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# CIS ISOMER OF CENTCHROMAN - A SELECTIVE LIGAND FOR THE MICROSOMAL ANTIESTROGEN BINDING SITE+

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Several compounds structurally related to the triarylethylene antiestrogens, but possessing weak estrogen receptor affinities, were assessed for their ability to interact with the microsomal antiestrogen binding site. While all the compounds tested did interact with this site their relative affinities were somewhat lower than that of tamoxifen. One of these, viz., the cis isomer of centchroman, has however emerged as a selective ligand for the antiestrogen binding site since its estrogen receptor affinity is nearly 50,000 times lower on a relative scale. 

The interaction of the triarylethylenes (TAEs), such as tamoxifen and clomiphene, with estrogen receptors (ER) is widely recognised as the critical step in their action as antiestrogens (1,2). It has however been demonstrated recently that these and similar other antiestrogens also interact with an estrogen noncompetable microsomal site, termed generally the Antiestrogen Binding Site (AEBS), which is distributed widely in the body with the largest concentration in liver (3-7). This finding has evoked considerable interest on account of the possibility that this site may have a role either in mediating some of the biological effects of TAEs or in the modulation of their antiestrogenic activities. The availability of selective

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ligands which can interact with the AEBS in preference to ER would be of immense help in probing some of these possibilities. There is enough evidence already that the AEBS and the ER possess some notable difference in their ligand specificities (3-5,8). While TAE antiestrogens interact with both, the characteristic tert-aminoalkoxy side chain is crucial for their AEBS but not ER interaction. A tamoxifen analog with a modified side chain has been reported to be a better ligand for AEBS than for ER with a modest dissociation in its relative affinity for the two sites (5). In order to look for even more selective AEBS ligands, we chose to investigate TAE analogs incorporating the requisite side chain, but which in our experience were known to or expected to possess weak ER affinities. One of these, as reported here, has indeed turned out to be a selective ligand for the AEBS.

## MATERIALS AND METHODS

[N-Methyl-$^{3}$H]-Tamoxifen (76 ci/mmol) and [2,4,6,7-$^{3}$H]-estradiol (114 ci/mmol) were purchased from the New England Nuclear, USA. Unlabelled tamoxifen was a gift from I.C.I. England, while unlabelled estradiol and diethylstilbestrol were purchased from Steraloids Inc. The female rats, 21-22 day old, of Charles foster strain, were from the rodent colony of this institute.

The TAE analogs used in this study, shown in Fig.1, were, centchroman (II) [trans-(±)-1-[2-[4-(3,4-dihydro-7-methoxy-2,2-dimethyl-3-phenyl-2H-1-benzopyran-4-yl)phenoxy] ethyl]pyrrolidine], the cis-chroman III [cis-(±)-1-[2-[4-(3,4-dihydro-7-methoxy-2,2-dimethyl-3-phenyl-2H-1-benzopyran-4-yl)phenoxy]ethyl]pyrrolidine], the coumarin IV [7-methoxy-3-phenyl-4-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]-2H-1-benzopyran-2-one], the benzofuran V [6-methoxy-2-phenyl-3-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]benzo[b]furan], the α-naphthofuran VI [2-phenyl-3-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl] naphtho[1,2-b]furan] and the β-naphthofuran VII [2-phenyl-1-[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]naphtho[2,1-b]furan]. The synthesis of centchroman and that of the cis-chroman has been reported earlier (9). The benzofuran and the isomeric naphthofurans were synthesised as described by Chawla et. al. (10). The coumarin was synthesised in the routine manner by reacting the parent phenol (11) with

pyrrolidinoethyl chloride in refluxing acetone in the presence of anh. $K_2CO_3$.

Tris-EDTA-sodium azide buffer (Tris-HCl 10 mmol; EDTA 1.5 mmol; sodium azide 0.02 %; pH 7.4) with 300 mmol sucrose (TES buffer), or without sucrose (TE buffer) was employed throughout the experiment. Dextran coated charcoal suspension (DCC) was prepared in TE buffer, as reported previously (12).

Preparation of the liver microsomal and the uterine cytosol fractions: The animals in groups of two each were sacrificed and their livers collected in ice cold saline. All subsequent operations were performed at 0-4°. The livers, washed with TES buffer, were minced and then homogenised gently in two volumes of the same buffer using a motor driven all glass homogeniser. The homogenates, adjusted to 10 ml per liver with TES buffer were centrifuged at 14,000 g for 30 min. The supernatants, designated as the microsomal fraction, were used directly for the competition experiments. The uterine cytosols were prepared from a different batch of animals using TE buffer, as reported earlier (12).

Competition experiments: The liver microsomal fractions were first incubated, at 4° for 2 hr, with 2 µm diethylstilbestrol, added in a small volume of DMF, to saturate the ER sites. 200 µl Aliquots of the fraction were then mixed, in pyrex glass tubes, with increasing amounts of the competitors, in triplicate, and fixed amount of radiolabelled tamoxifen, dissolved in 20 µl of 1:1 DMF-TES buffer, so as to have the final DMF concentration of 5 %, the radiolabelled tamoxifen concentration of $1x10^{-9}$ M and the competitor concentration varying between $1x10^{-9}$ and $3x10^{-6}$M, in the final volume of 220 µl. The tubes were incubated at 4° for 18-20 hr. DCC suspension (100 µl) was then added to each tube which were vortexed, kept for 15 min at 4° and then centrifuged at 1000 g for 15 min. The supernatants were counted for radioactivity. The data were plotted as the radioactivity bound versus log of the competitor concentration. The relative binding affinity (RBA) values were computed from these data as the ratio of the concentration of tamoxifen and that of a compound required to decrease the radioligand binding by 50 %, times 100.

The competition experiments with the uterine cytosol, using radiolabelled estradiol as the reference ligand, were performed at 4°, 18-20 hr incubation period, according to the microassay procedure reported in literature (13). The details will be reported elsewhere.

## RESULTS AND DISCUSSIONS

The chemical structures of the compounds used in this study are illustrated in Fig. 1. All these are closely related to the TAE antiestrogens, such as tamoxifen (I), in their chemical structure. The cis-chroman III, a geometric isomer of centchroman and the coumarin IV have previously

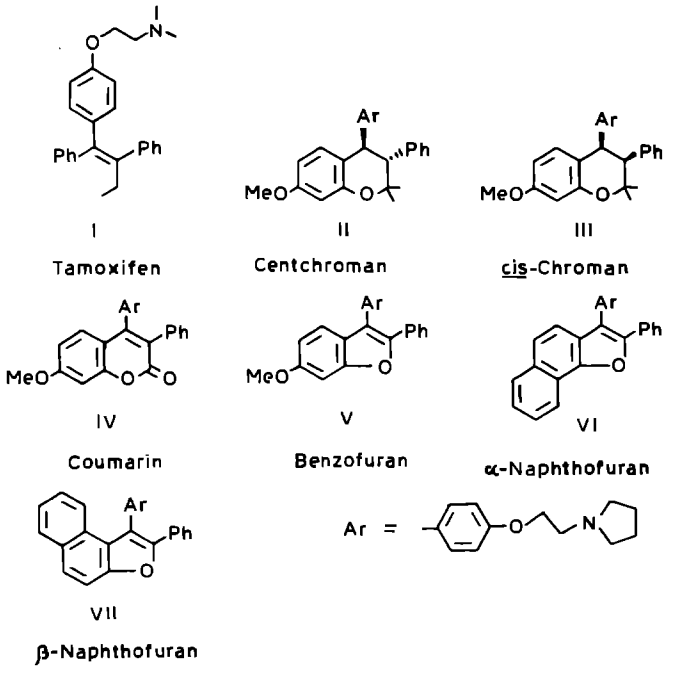


Figure 1 Chemical structures of the TAE antiestrogens and their analogs used.

been shown by us to be poor ER ligands (14,15). From their RBA values, now reevaluated and shown in Table 1, the cis-chroman is seen to be a poorer receptor ligand than the coumarin. Though a good receptor ligand, centchroman has been included here to illustrate the difference in the binding specificity of the AEBS and the ER. The benzofuran V and the isomeric naphthofurans VI and VII, also are relatively poor ER ligands (Table 1), though somewhat more potent than the cis-chroman. It is of note that the furans VI and VII, featuring an uncommon naphthalene moiety, are somewhat more potent receptor ligands than the benzofuran, which is related more closely to the TAE antiestrogens in its chemical structure.

A typical semilog plot, illustrating the competition offered by the test compounds to the AEBS interaction of

Table 1

Relative binding affinity of the test compounds for ER and AEBS

| Compound | | RBA-ER* | RBA-AEBS+ | RBA-AEBS / RBA-ER |
|---|---|---|---|---|
| I. | Tamoxifen | - | 100 | - |
| II. | Centchroman | 5.2† | 19± 5 | ~4 |
| III. | cis-Chroman | <0.001 | 46±17 | >46,000 |
| IV. | Coumarin | 0.01 | 32± 8 | ~3,200 |
| V. | Benzofuran | 0.01 | 23± 9 | ~2,300 |
| VI. | α-Naphthofuran | 0.04 | 19± 7 | ~500 |
| VII. | β-Naphthofuran | 0.2 | 8± 2 | ~40 |

* The values are expressed as percent of estradiol and are mean from at least three determinations.

\+ The values are mean ± SD from four independent determinations in each case.

† This value has been taken from Ref.14.

radiolabelled tamoxifen, is shown in Fig. 2. From their RBA values, shown in Table 1, it is apparent that all the compounds are reasonably good AEBS ligands, though somewhat

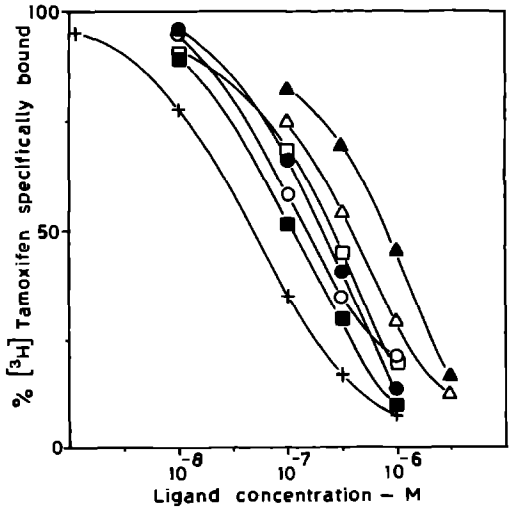


Figure 2 Competition of the TAE analogs for [$^3$H]-tamoxifen binding to AEBS in rat liver microsomes. Tamoxifen (+), centchroman (□), cis-chroman (■), coumarin (○), benzofuran (●), α-naphthofuran (△) and β-naphthofuran (▲).

less potent than tamoxifen. Among chromans, the cis-chroman is somewhat more potent, whereas the affinity of the coumarin is intermediate between that of the chromans. We have shown previously that the poor ER affinity of cis-chroman is due to the presence in this molecule of one of the methyl groups at C-2 and the attendant distortion in the conformation of its pyran ring, whereas it is the adverse effect of the carbonyl group present at the equivalent position which compromises the receptor affinity of the coumarin (15). These features are obviously not detrimental to AEBS interaction of the molecules. Among furans, the benzofuran and the α-naphthofuran are nearly equipotent, whereas the β-naphthofuran is marginally less potent than both these. It is of note that the trends in the AEBS and the ER affinity of the furans are opposite to each other.

From the ratio of their RBAs, for AEBS and ER, shown in Table 1, it is apparent that the cis-chroman is, for all practical purposes, a selective ligand for the AEBS since its relative affinity for this site is at least 46,000 times greater than for ER. The coumarin and the benzofuran on the other hand, with relatively higher ER affinities, show only a modest preference for AEBS interaction.

To conclude, we have presented here further evidence to substantiate that the ER and the AEBS possess a marked difference in their ligand specificities. The cis isomer of centchroman, having extremely poor ER affinity, has emerged as a good AEBS ligand. This compound can, for all practical purposes, be viewed as a selective ligand for the AEBS and could prove useful in the elucidation of the precise biochemical or physiological importance of this site.

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REFERENCES

1. Katzenellenbogen, B.S., Bhakoo, H.S., Ferguson, E.R., Lan, N.C., Tatee, T., Tsai, T.L.,and Katzenellenbogen, J.A. (1979) Rec. Prog. Horm. Res. 35, 259-300.
2. Sutherland, R.L., and Jordan, V.C. (1981) Nonsteroidal Antiestrogens, Academic Press, Sydney.
3. Sutherland, R.L., Murphy, L.C., Foo, M.S., Green, M.D., Whybourne, A.M., and Krozowski, Z.S. (1980) Nature 288, 273-275.
4. Murphy, L.C., and Sutherland, R.L. (1981) Biochem. Biophys. Res. Comm. 100, 1353-1360.
5. Sudo, K., Monsma, F.J., and Katzenellenbogen, B.S. (1983) Endocrinology 112, 425-434.
6. Kon, O.L. (1983) J. Biol. Chem. 258, 3173-3177.
7. Watts, C.K.W., and Sutherland, R.L. (1984) Biochem. Biophys. Res. Comm. 120, 109-115.
8. Pons, M., Michel, F., Crastes de Paulet, A., Gilbert, J., Miquel, J-F., Precigoux, G., Hospital, M., Ojasoo, T., and Raynaud, J-P., (1984) J. Steroid Biochem. 20, 137-145.
9. Ray, S., Grover, P.K., Kamboj, V.P., Setty, B.S., Kar, A.B., and Anand, N. (1976) J. Med. Chem. 19, 276-279.
10. Chawla, H.P.S., Grover, P.K., Anand, N., Kamboj, V.P., and Kar, A.B. (1970) J. Med. Chem. 13, 54-59.
11. Ray, S., Grover, P.K., and Anand, N. (1971) Indian J. Chem. 9, 619-623.
12. Garg, S., Bindal, R.D., Durani, S., and Kapil, R.S. (1983) J. Steroid Biochem. 18, 89-95.
13. Katzenellenbogen, J.A., Johnson, Jr., H.J., and Myers, H.N. (1973) Biochemistry 12, 4085-4092.
14. Durani, S., Agarwal, A.K., Saxena, R., Setty, B.S., Gupta, R.C., Kole, P.L., Ray, S., and Anand, N. (1979) J. Steroid Biochem. 11, 67-77.
15. Durani, S., and Anand, N. (1981) Internat. J. Quantum Chem. 20, 71-83.