Assesment of anti-HIV and Antiproliferative Activity of Homo-Aza-Steroidal Esters in Culture

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · JULY 1990

Impact Factor: 3.45 · DOI: 10.1016/0223-5234(90)90149-W

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Assessment of anti-HIV and antiproliferative activity of *homo*-aza-steroidal esters in culture

G Pairas, P Catsoulacos

University of Patras, School of Health and Sciences, Pharmacy Department, Laboratory of Pharmaceutical Chemistry, Patras, Greece

(Received 18 July 1989; accepted 6 December 1989)

synthesis of *homo*-aza-steroidal esters / assessment of anti-HIV activity

Introduction

Our activity relationship studies of modified steroidal esters of carboxylic derivatives of *N,N*-bis(2-chloroethyl)aniline suggest that in addition to an easily cleaved ester bond which is a basic requirement for antitumor activity the lactam moiety is required for activity in L1210 leukemia [1–4]. The above satisfactory results and the activity of modified steroidal esters in 3PS31 leukemia (unpublished results from this laboratory) prompted us to study the synthesis of A-*homo*-aza-steroidal esters of chloram-bucil and the possible anti-HIV activity.

Assessment of anti-HIV and antiproliferative activity of alkylating steroid derivatives in culture

All the alkylating steroid derivatives designated NSC 622556, NSC 622554 and NSC 622555 were tested for both anti-HIV and antiproliferative activity in cultures of CEM-V or CEV-Z cells. Assessment of these 2 parameters was carried out *in vitro*. Each agent was added in separate cultures at varying concentrations (ranging from 1.6×10^{-3} µg/ml (or molar) to 5.0 µg/ml (agents NSC: 622554, 622556) and from 3.0×10^{-3} to 9.4 µg/ml (agent NSC: 622555)) and tested for its ability to counteract the HIV-induced cytopathic effect on already HIV-infected CEM cells and to effect cell proliferation of uninfected CEM cells grown under similar culture conditions. The effect of each agent on cell growth of HIV, infected and uninfected cells was compared to that of uninfected cells incubated in the absence of drug (uninfected untreated control cultures). Measurement of cell growth was based on the number of cells remaining intact.

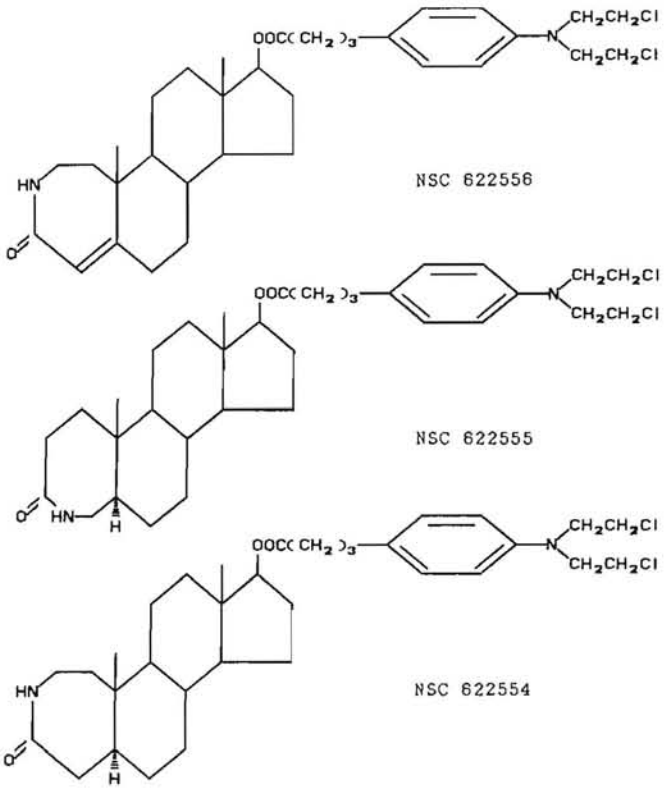

Table I. IR spectra of NSC 622554, 622555, 622556. Analyses: C, H, N. Analytical results obtained for those elements within ± 0.4 of the theoretical values.

Compd	*NH (cm⁻¹)*	*CO (cm⁻¹)*	*NHCO (cm⁻¹)*	*Ar ring (cm⁻¹)*
622554	3310, 3160	1725	1660–1610	800, 735
622555	3190, 3085	1720	1675–1610	800, 735
622556	3185, 3070	1720	1675–1630	800, 740

Results

All the agents (NSC 622554, 622555, 622556) failed to counteract the cytopathic effects of HIV virus on CEM cells, since cell growth of HIV-infected CEM cells remained close to 10% or < 10% of that of uninfected untreated cultures. Values of cell growth of HIV-infected cells of between 0–50% indicate lack of any substantial anti-HIV activity of these agents. In addition, the 3 alkylating steroid derivatives failed to suppress cell proliferation of uninfected CEM cells at concentrations as high as 0.5 μg/ml. Substantial inhibition of cell growth (> 90%), however, was observed at much higher concentrations (> 5.0 μg/ml). These results indicate that the 3 steroid derivatives lack both anti-HIV and antiproliferative activity at micromolar concentrations in culture. Why these steroid derivatives were found to be inactive despite the fact that they all carry mustargen bifunctional alkylating groups remains to be seen. The LD_{50} values for the 3 agents NSC 622554, 622555 and 622556 were found to be 2.5, 8.0 and 1.8 μg/ml, respectively.

Experimental protocols

Method A: General procedure for the synthesis of esters with p-[N,N-*bis(2-chloroethyl)amino]phenylbutyric acid with DCC*
A stirred mixture of 1 mmol of 17β-hydroxy-ε-lactam [5, 6] in 400 ml of dry dichloromethane, 1.05 mmol (320 mg) of *p*-[*N,N*-bis(2-chloroethyl)amino]phenylbutyric acid, 1.05 mmol (128 mg) of *p*-dimethylaminopyridine and 1.3 mmol (268 mg) of dicyclohexylcarbodiimide (DCC), was allowed to remain at room temperature for 5 days.

After the reaction time, a few drops of acetic acid were added to the mixture to destroy the excess DCC and the *N,N*-dicyclohexylurea precipitate was filtered off (from benzene). The solvent was removed under reduced pressure and the remaining residue was chromatographed on silica gel column. Elution with chloroform yielded the desired compound. The following compounds were obtained:
17β-Hydroxy-3-aza-A-*homo*-5α-androstan-4-one *p*-[*N,N*-bis(2-chloroethyl)amino]phenylbutyrate (NSC 622554) ester, mp 190°C (dec) ($CH_3COOC_2H_5$) Yield 30%. Analysis $C_{33}H_{48}N_2O_3Cl_2$: C, H, N.
17β-Hydroxy-4-aza-A-*homo*-5α-androstan-3-one *p*-[*N,N*-bis(2-chloroethyl)amino]phenylbutyrate (NSC 622555) ester, mp 190°C (dec) ($CH_3COOC_2H_5$) Yield 31%. Analysis $C_{33}H_{48}N_2O_3Cl_2$: C, H, N.
17β-Hydroxy-3-aza-A-*homo*-5α-androstan-4-one *p*-[*N,N*-bis(2-chloroethyl)amino]phenylbutyrate (NSC 622556) ester, mp 112–113°C [7] ($CH_3COOC_2H_5$. *n*-hexane) Yield 58%. Analysis $C_{33}H_{46}N_2O_3Cl_2$: C, H, N.

Method B: General procedure for the synthesis of esters with p-[N,N-*bis(2-chloroethyl)amino]phenylbutyryl chloride hydrochloride*
To a solution of 0.85 mmol of 17β-hydroxy-ε-lactam in 25 ml of dry benzene, was added 2.2 mmol (800 mg) of *p*-[*N,N*-bis(2-chloroethyl)amino]phenylbutyryl chloride hydrochloride. The mixture was heated under reflux for 30 h. Then, the reaction mixture was concentrated under reduced pressure. The remaining residue was dissolved in chloroform, chromatographed on a silica gel column and eluted with chloroform. After evaporation of the solvent the residue crystallized from the appropriate solvent to produce the esters in 50–58% yield.

The isolated esters were found to be identical by IR and melting points to the esters prepared by Method A.

Acknowledgment

These data are the result of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, USA.

References

1 Wampler GL, Catsoulacos P (1977) *Cancer Treat Rep* 61, 37
2 Catsoulacos P, Politis D, Wampler GL (1983) *Cancer Chemother Pharmacol* 10, 129
3 Catsoulacos P, Politis D, Wampler GL (1979) *Cancer Chem other Pharmacol* 3, 67
4 Pairas G, Camoutsis C, Catsoulacos P (1985) *Eur J Med Chem* 20, 287
5 Mazur RH (1963) *J Org Chem* 28, 248
6 Oka K, Hara S (1969) *Chem Ind Lond* 168
7 Dalmases P, Caselles JM, Serra J, Quintana J, Bonet JJ, Giner-Sorola AG, Schmidt FA (1985) *Eur J Med Chem* 20, 471