Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 7, pp. 903-908, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00141-2

BENZOPYRAN SELECTIVE ESTROGEN RECEPTOR MODULATORS (SERMS): PHARMACOLOGICAL EFFECTS AND STRUCTURAL CORRELATION WITH RALOXIFENE

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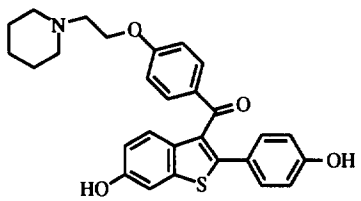
Abstract: Several 2,3-diarylbenzopyrans have been evaluated in an ovariectomized rat model and found to exhibit tissue selective estrogen agonist activity on bone and serum lipid parameters. A structural model that accounts for the pharmacological similarity of these benzopyrans and the benzothiophene SERM, raloxifene, is proposed.

The postmenopausal decline in the level of circulating ovarian steroids has been linked to a number of pathologies, particularly osteoporosis and coronary artery disease.[1,2] Although estrogen replacement therapy has demonstrated effectiveness in reducing the risks associated with these pathologies, concerns relating to the increased risk of endometrial[3] and breast cancer[4] have led to the search for treatment alternatives.



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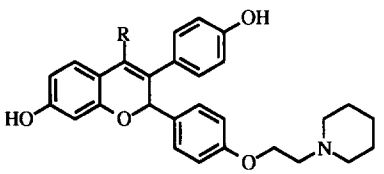


2 R = H
3 R = Me

Recently, several laboratories have described molecules which antagonize the effects of estrogen on uterine and breast tissue, while mimicking the effects of estrogen on bone and the cardiovascular system.[5-7] The term Selective Estrogen Receptor Modulator (SERM)[8] has been coined to describe these agents, and at least one such compound (raloxifene, **1**) is currently in advanced clinical trials for the prevention and treatment of osteoporosis.[9]

A structurally distinct series of estrogen receptor modulators containing a benzopyran nucleus (i.e. **2** or **3**) have recently been described in the literature.[10,11] Some of these compounds, which share a number of structural features with raloxifene, have been classified as "pure" estrogen antagonists.[12] Herein we describe the potent tissue-selective agonist effects of these compounds in an ovariectomized (OVX) rat model of post-menopausal osteoporosis which necessitates their reclassification as SERMs. We also propose a structural hypothesis that accounts for their pharmacological similarity to raloxifene.

The compounds were prepared as previously described.[10,11,13] Tissue-specific estrogen agonist effects were examined in an OVX rat model,[5] utilizing uterine weight, uterine eosinophil peroxidase (EPO)

activity,[14] serum cholesterol levels, and bone density as endpoints. Specifically, 75-day old OVX Sprague-Dawley rats were dosed daily (oral) for 4 days, commencing 2 weeks after ovariectomy. Vehicle (20% β-hydroxycyclodextrin) treated sham, OVX control, OVX/17α-ethynyl estradiol (100 mg/kg; PO) treated control groups were included in each experiment. The compounds were tested at 3 or 4 doses, with n = 5 for all experimental and control groups. Graphical representations of the dose/response for uterine weight increase, EPO activity, and percent decrease in serum cholesterol are provided in Figures 1a, b, and c. The compounds were then further evaluated in a 5 week, OVX rat model in which effects on skeletal parameters

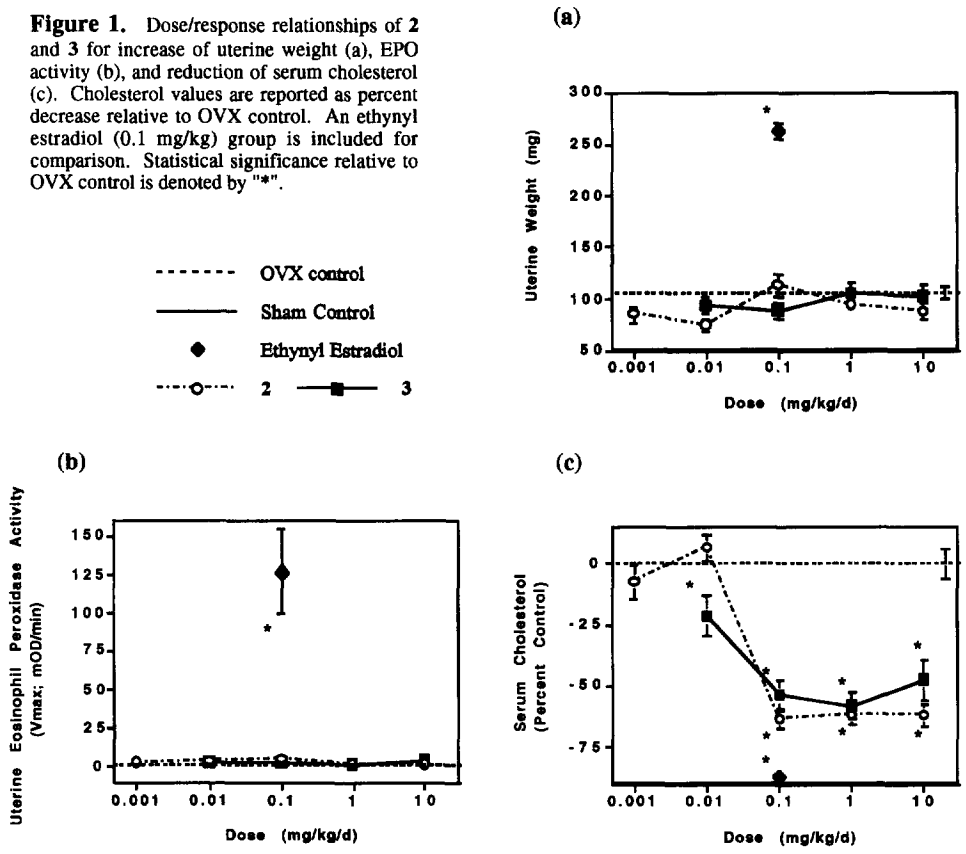


Figure 1. Dose/response relationships of **2** and **3** for increase of uterine weight (a), EPO activity (b), and reduction of serum cholesterol (c). Cholesterol values are reported as percent decrease relative to OVX control. An ethynyl estradiol (0.1 mg/kg) group is included for comparison. Statistical significance relative to OVX control is denoted by "*".

were also examined.[5,15] In this model, 75-day old Sprague-Dawley rats were dosed daily by oral gavage immediately post-OVX. Bone-mineral density (BMD), total bone-mineral content (TBMC), and cross-sectional area (X-area) were assessed at the distal metaphysis of femora by quantitative computed tomography (QCT). The effects on BMD , TBMC, and X-area are depicted in Figures 2a, b, and c.

Like 17α-ethynyl estradiol, both **2** and **3** provided significant, dose dependent reductions in serum cholesterol after 4 days post-OVX, with ED_{50}'s of 0.07 and 0.10 mg/kg, respectively. In contrast to the estrogen control however, neither compound showed any tendency to increase uterine weight or EPO activity. Similar trends in uterine weight and serum cholesterol were observed in the 5-week model (Table 1).

Furthermore, in the 5-week model **2** and **3** were found to significantly prevent OVX induced osteopenia, measured as BMD, at doses of 10 and 0.1 mg/kg respectively (Figure 2a). Compound **3** was also found to significantly enhance TBMC at all doses tested, an effect that was not noted for the estrogen controls or for compound **2** (Figure 2b). Interestingly, although at low doses **3** showed a significant increase in X-area with respect to OVX controls, this effect diminished in dose dependent fashion.

The ability of **3** to prevent OVX induced osteopenia was further evaluated in animals that were 6 months old at ovariectomy, where longitudinal growth of bone is less of a factor (Figure 3).[16] In these older animals, the effect on BMD was maintained at doses as low as

TABLE 1	Body Weight (g)	Uterine Weight (mg)	Serum Cholesterol (mg/dL)
Intact Sham	289 ± 12*	523.7 ± 34*	85.1 ± 6.3
OVX (CDX, PO)	330 ± 17	115.0 ± 11	81.7 ± 5.8
EE2 (0.1 mg/kg, PO)	274 ± 9*	319.0 ± 24*	37.1 ± 10.5*
2			
(0.01 mg kg, PO)	334 ± 5	105.7 ± 4	92.1 ± 11.0
(0.1 mg kg, PO)	314 ± 9	132.2 ± 9	69.3 ± 4.9
(1.0 mg kg, PO)	287 ± 9*	151.0 ± 16	52.6 ± 10.0*
(10.0 mg kg, PO)	284 ± 5*	124.2 ± 10	53.0 ± 6.8*
3			
(0.01 mg kg, PO)	306 ± 8	128.2 ± 5	79.0 ± 8.7
(0.1 mg kg, PO)	281 ± 4*	160.2 ± 14*	69.8 ± 8.4
(1.0 mg kg, PO)	295 ± 5*	134.2 ± 7	42.3 ± 4.6*
(10.0 mg kg, PO)	302 ± 9*	135.2 ± 5	57.4 ± 5.4*

Statistical significance relative to OVX control is denoted by "*".

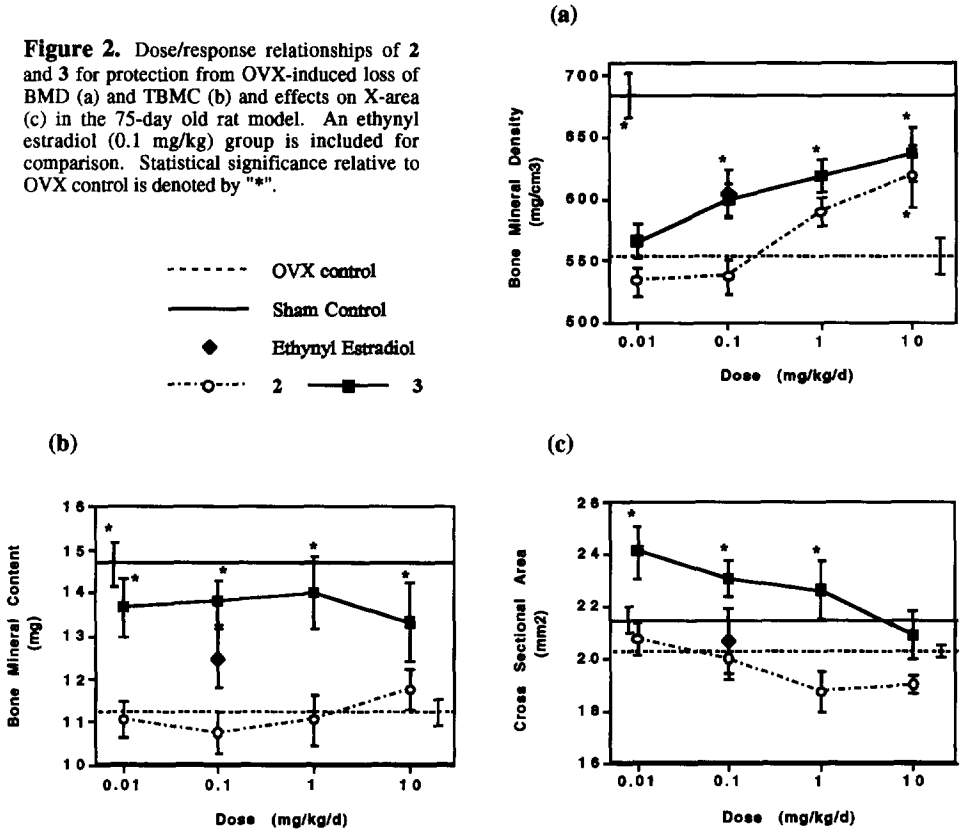
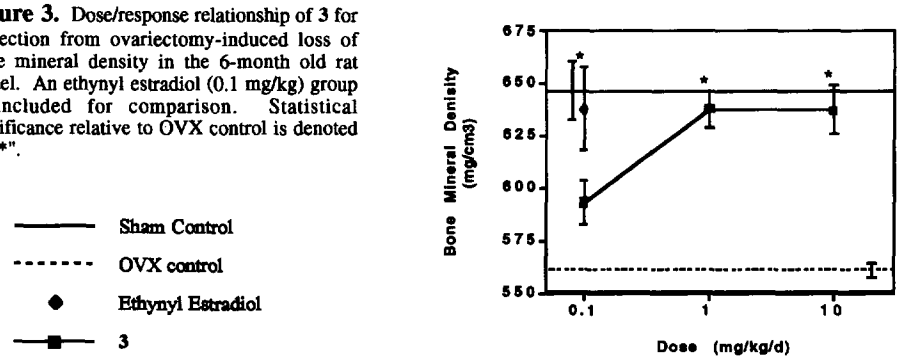


Figure 2. Dose/response relationships of **2** and **3** for protection from OVX-induced loss of BMD (a) and TBMC (b) and effects on X-area (c) in the 75-day old rat model. An ethynyl estradiol (0.1 mg/kg) group is included for comparison. Statistical significance relative to OVX control is denoted by "*".

1 mg/kg, however, significant effects on TBMC and X-area were not observed (data not shown). The age-related difference in effects on TBMC and the the inverted dose/response for X-area in 75-day old animals may indicate that **3** is influencing longitudinal bone growth, as well as bone turnover. The relevance of the effects on TBMC and X-area to the treatment of postmenopausal osteoporosis is therefore unclear.

Figure 3. Dose/response relationship of **3** for protection from ovariectomy-induced loss of bone mineral density in the 6-month old rat model. An ethynyl estradiol (0.1 mg/kg) group is included for comparison. Statistical significance relative to OVX control is denoted by "*".



The tissue-specific agonist activity of SERMs such as raloxifene (**1**), **2**, and **3** and the lack of uterine stimulation observed with these compounds contrasts with the effects of the partial agonist tamoxifen (**4**). Although tamoxifen also exhibits potent agonist effects in bone[17] and the cardiovascular system,[18] these are accompanied by strong uterotrophic activity.[19] A molecular biological assay that discriminates between these different classes of estrogen receptor modulators has been recently described.[20] We hypothesized that **2** and **3** may share structural features with raloxifene that differ from tamoxifen and which may be responsible for their tissue selectivity.

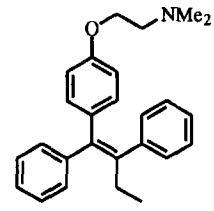


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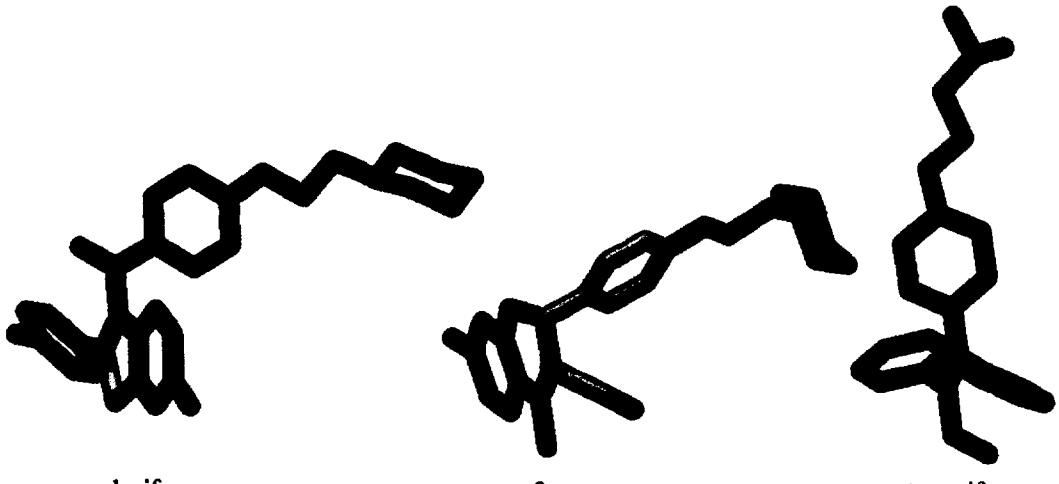
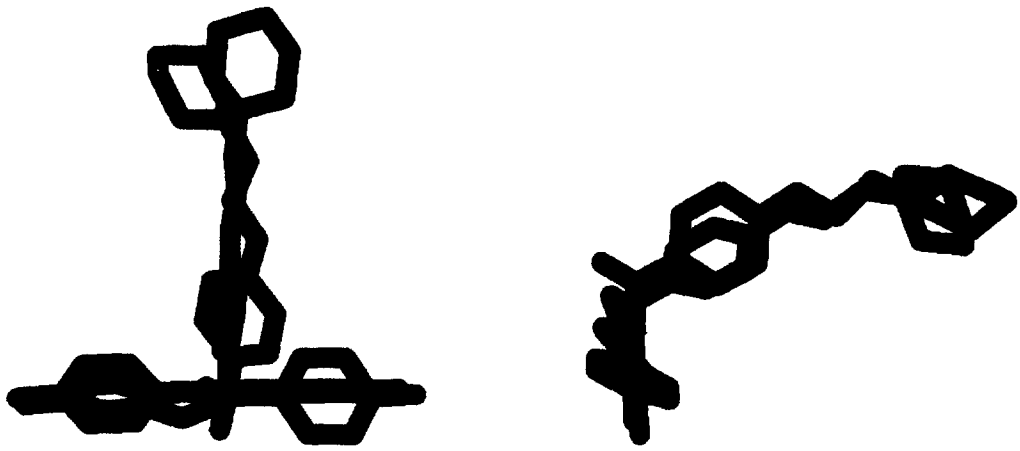
Figure 4. MOPAC/AM1 minimum energy conformations for raloxifene, **3**, and tamoxifen



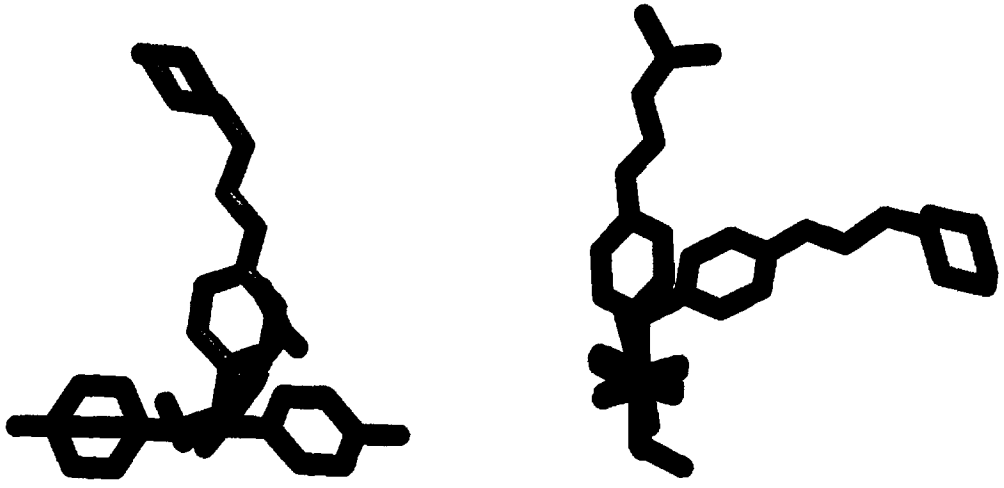
raloxifene **3** tamoxifen

Figure 5. Overlay of minimized structures of raloxifene (green carbons) and **3** (gray carbons).



In order to elucidate the structural features that distinguish raloxifene and **3** from tamoxifen and its analogs we compared their minimum energy conformations, as generated by MOPAC, utilizing the AM1 parameter set (Figure 4).[21] An overlay of the stilbene cores of **3** and raloxifene results in a very similar positioning of their basic amine containing side-chains (Figure 5). In both cases, the axis passing through the nitrogen, side-chain ether, and phenyl ring is roughly orthogonal to the plane of the dihydroxystilbene. In contrast, the basic side-chain of tamoxifen is constrained to lie within the plane of the stilbene moiety, and cannot achieve the conformation preferred by **3** and raloxifene without significant and energetically unfavorable distortion of the olefinic bond angles. An overlay of the structures of **3** and tamoxifen (Figure 6) demonstrates that different regions of space are occupied by the side-chains in these two systems. Since the amino-containing side-chain is thought to be associated with the antagonist properties of these molecules, we

Figure 6. Overlay of minimized structures of tamoxifen (green carbons) and **3** (gray carbons).



speculate that the lack of uterine stimulation by **3** and raloxifene relative to tamoxifen is due to this difference in side-chain orientation.[22]

In conclusion, we have demonstrated that benzopyrans **2** and **3** prevent OVX induced osteopenia and reduce serum cholesterol in a rat model in a dose-dependent manner, without concomitant stimulation of the uterus. This pharmacological profile demonstrates that they are more appropriately classified as SERMs rather than as "pure estrogen antagonists" as previously reported.[12] We have defined a novel structural feature shared by these benzopyrans and the clinical candidate raloxifene, which differentiates them from tamoxifen and may be responsible for their lack of uterotrophic activity. Further studies examining the structural characteristics which result in the SERM pharmacological profile are in progress. Clearly this class of compounds shows promise for the treatment and prevention of osteoporosis and other pathologies associated with the post-menopausal decline in ovarian hormones.

Acknowledgment: The authors wish to thank Mr. Leonard Weir and Mr. Bennie Foster for the preparation of test compounds and intermediates.

References and Notes

1. Lindsay, R. In *Osteoporosis: Etiology, Diagnosis, and Management*; Riggs, B. L.; Melton, L. J., Eds.; Raven: New York, 1988; p 333.
2. Ross, R. K.; Pike, M. C.; Mack, T. M.; Henderson, B. E. In *HRT and Osteoporosis*; Drife, J. O.; Studd, J. W. W., Eds.; Springer-Verlag: London, 1990; p 209.
3. Cust, M. P.; Gangar, K. F.; Whitehead, M. I. In *HRT and Osteoporosis*; Drife, J. O.; Studd, J. W. W., Eds.; Springer-Verlag: London, 1990; p 177.
4. Colditz, G. A.; Hankinson, S. E.; Hunter, D. J.; Willett, W. C.; Manson, J. E.; Stampfer, M. J.; Hennekens, C.; Rosner, B.; Speizer, F. E. *New Eng. J. Med.* **1995**, *332*, 1589.
5. Black, L. J.; Sato, M.; Rowley, E. R.; Magee, D. E.; Bekele, A.; Williams, D. C.; Cullinan, G. J.; Bendele, R.; Kauffman, R. F.; Bensch, W. R.; Frolik, C. A.; Termine, J. D.; Bryant, H. U. *J. Clin. Invest.* **1994**, *93*, 63.
6. Willson, T. M.; Henke, B. R.; Momtahen, Charifson, P. S.; Batchelor K. W.; Lubahn, D. B.; Moore, L. B.; Oliver, B. B.; Sauls, H. R.; Triantafillou, J. A.; Wolfe, S. G.; Baer, P. G. *J. Med. Chem.* **1994**, *37*, 1550.
7. Ke, H. Z.; Simmons, H. A.; Pirie, C. M.; Crawford, T.; Thompson, D. D. *Endocrinology* **1995**, *136*, 2435.
8. Termine, J. D. Presented at American Society of Bone and Mineral Research, 16th Annual Meeting, Kansas City, MO, 1994, Minisymposia A: *Estrogens and Anti-Estrogens.*
9. Draper, M. W.; Flowers, D. E.; Huster, W. J.; Neild, J. A. *Proceedings of the Fourth International Symposium on Osteoporosis* **1993**, 119.
10. Sharma, A. P.; Saeed, A.; Durani, S.; Kapil, R. S. *J. Med. Chem.* **1990**, *33*, 3222; and references therein.
11. Labrie, F.; Merand, Y. U.S. Patent 5 395 842, 1995; *Chem. Abstr.* **1995**, *123*, 83209..
12. Sreenivasulu, S.; Singh, M. M.; Setty, B. S.; Kamboj, V. P. *Contraception* **1993**, *48*, 597.
13. Grese, T. A.; Pennington, L. D. *Tetrahedron Lett.* **1995**, *36*, 8913.
14. Bryant, H. U.; Bendele, A. M.; Magee, D. E.; Cole, H. W.; Spaethe, S. W. *Pharmacologist* **1992**, *34*, 194.
15. Wronski, T. J.; Yen, C.-F. *Cells Mater.* **1991**, Suppl. 1, 69.
16. Westerlind, K. C.; Wakley, G. K.; Evans, G. L.; Turner, R. T. *Endocrinology* **1993**, *133*, 2924.
17. Turner, R. T.; Wakley, G. K.; Hannon, K. S.; Bell, N. H. *J. Bone Min. Res.* **1987**, *2*, 449.
18. Love, R. R.; Wiebe, D. A.; Newcomb, P. A.; Cameron, L.; Leventhal, H.; Jordan, V. C.; Feyzi, J.; DeMets, D. L. *Ann. Intern. Med.* **1991**, *115*, 860.
19. Dodge, J. A.; Stocksdale, M. G.; Black, L. J.; Rowley, E. R.; Bekele, A.; Cole, H. W.; Brown, C. E.; Magee, D. E.; Dehoney, G. A.; Bryant, H. U. *J. Bone. Min. Res.* **1993**, *8*, S278, abstract 648.
20. McDonnell, D. P.; Clemm, D. L.; Hermann, T.; Goldman, M. E.; Pike, J. W. *Mol. Endocrinology* **1995**, *9*, 659.
21. By James J. P. Stewart, Frank J. Seller Research Laboratory, U.S. Air Force Academy, Colorado 80840. QCPE #455 and references therein.
22. Previous studies have demonstrated that the conversion of the dimethylamino moiety to piperidino is not sufficient to account for this effect: Robertson, D. W.; Katzenellenbogen, J. A.; Hayes, J. R.; Katzenellenbogen, B. S. *J. Med. Chem.* **1982**, *25*, 167. Jones, C. D.; Jevnikar, M. G.; Pike, A. J.; Peters, M. K.; Black, L. J.; Thompson, A. R.; Falcone, J. F.; Clemens, J. A. *J. Med. Chem.* **1984**, *27*, 1057.

(*Received in USA* 8 *February* 1996; *accepted* 7 *March* 1996)