

NSC 290205-based Therapy in Murine Pancreatic Adenocarcinoma PAN02 in Combination with Adriamycin (ADR)

ATHANASIOS PAPAGEORGIOU¹, CHARALAMBOS ANDREADIS², ANASTASIOS BOUTIS², THEODOROS S. LIALIARIS³ and DESPINA MOURATIDOU²

¹Department of Experimental Chemotherapy, Symeonidion Research Center and

²Third Department of Clinical Oncology, Theagenion Cancer Hospital, 54007 Thessaloniki;

³Department of Genetics, Democritus University of Thrace, 68100 Alexandroupolis, Greece

Abstract. *Background:* NSC 290205 (A) is a hybrid synthetic antitumor ester, which combines a D-lactam derivative of androsterone and nitrogen mustard. In this study, the antitumor activity of A in combination with ADR (AHOP) was investigated in comparison with the standard CHOP regimen. *Materials and Methods:* PAN02 adenocarcinoma was used in this study. C₅₇Bl mice were used for chemotherapy evaluation. The activity was assessed from the inhibition of tumor growth and the oncostatic parameter T/C%. *Results:* Treatment with A or cyclophosphamide produced almost equal borderline activity. Moreover, both the CHOP and AHOP regimens showed significant and comparable antitumor effects. AHOP caused the maximum effect, inhibiting tumor growth by 56.8%. CHOP was less effective, producing 47.7% tumor inhibition. *Conclusion:* It is very likely that the D-lactamic steroid (androstan) alkylator for A, containing the amide group -NH-CO-, combined with ADR which intercalates between DNA base-pairs, is the explanation for the higher activity of AHOP as compared to CHOP.

In order to reduce toxicity and to increase antineoplastic activity, steroid molecules have been utilized as biological vectors for chemotherapeutic agents. Studies on modified homo-aza-steroid esters of the carboxylic derivatives of N,N-bis (2-chloroethyl) aniline have shown that these hybrid compounds combine two molecules in one, exhibit reduced toxicity and increased antineoplastic activity and specificity.

These modified steroid hormones are employed as carriers of alkylating agents to specific targeted tissues. The presence of the characteristic amide group -NH-CO- of the homo-aza-steroid molecule is important in order to lower systemic toxicity and improve activity. As a consequence of this important observation, several lactam steroid alkylators were synthesized (1, 2).

Non-modified esters have been inactive in murine L1210 lymphoid (3) and in P388 lymphocytic leukemia (4). Unlike non-modified esters, homo-aza-steroidal alkylators, with substitution for an easily cleaved ester bond of the A- or D- steroid nucleus, have produced significant results in experimental leukemia models (4-6), as well as in rodent solid tumors including human xenografts (7-9).

The D-lactam steroid alkylator of androsterone, 13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-p-N,N-bis (2-chloroethyl) amino phenyl acetic acid (NSC 290205), has been extensively tested by our research team and by the National Cancer Institute (NCI) in preclinical studies against 56 human cancer cell lines (unpublished data), 11 rodent tumors and 3 human tumor xenografts (9), giving very satisfactory results. In particular, NSC 290205 was highly effective against lymphoid leukemia (4). Further, NSC 290205 produced low acute and favorable subacute toxicity in relation to currently used chemotherapeutic agents (10, 11). Moreover, combinations of NSC 290205 with several chemotherapeutic agents (over 15) were investigated for anticancer activity *in vitro* (unpublished data). The results displayed a significant synergistic antineoplastic effect when NSC 290205 was combined with anthracyclines (Adriamycin, Idarubicin, Daunorubicin). Based on the above results, and in order to validate a liable advantage of NSC 290205 in combination with anthracyclines in the treatment of murine adenocarcinoma PAN02, NSC 290205 was tested alone or in combination with Adriamycin. With the aim of introducing NSC 290205 into further clinical development, we investigated

Correspondence to: Athanasios Papageorgiou, Ph.D., Director, Symeonidion Research Center, Theagenion Cancer Hospital 2, Al. Symeonidis Str., 54007 Thessaloniki, Greece. Tel: +30 2310 898221, Fax: +30 2310 845514, e-mail: gerom@chem.auth.gr

Key Words: CHOP, NSC 290205, pancreatic adenocarcinoma, adriamycin, mice.

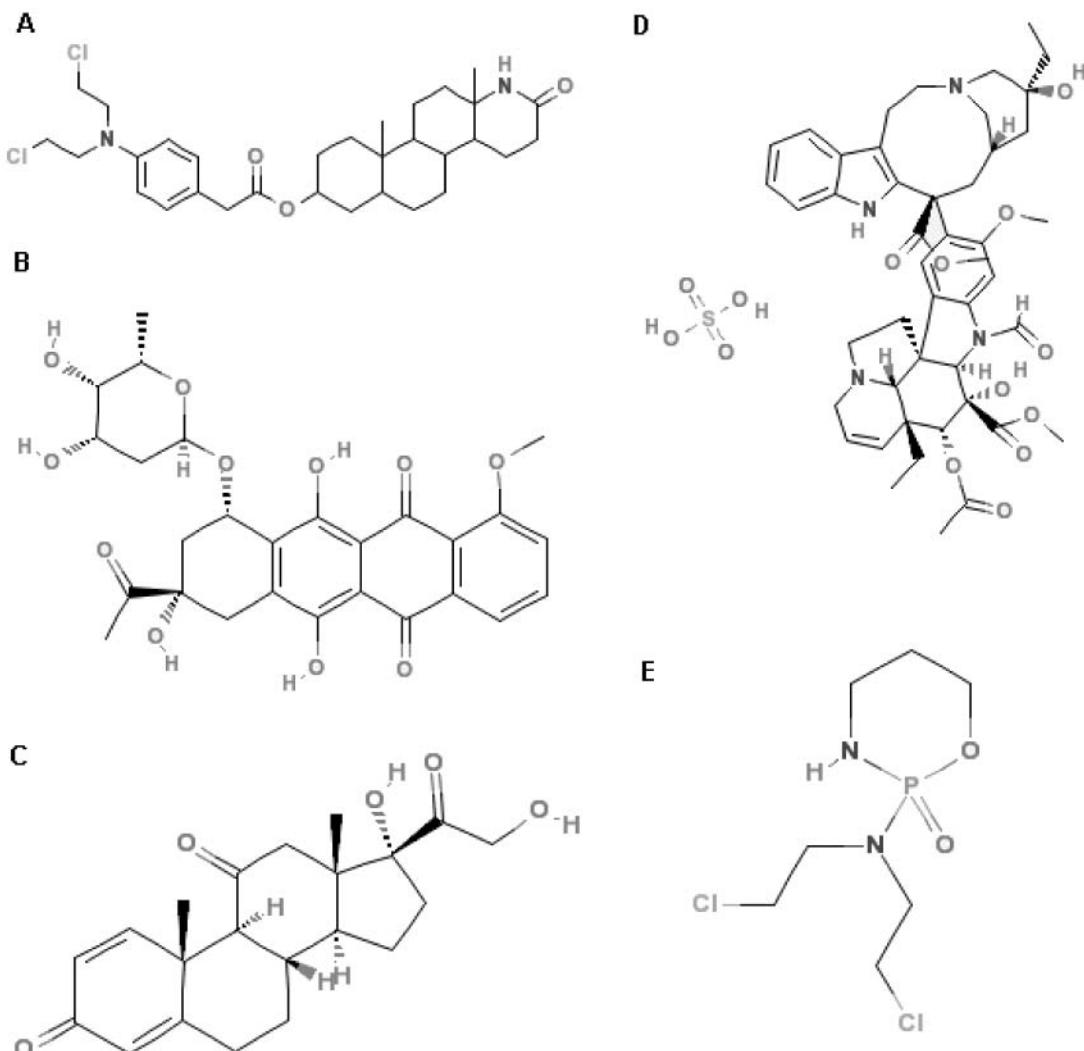


Figure 1. Chemical structures of NSC 290205 (A), Hydroxydaunorubicin (B), Prednisone (C), Vincristine (D) and Cyclophosphamide (E).

its *in vivo* activity against adenocarcinoma PAN02 in combination with Hydroxydaunorubicin (Adriamycin), Vincristine, Prednisone and in combination with the standard CHOP (Cyclophosphamide, Hydroxydaunorubicin, Vincristine, Prednisone) regimen.

Materials and Methods

Chemicals. NSC 290205 was synthesized as previously described (12) (Figure 1A). A stock solution of the test compound was made immediately before use. The compound was initially dissolved in a small amount of dimethyl sulphoxide (10% DMSO). Suspension in corn oil at the desired concentration followed prior to the intraperitoneal (*i.p.*) administration.

Cyclophosphamide, Hydroxydaunorubicin, Vincristine and Prednisone were obtained from the commercially available generic drugs (Baxter, Germany, Daunoblastina® (Gilead, USA), Oncovin® (PCH Pharmacheme, Holland), Prezolon® (Nycomed,

Linz-Austria) respectively). The chemical structures of the compounds are shown in Figure 1 B-E.

Animals. Male and female C₅₇Bl mice, 4-6 weeks of age and weighing 12-28 g, were used for antitumor evaluation. The mice were provided by the Experimental Animal Production Laboratory and were under conditions of constant temperature and humidity, with sterile bedding, water and food.

Tumor. The pancreatic ductal adenocarcinoma PAN-02 was used in this study. The tumor was purchased from the DCTD, NCI, Frederick, MD, USA. C₅₇Bl mice of both sexes were used for chemotherapeutic evaluation. The tumor was implanted in the axillary region with puncture in the inguinal region. The sacrificed donor animal was pinned to a dissecting board, dorsal surface up. The tumor was transferred to a sterile Petri dish placed over ice and cleared of any necrotic material. The tumor was cut into cubes, usually about 2x2x2 mm. With forceps, the tumor fragments were placed into the bevel end of the 13-gauge trocar. The area of the

Table I. In vivo antitumor activity of NSC 290205 and Cyclophosphamide against adenocarcinoma PAN 02.

Compound	Treatment schedule	MST*±SD (days)	T/C** (%)	Cures
Controls	Saline	33.7±4.24	100.0	0/6
	Day 1 (D)	43.0±4.85	127.6	0/6
Cyclo phosphamide	Days 1, 5, 9 (D/2 X 3)	51.8±4.28	153.7	0/6
	Days 1-9 (D/4 X 9)	46.9±3.74	139.0	0/6
NSC 290205	Day 1 (D)	39.4±5.45	116.9	0/6
	Days 1, 5, 9 (D/2 X 3)	49.1±5.30	145.6	0/6
	Days 1-9 (D/4 X 9)	43.4±5.8	128.8	0/6

*MST: mean survival time

**T/C: tumor-bearing mice/controls

implant was swabbed with 70% ethanol and the tumor fragment was inoculated subcutaneously. The animals were allowed to rest for 24 hours and subsequently divided into the indicated number of groups, which received *i.p.* treatment.

Antitumor evaluation. Standard propagation methods were used for testing the cytostatic effect of the compounds. The antitumor activity against PAN-02 was assessed from the inhibition of tumor growth by volume in cubic centimeters (cm^3), according to the protocol of the experimental evaluation of antitumor drugs of the NCI. The tumor size was measured on days 22, 25, 28 with a microvernier and calculated from 3-dimensional measurements using the formula $a \times b \times c / 2 \text{ cm}^3$, where a =length, b =width, c =depth at the site of transplantation (Average Tumor Volume, ATV). The oncostatic parameter T/C%, the mean of median survival time of drug-treated animals (T) versus saline-treated controls (C), was also used.

Treatment. Experimental groups of six mice in each drug-treated group and eight mice in the control group were used. The experiments were initiated by implanting the mice with the tumor cells. Treatments were given as a single dose (LD_{10}) on day 1 after transplantation; intermittent doses ($\text{LD}_{10/2} \times 3$) were given on days 1, 4, 7 or on days 1-9 ($\text{LD}_{10/4} \times 9$).

Results

Therapy with single drugs. The results of the single agent antitumor activity in a number of treatment schedules and doses are shown in Table I and Figure 2 (A, B). Treatment with NSC 290205 or Cyclophosphamide produced almost equal antitumor effects, inhibiting the tumor growth by 21.1% and producing T/C values of 145.6% (NSC 290205) and inhibition of tumor growth by 23.4% with T/C values of 153.7% (cyclophosphamide).

Therapy with drug combination. The results of the combination therapy in a number of treatment schedules and doses are shown in Table II and Figure 2 (C, D). It can

Table II. In vivo antitumor activity of CHOP and AHOP against adenocarcinoma PAN 02.

Compound	Treatment schedule	MST*±SD (days)	T/C** (%)	Cures
CHOP	Day 1 (D)	52.0±4.69	143.1	0/6
	Days 1, 5, 9 (D/2 X 3)	60.1±3.86	165.5	0/6
	Days 1-9 (D/4 X 9)	50.0±3.57	139.8	0/6
AHOP	Day 1 (D)	64.5±3.50	177.7	0/6
	Days 1, 5, 9 (D/2 X 3)	78.5±2.40	216.6	0/6
	Days 1-9 (D/4 X 9)	55.0±4.50	151.5	0/6

*MST: mean survival time

**T/C: tumor-bearing mice/controls

be seen that both the CHOP and AHOP regimens showed significant and comparable antitumor effects ($p < 0.05$ by the Wilcoxon test). All the treatment schedules caused inhibition of the tumor growth and increased the lifespan of the tumor-bearing mice. AHOP caused the maximum effect, inhibiting the tumor growth by 56.8% and producing T/C values of 216%, when the intermittent treatment schedule was used. CHOP was less effective, inhibiting the tumor growth by 47.7% and producing T/C values of 165%.

Discussion

In this study, cyclophosphamide in the standard CHOP chemotherapeutic regimen was replaced with NSC 290205 (AHOP) and the efficacy of these two regimens against ductal adenocarcinoma PAN02, transplanted into C_{57}Bl mice, was compared. Although the treatment of PAN02-bearing mice with cyclophosphamide or NSC 290205 yielded equivalent results, AHOP showed higher potency against PAN02. Cytogenetic experiments showed that AHOP was a higher inducer of genotoxicity (SCEs) and a higher depressor of cell proliferation (PRI) than CHOP. Previous experiments revealed that NSC 290205, as well as Cyclophosphamide are high SCE inducers and substantial chemotherapeutic agents (13-16). These findings coincide with the survival experiments, further confirming the activity of NSC 290205 and Adriamycin.

CHOP is a first-generation regimen and represents the "gold standard" chemotherapy for non-Hodgkin lymphomas. The lack of superiority of new generation chemotherapy schemes necessitated the comparison of newer therapies with CHOP (17, 18). CHOP is the combination of three well-established and widely-used chemotherapeutic agents and Prednisone; cyclophosphamide is a typical alkylating nitrogen mustard that alkylates DNA and inhibits its

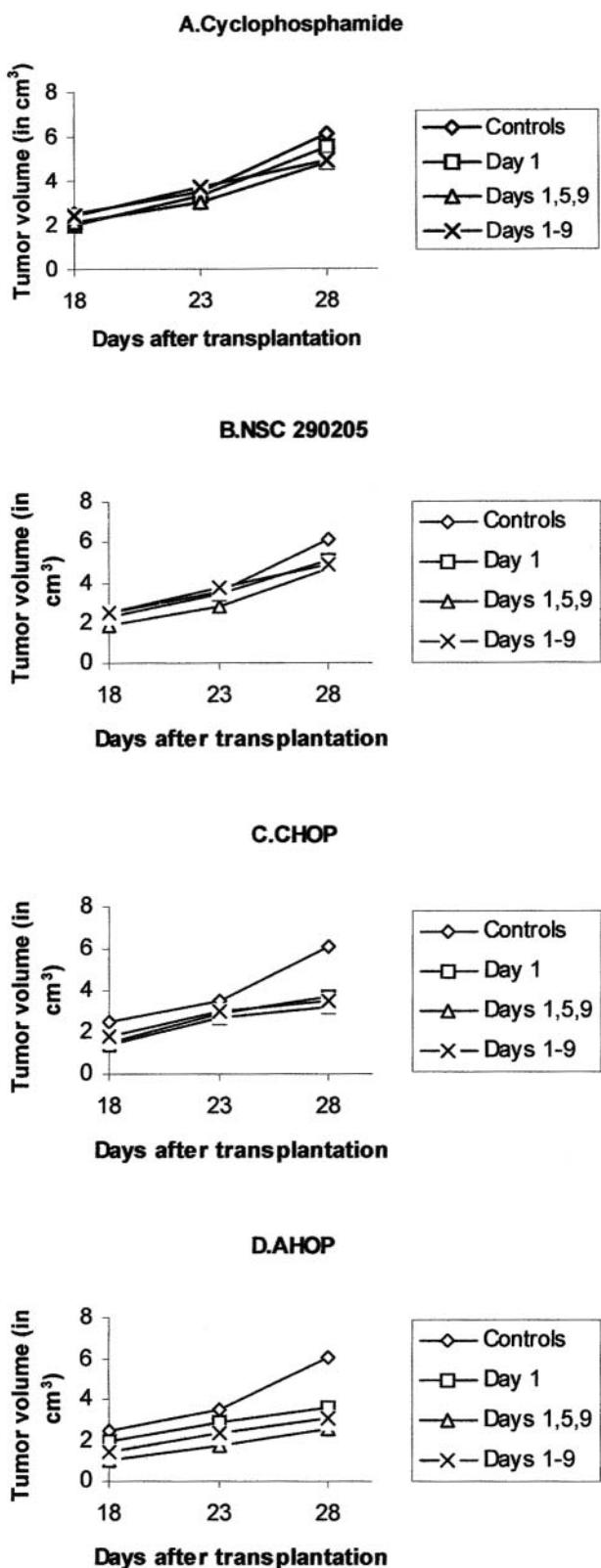


Figure 2. Antitumor effect of Cyclophosphamide (A), NSC 290205 (B), CHOP (C) and AHOP (D) in pancreatic adenocarcinoma PAN02. Growth curves of single and multiple treatment schedules.

replication (19), while Adriamycin (Hydroxydaunorubicin) is an antitumor antibiotic that intercalates between DNA base-pairs and suppresses DNA synthesis and repair (20).

The D-lactamic steroid (androstan) alkylator NSC 290205 combined with Adriamycin (ADR) produced significantly increased antitumor activity. NSC 290205 contains a similar alkylating moiety to cyclophosphamide, is the *N,N*-bis-2-chloroethyl agent. The presence of the characteristic group -NHCO- of the homo-aza-steroid molecule was proven to be important, in order to lower toxicity and improve antitumor activity (2, 8). Possibly the antineoplastic effects of these steroid esters may be due to the multiple interactions of the -NHCO- lactam group with similar groups or with structural specific domains which exist in DNA and proteins. It was suggested that the -NHCO- lactam group is transformed by a metabolic process, or at least by an enzymatically-catalyzed reaction, to active species which strongly interact with similar groups existing in the DNA and proteins (-NHCO- → -NH- + -C=O⁺). Furthermore, modifications of the -NHCO- lactam group by NH methylation (-NCH₃CO-) or by -CO-reduction led to derivatives with lower anticancer activity than that of the parent compounds (21, 22).

Later studies indicated that the lactam ring of the aza-steroids can react as an antagonist or agonist by binding to certain cellular enzymes in a similar way to the indo-benzene or other steroid lactams, which effect on protein kinase C (PKC), enzymes with a relative specificity (23, 24). The exact mechanism of the action of the homo-aza-steroid alkylators is still unknown. The alkylating component of these esters acts via the same biochemical pathway as other bifunctional alkylating mustards (25). It is believed that these compounds can generate high intracellular concentrations due to the lipophilic nature of the steroid carrier. It has been reported, for other steroid alkylators, that a rate-limiting hydrolysis of the ester bond liberates the two active moieties (one steroid and one alkylating) into the cellular microenvironment (26). Comparative studies on the antineoplastic activity of homo-aza-steroid esters against experimental systems with alkylating agents used in current chemotherapy, such as Melphalan, Chlorambucil, Cyclophosphamide, Mechlorethamine, Thiotepa and Mitomycin C, showed that the tested homo-aza-steroid esters hold a superior or, at least, an equal anticancer activity (5, 14). It has been previously reported that the stereo-isomeric form and the chemical structure of the steroid vectors and the alkylating components of the esters determine the antitumor effect of these compounds (27, 28). Steroid esters similar to NSC 290205, that contain the alkylating moiety *p-N,N*-bis(2-chloroethyl) aminophenyl acetic acid and/or bear the nitrogen-containing derivative of 3-hydroxy-5-androsten as a steroid vector, appeared significantly more active.

Preclinical research supports that the aza-steroidal alkylator NSC 290205 demonstrates favorable acute and subacute

toxicity, as well as superior antitumor activity. The significant antineoplastic effect of NSC 290205 combined with Adriamycin against murine ductal adenocarcinoma PAN02 justifies further clinical studies.

References

- 1 Catsoulacos P and Catsoulacos D: Antitumor activity of homo-aza-steroidal esters of *p*-*N,N*-bis(2-chloroethyl)aminophenoxyacetic acid. *Anticancer Res* 13(4): 1203-1208, 1993.
- 2 Catsoulacos P and Catsoulacos D: Conjugated system of homo-aza-steroidal esters in cancer chemotherapy. *Anticancer Res* 14(6B): 2525-2528, 1994.
- 3 Wall ME, Abernethy GS Jr, Carroll FI and Taylor DJ: The effects of some steroidalkylating agents on experimental animal mammary tumor and leukemia systems. *J Med Chem* 12(5): 810-818, 1969.
- 4 Wampler GL and Catsoulacos P: Antileukemic effect of homo-aza-steroidal ester of [*p*-[bis(2-chloroethyl)amino]phenyl]acetic acid. *Cancer Treat Rep* 61(1): 37-41, 1977.
- 5 Catsoulacos P, Politis D and Wampler GL: A new steroidalkylating agent with improved activity in advanced murine leukemias. *Cancer Chemother Pharmacol* 3(1): 67-70, 1979.
- 6 Catsoulacos P, Camoutsis C and Wampler GL: Effect of a delta 5-homo-aza-steroidal ester in P388 and L1210 murine leukemias. *Oncology* 39(1): 59-60, 1982.
- 7 Catsoulacos P and Wampler GL: Activity of 3 beta-hydroxy-13 alpha-amino-13,17-seco-5-alpha-androstan-17-oic-13,17-lactam(*p*-[bis(2-chloroethyl) amino]-phenyl) acetate (NSC 290205) in murine solid tumors. *Oncology* 39(2): 109-112, 1982.
- 8 Catsoulacos P: Activity of 3 beta-hydroxy-13 alpha-amino-13,17-seco-5 alpha-androstan-17-oic-13,17-lactam-*p*-bis (2 chloroethyl) aminophenoxy-acetate (NSC 294859) on experimental animal tumor and leukemia systems. *Oncology* 40(4): 290-292, 1983.
- 9 Catsoulacos P: Further studies on the anti-neoplastic activity of 3 beta-hydroxy-13 alpha-amino-13,17-seco-5 alpha-androstan-17-oic-13,17-lactam [*p*-[bis(2-chloroethyl)amino]-phenyl]acetate (NSC 290205). *Cancer Lett* 22(2): 199-202, 1984.
- 10 Pispirigos K, Catsoulacos P and Karakiulakis G: Evaluation of kidney and liver subacute toxicity of antitumor agents using serum biochemical parameters in rats. *Biochem Mol Biol Int* 31(3): 565-573, 1993.
- 11 Pispirigos K, Paradelis AG and Karakiulakis G: Evaluation of cardiac subacute toxicity of epirubicin, chlorambucil, cisplatin, methotrexate and a homo-aza-steroid ester with antitumor activity in rats using serum biochemical parameters. *Arzneimittelforschung* 47(1): 92-96, 1997.
- 12 Catsoulacos P and Boutis L: Aza-steroids. Beckman rearrangement of 3β-acetoxy-androstan-17-one oxime acetate with boron fluoride. Alkylating agents. *Chim Ther* 8: 215-217, 1973.
- 13 Athanasiou K, Demopoulos NA and Catsoulacos P: Chromosome damage and SCE induced by the cytostatic factor homo-aza steroid ester of *P*-bis (2-chloro-ethyl) amino phenyl acetic acid in CHO cells in culture. *Environ Mutagen* 5(3): 279-283, 1983.
- 14 Mourelatos D, Petrou C, Boutis L, Papageorgiou A, Catsoulacos P and Dozi-Vassiliades J: Induction of cytogenetic damage by modified steroidalkylating derivatives of *p*-bis(2-chloroethyl)aminophenoxyacetic acid in human lymphocytes. *Mutat Res* 190(3): 205-210, 1987.
- 15 Catsoulacos P, Papageorgiou A, Margaritis E, Mourelatos D and Mioglou E: Comparison of current alkylating agents with a homo-aza-steroidal ester for antineoplastic activity. *Oncology* 51(1): 74-78, 1994.
- 16 Nikolaropoulos SS, Arsenou ES, Papageorgiou A and Mourelatos D: Antitumor and cytogenetic effects of esteric (ASE) and amidic (ASA) steroid derivative of *p*-bis (2-chloroethyl) amino phenylacetic acid (CAPA). A comparative study. *Anticancer Res* 17(6D): 4525-4529, 1997.
- 17 Fisher RI, Gaynor ER, Dahlberg S, Oken MM, Grogan TM, Mize EM, Glick JH, Coltman CA Jr and Miller TP: Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. *N Engl J Med* 328(14): 1002-1006, 1993.
- 18 Linch DC, Vaughan Hudson B, Hancock BW, Hoskin PJ, Cunningham DC, Newland AC, Milligan DW, Stevenson PA, Wood JK, MacLennan KA, Anderson L, Gregory WM and Vaughan Hudson G: A randomised comparison of a third-generation regimen (PACEBOM) with a standard regimen (CHOP) in patients with histologically aggressive non-Hodgkin's lymphoma: a British National Lymphoma Investigation report. *Br J Cancer* 74(2): 318-322, 1996.
- 19 Cox PJ, Farmer PB and Jarman M: Proceedings of the symposium on the metabolism and mechanism of action cyclophosphamide. *Cancer Treat Rep* 60: 229-525, 1976.
- 20 Bachur NR, Benjamin RS, Hall TC: Proceedings of the fifth new drug seminar on adriamycin. *Cancer Chemother Rep* 6: 83-419, 1975.
- 21 Dalmases P, Gomez-Belinchon JI, Bonet J-J, Giner-Sorolla A and Schmid FA: Antineoplastic agents. II. A nitrogen mustard derivative of N-methylated steroid lactam. *Eur J Med Chem* 18: 541-543, 1983.
- 22 Dalmases P, Cervantes G, Quintana J and Bonet J-J: Antineoplastic agents. V. Nitrogen mustards of systematically modified steroid ring A lactams. *Eur J Med Chem* 19: 465-467, 1984.
- 23 Ma D, Wang G, Wang S, Kozikowski AP, Lewin NE and Blumberg PM: Synthesis and protein kinase C binding activity of benzolactam-V7. *Bioorg Med Chem Lett* 9(10): 1371-1374, 1999.
- 24 Endo Y and Yokoyama A: Role of the hydrophobic moiety of tumor promoters. Synthesis and activity of 2-alkylated benzolactams. *Bioorg Med Chem Lett* 10(1): 63-66, 2000.
- 25 Papageorgiou A, Ivanov IG, Markov GG, Kolaias SI, Boutis L and Catsoulacos P: Interaction of homo-aza-steroidal ester of [*p*-[bis(2-chloroethyl) amino]phenyl]acetic acid (ASE) with DNA of Ehrlich ascites tumor cells. *FEBS Lett* 153(1): 194-198, 1983.
- 26 Shepherd RE, Huff K and McGuire WL: Estrogen receptor interaction with the antitumor agent estradiol mustard. *J Natl Cancer Inst* 53(3): 895-897, 1974.
- 27 Catsoulacos P and Catsoulacos D: Hybrid anticancer compounds. Steroidal lactam esters of carboxylic derivatives of *N,N*-bis (2-chloroethyl) aniline (review). *Anticancer Res* 11(5): 1773-1777, 1991.
- 28 Camoutsis C and Trafalis DT: An overview on the antileukemic potential of D-homo-aza- and respective 17beta-acetamido-steroidal alkylating esters. *Invest New Drugs* 21(1): 47-54, 2003.

Received August 10, 2005

Accepted December 1, 2005