Synthesis and Biological Behavior of a Boronated Analogue of the Antiestrogen U 23,469-M

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A boronated analogue of the antiestrogen U 23,469-M (D. Lednicer, D. W. Emmert, S. C. Lyster, and G. W. Duncan, J. Med. Chem. **12,** 881 (1969)) was prepared, for possible use in neutron capture therapy of estrogen receptor-positive tumors. In this analogue, the terminal OH group was replaced by a B-decachloro-*o*-carboranyl residue. This compound showed a large, non-specific uptake in ZR 75-1 breast cancer-derived cells. It could partially inhibit the uptake of estradiol in these cells. Accumulation in the cells at physiologically obtainable concentrations was, however, too low to envisage a therapeutic effect following thermal neutron irradiation.

Introduction

The neutron capture reaction in boron is, in principle, very efficient in killing tumor cells. The two particles emerging from the $^{10}B(n,\alpha)^{7}Li$ reaction disperse their combined energy for ionization of 2.34 MeV within a short distance of 4.9 (^{7}Li) and 8.9 µm (^{4}He), corresponding to cellular dimensions, thereby transferring enough energy to lethally damage the cell traversed [1, 2]. Whereas this reaction is used presently to treat brain tumors [3], its general applicability to tumor treatment is greatly hindered by the unavailability of boronated analogues of tumor-localizing compounds. Such compounds would include antibodies, porphyrins, amino acids, nucleosides, melanin-affinic substances, and hormones [22].

All of these compounds would be administered systematically, and irradiation procedures could be started only after uptake in the tumor has been established and a sufficient clearance of boron from the surrounding tissue and especially the blood would be achieved. Thus, suitable boron compounds would have to show a sufficient retention time in the target tissue, as well as clear adequately from the surrounding.

Estrogen-influenced tumors are found primarily among breast and prostate cancers [4]. Here, breast tumors sometimes are dependent on estrogens for their growth, whereas the growth of prostate cancers often can be reduced or halted by estrogens.

In the past, boron-containing analogues of estrogens and antiestrogens have been suggested repeatedly for use in boron neutron capture therapy. Boronated estrogen derivatives intended for this therapy have been described by Hadd [5], Kahl [6], and Sweet [7]. No reports have appeared on their accumulation in cells or *in vivo*.

Here, we describe the synthesis and biological behavior of a boronated analogue of the antiestrogen U 23,469-M, originally described by Lednicer [8] and Katzenellenbogen [9]. In contrast to estrogens, antiestrogens show a much prolonged uptake in the nucleus of cell in cell culture [10] and *in vivo* [11]. Thus, it was hoped that a suitable compound with sufficient cell retention could be obtained.

The pathway for the synthesis of the compound (see Scheme 1) followed that described by Lednicer [8].

For the introduction of the boron moiety, B-decachlorocarborane, originally described by Zakharkin [12] was used. Its anion reacts readily with epoxides [13]. Thus, the easily prepared epoxide **I** could be opened selectively through a nucleophilic attack at the terminal carbon [13] to yield the desired product **III.** As described by Katzenellenbogen [9], the methyl group in the 6-position could be removed selectively.

The binding of this compound to cells in cell culture was analyzed by direct boron analysis of the

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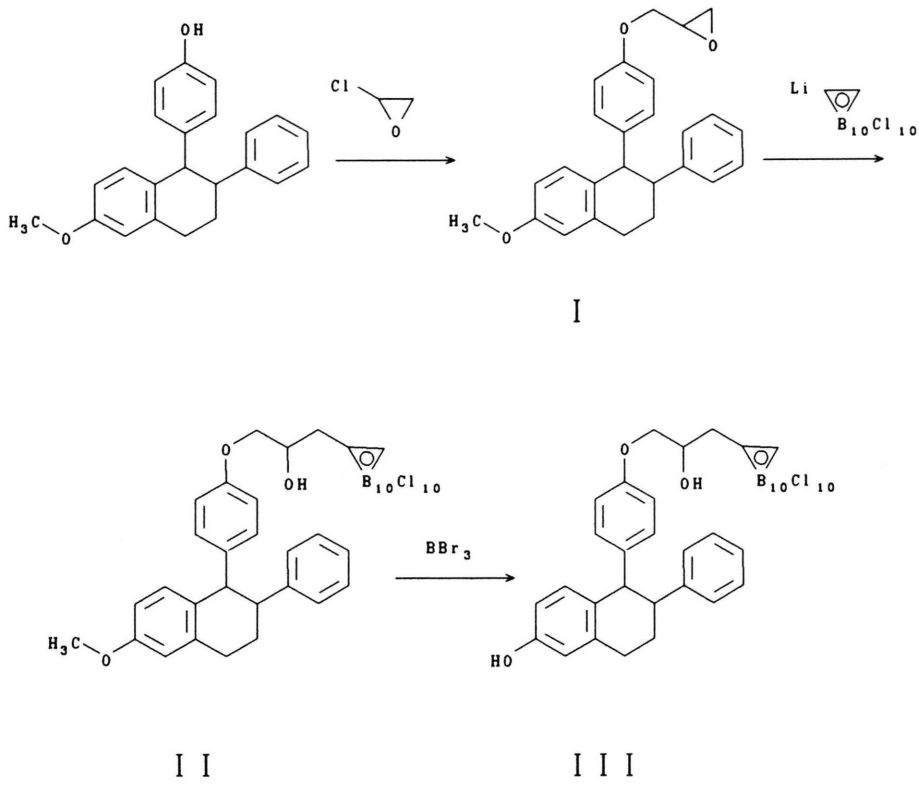

Scheme 1.

incubated cells [14]. This method allows to analyze for boron down to ppm concentrations, with an error of about 20%. The inhibition of estradiol uptake by U 23,469-M and its boronated analogue **III** was also investigated.

Results and Discussion

Figure 1 shows the accumulation of boron by in ZR 75-1 cells. No saturation of boron binding occurs up to 100 µmol/l, indicating a large non-specific binding or, less likely, a small binding constant.

Figure 2 shows that the binding of [^{3}H]estradiol can be partially inhibited by **III.** For the inhibitable part of estradiol binding, an apparent inhibition constant of around 10^{-8} mol/l is obtained, similar to that of U 23,469-M. Both antiestrogens are less good inhibitors compared to diethylstilbestrol.

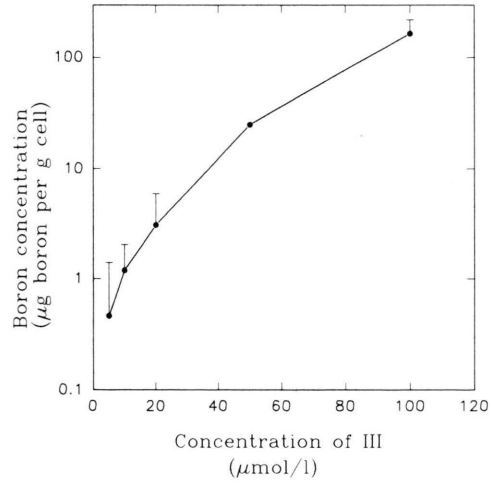

Fig. 1. Boron uptake by ZR 75-1 cells upon incubation with **III.** The points are means ± S.D. of up to 7 determinations.

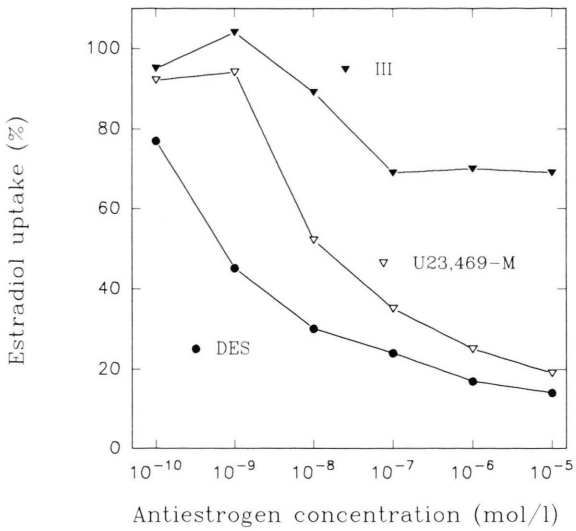

Fig. 2. Inhibition of [^{3}H]estradiol uptake by diethylstilbestrol (●), U 23,469-M (▽), and **III** (▼). The points are means of quadruplicate determinations.

The stereochemistry of low-molecular weight substances is often of great importance for their interaction with their receptors. In **III,** three chiral carbon atoms are present. As previously Tatee *et al.* [9], we have used the racemic mixture of the tetrahydronaphthaline moiety. As racemic epichlorohydrine was used for the introduction of the glycerol side chain, the stereochemistry of the hydroxyl group would be *R,S* in equal proportions. This is also true for the compound used by Tatee *et al.* [9]. No effort was made to prepare one of the stereoisomers.

The number of receptors present in estrogen-receptor containing cells has been estimated to be in the order of 10^4 to 10^5 per cell [15]. For the cells used here, we found a number of 4×10^4 receptors. If only these receptors were used for boron accumulation, much less than 1 μg boron per gram cell mass [16] could be obtained. This is far too little for useful neutron capture therapy [17], where several micrograms per gram would be required for delivering a relevant tumor dose and at the same time-sparing normal tissue.

The non-specific binding of the boronated antiestrogen at concentrations above 10 μmol/l might accumulate enough boron in cell culture to perform radiobiological experiments. It is doubtful, however, that there might be any differentiation *in vivo* of tumor and normal cells, when non-specific binding must be used to accumulate boron in a target organ.

In order to increase the binding of boronated antiestrogen analogues, other binding sites than the estrogen receptors might be considered. Saturable antiestrogen-specific binding sites in the cytosol of hormone-dependent tissues have been described [18]. These antiestrogen binding sites (AEBS) are different from the estrogen receptors insofar as they bind only triphenylethylene antiestrogens. Their natural significance is not yet fully understood. In contrast to the estrogen receptors, the AEBS are not translocated to the nucleus after binding of the antiestrogen. They are found in estrogen-dependent tumors and, at relatively high levels, in other tissues of the rat, *e.g.* liver, esophagus, uterus, ovary, and brain [19]. The AEBS found in the microsomal fraction of the human MCF-7 breast carcinoma cell line has a high affinity (K_d = 0.97 nM) and a narrow specificity for basic triphenylethylene aminoether derivatives. The basic aminoether is essential for binding to the AEBS [20]. The antiestrogen U 23,469-M is thus not suitable for binding to AEBS. In order to reach a relatively high level of boron in estrogen-receptor positive tumor cells, a boronated derivative of an antiestrogen with a basic aminoether side chain, like tamoxifen, should be more suitable. However, the number of AEBS found in cells (40,000 [21] to 140,000 [22] for MCF-7 cells and 330,000 for ZR 75-1 cells [23]) might still be too low for attaining therapeutically useful boron concentrations.

The synthesis of boronated estrogens and antiestrogens is possible. It must be concluded, however, that their accumulation in estrogen-dependent cells even with retained optimal properties makes them less likely candidates for targeting boron successfully for boron neutron capture therapy.

It should be noted, however, that specific accumulation of boron in tumors has been achieved for other compounds [27–29], thus allowing successful boron neutron capture therapy to be carried out.

Experimental

^{1}H NMR spectra were recorded on a Bruker WH 360, mass spectra on a Finnigan MAT 8222. The mass spectra recorded were in good agreement

with the isotope distribution patterns calculated from the natural abundance of chlorine-35 and -37 and boron-10 and -11. Elemental analysis was carried out by Beller, Göttingen. The results of elemental analysis of the B-decachloro-*o*-carborane derivatives described here and of other derivatives were often found not be in good agreement with the calculated values.

*cis-1-(p-(2,3-Epoxypropyloxy)-phenyl)-2-phenyl-6-methoxy-1,2,3,4-tetrahydronaphthalin (**I**)*

A solution of 1.65 g (5 mmol) of 1-(*p*-hydroxyphenyl-2-phenyl-6-methoxy-1,2,3,4-tetrahydronaphthalin [8] in 50 ml freshly distilled epichlorohydrin was refluxed, and water removed with a Dean-Stark trap. When boiling, 5 mmol NaOH as a 20 percent solution in water was added during 30 min. The excess epichlorohydrin was removed *in vacuo*, the residue was washed with *n*-hexane and purified by chromatography on silica gel in petrol ether/acetone 100/15. The colorless oil, still containing solvent, was used directly for the next step.

*cis-1-(p-(3-(Decachloro-o-carboranyl)-2-hydroxylpropyloxy)-phenyl)-2-phenyl-6-methoxy-1,2,3,4-tetrahydronaphthalin (**II**)*

B-decachloro-*o*-carboran (2.4 g = 5 mmol) was dissolved in 30 ml dry diethyl ether. At 0 °C, an equimolar amount of a 15% solution of butyllithium in *n*-hexane was added dropwise. After 30 min, the cooling mixture was removed and **I**, dissolved in 20 ml diethyl ether, was added together with 100 ml *n*-hexane. After 20 h of stirring at room temperature, water was added and the phases were separated. The aqueous phase was acidified and extracted with diethyl ether. After drying, the ether was removed, and the residue was chromatographed on silica gel with cyclohexane/CH_2Cl_2 1:1. Yield 72%; m.p. 115–118 °C.

Anal ($C_{28}H_{28}B_{10}Cl_{10}O_3$) C: calc., 38.42; found 33.40, H, B; Cl: calc. 40.51; found 41.28.

^{1}H NMR ($CDCl_3$ITMS): δ = 1.85, 2.15 (m, 2H, C^3H_2); 2.65, 2.98 (m, 2H, $C^{3'}H_2$); 2.75 (d, 1H, –OH); 3.1 (m, 2H, C^4H_2); 3.4 (m, 1H, $-C^2H$); 3.82 (s, 3H, CH_3O); 3.85, 4.05 (m, 2H, $C^{1'}H_2$); 4.27 (d, 1H, C^1H); 4.85 (m, 1H, $C^{2'}H$); 5.6 (s, 1H, $-CB_{10}Cl_{10}CH$); 6.25, 6.45 (d, 4H, *p*-phenylene); 6.55–7.1 ppm (m, 8H, phenyl, 1,2,4-trisubstituted aromate).

IR (KBr): (cm^{-1}) = 3451s, 3015m, 2931m, 1609m, 1504s, 1455w, 1258s, 1237s, 1180w, 1131s, 1090s, 1026s, 906w, 843w, 829m, 801m, 758w, 730w.

MS (FAB, negative, MeOH + TEG): *m/e* (rel. int. %) = 874 (M^-, 100), 838 (33), 503 (18), 467 (13).

*cis-1-(p-(3-(Decachloro-o-carboranyl)-2-hydroxylpropyloxy)-phenyl)-2-phenyl-6-hydroxy-1,2,3,4-tetrahydronaphthalin (**III**)*

II (745 mg = 0.85 mmol) was dissolved in 20 ml dry CH_2Cl_2. At 0 °C, BBr_3 (5 drops at a time) was added in 15 min intervals. After 40 drops, the mixture was carefully hydrolyzed and the CH_2Cl_2 phase was separated. After drying and evaporation, the residue was chromatographed on silica gel with cyclohexane/CH_2Cl_2/ethanol 40:52:3. Yield 85%; m.p. 142–153 °C.

Anal ($C_{27}H_{26}{}^{10}B_{10}Cl_{10}O_3$) C: calc., 36.62; found, 36.19; H: calc., 3.08; found, 3.49; B; Cl: calc., 41.17; found, 41.56.

^{1}H NMR ($CDCl_3$ITMS): δ = 1.75, 2.05 (m, 2H, C^3H_2); 2.55, 2.85 (m, 2H, $C^{3'}H_2$); 2.7 (d, 1H, propyl-OH); 2.95 (m, 2H, C^4H_2); 3.3 (m, 1H, C^2H); 3.75, 3.93 (m, 2H, $C^{1'}H_2$); 4.17 (d, 1H, C^1H); 4.7 (m, 1H, $C^{2'}H$); 5.21 (s, 1H, aryl-OH); 5.6 (s, 1H, $-CB_{10}Cl_{10}CH$); 6.27, 6.45 (d, 4H, *p*-phenylene); 6.48–7.1 ppm (m, 8H, phenyl, 1,2,4-trisubstituted aromate).

IR (KBr): (cm^{-1}) = 3430sbr, 3016w, 2931m, 2882m, 1609m, 1504s, 1455m, 1307w, 1265m, 1237s, 1180s, 1152s, 1103s, 1060s, 920w, 850w, 822m, 758m, 702m.

MS (FAB, negative, MeOH + TEG): *m/e* (rel. int. %) = 852 (M^-, 100), 816 ($M^- - Cl$, 52), 772 (14), 493 (50), 455 (63), 410 (57), 378 (41).

Cell growth

The human breast cancer cell line ZR 75-1 [24], obtained through the American Type Culture Collection, was grown on Petri dishes in RPMI growth medium supplemented with 10% fetal calf serum, 2 mM glutamin, 10 mg/ml penicillin/streptomycin. For all cell-binding assays, the cells were grown for 10–14 days in medium supplemented with charcoal-stripped serum [25].

Determination of estrogen receptors

Confluent Petri dishes were incubated at 37 °C for 60 min with tritiated estradiol (Amersham-Buchler) at concentrations of 10^{-10} to 10^{-9} M and, when indicated, in the presence of diethylstilbestrol (10^{-7} M) as an inhibitor. The cells were extensively washed with cold phosphate-buffered saline (PBS), trypsinized, and suspended in PBS. The number of cells was counted, and the cell suspension was counted for tritium in a liquid scintillation counter. The number of receptors was determined from a Scatchard plot.

Cell binding of boronated antiestrogen

Confluent Petri dishes were incubated with **III** at concentration of 2×10^{-6} to 10^{-4} M. After incubation at 37 °C for 60 min, the cells were washed, trypsinized, resuspended in PBS and counted. Boron concentration in the cells was determined according to Gabel [26] and Fairchild [14].

Inhibition of estradiol uptake

Confluent Petri dishes were incubated at 37 °C with 10^{-10} M tritiated estradiol, in the presence of diethylstilbestrol, **III**, and U 23,469-M (the active form of this antiestrogen, containing a free phenolic group) at concentrations between 10^{-9} and 10^{-4} M. All other conditions were kept as described above.

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