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Antitumor Activity of Homo-aza-Steroidal Esters of [*p*-[Bis(2-chloroethyl)amino]phenyl]acetic Acid and [*p*-[Bis(2-chloroethyl)amino]phenyl]butyric Acid

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Summary. *Three new modified steroidal alkylating agents, 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-p-bis(2-chloroethyl)aminophenylacetate, 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-p-bis-(2-chloroethyl)aminophenylbutyrate, and 17β-hydroxy-3-aza-A-homo-4α-androsten-4-one-p-N,N-bis(2-chloroethyl)-aminophenylacetate are active in treatment of L1210 and P388 leukemias. A stereoisomer of the first compound, 3α-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13, 17-lactam-p-bis(2-chloroethyl)aminophenylacetate, was tested in L1210 leukemia. This stereoisomer, in which the alkylating agent is linked to the modified steroid in the axial position, is active only at much higher doses in L1210 leukemia. The results of testing these compounds and previous results from similar compounds allow certain conclusions to be drawn regarding structure-activity relationships. The presence of the lactam moiety is the major structural feature that confers activity in the murine leukemias. The steric arrangement of the alkylating moiety at position 3 and the hydrogen atom at position 5 influence toxicity and antileukemic activity.*

Introduction

The results of our previous work on modified steroid-containing alkylating agents, e.g., the carboxylic derivative of bis-(2-chloroethyl) aniline [1, 3–5] and 3β-hydroxy-13α-amino-13, 17-seco-5α-androstan-17-oic-13, 17-lactam-*p*-bis (2-chloroethyl)aminophenylacetate (ASE) [7], prompted us to study compounds with similar structures.

The stereoisomer lactams, 3β-hydroxy-13α-amino-13, 17-seco-5β-androstan-17-oic-13,17-lactam; 3α-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam; and 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam and the testosterone lactam have been used as biologically acceptable platforms for transporting the alkylating agent to the target tissue in a rather specific manner.

We synthesized the homo-aza-steroidal esters I, II, III, and V by the action of acid chloride hydrochloride on the corresponding lactams [2] and tested them against the P388 and L1210 leukemias. The formulas of the prepared compounds are shown in Fig. 1.

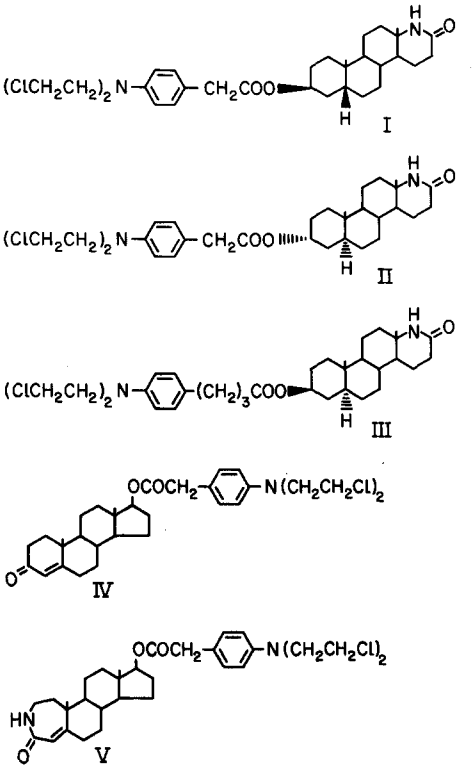

Fig. 1. Formulas of the prepared compounds: *I* 3β-hydroxy-13α-amino-13,17-seco-5β-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)-aminophenylacetate; *II* 3α-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)aminophenylacetate; *III* 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam-*p*-bis(2-chloroethyl)aminophenylbutyrate; *IV* 17-hydroxy-4-androsten-3-one-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate; *V* 17β-hydroxy-3-aza-*A*-homo-4α-androsten-4-one-*p*-*N*,*N*-bis(2-chloroethyl)-aminophenylacetate

The structures of the reported compounds are confirmed by elemental analysis, nuclear magnetic resonance, infrared and mass spectra.

Because of our success in preparing D-ring-modified steroids having antileukemic activty, it was decided also to prepare steroids in which the A-ring was modified to a seven-member-ring lactam. Compound V (Fig. 1) is the first such compound, which is a congener of 17-hydroxy-4-androsten-3-one-*p*-*N*,*N*-bis(2-chloroethyl)aminophenylacetate (IV). Compound IV was studied by Wall et al. [6], who found it was

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inactive in L1210 leukemia according to the criteria of the National Cancer Institute.

Materials and Methods

Compounds used in this study were prepared according to previously reported procedures [2]. A summary of the physical properties is presented in Table 1.

The esters were dissolved in ethanol in a concentration of 10 mg/ml. A small amount of Tween 80 was added (equal to 0.1% of the final suspension), and then saline was added to give a final concentration of drug of 1, 2, or 3 mg/ml. This produced a milky suspension which was freshly made for each separate experiment from the stock chemicals.

Compound in doses of 1 ml/injection or less was administered IP in several schedules.

L1210 leukemia was maintained in our laboratory by weekly IP passage of 10^5 L1210 cells in DBA/2 mice. P388 leukemia was maintained by weekly IP passage of 10^6 P388 cells into DBA/2 mice. C57BL/6 × DBA/2 ($B6D2F_1$) or BALB/c × DBA/2 ($C3D2F_1$) female mice, average weight 20–22 g each, were used as test animals. L1210 (10^5) or P388 (10^6) cells were inoculated IP into each test animal and the life-span was recorded. For all experiments, mice (uniform as to sex and age) were kept in groups of eight in an air-conditioned, light-controlled environment.

Results

Compounds I, III, and V were active against both L1210 and P388 leukemia. Compound II, active in treatment of L1210 leukemia at five times the optimum dose of the stereoisomer (compound I), was not tested in P388 leukemia. Tables 2 and 3 list the results of treatment of ascitic L1210 leukemia. Compound I reliably produced T/C values > 150% over a 0.35–0.5 log range of doses. Compound V gave the best individual T/C values in L1210 leukemia, 213% in $B6D2F_1$ mice and 207% in $C3D2F_1$ mice. Compounds II and III gave maximum T/C values of 179% and 175%, respectively.

In P388 leukemia compound I gave 667% T/C and compound V gave 528% T/C. Both optimum results were obtained in a days 1, 5, and 9 schedule of treatment. Compound III gave 333% T/C on a daily × 8 schedule of treatment (Tables 4 and 5).

Optimal doses for compounds I, III, and V were similar, ranging from 22.5 to 33.8 mg/kg in L1210 leukemia when used on a daily × 8 treatment schedule. Optimal doses in P388 leukemia tended to be slightly lower. In the intermittent treatment schedule higher doses were optimal but lower total cumulative doses were used.

Weight loss ranged from 14% to 32% at optimal doses if T/C values were > 150% and weights were taken at the end of the courses of treatment.

Discussion

Compounds I and II are stereoisomers of a third compound that was previously reported to be active in L1210 and P388 leukemias [7]. These compounds represent three of four possible stereoisomeric permutations at the three and five positions. The major difference in these stereoisomers is a difference in potency, with compound I equally active with ASE, the original stereoisomer, at twice the dose and compound II optimally active at about ten times and five times the optimal doses of ASE and compound I respectively. Compound II is also less active than compound I according to the range of doses of compound I that will give T/C values ≥ 150% and the higher maximum T/C values observed.

The activity of compound III, which contains a chlorambucil moiety, was consistently slightly less than that of compounds I and V. We had expected that the results might be superior in view of the activity of the anticipated hydrolytic product. The steric arrangement at the three and five positions is the same as for the originally reported active congener ASE [7].

Compound V is the first A-ring-modified steroid tested. Results were favorable with T/C values greater than 200% in L1210 leukemia and up to 528% in P388 leukemia with long-term survivors in two separate experiments using the intermittent treatment schedule.

We originally reported that the daily and intermittent treatment schedules were essentially equally effective for ASE

Table 1. Physical properties of the homo-aza-steroidal esters

Compound	Yield (%)	Mpt	Recrystal. solvent	Analysis[a]	Infrared (cm^{-1})	Molecular weight (mass spectrometry)
I	66	165	Ethylacetate-*n*-hexane	C, N, H	3,200, 3,060 (NH) 1,720 (COO-) 1,650, 1,610 (NHCO) 800, 740 (aromatic ring)	563
II	76	158	Ethylacetate-*n*-hexane	C, H, N	3,170, 3,040 (NH) 1,724 (COO-) 1,660, 1,615 (NHCO) 800, 730 (aromatic ring)	563
III	62	121	Ethylacetate-*n*-hexane	C, H, N	3,145, 3,030 (NH) 1,715 (COO-) 1,670 (NHCO) 800, 700 (aromatic ring)	591
V	50	163–165	Ethylacetate-*n*-hexane	C, H, N	3,300, 3,200 (NH) 1,720 (COO-) 1,650 (NHCO) 800, 750 (aromatic ring)	560

[a] Elemental analysis for elements listed was within ± 0.4% of the expected value

Table 2. Activity in L1210 leukemia

Compound	Dose	Schedule	Median survival	T/C (%)[b]	AWC (%)[c] D 8
I	10	QD × 8	10.5	131	+ 4.2
I	15	QD × 8	12.0	150	− 7.8
I	22.5	QD × 8	14.0	175	−14.8
I	25	QD × 7	12.5	156	−
I	33.8	QD × 7	13.0	163	−
I	25	D 1 and 4	11.5	144	−
I	50	D 1 and 4	15.5	194	−
I	75	D 1 only	2.0	23	−
II	10	QD × 8	8.0	100	−
II	15	QD × 8	8.0	100	−
II	22.5	QD × 8	8.0	100	−
III	20	QD × 8	12.0	150	− 9.6
III	30	QD × 8	14.0	175	−17.8
III	45	QD × 8	3.0	38	−
III	25	QD × 7	11.5	144	−
III[a]	25	D 1 and 4	10.0	125	−
III	50	D 1 only	6.0	75	−
III	75	D 1 only	2.0	25	−
V	20	QD × 8	14.5	181	−13.8
V	30	QD × 8	11.5	144	−23.0
V	45	QD × 8	7.0	88	−
V	25	QD × 7	13.5	169	−
V	25	D 1 and 4	11.0	138	−
V	50	D 1 and 4	17.0	213	−
V	50	D 1 only	14.0	175	−
V	75	D 1 only	2.0	25	−

Each group consisted of eight $B6D2F_1$ female mice except where indicated otherwise. Control life-span was 8 days

[a] Seven animals

[b] Ratio of test evaluation (median survival) to control evaluation, expressed as a percentage

[c] Average weight change of tumored animals on D 8 compared with an initial weight

Table 3. Activity in L1210 leukemia

Compound	Dose	Schedule	Median survival	T/C (%)[a]	AWC (%)[b]
I	10	QD × 8	12.0	150	− 1.2
I	15	QD × 8	12.5	156	−14.7
I	22.5	QD × 8	16.0	200	−19.9
I	33.8	QD × 8	14.0	175	−33.3
I	17.6	QD × 8	12.5	179	−
I	26.4	QD × 8	13.0	186	−30.2
I	39.6	QD × 8	9.0	129	−33.9
II	25	QD × 8	10.0	125	+ 9.7
II	50	QD × 8	10.5	131	+ 9.8
II	75	QD × 8	11.0	138	−
II	53	QD × 8	9.5	136	+10.7
II	79	QD × 8	11.0	157	− 7.3
II	119	QD × 8	12.5	179	−29.3
II	178	QD × 8	3.5	50	−
III	17.6	QD × 8	8.5	121	+ 6.6
III	26.4	QD × 8	8.5	121	+ 9.9
III	39.6	QD × 8	8.0	114	−
V	17.6	QD × 8	12.0	171	−22.3
V	26.4	QD × 8	14.5	207	−32.1
V	39.6	QD × 8	12.0	171	−36.3

Each group consisted of eight $C3D2F_1$ female mice. Control life-span was 7–8 days

[a] Ratio of test evaluation (median survival) to control evaluation, expressed as a percentage

[b] Average weight change of tumored animals on D 8 compared with an initial weight

Table 4. Activity in P388 leukemia

Compound	Dose	Schedule	60-day survivors	Median survival	T/C (%)[a]	AWC (%)[b]
I	9.4	QD × 8	0	26	289	−13.5
I	14.1	QD × 8	0	31.5	350	−13.9
I	21.1	QD × 8	0	30.0	333	−17.6
I	28	D 1, 5, and 9	1	35.5	394	− 1.9
I	42	D 1, 5, and 9	5	60 +	661 +	− 4.7
I	63	D 1, 5, and 9	0	31.0	344	− 7.0
I	28	D 7, 11, and 15	0	9.0	100	+12.9
I	42	D 7, 11, and 15	0	12.5	139	− 8.5
I	63	D 7, 11, and 15	0	16.5	183	− 1.8
III	14.1	QD × 8	0	21.0	233	−17.0
III	21.1	QD × 8	0	21.0	333	−20.0
III	31.7	QD × 8	0	21.5	239	−24.0
III	28	D 1, 5, and 9	0	14.0	156	+ 5.1
III	42	D 1, 5, and 9	0	17.5	194	+ 2.2
III	63	D 1, 5, and 9	0	21.5	239	− 1.8
III	28	D 7, 11, and 15	0	9.0	100	+ 0.6
III	42	D 7, 11, and 15	0	15.0	167	+ 0.4
III	63	D 7, 11, and 15	0	11.5	128	+ 7.1
V	9.4	QD × 8	0	23.0	256	−10.6
V	14.1	QD × 8	0	27.5	306	−17.5
V	21.1	QD × 8	0	30.0	333	−21.7
V	28	D 1, 5, and 9	1	47.5	528	− 2.5
V	42	D 1, 5, and 9	2	34.0	378	− 7.2
V	63	D 1, 5, and 9	0	11.0	122	− 7.0
V	28	D 7, 11, and 15	0	22.0	244	− 2.5
V	42	D 7, 11, and 15	0	13.0	144	−11.1
V	63	D 7, 11, and 15	0	8.0	89	− 7.0

Each group consisted of eight $B6D2F_1$ female mice per group. Control life-span was 9 days

[a] Ratio of test evaluation (median survival) to control evaluation, expressed as a percentage

[b] Average weight change of tumored animals on D 5 (QD × 8 and D 1, 5, and 9 schedule) or D 11 (D 7, 11, and 15 schedule) compared with an initial weight

Table 5. Activity in P388 leukemia

Compound	Dose	Schedule	50-day survivors	Median survival	T/C (%)[a]	AWC (%)[b]
I	31.6	D 1, 5, and 9	0	16	178	−14.2
I	47.3	D 1, 5, and 9	0	11	122	−23.6
I	71.0	D 1, 5, and 9	0	6	66	−
I	31.6	D 7, 11, and 15	0	14	156	− 8.6
I	47.3	D 7, 11, and 15	0	16.5	183	−14.5
I	71.0	D 7, 11, and 15	0	11.5	128	−17.1
V	21.0	D 1, 5, and 9	2	28.5	317	−19.7
V	31.6	D 1, 5, and 9	0	21.5	239	−26.6
V	47.3	D 1, 5, and 9	0	15.5	172	−
V	21.0	D 7, 11, and 15	0	17.0	189	−13.2
V	31.6	D 7, 11, and 15	0	9.0	100	−
V	47.3	D 7, 11, and 15	0	18.0	200	−23.6

Each group consisted of eight $C3D2F_1$ female mice. Control life-span was 9 days

[a] Ratio of test evaluation (median survival) to control evaluation, expressed as a percentage

[b] Average weight change of tumored animals on D 8 (D 1, 5, and 9 schedule) or D 16 (D 7, 11, and 15 schedule) compared with an initial weight

[7], while the single larger dose treatment on day 1 was inferior. This statement is generally true for all active compounds; however, it is worth noting that the only long-term survivors (one with ASE [7], six with compound I, and five with compound V) were P388 tumor-bearing mice treated on an intermittent schedule. A consideration of the kinetics of cell kill will indicate why larger doses given intermittently might give cures if the tumor burden is being reduced to low numbers of viable cells with each treatment. If the drug is not so effective, repeating the treatment frequently would give similar results.

Since it is important to achieve a remission in advanced leukemia with the first treatment if possible, we chose the intermittent treatment schedule and treated the mice on days 7, 11, and 15. This treatment was the same as the days 1, 5, and 9 treatment except that it was started 6 days later. In advanced P388 leukemia T/C values $\geq$ 200% were obtained with compound V, 183% with compound I, and 167% with compound III.

Most steroidal alkylating agents have been inactive in L1210 leukemia [6], but most homo-aza-steroidal esters have been active with substitutions to date in either the D-ring or the A-ring. The lactam moiety appears to confer this activity. The steric arrangement of the alkylating moiety greatly affects toxicity and activity of the drugs, while the selectivity on tumor cell kill vs normal cell kill as reflected in life-span at optimal dose is influenced to a lesser extent. The steric arrangement of the hydrogen atom at position 5 influences these parameters to a smaller extent. The nature of the alkylating moiety does not appear to be critical, and in the limited group of compounds studied does not appear to control activity in any predictable way. Previous studies have shown that for activity the compound must contain an easily cleaved ester or heterocyclic ether bonding of the alkylating moiety to the steroid [6].

Further studies of these compounds and newer congeners are warranted to refine structure-activity relationships and to identify promising drugs for clinical trial.

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