Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 16, pp. 2131-2136, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00379-X

EVALUATION OF PIPERIDINOETHOXY MOIETY AS AN ANTIESTROGENIC SUBSTITUENT IN NON-STEROIDAL ANTI-ESTROGENS: FERTILITY REGULATION^

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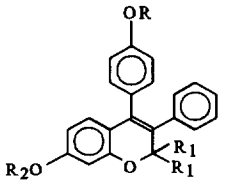
Abstract: A piperidinoethoxy substituent in non-steroidal antiestrogens has a relatively higher antiestrogenic effect as compared to a pyrrolidinoethoxy moiety. However, the antagonistic activity is more depended on the molecular geometry than the nature of the basic chain. No significant difference in the antifertility activity in these two sets of compounds was observed. © 1997 Elsevier Science Ltd.

A non-steroidal antiestrogenic molecule generally has both agonistic and antagonistic properties. The ratio of these two activities is very crucial for their different pharmaceutical uses. Whereas a potent antagonistic effect is needed for the treatment of breast cancer , a significant estrogenic property for a fertility inducer, a mild estrogenic effect on bone estrogen receptor for the treatment of osteoporosis, an appropriate ratio of the two is possibly required for a pregnancy inhibitor.Thus, in the molecular designing of non-steroidal antiestrogens as a pharmaceutical, a knowledge of the impact of various substituents on their agonist-antagonist activities is very important. A number of non-steroidal antiestrogens substituted with various aminoalkoxy side chains, wherein it plays a pivotal role, have been reported in the literature[1].The pyrrolidinoethoxy chain has been claimed to be superior to others, by different groups[2,3], whereas two of the potent antiestrogenic compounds raloxifene[4] (**22**) and 85/287[5] (**19**) carry a piperidine moiety in their antiestrogenic (aminoalkoxy) side chain. To study the effect of the piperidinoethoxy moiety toward antiestrogenic and antifertility activities of a molecule, the pyrrolidine residue from earlier reported[6] benzopyran derivatives has now been substituted with a piperidine moiety and their biological activities compared.

Preparation of compounds: Compound studied are given in Fig.1. 2,2-Dimethyl-3-phenyl-4-(p-hydroxy phenyl)-7-methoxychromene (**1**)[6] was alkylated with 2-chloroethyl piperidine under basic conditions to furnish piperidinoethyl ether derivative **4**. Similarly alkylation of corresponding 3,4- *cis* chroman **2**[6] & 3,4-*trans* chroman **11**[6] gave piperidinoethoxy derivatives **8** and **13** respectively. Demethylation of the 7-methoxy group was achieved by treatment with strong alkali in the presence of hydrazine hydrate in refluxing diethylene glycol[7]. Similarly hydroxy chromene derivatives **5** & **6** and *trans* hydroxychromans **14** & **15** were obtained. This latter step however, led to simultaneous isomerisation of the *cis* chroman to *trans* chroman. Therefore, preparation of 3,4-*cis*-2,2-dimethyl-3-phenyl-4-(4-(2-pyrrolidinoethoxyphenyl))-7-hydroxychroman (**9**) and 3,4-*cis*-2,2-dimethyl-3-phenyl-4-(4-

^ CDRI communication no. 5621



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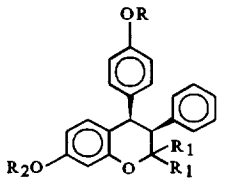
5. R=$(CH_2)_2NC_4H_8$ R_1=Me ; R_2=H
6. R=$(CH_2)_2NC_5H_{10}$ R_1=Me ; R_2=H
16. R=$(CH_2)_2NC_4H_8$ R_1=H ; R_2=Me
17. R=$(CH_2)_2NC_5H_{10}$ R_1=H ; R_2=Me



2. R=H R_1=R_2=Me
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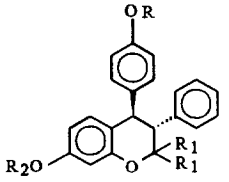
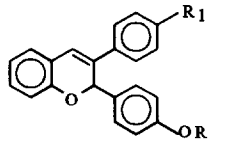
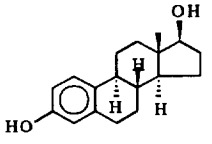
8. R=$(CH_2)_2NC_5H_{10}$ R_1=R_2=Me
9. R=$(CH_2)_2NC_4H_8$ R_1=Me ; R_2=H
10. R=$(CH_2)_2NC_5H_{10}$ R_1=Me ; R_2=H



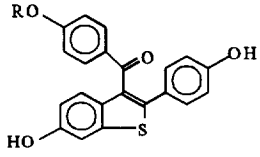
11. R=H R_1=R_2=Me
12. R=$(CH_2)_2NC_4H_8$ R_1=R_2=Me
13. R=$(CH_2)_2NC_5H_{10}$ R_1=R_2=Me
14. R=$(CH_2)_2NC_4H_8$ R_1=Me ; R_2=H
15. R=$(CH_2)_2NC_5H_{10}$ R_1=Me ; R_2=H



18. R=$(CH_2)_2NC_4H_8$ R_1=H
19. R=$(CH_2)_2NC_5H_{10}$ R_1=H
20. R=$(CH_2)_2NC_5H_{10}$ R_1=OMe



Estradiol



21. R=$(CH_2)_2NC_4H_8$
22. R=$(CH_2)_2NC_5H_{10}$

Figure 1 : Identity of compounds studied.

(2-piperidino ethoxyphenyl))-7-hydroxychroman (**10**) was achieved through catalytic hydrogenation of corresponding chromene derivatives **5** and **6**, respectively. All new compounds gave desired elemental analyses and were further characterized on the basis of their spectral data (^{1}HNMR, IR, Mass). Computergraphic study was done using Alchemy III, purchased from Aldrich.

Biological Assays: Antifertility and estrogenic activities were assessed according to the procedure adopted by Dhar et al [8]. In short, adult female rats of Sprague-Dawley strain were caged overnight with coeval males of proven fertility. The day vaginal smear showed presence of sperm was considered as day 1 of pregnancy. Different groups of mated females were fed with different compounds, as gum acacia aqueous suspension, either on day 1 or days 1-5/1-7 post coitum. Control group was administered the vehicle alone. At the time of laparotomy, on day 11, number of implantation sites and corpora-lutea were recorded.

For estrogenic activity, 21 days old female rats were bilaterally ovariectomized and after a rest period of 7 days, graded doses of compounds were administered to these rats for three consecutive days except for animals of one group which were fed with the vehicle alone and served as control. Animals of all groups were autopsied 24 h after the last treatment. Their uterine weight and status of vaginal opening/vaginal smears noted.

Table- 1

Compd. No.	Class of Compound	R	R_1	R_2	Antifertility activity MED_{100} (rat) mg/kg	RBA
3.	Chromene	$-(CH_2)_2NC_4H_8$	Me	Me	5	0.86 ± 0.10
4.	Chromene	$-(CH_2)_2NC_5H_{10}$	Me	Me	10	0.53 ± 0.04
5.	Chromene	$-(CH_2)_2NC_4H_8$	Me	H	15	9.2 ± 1.16
6.	Chromene	$-(CH_2)_2NC_5H_{10}$	Me	H	Inactive at 5	2.11 ± 0.15
7.	3,4-cis Chroman	$-(CH_2)_2NC_4H_8$	Me	Me	Inactive at 10	0.16 ± 0.02
8.	3,4-cis Chroman	$-(CH_2)_2NC_5H_{10}$	Me	Me	90% active at 10	N.D[a]
9.	3,4-cis Chroman	$-(CH_2)_2NC_4H_8$	Me	H	Inactive	N.D[a]
10.	3,4-cis Chroman	$-(CH_2)_2NC_5H_{10}$	Me	H	Inactive	N.D[a]
12.	3,4-trans Chroman	$-(CH_2)_2NC_4H_8$	Me	Me	0.25	5.24 ± 1.45
13.	3,4-trans Chroman	$-(CH_2)_2NC_5H_{10}$	Me	Me	0.1	3.77 ± 0.25
14.	3,4-trans Chroman	$-(CH_2)_2NC_4H_8$	Me	H	0.25	112 ± 18
15.	3,4-trans Chroman	$-(CH_2)_2NC_5H_{10}$	Me	H	0.2	50.11±12.5
16.	Chromene	$-(CH_2)_2NC_4H_8$	H	Me	0.1	7.35 ± 1.8
17.	Chromene	$-(CH_2)_2NC_5H_{10}$	H	Me	0.25	1.45 ± 0.15

a = not detectable

In antiestrogen assay, different groups of rats were given daily dose of estradiol-17β (E_2, 0.1 or 1.0 μg, *s.c.*) or ethynyl estradiol (EE, 1μg, *p.o.*) and they were subjected to simultaneous treatment with test compound. One group of rats served as control and two groups of rats received E_2/EE alone. On day 4, i.e., 24 h after the last treatment, the animals were examined for vaginal opening, smears taken and then autopsied. The uterine tissue was removed, freed of fat and, after expulsion of fluid, weighed. Inhibition of uterotrophic activity (% inhibition) was computed as(E-Ce/E-V)x100, where V, E and Ce refer to the mean uterine weights from animals treated with the vehicle alone, with E_2/EE alone, and with a given compound along with E_2/ EE respectively.

Results and discussion: Antifertility activity and Relative Binding Affinity (RBA) values of compounds are given in the Table-1. Apparently there is no significant difference in the antiimplantation activity and RBA of the piperidinoethoxy compounds as compared to their corresponding pyrrolidinoethoxy counterparts. In the 2,2 dimethyl series, as reported[6] earlier for pyrrolidinoethoxy compounds, higher order of antifertility activity was observed in 3,4- *trans* chromans (**12 - 15**) which was followed by chromenes (**3-5**). 3,4- *cis* chromans (**7 - 10**) were in general inactive or marginally active. Compounds with free phenolic groups (**5, 6, 14, 15**) showed significant RBA which were many fold higher than their corresponding methylated derivatives (**3, 4, 12, 13**). This would suggest that a free phenolic group at C-7 is essential for an effective binding to the estrogen receptor.

Compound **13** which corresponds to centchroman (**12**), a recently introduced oral contraceptive[9], was studied in greater details. At the contraceptive dose (0.1mg/kg), on day 1-5 schedule, it failed to stimulate the uterine weight gain but in a single day dose schedule (1.5 mg/ kg), there were 3 fold increases in the uterine weight. At none of the doses, compound **13**, induced vaginal cornification as it occurs with centchroman.

The desmethyl derivative **16** having a pyrrolidine residue, reported by Merck group has been found to be estrogenic[6]. It inhibits implantation in rats at 0.1 mg/kg dose in a 1-5 day schedule. The corresponding piperidinoethoxy compound **17,** now prepared, though marginally less active in preventing implantation, did not cause cornification even up to 1.0 mg/kg /day doses. At the contraceptive dose (0.25 mg/kg) it produced 16% lowering of the uterine weight gain induced by ethynyl estradiol(1 μg/day, *p.o.*).

Similarly, profound antiestrogenic effect of the piperidine residue containing molecule raloxifene[4] (**22**) has been reported. Raloxifene is being developed by Eli Lilly & Co., USA, for the treatment of osteoporosis. Structure activity relationship[10] (SAR) study with Raloxifene related compounds showed that it was less uterotrophic and more antiuterotrophic as compared to the corresponding pyrrolidino compound **21**.

Another potent antiestrogen 85/287 (**19**), developed by this Institute, also elicited lesser uterotrophic activity and more pronounced antiestrogenic effect as compared to its corresponding pyrrolidinoethoxy compound **18**[5].

Interestingly, compound **20**, which is a positional isomer of compound **17**, with a methoxy group attached to a pendant phenyl ring, has been reported [11] to cause 76% inhibition of uterine weight gain. A computer graphic study of these two compounds by overlapping them onto estradiol molecule separately (Fig. 2) would suggest that

for the compounds **17** & **20** to bind to the receptor their methoxy substituents would occupy the position onto the receptor where the phenolic group of estradiol binds, thereby projecting the piperidinoethoxy group in space in the antiestrogen binding region as proposed in our receptor model[12].

Superimposition of compounds **17** and **20**, keeping their estrogenic binding regions in similar orientation, shows considerable similarity in their molecular geometry and therefore similar hormonal activity. A better molecular fit at the estradiol binding region and proper disposition of the basic residue at the antiestrogenic subunit of the receptor, determines the potency of estrogen antagonistic activity of a molecule.

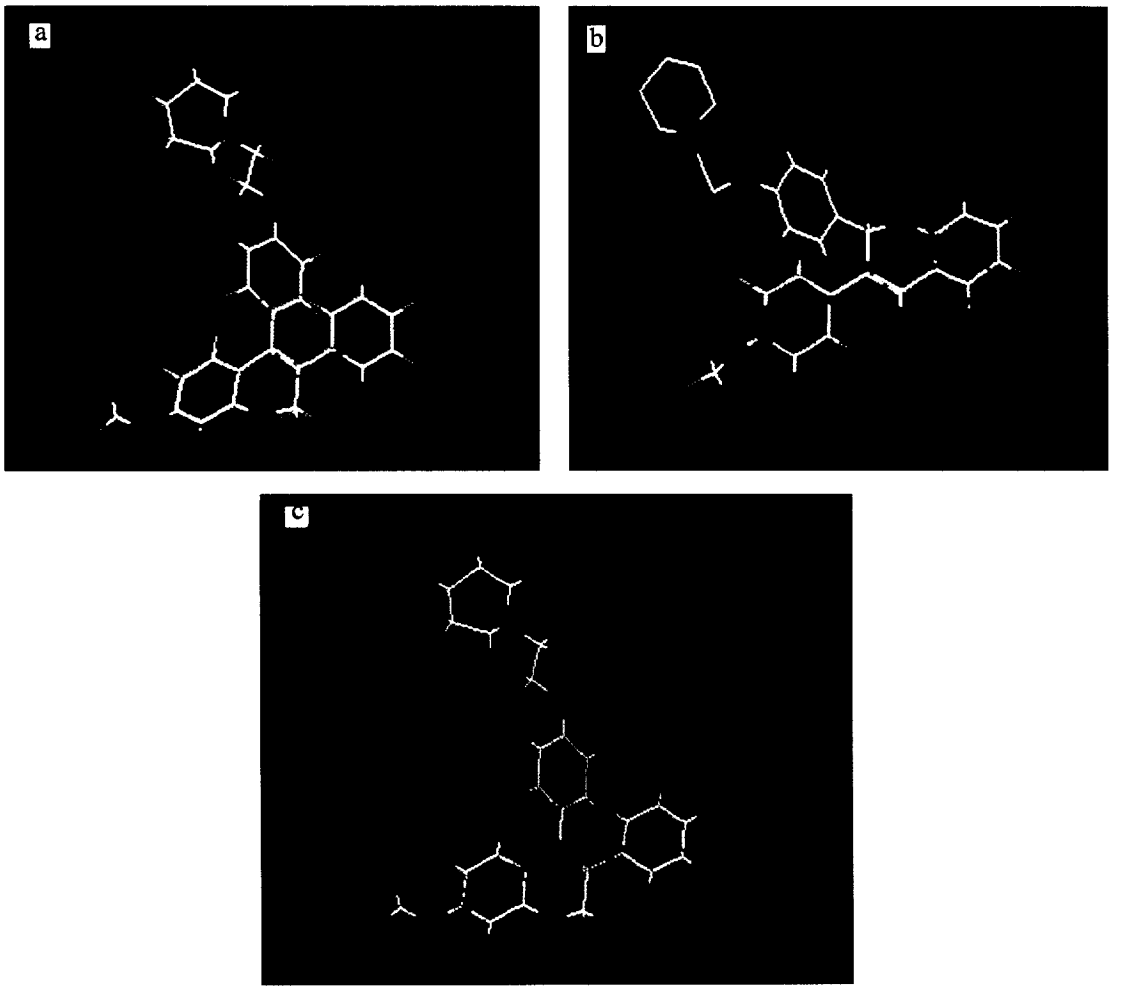


Fig.2 Superimposition of molecules showing common binding regions a. compound **17** & estradiol, b. compound **20** & estradiol and c. compounds **17** & **20**.

Out of a large number of non-steroidal antiestrogens , carrying various substituted aminoalkyl residues, only centchroman has been accepted as a drug for fertility regulation. The reason for such fall outs is their unacceptable side effects for which a major reason is possibly related to the ratio of their estrogenic and antiestrogenic properties. Since estrogen antagonistic activity of a molecule is due to the presence of the amino alkyl chain, its nature plays a key role. This present study shows that a piperidinoethoxy chain does not lower the antifertility potential of a compound when substituted in place of the commonly used pyrrolidinoethoxy residue. However, this chain produces more pronounced antiestrogenic effect. Whether this difference is of advantage in designing contraceptive agents is to be seen.

Acknowledgment : Financial support received to ST from ZymoGenetics is acknowledged.

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(*Received in Japan* 7 *April* 1997; *accepted* 14 *July* 1997)