Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 4317–4320

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

4-(4-Alkylpiperazin-1-yl)phenyl Group: A Novel Class of Basic Side Chains for Selective Estrogen Receptor Modulators

Nobuhide Watanabe,[a,*] Hiroshi Nakagawa,[a] Akihisa Ikeno,[b] Hisao Minato,[b] Chie Kohayakawa[b] and Jun-ichi Tsuji[b]

[a]*Chemistry Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki-cho 33-94, Suita, Osaka 564-0053, Japan*
[b]*Pharmacology & Microbiology Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki-cho 33-94, Suita, Osaka 564-0053, Japan*

Received 2 September 2003; revised 20 September 2003; accepted 22 September 2003

Abstract—In the structure–activity relationships of spiro[indene-1,1′-indane] series, the 4-(4-alkylpiperazin-1-yl)phenyl group was found to be functionally equipotent to the 4-(1-piperidinoethoxy)phenyl group, the most widely used basic side chain in selective estrogen receptor modulators.

Introduction

With the advent of tamoxifen (TAM) and raloxifene (RAL), selective estrogen receptor modulators (SERMs) have shown high therapeutic potential for estrogen-related diseases.[1] Thus, TAM, the first SERM to be approved for the prevention of breast cancer in high-risk women, is effective for hormone-dependent breast cancer, whereas RAL is prescribed for the prevention and treatment of osteoporosis in postmenopausal women.[2] Along this line, several other SERMs such as lasofoxifene,[3] EM-652,[4] and ERA-923[5] are enjoying the confidence of good efficacy in clinical trials, and further investigations for additional diversities and new scaffolds that confer improved pharmacological profiles are continuing (Fig. 1).

A key to identifying new SERMs is to design a pharmacophore that can be responsible for estrogen antagonist activity in reproductive tissues, as is evident from clinical data showing that HRT and TAM have increased risks of breast and endometrial cancers respectively.[2b,6] It has been reported that the basic side chain (BSC) in RAL plays a critical role in RAL's estrogen antagonist activity in the uterus and that the nature of substituents on the nitrogen atom of BSC considerably affects this antagonism.[7] Moreover, X-ray crystal structure analysis of ERα ligand binding domain complexed with RAL or 4-hydroxyTAM, an active metabolite of TAM, seems to elucidate the mechanism of these SERMs antagonist activity.[8] Thus, BSC has been demonstrated to protrude out of the ligand binding pocket formed by helices 3,5/11, thereby inducing

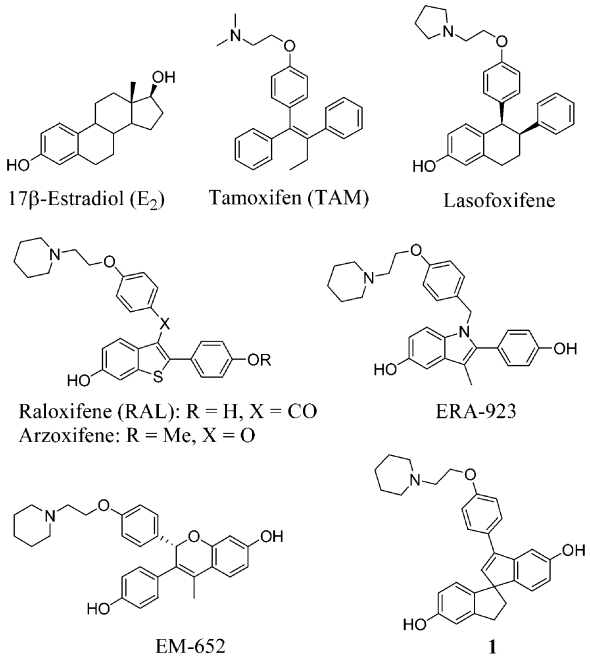


Figure 1. Structure of 17β-estradiol and SERMs.

*Corresponding author. Tel.: +81-663-375-906; fax: +81-663-387656; e-mail: nobuhide-watanabe@dainippon-pharm.co.jp

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.09.049

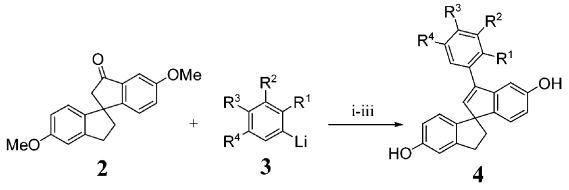
conformational changes that block interaction of ER with coactivator proteins. Despite the importance of BSC, efforts for the development of new SERMs, have only been focused on finding scaffolds such as indoles and tetrahydronaphthalenes.[3–5,9]

We have recently found (+)-3-[4-(1-piperidinoethoxy) phenyl]-spiro[indene-1,1′-indane]-5,5′-diol hydrochloride [(+)-**1**] as a promising non-steroidal estrogen receptor agonist for the treatment of hot flush.[10] In order to enhance the antagonist activity of (+)-**1** while maintaining its unique pharmacological profile, logical extension of our structure–activity relationship (SAR) studies was centered on modification of the 4-(1-piperidinoethoxy)phenyl moiety, the most widely used BSC. Herein, we report the results of our SAR studies and a novel class of BSCs, modification of which showed wide tolerance for ER in terms of in vivo potency as well as binding affinity.

Results and Discussion

Chemical synthesis

The starting ketone **2**, **1** and (+)-**1** were prepared as described previously.[10] BSCs were incorporated by addition of the corresponding aryllithium reagents **3a–r** and subsequent dehydration with TsOH followed by demethylation with Ph_2PLi to give **4a–r** in yields ranging from 32 to 56% in three steps (Scheme 1).



a: $R^1 = R^2 = R^4 = H$, R^3 = 1-(4-methylpiperidinyl)CH_2CH_2O
b: $R^1 = R^2 = R^4 = H$, R^3 = 1-piperidino$CH_2CH_2CH_2O$
c: $R^1 = R^2 = R^4 = H$, R^3 = 1-pyrrolidinoCH_2CH_2O
d: $R^1 = R^2 = R^4 = H$, $R^3 = Me_2NCH_2CH_2O$
e: $R^1 = R^2 = R^4 = H$, $R^3 = Et_2NCH_2CH_2O$
f: $R^1 = R^2 = R^4 = H$, R^3 = 1-piperidino$CH_2CH_2CH_2$
g: $R^1 = R^4 = H$, R^2 = Me, R^3 = 1-piperidinoCH_2CH_2O
h: R^1 = Me, $R^2 = R^4 = H$, R^3 = 1-piperidinoCH_2CH_2O
i: $R^1 = H$, $R^2 = R^4$ = Me, R^3 = 1-piperidinoCH_2CH_2O
j: $R^1 = R^4 = H$, R^2 = F, R^3 = 1-piperidinoCH_2CH_2O
k: $R^1 = R^3 = R^4 = H$, R^2 = 1-piperidinoCH_2CH_2O
l: $R^1 = R^2 = R^4 = H$, R^3 = 1-(4-methylpiperazinyl)
m: $R^1 = H$, $R^2 = H$, R^3 = 1-(4-ethylpiperazinyl)
n: $R^1 = R^2 = R^4 = H$, R^3 = 1-(4-n-propylpiperazinyl)
o: $R^1 = R^2 = R^4 = H$, R^3 = 1-(4-i-propylpiperazinyl)
p: $R^1 = R^2 = R^4 = H$, R^3 = 1-(4-i-butylpiperazinyl)
q: $R^1 = R^2 = R^4 = H$, R^3 = 4-(1-piperidinyl)
r: $R^1 = R^2 = R^4 = H$, R^3 = 1-(4-ethylhomopiperazinyl)

Scheme 1. Synthesis of **4a–r**: (i) THF, −78 °C; (ii) TsOH, toluene, reflux; (iii) Ph_2PH, *n*-BuLi, THF, reflux.

Binding assay

Compounds binding affinity for ER was determined by a competitive radiometric binding assay in human breast cancer (MCF-7) cytosol, using [^{3}H]-17β-estradiol as tracer. Each binding affinity value is expressed as relative binding affinity (RBA), where the affinity of the parent compound **1** (K_i = 25.3 nM) is considered to be 100%.

Cholesterol-lowering effect

Compounds cholesterol-lowering effects were estimated from the mean percent decrease of plasma cholesterol level relative to vehicle as follows: Sprague–Dawley (SD) rats (6–8 weeks old) were ovariectomized and orally treated for 4 days with test compounds at a dose of 1 mg/kg/day.

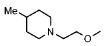
Uterotrophic effect

Compounds uterotrophic effects were estimated from the mean percent increase in uterine wet weight relative to vehicle (method 1) or sham (method 2) in two ways: (**1**) SD rats (14 weeks old) were ovariectomized and orally treated for 4 days with test compounds at a dose of 1 mg/kg/day (Tables 1 and 2). (2) SD rats (21 day old) were orally treated for 4 days with test compounds at the indicated doses. Uteri were removed and weighed (Fig. 2).

Among the complex biological activities of SERMs, their cholesterol-lowering ability seems to be the primary criterion for compounds selection not only because SERMs are expected to have beneficial effects on lipid metabolism but also because ED_{50} values for RAL analogues activity have been reported to highly correlate with RBA for ER.[11] Therefore, in this study, we used cholesterol-lowering ability to evaluate in vivo beneficial effects of compounds. As can been seen from Table 1, an alkyl group on the nitrogen atom and a methylene tether in the BSC moiety were well tolerated in terms of binding affinity (**1**, **4a–f**). In particular, replacement of the oxygen atom by a methylene led to approximately 10-fold increase in binding affinity (**4f**). However, as was not anticipated from SARs of other SERMs,[12] these subtle modifications resulted in significant reduction in cholesterol-lowering effect compared to the prototype **1**. As for the pendant phenyl group, additional substitution by a methyl or fluoro group and alternate placement of the 1-piperidinoethoxy moiety at the R^2 position led to reduction in cholesterol-lowering effect (**4g–k**). The inconsistent correlation between binding affinity and in vivo activity for this series prompted us to identify a new class of BSCs that have distinct physical properties to improve pharmacokinetic profiles of compounds.

Given the significance of the tertiary basic nitrogen atom and the ethoxy tether length in the BSC,[13] we became interested in piperazine structure as a constrained aminoethoxy moiety, and then tested **4l–r** for binding affinity (Table 2). As expected, all compounds

Table 1. Binding affinity and in vivo activity of compounds **1** and **4a–k**

Compd	R^1	R^2	R^3	R^4	RBA	Plasma cholesterol (%decr.)
1	H	H		H	100	44[a]
4a	H	H		H	428	18
4b	H	H		H	460	8.5
4c	H	H		H	118	17
4d	H	H		H	85	6.0
4e	H	H		H	153	18
4f	H	H		H	956	6.0
4g	H	Me		H	122	4.5
4h	Me	H		H	70	−12
4i	H	Me		Me	22	−9.1
4j	H	F		H	247	16
4k	H		H	H	47	NT[b]

[a]statistically significant difference ($p < 0.05$).
[b]NT, not tested.

Table 2. Binding affinity, in vivo activity and uterotrophic effect of compounds **4l–r**

Compd	R^{3a}	RBA	Plasma cholesterol[b]	Uterotropic effect[c]
4l		207	NT[d]	NT[d]
4m		181	52*	208
4n		186	55*	223
4o		103	21	NT[c]
4p		42	87*	185
4q		125	51*	139[e]
4r		387	32[f]*	114
1				220
RAL				155
TAM				244

[a]$R^1 = R^2 = R^4 = H$.
[b]The values are expressed as mean percent decrease relative to vehicle. Statistically significant ($p < 0.001$) differences are denoted by an asterisk (*).
[c]Fourteen-week-old SD rats were used in this experiment (see text).
[d]NT, not tested.
[e]An increase dose (10 mg/kg/day) of **4q** produced higher increase (26.5%) in uterine weight than that of **1** (211%).
[f]No standard dose–response curve was observed.

with small alkyl substituents at N(4) showed good RBA and high potency in cholesterol-lowering effect (**4l–n**). Substitution with bulky alkyl groups such as *iso*-propyl and *iso*-butyl led to a modest decrease in binding affinity (**4o–p**). Interestingly, in contrast to its RBA, **4p** showed the highest potency in cholesterol-lowering effect with 87% decrease at a dose of 1 mg/kg/day. In addition, enlargement of the piperazine ring size or replacement of N(1) nitrogen by a methylene maintained a good binding affinity (**4q–r**). With these results in hand, we next examined estrogen antagonist effects of these compounds on the uterus. In ovariectomized rat model, oral administration (1 mg/kg/day) of test compounds produced acceptable estrogen antagonist effects on the uterus compared to RAL and TAM. Of the new compounds, **4r** was the most potent estrogen antagonist with only 14% increase in uterine weight relative to controls. However, the cholesterol-lowering effect of **4r** was weaker than what was anticipated from its RBA, and did not conform to a standard dose–response curve, which may indicate the involvement of an unidentified pathway. On the other hand, while **4q** showed potent estrogen antagonist activity at 1 mg/kg/day, an increase in its dose (10 mg/kg/day) produced higher increase in uterine weight than that of **4m**. Taken together, **4m** was chosen as a promising alternative to (+)-**1**, an orally bioavailable enantiomer of **1**, and was finally tested in an immature rat model. While **4m** showed no improvement over (+)-**1** in terms of intrinsic estrogen antagonistic activity (Fig. 2), all data for **4m** demonstrated that the 4-(4-ethylpiperazin-1-ly)phenyl moiety was

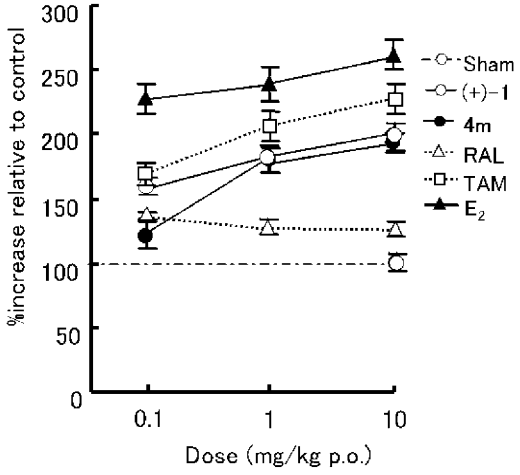


Figure 2. Uterotrophic effect of selected compounds in an immature rat model.

functionally equipotent to the 4-(1-piperidinoethoxy)-phenyl moiety in the spiro[indene-1,1′-indane] series. As the compounds tested in this study were in a racemic form, rationalization of the results obtained should be taken into account for the possibility that each enantiomer has different biological activity. Our studies with **1** and (+)-**1** have, thereby, indicated that the racemate **1** displays the same qualitative profile of activity, with one enantiomer [i.e., (+)-**1**] possessing the majority of the biological effects.[10] Further studies on the biological effects of both enantiomers of these compounds will be reported in due course.

In conclusion, we have investigated SARs of **1** centered on modification of its BSC and devised 4-(4-alkylpiperazin-1-yl)phenyl group as a constrained aminoethoxy side chain. Our SAR studies demonstrated considerable tolerance for modification around the piperazine ring region in this series of compounds. Of the compounds tested, **4m** was found to have beneficial effect on lipid metabolism while maintaining marginal estrogen antagonist effect on the uterus. These findings imply that 4-(4-alkylpiperazin-1-yl)phenyl group may serve as novel BSCs that elicit reasonable pharmacological profiles in other classes of SERMs.[11]

References and Notes

1. (a) Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 883. (b) Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 1081. (c) Riggs, B. L.; Hartmann, L. C. *N. Engl. J. Med.* **2003**, *348*, 618.
2. (a) Fisher, B.; Costantino, J. P.; Wickerham, D. L.; Redmond, C. K.; Kavanah, M.; Cronin, W. M.; Vogel, V.; Robidoux, A.; Dimitrov, N.; Atkins, J.; Daly, M.; Wieand, S.; Tan-Chiu, E.; Ford, L.; Wolmark, N., other National Surgical Adjuvant Breast and Bowel Project Investigators *J. Natl. Cancer Inst.* **1998**, *90*, 1371. (b) Plouffle, L., Jr.; Facog, C. M. *J. Soc. Gynecol. Invest.* **2000**, *7*, S38.
3. Rosati, R. L.; Jardine, P. D. S.; Cameron, K. O.; Thompson, D. D.; Ke, H. Z.; Toler, S. M.; Brown, T. A.; Pan, L. C.; Ebbinghaus, C. F.; Reinhold, A. R.; Elliot, N. C.; Newhouse, B. N.; Tjoa, C. M.; Sweetman, P. M.; Cole, M. J.; Arriola, M. W.; Gauthier, J. W.; Crawford, D. T.; Nickerson, D. F.; Pirie, C. M.; Qi, H.; Simmons, H. A.; Tkalcevic, G. T. *J. Med. Chem.* **1998**, *41*, 2928.
4. Labrie, F.; Labrie, C.; Belanger, A.; Simard, J.; Giguere, V.; Tremblay, G. J. *Ster. Biochem. Mol. Biol.* **2001**, *79*, 213.
5. Miller, C. P.; Harris, H. A.; Komm, B. S. *Drugs* **2002**, *27*, 117.
6. Collaborative group on hormonal factors in breast cancer and hormone replacement therapy *Lancet* **1997**, *350*, 1047.
7. Grese, T. A.; Sluka, J. P.; Bryant, H. U.; Cullinan, G. J.,; Glasebrook, A. L.; Jones, C. D.; Matsumoto, K.; Palkowitz, A. D.; Sato, M.; Termine, J. D.; Winter, M. A.; Yang, N. N.; Dodge, J. A. *Proc. Natl. Acd. Sci. U.S.A.* **1997**, *94*, 14105.
8. (a) Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engström, O.; Öhman , L.; Greene, G. L.; Gustafsson, J.-A.; Carlquist, M. *Nature* **1997**, *389*, 753. (b) Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. *Cell* **1998**, *95*, 927.
9. For recent other examples, see: (a) Jørgensen, A. S.; Jacobsen, P.; Christiansen, L. B.; Bury, P. S.; Kanstrup, A.; Thorpe, S. M.; Bain, S.; Nærum, L.; Wassermann, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 399. (b) Kim, Y.-W.; Mobley, J. A.; Brueggemeier, R. B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1475, and references cited therein.
10. Watanabe, N.; Ikeno, A.; Minato, H.; Nakagawa, H.; Kohayakawa, C.; Tsuji, J. *J. Med. Chem.* **2003**, *46*, 3961.
11. Kauffman, R. F.; Bensch, W. R.; Roudebush, R. E.; Cole, H. W.; Bean, J. S.; Phillips, D. L.; Monroe, A.; Cullinan, G. J.; Glasebrook, A. L.; Bryant, H. U. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 146. In addition, most of current SERMs have similar beneficial effects on lipid and bone metabolism. Therefore, the potency in cholesterol-lowering effect may correlate to that in preventive effect on osteoporosis; see: Levenson, A. S.; Kliakhandler, I. L.; Svoboda, K. M.; Pease, K. M.; Kaiser, S. A.; Ward, J. E., III; Jordan, V. C. *Br. J. Cancer* **2002**, *87*, 449.
12. (a) Grese, T. M.; Cho, S.; Finley, D. R.; Godfrey, A. G.; Jones, C. D.; Lugar, C. H., III; Martin, M. J.; Matsumoto, K.; Pennington, L. D.; Winter, M. A.; Adrian, M. D.; Cole, H. W.; Magee, D. E.; Phillips, D. L.; Rowley, E. R.; Short, L. L.; Glasebrook, A. L.; Bryant, H. U. *J. Med. Chem.* **1997**, *40*, 146. (b) Grese, T. M.; Pennington, L. D.; Sluka, J. P.; Adrian, M. D.; Cole, H. W.; Fuson, T. R.; Magee, D. E.; Phillips, D. L.; Rowley, E. R.; Shetler, P. K.; Short, L. L.; Venugopalan, M.; Yang, N. N.; Sato, M.; Glasebrook, A. L.; Bryant, H. U. *J. Med. Chem.* **1998**, *41*, 1272.
13. (a) Levenson, A. S.; Jordan, V. C. *Cancer Res.* **1998**, *58*, 1872. (b) Jordan, V. C.; Schafer, J. M.; Levenson, A. S.; Liu, H.; Pease, K. M.; Simons, L. A.; Zapf, J. W. *Cancer Res.* **2001**, *61*, 6619. (c) Fahrnow, M.; Egner, A.; Curr, U. *Opin. Biotech.* **1999**, *10*, 550.