Eur J Med Chem (1991) 26, 659–661

Antileukemic activity of homo-aza-steroidal esters of the isomers of *N*, *N*-bis(2-chloroethyl)aminocinnamic acid

P Catsoulacos, C Camoutsis, M Pelecanou

Laboratory of Pharmaceutical Chemistry, University of Patras, Patras, Greece

(Received 22 June 1990; accepted 25 February 1991)

heterocyclic steroids / alkylating agents / P388 leukemia

Introduction

Members of the class of homo-aza-steroidal esters of carboxylic derivatives of the nitrogen mustard *N,N*-bis(2-chloroethyl)aniline have demonstrated interesting results against a variety of experimental animal tumors and leukemias [1–4]. In this paper we report the synthesis and the evaluation of antineoplastic activity against lymphoid leukemia P388 of the isomeric esters **1**, **2**, and **3**. In these compounds, 3β-hydroxy-17a-aza-D-homo-5α-androstan-17-one has been joined through an ester bond with the 3 isomeric nitrogen mustards of cinnamic acid, namely the *ortho*-, *meta*-, and *para*-[*N,N*-bis(2-chloroethyl)amino]cinnamic acid. These esters, due to the presence of an extended conjugation system, are suitable for the study of steric and electronic effects on the anticancer activity of this class of compounds.

Results

Chemistry

The isomeric mustards of cinnamic acid were prepared by a previously described method [5]. The condensation reaction of the 3β-hydroxy group of the steroidal lactam 3β-hydroxy-17a-aza-D-homo-5α-androstan-17-one with each mustard was effected in dichloromethane in the presence of *p*-dimethylaminopyridine as catalyst and of presence of *p*-dimethylaminopyridine as catalyst and of dicyclohexylcarbodiimide (DCC) as dehydrating agent [6]. Physical and spectral properties of the esters prepared are given in table I.

The percent hydrolysis (table II) of compounds **1–3** in H_2O/CH_3CN 6/4 mixture under standard conditions (incubation for 1/2 h at 66°C) set by Ross [7] was determined by HPLC by the decrease in the peak area

Table I. Physical and spectral properties of the esters **1**, **2**, **3**. C, H, N analytical results obtained for the esters **1**, **2**, and **3** were within ± 0.4% of the theoretical values. Melting points were determined on a Fisher–Johns apparatus and are uncorrected.

Ester	*Formula*	*Yield (%)*	*mp (°C)*	*Recryst solvent*	*IR (cm^{-1}) C=O*	*C=C*	*NHC=O*
1	$C_{32}H_{44}Cl_2N_2O_3$	62	215–217	$CH_3COOC_2H_5$	1705	1630	1660
2	$C_{32}H_{44}Cl_2N_2O_3$	80	221–222	$CH_3COOC_2H_5$-$CHCl_3$	1700	1635	1675
3	$C_{32}H_{44}Cl_2N_2O_3$	70	230–231	$CH_3COOC_2H_5$	1695	1625	1655

Table II. % Hydrolysis (average of 2 runs) of compounds **1**, **2**, **3** (concentration of **1**, **2**, and **3** was *c/a* 1.5 x 10^{-4} M) after 1/2 h in H_2O/CH_3CN 6/4 at 66°C as determined by HPLC (C-18 μ-Bondapak column, eluent CH_3OH/H_2O 9/1, UV absorbance monitored at 254 nm).

1	40 ± 3
2	16 ± 2
3	< 3

corresponding to starting material. CH_3CN was used as a co-solvent due to the limited solubility of the esters in H_2O. In all cases the product of hydrolysis was isolated and characterized as the corresponding *N,N*-bis(2-hydroxyethyl)amino derivative **4**. Under the reaction conditions no hydrolysis of the ester bond was observed.

Pharmacology

Screening against P388 leukemia was carried out by the National Cancer Institute (Bethesda, MD, USA). Compounds were administered intraperitoneally suspended in saline stabilized by Tween 80. Schedule and dosage details are given in table III. P388 leukemia was maintained by weekly IP passage of 10^6 cells in CDF_1 male and female mice.

Out of the 3 isomers tested, only **1** was active against P388 leukemia with a maximum activity of T/C = 140% achieved at the dose level of 400 mg/kg. At the same dose level, compound **2** gave T/C of 111% and compound **3** gave T/C of 110%. Table III summarizes the screening data for compound **1**.

The LD50's for compounds **1**, **2**, and **3** are 900, 450 and 900 mg/kg respectively.

Discussion

A good correlation exists between antineoplastic activity and chemical reactivity assessed by determining the % hydrolysis of the nitrogen mustard moiety under standard conditions. Specifically, compound **1** bearing the nitrogen mustard moiety in the *ortho* position is active against leukemia P388 and also hydrolyzes to the corresponding hydroxy analogue considerably faster than the nonactive isomers **2** and **3**.

The higher chemical reactivity of the *ortho*-substituted nitrogen mustards as compared to their *meta* and *para* isomers is not uncommon and has been attributed to steric hindrance of mesomerism between the aromatic ring and the nitrogen of the mustard group [8]. It has been established that in the aromatic nitrogen mustard series an increase in the basicity of the nitrogen results in an increase in chemical reactivity towards hydrolysis [9]. It is important to note that the chemical reactivity of the mustard group towards hydrolysis cannot account alone for the observed antineoplastic behavior of the isomers since special enzymatic mechanisms are probably involved during transport, penetration into the malignant cell, activation etc. However, structure–activity studies indicate that the ease of hydrolysis may be an important factor in antileukemic efficacy [10].

Table III. Activity of compound **1** against P388 lymphocytic leukemia in CDF_1 mice.

Dose injection [1] *(mg/kg)*	*Schedule*	*Median survival time (d)*	*T/C* [2] *(%)*	*Animal weight (g) difference* [3]	*Survivors*
Experiment I, male mice					
400	Day 1	14.3	140	–2.4	6/6
200	Day 1	12.8	125	–0.7	6/6
100	Day 1	12.3	120	0.2	6/6
0	–	10.2	–	1.2	–
Experiment II, female mice					
400	Day 1	14.0	134	–1.9	6/6
200	Day 1	12.3	118	–1.3	6/6
100	Day 1	11.0	105	–1.1	6/6
0	–	10.4	–	1.1	–

[1]Six male or female mice in each group. [2]Ratio of test (T) evaluation to control (C) evaluation expressed in %. [3]Average weight change (weight on toxicity evaluation day minus weight on initial day of treatment) of test group minus average weight change of control.

Experimental protocols

*3-β-Hydroxy-17a-aza-D-homo-5α-androstan-17-one ortho-[*N,N-*bis(2-chloroethyl)amino]cinnamate (**1**), 3-β-Hydroxy-17a-aza-D-homo-5α-androstan-17-one meta-[*N,N-*bis(2-chloroethyl)amino]-cinnamate (**2**), 3-β-Hydroxy-17a-aza-D-homo-5α- androstan-17-one para-[*N,N-*bis(2-chloroethyl)amino]cinnamate (**3**)*
Compounds **1**, **2**, and **3** were prepared according to published procedure [6]. Physical and spectral properties of the esters prepared are given in table I.

*Hydrolysis of the esters **1**, **2**, and **3** in H_2O/CH_3CN 6/4 solution*
A 2-ml aliquot of a 3.75 x 10^{-3} M stock solution of starting material in purified CH_3CN was brought to a volumetric flask and 18 ml of CH_3CN was added followed by 30 ml of deionized H_2O to achieve a final concentration of 1.5 x 10^{-4} M (a lower concentration was employed in the case of the *meta* isomer due to limited solubility). The solution was immediately incubated in a water bath at 66°C and a 5-μl aliquot was subjected to HPLC analysis (μ-Bondapak C-18 reverse-phase column, CH_3OH/H_2O 9/1 eluent, UV absorbance monitored at 254 nm). After 30 min another 5-μl aliquot was subjected to HPLC analysis and the % hydrolysis was determined by the decrease in the peak area corresponding to starting material as compared to the peak area recorded at the beginning of the reaction.

The reaction was allowed to reach completion and for all isomers HPLC analysis indicated the formation of 1 main product. The reaction mixture was cooled and extracted with $CHCl_3$. The organic extract was evaporated and the residue was chromatographed on silica gel column with $CHCl_3$ as eluent. In all cases the main product was characterized by IR and elemental analyses as being the corresponding *N,N*-bis(hydroxyethyl)-derivative.

3β-Hydroxy-17a-aza-D-homo-5α-androstan-17-one o-*[*N,N-*bis(2-hydroxyethyl)amino]cinnamate **4a***. mp = 217–219°C. IR (KBr): 3500–3100, 1700, 1640, 1593, 1166, 755.

3β-Hydroxy-17a-aza-D-homo-5α-androstan-17-one m-*[*N,N-*bis(2-hydroxyethyl)amino]cinnamate **4b***. mp = 221–223°C. IR (KBr): 3500–3100, 1710, 1635, 1597, 1503, 1180, 840, 780, 587.

3β-Hydroxy-17a-aza-D-homo-5α-androstan-17-one p-*[*N,N-*bis(2-hydroxyethyl)amino]cinnamate **4c***. mp = 260–262°C. IR (KBr): 3500–3100, 1700, 1640, 1595, 1519, 1165, 812.

References

1 Catsoulacos P, Politis D, Wampler GL (1983) *Cancer Chemother Pharmacol* 10, 129–132
2 Catsoulacos P (1984) *Cancer Lett* 22, 199–202
3 Catsoulacos P, Boutis L (1973) *Cancer Chem Rep* 57, 365–367
4 Wall ME, Abernethy GS Jr, Carrol FI, Taylor DJ (1969) *J Med Chem* 12, 810–818
5 Skinner WA, Schelstraete MGM, Baker BR (1961) *J Org Chem* 26, 1674–1676
6 Pairas G, Camoutsis P, Catsoulacos P (1985) *Eur J Med Chem* 20, 287–288
7 Ross WCJ (1949) *J Chem Soc* 183–191
8 Niculescu DI, Ionescu M, Cambanis A, Vitan M, Feyns V (1968) *J Med Chem* 11, 500–503
9 Bardos TJ, Datta-Gupta N, Hebborn P, Triggle DJ (1965) *J Med Chem* 8, 167–174
10 Panthananickal A, Hansch C, Leo A (1978) *J Med Chem* 21, 16–26