the compound on the DNA. In the orientation that is suitable for DNA alkylation, the benzyl group needs to protrude from the minor groove, and it is assumed that this causes significant energy loss. Therefore, the introduction of a benzyl group decreased the alkylating activity and also the corresponding cytotoxicity. In contrast to the case of hybrids **18–20**, hybrids **15–17** show unique DNA sequence-selective alkylation in AT-rich sequences. High-resolution denaturating gel electrophoresis indicated that **17** selectively alkylates the third adenine of the 5′-ACAAAAATCG-3′ motif within a 400 bp DNA fragment, the strongest and most highly sequence-specific DNA alkylation activity observed. This compound elicited the strongest and most highly sequence-specific DNA alkylation activity. For compound **17**, DNA alkylation was observed even at 50 nM. Results from this investigation suggest a promising approach for developing a new generation of DNA-alkylating agents based on CPI analogs and a lexitropsin hybrid system that can alkylate purine bases in a sequence-selective fashion. Because of the high efficiency of alkylation, results from the present investigation suggest that these molecules should be useful in the design of compounds that target a single gene. Further studies on the generality and the optimization of this new class of DNA alkylation systems are currently in progress.

CONCLUSIONS

In the last few years, several hybrid compounds in which known antitumor activity has been tethered to a distamycin frame, have been described in the literature. The nature of antitumor agents and, therefore, also the rationale that led to these compounds were different. In general, the interaction with DNA tends to be dominated by the minor-groove binding moiety, i.e., the conjugates bind to the minor groove with preferential interaction with AT-rich sequences. Until the end of the last century, the hybrid approach did not receive much attention because it failed to present practical significant advantages in terms of activity. In fact, in some cases, these hybrid derivatives even lost the activity of the antitumor moiety, as occurred when the anticancer drug mitomycin C was joined to a distamycin-like frame [24]. The present work demonstrated the validity of the hybrid approach between distamycin A (as minor-groove binder) and derivatives of naturally occurring antitumor antibiotics with DNA-alkylating properties. It may be noted that, in general, the cytotoxicity of these hybrid derivatives was much greater than that of the alkylating units alone.

ACKNOWLEDGMENTS

We wish to thank Pharmacia & Upjohn for providing distamycin A and Progetto Giovani Ricercatori (1999) for financial support of this work. We wish also to thank Ministero Università e Ricerca Scientifica (MIUR) (40 and 60 %) for generous financial support of this work.

REFERENCES AND NOTES

1. P. B. Dervan. *Bioorg. Med. Chem.* **9**, 2215 (2001).
2. F. Arcamone, S. Penco, P. G. Orezzi, V. Nicolella, A. Pirelli. *Nature* **203**, 1064 (1964).
3. J. G. Pelton and D. E. Wemmer. *J. Am. Chem. Soc.* **112**, 1393 (1990).
4. S. Marchini, M. Broggini, C. Sessa, M. D'Incalci. *Expert Opin. Invest. Drugs* **10**, 1703 (2001).
5. P. G. Baraldi, B. Cacciari, G. Spalluto, R. Romagnoli. *Exp. Op. Ther. Pat.* **6**, 891 (2000).
6. D. E. Thurston and D. S. Bose. *Chem. Rev.* **94**, 433 (1994).
7. M. L. Kopka, D. S. Goodsell, I. Baikalov, K. Grzeskowiak, D. Cascio, R. E. Dickerson. *Biochemistry* **33**, 13593 (1994).
8. P. G. Baraldi, B. Cacciari, A. Guiotto, A. Leoni, R. Romagnoli, G. Spalluto, N. Mongelli, D. E. Thurston, N. Bianchi, R. Gambari. *Bioorg. Med. Chem. Lett.* **8**, 3019 (1998).
9. P. G. Baraldi, G. Balboni, B. Cacciari, A. Guiotto, S. Manfredini, R. Romagnoli, G. Spalluto, P. H. Howard, D. E. Thurston, N. Bianchi, C. Rutigliano, C. Mischiati, R. Gambari. *J. Med. Chem.* **42**, 5131 (1999).
10. Y. Damayanthi, B. S. Praveen Reddy, J. W. Lown. *J. Org. Chem.* **64**, 290 (1999).
11. P. G. Baraldi, B. Cacciari, A. Guiotto, R. Romagnoli, G. Spalluto, A. Leoni, N. Bianchi, G. Feriotto, C. Rutigliano, C. Mischiati, R. Gambari. *Nucleosides, Nucleotides* **19** (8), 1219 (2000).
12. Y. Li, G. Mak, B. R. Franza. *J. Biol. Chem*. **269**, 30616 (1994).
13. R. Ray, R. C. Snyder, S. Thomas, C. A. Koller, D. M. Miller. *J. Clin. Invest.* **83**, 2003 (1989).
14. R. C. Snyder, R. Ray, S. Blume, D. M. Miller. *Biochemistry* **30**, 4290 (1991).
15. P. G. Baraldi, G. Balboni, M. G. Pavani, G. Spalluto, M. A. Tabrizi, E. De Clercq, J. Balzarini, T. Bando, H. Sugiyama, R. Romagnoli. *J. Med. Chem.* **44**, 2536 (2001).
16. L. J. Hanka, A. Dietz, S. A. Gerpheide, S. L. Kuentzel, D. G. Martin. *J. Antibiot.* **31**, 1211 (1978).
17. D. G. Martin, C. Biles, S. A. Gerpheide, L. J. Hanka, W. C. Kroeger, J. P. Mc Govren, S. A. Mizsk, G. L. Neil, J. C. Stewart, J. Visser. *J. Antibiot.* **34**, 1119 (1981).
18. B. K. Bhuyan, K. A. Newell, S. L. Crampton, D. D. Von Hoff. *Cancer Res.* **42**, 3532 (1982).
19. V. L. Reynolds, I. J. Molineaux, D. J. Kaplan, D. H. Swenson, L. H. Hurley. *Biochemistry* **24**, 6220–6237 (1985).

20. J. P. Mc Govren, G. L. Clarke, E. A. Pratt, T. F. De Koning. *J. Antibiot.* **37**, 63 (1984).
21. P. G. Baraldi, B. Cacciari, M. J. Pineda de Las Infantas, R. Romagnoli, G. Spalluto, P. Cozzi, N. Mongelli. *Anti-Cancer Drug Des.* **12**, 67 (1997).
22. P. G. Baraldi, B. Cacciari, R. Romagnoli, G. Spalluto, R. Gambari, N. Bianchi, M. Passadore, P. Ambrosino, N. Mongelli, P. Cozzi, C. Geroni. *Anti-Cancer Drug Des.* **12**, 555 (1997).
23. P. G. Baraldi, B. Cacciari, A. Guiotto, R. Romagnoli, G. Spalluto, A. N. Zaid, L. Capolongo, P. Cozzi, C. Geroni, N. Mongelli. *Il Farmaco* **52**, 717 (1997).
24. M. M. Paz, A. Das, M. Tomasz. *Bioorg. Med. Chem.* **7**, 2713 (1999).