ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 55 (2000) 168–173

IL FARMACO

Recent outcome in the field of distamycin-derived minor groove binders

Paolo Cozzi

Department of Chemistry, Discovery Research, Oncology, Pharmacia & Upjohn, Viale Pasteur 10, 20014 Nerviano, Milan, Italy

Abstract

DNA minor groove binders represent a class of cytotoxic antitumor agents whose DNA sequence specificity may lead to a high selectivity of action. Tallimustine, benzoyl nitrogen mustard derivative of distamycin A, showed excellent antitumor activity in the preclinical tests, but as other minor groove binders, showed severe myelotoxicity. Novel nitrogen mustard derivatives of distamycin showing improved activity profile, have been identified recently. Moreover, a series of α-halogenoacrylamido derivatives of distamycin-like frames, in which the typical amidino moiety has been replaced with other moieties, was found to show cytotoxic and antitumor activity and cytotoxicity/myelotoxicity ratio improved significantly in comparison to tallimustine. The structural features of the alkylating moieties and binding frames, of distamycin and distamycin-like derivatives disclosed recently are discussed. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Minor groove binders; Tallimustine; Distamycin; Nitrogen mustards; α-Halogenoacrylamides

1. Introduction

Cancer is a heterogeneous group of diseases, characterized by uncontrolled growth of the malignant cell population. The incidence and prevalence of cancer differ by sex and by geography, but as a whole one in three people develops cancer and the estimated worldwide prevalence is around 10 million. Currently, although a large number of chemotherapeutic agents are available, the medical need is largely unmet and the 5-year survival rate for the average of the more common tumors is about 60%, while it is as low as 13% for lung cancer. This situation arises from the inadequacy of current diagnosis and therapy for cancer, which in most cases address only the latter stages of disease.

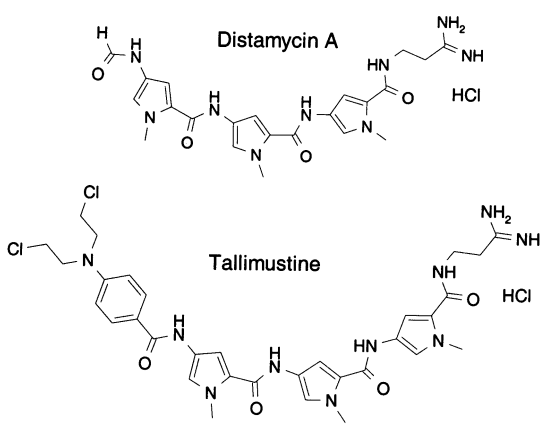


Fig. 1. Distamycin A and its benzoyl nitrogen mustard tallimustine.

A number of innovative technologies are in development, aimed to target the malignant abnormalities of tumor cells. The targets include pathways for oncogenes, tumor suppressor genes, components of the cell cycle, regulation of apoptosis and cell senescence. In alternative, angiogenesis inhibition may prevent the formation of tumor blood vessels, thus limiting the tumor's ability to grow and spread into metastases.

Despite the long-term potential of these innovative agents, it will likely take years to define their role in preventing or delaying disease progression. Therefore cytotoxic agents will continue to represent the chief part of the therapy over the next decade, possibly in combination with novel agents. This implies a need for new cytotoxics, either with greater or broader activity.

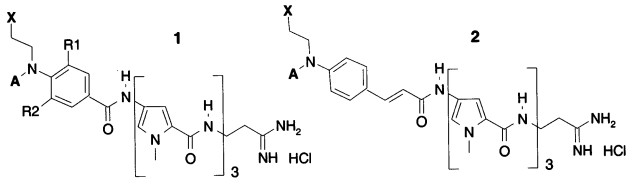
2. Distamycin-derived nitrogen mustards: tallimustine

The putative mode of action of many antitumor agents involves DNA damage, either by direct binding

E-mail address: paolo.cozzi@eu.pnu.com (P. Cozzi)

PII: S0014-827X(00)00013-6

Table 1
Cytotoxic activity (L1210 leukemia) and NBP alkylation constants of distamycin-derived benzoic/cinnamic mustards and half-mustards



Comp. [a]	X	A	R_1	R_2	In vitro IC_{50} (ng ml^{-1})	κ_{alkyl} (s^{-1})
1a [b]	Cl	CH_2CH_2Cl	H	H	50.3 ± 5.9	6.2×10^{-4}
1b	OH	CH_2CH_2OH	H	H	>10000	nd
1c	F	CH_2CH_2F	H	H	>10000	nd
1d	Br	CH_2CH_2Br	H	H	0.6 ± 0.1	6.2×10^{-3}
1e	Cl	CH_2CH_2Cl	F	H	222.5 ± 17.5	5.8×10^{-4}
1f	Cl	CH_2CH_2Cl	CH_3	H	11.1 ± 1.9	1.4×10^{-2}
1g	Cl	CH_2CH_2Cl	CH_3	CH_3	227.6 ± 13.4	9.7×10^{-3}
1h [c]	Cl	CH_2CH_2Cl	H	H	208.0 ± 4.4	5.5×10^{-4}
1j	Cl	CH_2CH_3	H	H	42.0 ± 9.0	5.5×10^{-4}
2a	Cl	CH_2CH_2Cl	–	–	7.2 ± 2.1	1.3×10^{-3}
2b	Cl	CH_2CH_3	–	–	2.9 ± 0.2	nd

[a] All reported compounds are hydrochloride salts except **1d** which is a hydrobromide.
[b] Tallimustine.
[c] *Meta* isomer. IC_{50} = 50% inhibitory concentration as the mean $\pm$SE from dose–response curves of at least two experiments, drug sensitivity was determined after 48 h of continuous exposure against L1210 cells. κ_{alkyl}, alkylation of 4-(4-nitrobenzyl)pyridine (NBP); $T = 66°C$.

of the drug to DNA or to DNA-binding proteins such as topoisomerase enzymes. Most of DNA interacting agents have only a limited degree of sequence specificity, which imply that they may hit all the cellular genes. A new class of cytotoxics, such as the DNA minor groove binders, could provide significant improvement in cancer management due to very high selectivity of interaction with thymine–adenine (TA)-rich sequences [1].

The main representatives of this class are adozelesin, carzelesin, bizelesin, structurally derived from antibiotic CC-1065 [2], and tallimustine (TAM) [3], a benzoic acid nitrogen mustard derivative of distamycin A (DST), (Fig. 1), an antibiotic characterized by an oligopeptidic pyrrolocarbamoyl frame ending with an amidino moiety [4].

Known minor groove binders showed excellent antitumor activity in the preclinical tests and were selected for clinical investigation; however their common limiting factor was myelotoxicity. TAM, for instance, advanced in the clinical development up to phase II, however its clinical evaluation showed a severe myelotoxicity [5], preliminary results of activity were disappointing [6,7] and its development was discontinued.

Nevertheless TAM has represented an important model compound for the design of new minor groove alkylating agents and a number of nitrogen mustard derivatives of DST or DST-like frames were reported by several authors [8]. In fact TAM was the result of a drug design rationale which disclosed the possibility of obtaining cytotoxic and antitumor agents by combining a chemically reactive moiety with a DNA binding-frame, acting as a sequence-selective vector. In the case of TAM, the rationale implied also, the tethering to DST of a very mild alkylating moiety, the benzoic acid nitrogen mustard, with the aim of avoiding as much as possible a specific alkylation of biological nucleophiles [9].

3. Recent distamycin-derived nitrogen mustards

The cytotoxicity of nitrogen mustard derivatives of DST depends on the presence of a reactive mustard moiety, as demonstrated by the inactivity of diol and difluoro-mustard analogs of TAM and by the outstanding activity of dibromo-mustard analog. However the chemical reactivity of the mustard, as determined from the kinetics of alkylation of 4-(4-nitrobenzyl) pyridine (NBP), is not the sole determinant of cytotoxic activity. For instance, *ortho*-fluorophenyl analogue of TAM and TAM show substantially equivalent chemical reactivities but significantly different cytotoxicities. The same occurs for the couple represented by *ortho*-methylphenyl and *ortho*-dimethylphenyl analogues of TAM. The relatively low cytotoxicity of the latter, in spite of its reactivity, suggests that the conformation, which the phenyl ring could assume in the DNA minor groove, due to *ortho*-dimethyl substitution, may play a significant role in the DNA binding. Similarly the re-

duced cytotoxicity of the *meta* isomer of TAM, despite increased chemical reactivity, underlines the role which may be played by the spatial relationship between the nitrogen mustard moiety and the DST frame [9] (Table 1).

Cinnamic acid mustard derivative of DST, a vinylogue of TAM, is characterized by an increased distance between the alkylating moiety and the DNA-binding frame of DST and by an increased chemical reactivity, in accordance with long-range nitrogen–carbonyl conjugation via the vinylic double bond. This compound appears to be significantly more cytotoxic than TAM [9].

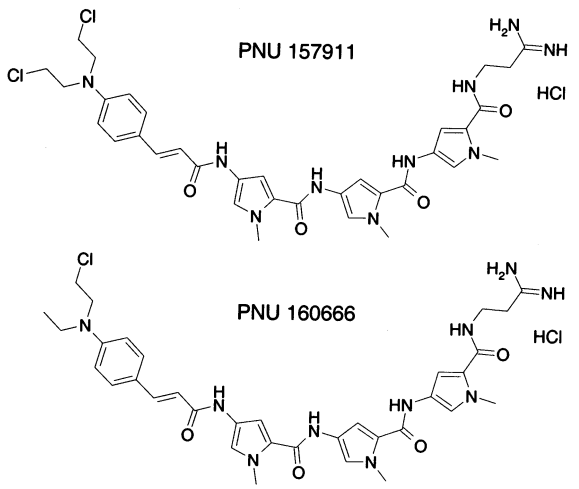
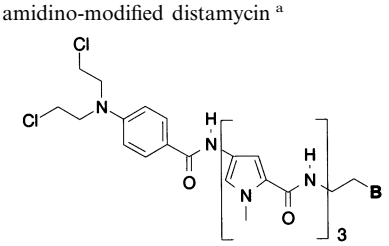


Fig. 2. Cinnamic mustard and half-mustard derivatives of distamycin.

Table 2
Cytotoxic activity (L1210 leukemia) of benzoyl nitrogen mustard derivatives of amidino-modified distamycin [a]



B	In vitro IC_{50} (ng ml^{-1})
$C(NH)NH_2 \cdot HCl$	50.3 ± 5.9
$C(NOH)NH_2$	1476.7 ± 163.1
$C(NNH_2)NH_2$	738.1 ± 36.2
$C(NCN)NH_2$	37.9 ± 5.6
$C(NCH_3)NH_2 \cdot HCl$	38.5 ± 11.3
$C(NCH_3)NHCH_3 \cdot HCl$	36.5 ± 11.3
C-imidazolin-2-yl·HCl	142.5 ± 17.5
C-imidazol-2-yl·HCl	69.0 ± 16.0
$NHC(NH)NH_2 \cdot HCl$	64.4 ± 3.6
$CONH_2$	130.0 ± 33.0
CN	94.0 ± 6.0
COOH	959.7 ± 212.0
CH_2OH	> 2000

[a] IC_{50} = 50% inhibitory concentration as the mean ± SE from dose–response curves of at least two experiments, drug sensitivity was determined after 48 h of continuous exposure against L1210 cells.

Ethyl–chloroethyl half-mustard analogues of TAM and cinnamic congener show cytotoxicities substantially equivalent to, or better than, those of corresponding two-arm mustards [9] (Table 1).

It is known that in the case of the classical nitrogen mustards, the corresponding half-mustards are substantially non cytotoxic [10], possibly due to the impossibility of crosslinking the two DNA strands after twin N(7)–guanine alkylation [11]. The activity of DST half-mustard derivatives underlines once more the mechanistic diversity of these compounds.

Cinnamic mustard (PNU 157911) and half-mustard (PNU 160666) derivatives (Fig. 2), show very good antileukemic activity and appear to be significantly less myelotoxic than TAM against murine and human hematopoietic progenitor cells [12].

Little attention was paid in the past to the possible role of the strongly basic amidino moiety, which is typical of DST and of other DNA minor groove binders with TA-rich sequence selectivity, such as netropsin and diarylamidines. This role, due to the strong basic nature of the amidino group, which implies its total protonation at any physiological pH may concern both the DNA binding and cell or tissue bioavailability.

Novel benzoyl nitrogen mustard derivatives of DST, in which the amidino moiety has been replaced by various amidine-like groups, such cyanoamidine, *N*-methylamidine, *N*,*N*-dimethylamidine, and guanidino moieties, maintain cytotoxicities substantially equivalent to that of TAM. While *N*-methylamidine, *N*,*N*-dimethylamidine and guanidino derivatives are strongly basic, cyanoamidine is not. Moreover, the weakly basic imidazol-2-yl derivative, which only formally can be considered an amidine, shows cytotoxicity better than that of imidazoline, a strongly basic cyclic amidine. Non basic, non amidine-like, carbamoyl and cyano derivatives show lower but still significant cytotoxicities.

On the other hand, the low cytotoxicity of amidoxime and amidrazone analogs indicates that the presence of an amidino-like structure does not guarantee per se a significant activity [13]. (Table 2).

The preservation of the activity with the modification of the amidino moiety in the benzoyl mustard series, is confirmed also in the case of half-mustard and cinnamic mustard derivatives, suggesting a general behavior of DST-like compounds as regards the amidine modifications [13].

It appears therefore that the presence of the amidino moiety or even of any basic moiety is not an absolute requirement for in vitro and in vivo activity of DST mustard derivatives. This contrasts with the established opinion that electrostatic interaction between the cationic moiety and the negatively charged DNA phosphate residues represents one of the main contributions

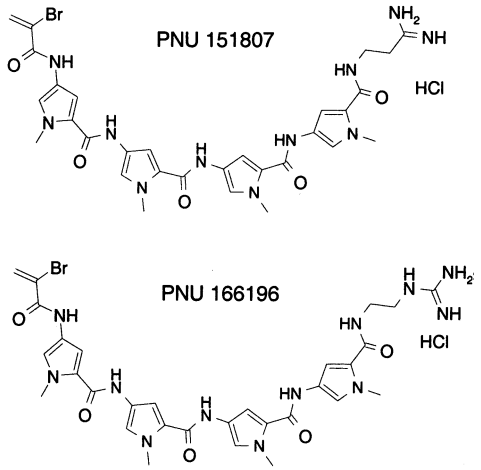


Fig. 3. α-Bromoacrylic derivative of four pyrrolecarbamoyl oligomers.

to molecular recognition of DST and DST-like derivatives [14].

4. Distamycin-derived α-halogenoacrylamides

Recently a new class of cytotoxic minor groove binders showing α-bromo or chloroacrylamide moieties linked to DST or DST-like derivatives has been identified [15]. The first lead of this class, the α-bromoacrylamido derivative of four-pyrrole distamycin homologue (PNU 151807) (Fig. 3), was found endowed with significant cytotoxicity and in vivo activity. Very recently PNU 151807, at variance with TAM and congeners, was found to bind to the DNA minor groove but unable to alkylate minor groove AT-rich sequences [16]. Also α-bromooacrylic derivatives of isosteric analogs of distamycin in which one or more pyrrole rings were replaced by pyrazole or imidazole rings were described [17].

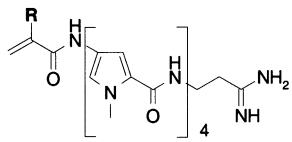
Noteworthy while α-bromo and chloroacrylamido derivatives show a relevant cytotoxicity, α-fluoroacrylamido and acrylamido derivatives appear devoid of significant activity [18] (Table 3).

These data suggest a key role of the reactivity of the α-halogenoacrylic moiety for cytotoxicity, in spite of the fact that PNU 151807 was found unable to alkylate DNA minor groove AT-rich sequences.

We hypothesize that α-halogenoacrylic moiety reactivity, due to the low reactivity of the vinylic halogen, could be based on a first-step Michael-type nucleophilic attack, followed by a further reaction of the no more vinylic halogen leading to a second nucleophilic substitution or to beta elimination.

Moreover we had the experimental evidence, of a dramatically different reactivity toward nucleophilic attack of α-bromoacrylamido and of α-fluoroacrylamido

Table 3
Cytotoxic activity (L1210 leukemia) of α-halogenoacrylic derivatives of distamycin and congeners



R	In vitro [a]	In vivo [b]	
	IC_{50} (ng ml^{-1})	OD (mg kg^{-1})	T/C
Br	4.7 ± 1.0	1.56	200
Cl	2.7 ± 1.0	1.56	133
F	>500	nd	nd
H	>2500	nd	

[a] All reported compounds are hydrochloride salts; IC_{50} = 50% inhibitory concentration as the mean ± SE from dose–response curves of at least two experiments, drug sensitivity was determined after 48 h of continuous exposure against L1210 cells.

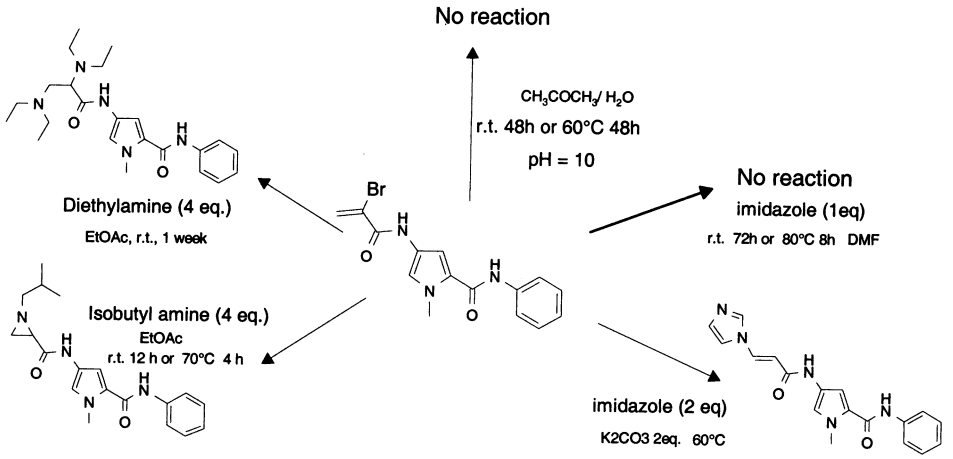
[b] For in vivo studies cells were injected i.v. at day 0 and mice were treated i.v. the day after tumor injection; OD, optimal non-toxic dose < LD10; %T/C, median survival time of treated vs. untreated mice × 100.

moieties. The former reacts with an excess of primary and secondary amines undergoing double nucleophilic substitution, and with the imidazole ring, undergoing nucleophilic substitution and beta-elimination, while the latter gives no reaction in the same or harder conditions (Scheme 1).

The replacement of the amidino moiety of the parent PNU 151807 with basic amidino-derived analogues and with non-basic groups of different nature gives results similar to those obtained in the case of distamycin nitrogen mustard derivatives [19].

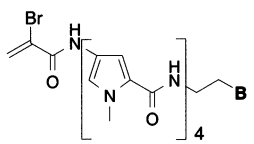
The activity of the parent amidino derivative PNU 151807 is fully maintained not only by basic amidino-derived compounds of different lipophilicity and bulk, such as *N*-methylamidine, *N*,*N*′-dimethylamidine, *N*,*N*-dimethylamidine, 2-imidazoline and by guanidine derivative, but also by non-basic amidino-derived compounds such as amidoxime and cyanoamidine, and even by, carboxyamide derivative. Also, in vivo antileukemic activities appear equivalent to, or better than, those of parent PNU 151807. (Table 4). These data indicate, as in the case of nitrogen mustard derivatives, that neither the amidino moiety nor a basic moiety is an absolute requirement for activity.

Some of these compounds, which appear significantly more cytotoxic than tallimustine and show a favorable myelotoxicity/cytotoxicity ratio, have been selected for further extensive evaluation on murine solid tumors and human xenografts. For instance, PNU 166196 (Fig. 3) shows cytotoxicity on tumor cells resistant to alkylating agents and camptothecin, broad antitumor activity, and myelotoxicity on human hematopoietic progenitor cells radically reduced in comparison to tallimustine.



Scheme 1. Reactivity of α-bromo/fluoroacrylamido moiety.

Table 4
In vitro and in vivo activity (L1210 leukemia) of α-bromoacrylic derivatives of distamycin-like tetrapyrrolecarbamoyl oligomers



B	In vitro [a]	In vivo [b]	
	IC_{50} (ng ml^{-1})	OD	T/C (%)
$C(NH)NH_2 \cdot HCl$	4.70 ± 1.00	1.56	200
$C(NCH_3)NH_2 \cdot HCl$	2.03 ± 0.59	3.13	150
$C(NCH_3)NCH_3 \cdot HCl$	1.44 ± 0.14	1.56	258
$CNHN(CH_3)_2 \cdot HCl$	1.15 ± 0.65	nd	nd
C-imidazolin-2-yl·HCl	1.90 ± 0.40	nd	nd
$NHC(NH)NH_2 \cdot HCl$	1.41 ± 0.13	1.56	196
$C(NOH)NH_2$	6.22 ± 1.12	6.25	186
$C(NCN)NH_2$	3.03 ± 0.20	3.13	157
$CONH_2$	3.53 ± 0.06	6.25	169
CN	14.60 ± 4.50	12.5	157

[a] All reported compounds are hydrochloride salts; IC_{50} = 50% inhibitory concentration as the mean ± SE from dose–response curves of at least two experiments, drug sensitivity was determined after 48 h of continuous exposure against L1210 cells.

[b] For in vivo studies cells were injected i.v. at day 0 and mice were treated i.v. the day after tumor injection; OD, optimal non-toxic dose < LD10; %T/C, median survival time of treated vs. untreated mice × 100.

This compound, as the parent PNU 151807, appears unreactive in DNA alkylation assays [20], and results thirtyfold more active than tallimustine in inducing apoptosis in A2780 human ovarian carcinoma cells [21].

5. Conclusions

In conclusion, these studies led to the identification of a new class of minor groove binders endowed with potent cytotoxic and antitumor activity, and reduced myelotoxicity. Compounds of this class interact with TA-rich sequence of DNA, but at variance with tallimustine or CC-1065-derived agents, appear unreactive in DNA alkylation assays, suggesting a different mechanism of action.

References

[1] See e.g. M. D'Incalci, C. Sessa, Exp. Opin. Invest. Drugs 6 (1997) 875–884.
[2] See e.g. P.A. Aristoff, in: Advances in Medicinal Chemistry, vol. 2, JAI, 1993, pp. 67–110, and references therein.
[3] G. Pezzoni, M. Grandi, G. Biasoli, L. Capolongo, D. Ballinari, F.C. Giuliani, B. Barbieri, A. Pastori, E. Pesenti, N. Mongelli, F. Spreafico, Br. J. Cancer 64 (1991) 1047.
[4] F. Arcamone, S. Penco, P.G. Orezzi, V. Nicolella, A. Pirelli, Nature 203 (1964) 1064.
[5] A. Hageboutros, A. Mooneyham, T. De Maria, D. VonHoff, L. Vitek, P.J. O'Dwyer, G.R. Weiss, Proc. Am. Assoc. Cancer. Res. 35 (1994) 1467.
[6] See e.g. P. Cozzi, N. Mongelli, Curr. Pharm. Des. 4 (1998) 81, and references therein.
[7] C.J.A. Punt, Y. Humblet, E. Roca, L.J. Dirix, R. Wainstein, A. Polli, I. Corradino, Br. J. Cancer 73 (1996) 803.
[8] J. Viallet, D. Stewart, F. Shepherd, J. Ayoub, Y. Cormier, N. Di Pietro, W. Steward, Lung Cancer 15 (1996) 67.
[9] P. Cozzi, I. Beria, G. Biasoli, M. Caldarelli, L. Capolongo, R. D'Alessio, C. Geroni, S. Mazzini, E. Ragg, C. Rossi, N. Mongelli, Biorg. Med. Chem. Lett. 7 (1997) 2985.
[10] M. Brendel, A. Ruffland, Mutat. Res. 133 (1984) 51.
[11] A. Sunters, C.J. Springer, K.D. Bagshave, R.L. Souhami, J.A. Hartley, Biochem. Pharmacol. 44 (1992) 59.
[12] L. Giromini, D. Moneta, M. Tammiso, G. Pennella, C. Geroni, G. Mazué, Tenth NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, Abstract 203, 1998.
[13] P. Cozzi, I. Beria, G. Biasoli, M. Caldarelli, L. Capolongo, C. Geroni, N. Mongelli, Biorg. Med. Chem. Lett. 7 (1997) 2979.
[14] See e.g. J.W. Lown, Chemtracts-Org. Chem. 6 (1993) 205.

[15] R. D'Alessio, C. Geroni, G. Biasoli, E. Pesenti, M. Grandi, N. Mongelli, Bioorg. Med. Chem. Lett. 4 (1994) 1467.
[16] M. Marcaini, M. Ciró, F. Gallinari, C. Geroni, P. Cozzi, M. D'Incalci, M. Broggini, Br. J. Cancer 80 (1999) 991.
[17] P.G. Baraldi, I. Beria, B. Cacciari, L. Capolongo, P. Cozzi, N. Mongelli, R. Romagnoli, G. Spalluto, Bioorg. Med. Chem. Lett. 6 (1996) 1247.
[18] I. Beria, M. Caldarelli, L. Capolongo, P. Cozzi, C. Geroni, N. Mongelli, The Eighth Cyprus Conference on New Methods in Drug Design, Limassol, Abstract 40, 1999.
[19] P. Cozzi, I. Beria, M. Caldarelli, L. Capolongo, C. Geroni, N. Mongelli, Tenth RSC-SCI Medicinal Chemistry Symposium, Cambridge, UK, 1999.
[20] Unpublished results.
[21] M. Ciomei, W. Pastori, P. Cozzi, C. Geroni, Proceedings of AACR, Philadelphia, vol. 40, p. 224, Abstract 1490, 1999.