Published in final edited form as:
Bioorg Med Chem Lett. 2009 January 1; 19(1): 108–110. doi:10.1016/j.bmcl.2008.11.006.

Analogs of Methyl-Piperidinopyrazole (MPP): Antiestrogens with Estrogen Receptor α Selective Activity

Hai-Bing Zhou[a,c], **Kathryn E. Carlson**[a], **Fabio Stossi**[b], **Benita S. Katzenellenbogen**[b], and **John A. Katzenellenbogen**[a,*]

[a]Department of Chemistry, University of Illinois, Urbana, IL 61801, USA

[b]Departments of Molecular and Integrative Physiology, Cell and Developmental Biology, University of Illinois, Urbana, IL 61801, USA

Abstract

Methyl-piperidino-pyrazole (**MPP**), an estrogen receptor α (ERα)-selective antagonist we developed, has a basic side chain (BSC) attached to an ERα-selective agonist ligand, methyl pyrazole triol (**MPT**) through an ether linkage. To remove the possibility that metabolic cleavage of the BSC in **MPP** would regenerate **MPT**, we have replaced the N-piperidinylethoxy moiety with an N-piperidinylpropyl group, giving **MPrP**. This new analog retains the high ERα-selective binding affinity and antagonist potency of **MPP**.

Keywords

pyrazoles; SERMs; binding affinity; antagonist; estrogen receptor

Estrogens can have remarkable tissue-selective effects, and this has led to the development of compounds termed selective estrogen receptor modulators (SERMs), which function as estrogen agonists in some tissues (bone, brain and the cardiovascular system) but as antagonists in others (uterus and breast).[1,2] Estrogen receptors (ERs) can bind a variety of steroidal and non-steroidal ligands, and the search for better SERMs has driven efforts to increase the chemical diversity of these compounds, especially the non-steroidal ones. In fact, subtle changes in ligand structure can have a dramatic impact on receptor conformation and the resulting biological activities.[3-7] A prominent feature of SERMs is a basic side chain (BSC), typically an aminoethyl group, appended to a core non-steroidal ER ligand by a phenyl ether linkage. The precise structure and orientation of the BSC can modulate SERM activity.[8,9]

Estrogen action is mediated through two ER subtypes, ERα and ERβ, which have distinct target tissue distributions and functional activities.[10-14] Classical SERMs (e.g., tamoxifen and raloxifene), however, have essentially no selectivity for either ER subtype. Compounds capable of stimulating ERα very selectively have been developed,[15-17] and we found that members of the triarylpyrazole class, such as propyl-pyrazole-triol (**PPT**), have ca. 1000-fold higher affinity and agonist potency on ERα than on ERβ.[16,17] By attaching a BSC onto members of the pyrazole triol family of non-steroidal ER ligands, we obtained antagonist compounds that retained this affinity and potency preference for ERα.[18] Of the seven BSC-pyrazole combinations that we investigated, the most selective was a methyl-piperidino-pyrazole, which

*Corresponding author. Tel.: +1 217 333 6310; fax: +1 217 333 7325; e-mail: E-mail: jkatzene@uiuc.edu.
[C]Current Address: State Key Laboratory of Virology, College of Pharmacy, Wuhan University, Wuhan 430072, China

we termed **MPP** (Scheme 1). In binding and transcription activation assays, **MPP** is very ERα selective, with its antagonistic activity on ERα being complete at concentrations at which it has neither agonist nor antagonist activity on ERβ.[18]

While **MPP** appeared to be a complete ERα antagonist in cell-based assay systems and has been widely used by others to evaluate the role of ERα in various estrogen-responsive systems, it resembles a SERM. Therefore, it was not surprising that we (unpublished) and others[19] found **MPP** to have some agonist activity in certain animal models of estrogenic activity.

Structurally, **MPP** is based on and prepared from a pyrazole triol, methyl-pyrazole-triol (**MPT**; Scheme 1), which is an ERα agonist, though of modest potency.[16] Thus, in principle, metabolic cleavage of the BSC might reveal latent agonist activity in an **MPT** metabolite. To investigate this possibility, we synthesized two **MPP** analogs in which the side chain was modified so as to preclude an ether metabolic cleavage that could convert **MPP** to **MPT**. The best of these analogs, **MPrP**, maintains excellent selectivity for ERα in terms of binding affinity and antagonist potency in transcription activation assays.

Notably, other SERMs having aminoethoxy BSCs could, in principle, also be metabolized to more agonistic phenols,[20] and while this possibility has been considered as a mechanism for tamoxifen resistance in breast cancer,[21,22] we have found only one case in which the propyl for ethoxy group substitution in a SERM has been made.[23] This compound, however, was characterized only as a potent antifertility agent, similar in activity to the SERM nafoxidine.[23]

To prepare the BSC pyrazole **1** (**MPrP**) and its analog **2**, we first performed an aldol condensation of 1-(4-methoxy-phenyl)-propan-1-one **3** and *p*-hydroxybenzaldehyde **4**, according to a modified literature procedure (Scheme 2).[24] The highly crystalline enone **5** underwent reaction with 4-methoxylphenylhydrazine **6** under vigorous conditions to give pyrazole **7** from which the triflate **8** was obtained. Heck-type coupling with ethyl acrylate and a catalytic amount of $(PhCN)_2PdCl_2$ in toluene afforded the desired pyrazole ester **10** in 82% yield. Piperidinolysis with stoichiometric dimethyl-aluminum chloride in CH_2Cl_2 then gives compound **11** in good yield. Hydrogenation gave the saturated amide **12**, which was reduced by borane to the corresponding amine **13**. Methyl ether cleavage with $BF_3{\cdot}Me_2S$ gave from **13** the desired product **1** (**MPrP**) in 81% yield and from **11**, the unsaturated amide **2**.

The ERα and ERβ binding affinities, determined by a competitive radiometric binding assay,[25,26] shown in Scheme 1, are expressed as relative binding affinity (RBA) values (estradiol = 100%). The nature of the BSC affects binding affinity, and compound **1** (**MPrP**) has an ERα binding affinity (5.1%) slightly lower than that of **MPP** (12%), but because its ERβ binding is further reduced from that of **MPP**, **MPrP** has a somewhat increased ERα binding selectivity (320-fold). The ERα selectivity of **MPrP** is also about 2.3-fold greater than that of the triol agonist **MPT**, which was the parent of **MPP**. The binding of the unsaturated amide (**2**) is markedly lower.

The ERα and ERβ transcriptional activity of **MPP** and compounds **1** (**MPrP**) and **2** was determined by estrogen-responsive reporter gene cotransfection assays in human endometrial cancer cells (HEC-1; Figure 1; IC_{50} values are given in the legend).[27] All three compounds are ERα antagonists with no significant agonist or antagonist activity on ERβ. The potency of analog **1** (**MPrP**) appears to be somewhat higher than that of **MPP** itself, and it also lacks the residual, low ERα partial agonist activity of **MPP**. The potency of amide (**2**) is somewhat lower.

In this study, we have developed a synthetic strategy to generate **1** (**MPrP**), a novel analog of our ERα-selective antagonist **MPP**, in which the 2-(*N*-piperidino)ethoxy moiety has been replaced by the 3-(*N*-piperidino)propyl moiety, removing a potential metabolic liability that

might engender agonist activity. This new analog retains the high affinity and antagonist potency selectivity for ERα of the parent ligand and should be a useful probe for the biological activity of ERα.

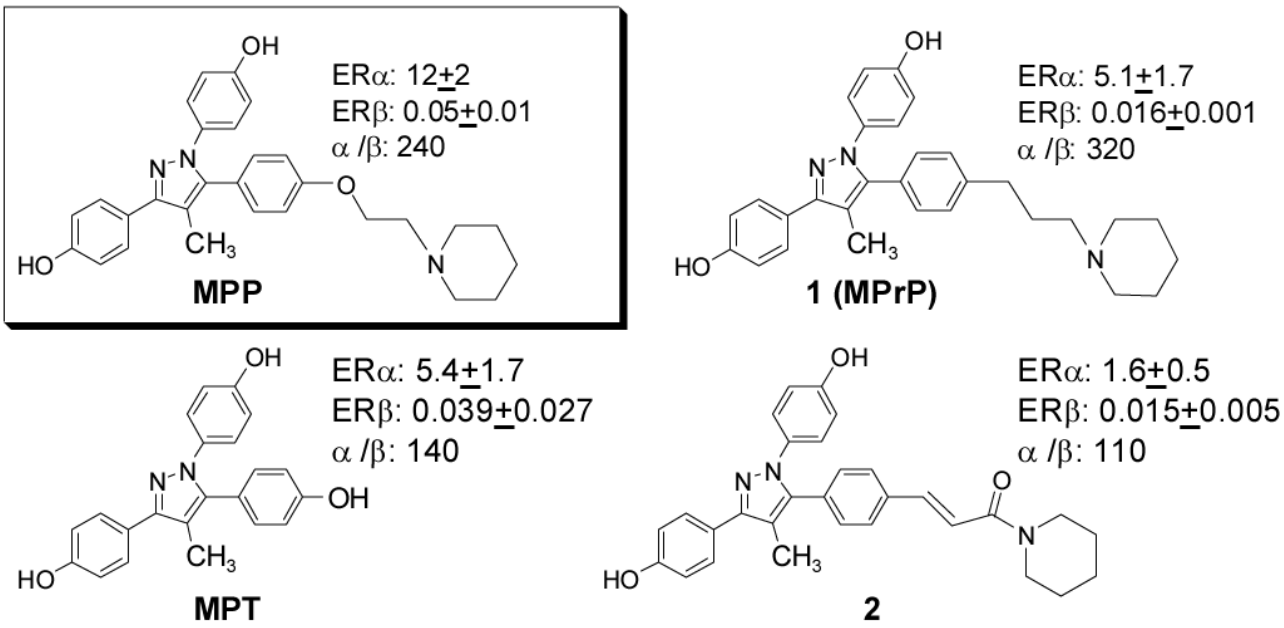
Acknowledgments

Supported by grants from the NIH (PHS 5R37 DK15556 to JAK and 5R01 CA11819 to BSK). Funding for NMR and MS instrumentation is from the Keck Foundation, NIH and NSF. We are grateful to Dr. Sung Hoon Kim for helpful comments.

References

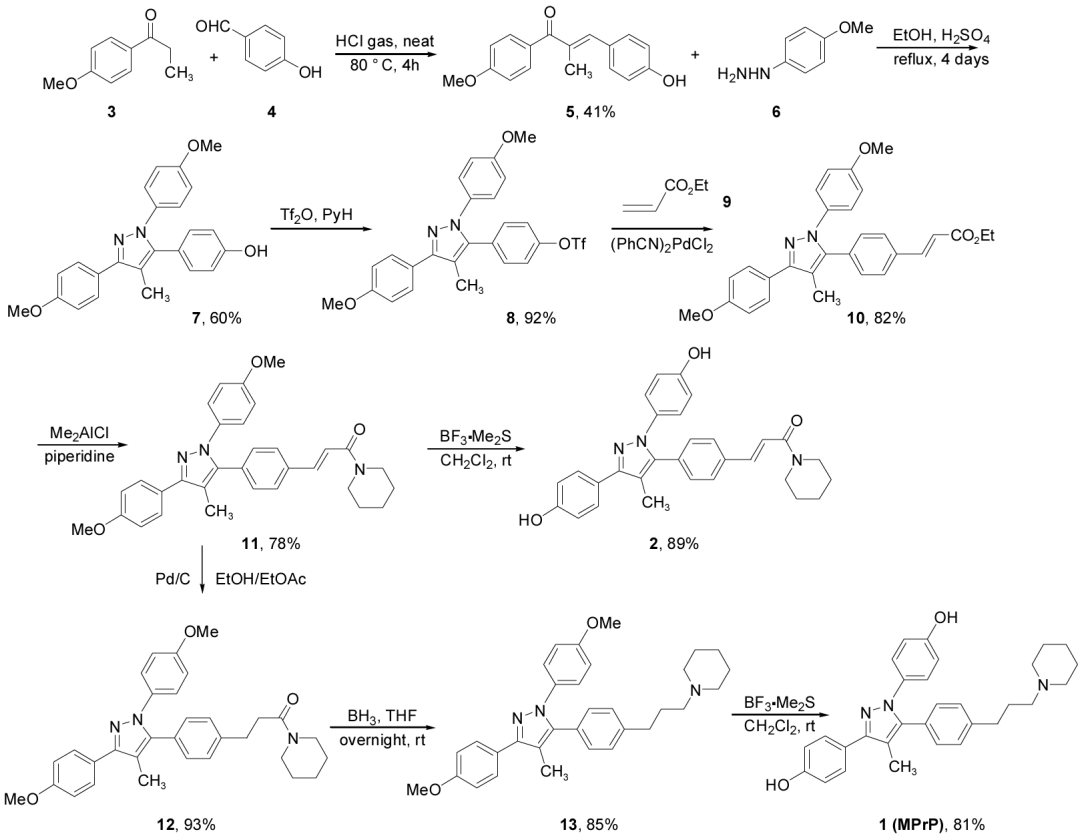
1. Park WC, Jordan VC. Trends Mol. Med 2002;8:82. [PubMed: 11815274]
2. Sato M, Grese TA, Dodge JA, Bryant HU, Turner CH. J. Med. Chem 1999;42:1. [PubMed: 9888829]
3. Zhou HB, Nettles KW, Bruning JB, Kim Y, Joachimiak A, Sharma S, Carlson KE, Stossi F, Katzenellenbogen BS, Greene GL, Katzenellenbogen JA. Chem Biol 2007;14:659–669. [PubMed: 17584613]
4. Fink BE, Mortensen DS, Stauffer SR, Aron ZD, Katzenellenbogen JA. Chem. Biol 1999;6:205. [PubMed: 10099132]
5. Barlaam, B.; Bernstein, P.; Dantzman, C.; Warwick, P. PCT Int. Appl. Astrazeneca AB; Swed.: 2002. p. 71Wo
6. Teo CC, Kon OL, Sim KY, Ng SC. J. Med. Chem 1992;35:1330. [PubMed: 1573630]
7. Palkowitz AD, Glasebrook AL, Thrasher KJ, Hauser KL, Short LL, Phillips DL, Muehl BS, Sato M, Shetler PK, Cullinan GJ, Pell TR, Bryant HU. J. Med. Chem 1997;40:1407. [PubMed: 9154963]
8. Zhou HB, Sheng S, Compton DR, Kim Y, Joachimiak A, Sharma S, Carlson KE, Katzenellenbogen BS, Nettles KW, Greene GL, Katzenellenbogen JA. J. Med. Chem 2007;50:399. [PubMed: 17228884]
9. Wallace OB, Bryant HU, Shetler PK, Adrian MD, Geiser AG. Bioorg. Med. Chem. Lett 2004;14:5103–5106. [PubMed: 15380208]
10. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Proc. Natl. Acad. Sci. U. S. A 1996;93:5925. [PubMed: 8650195]
11. Mosselman S, Polman J, Dijkema R. FEBS Lett 1996;392:49. [PubMed: 8769313]
12. Pettersson K, Gustafsson JA. Annu. Rev. Physiol 2001;63:165. [PubMed: 11181953]
13. Dechering K, Boersma C, Mosselman S. Curr. Med. Chem 2000;7:561. [PubMed: 10702625]
14. Katzenellenbogen BS, Montano MM, Ediger TR, Sun J, Ekena K, Lazennec G, Martini PG, McInerney EM, Delage-Mourroux R, Weis K, Katzenellenbogen JA. Recent Prog. Horm. Res 2000;55:163–193. [PubMed: 11036937]discussion 194
15. Larrea F, Garcia-Becerra R, Lemus AE, Garcia GA, Perez-Palacios G, Jackson KJ, Coleman KM, Dace R, Smith CL, Cooney AJ. Endocrinology 2001;142:3791. [PubMed: 11517155]
16. Stauffer SR, Coletta CJ, Tedesco R, Nishiguchi G, Carlson K, Sun J, Katzenellenbogen BS, Katzenellenbogen JA. J. Med. Chem 2000;43:4934. [PubMed: 11150164]
17. Kraichely DM, Sun J, Katzenellenbogen JA, Katzenellenbogen BS. Endocrinology 2000;141:3534. [PubMed: 11014206]
18. Sun J, Huang YR, Harrington WR, Sheng S, Katzenellenbogen JA, Katzenellenbogen BS. Endocrinology 2002;143:941. [PubMed: 11861516]
19. Davis AM, Ellersieck MR, Grimm KM, Rosenfeld CS. Mol. Reprod. Dev 2006;73:1034. [PubMed: 16688783]
20. Murphy CS, Langan-Fahey SM, McCague R, Jordan VC. Mol. Pharmacol 1990;38:737. [PubMed: 2233701]
21. Osborne CK, Jarman M, McCague R, Coronado EB, Hilsenbeck SG, Wakeling AE. Cancer Chemother. Pharmacol 1994;34:89. [PubMed: 8194171]
22. Wolf DM, Langan-Fahey SM, Parker CJ, McCague R, Jordan VC. J. Natl. Cancer Inst 1993;85:806. [PubMed: 8487325]
23. Lednicer D, Lyster SC, Duncan GW. J. Med. Chem 1967;10:78. [PubMed: 6031707]

24. Huang YR, Katzenellenbogen JA. Org. Lett 2000;2:2833. [PubMed: 10964377]
25. Carlson KE, Choi I, Gee A, Katzenellenbogen BS, Katzenellenbogen JA. Biochemistry 1997;36:14897. [PubMed: 9398213]
26. Katzenellenbogen JA, Johnson HJ Jr. Myers HN. Biochemistry 1973;12:4085–4092. [PubMed: 4745660]
27. Sun J, Meyers MJ, Fink BE, Rajendran R, Katzenellenbogen JA, Katzenellenbogen BS. Endocrinology 1999;140:800. [PubMed: 9927308]



Scheme 1.

Structures and ERα and ERβ relative binding affinity (RBA, estradiol = 100%) values of **MPP**, its precursor **MPT**, and analogs **1** (**MPrP**) and compound **2**. RBA values are the average of duplicate or triplicate determinations ± SD.



Scheme 2.
Synthesis of **MPP** analogs **1** (**MPrP**) and **2**.

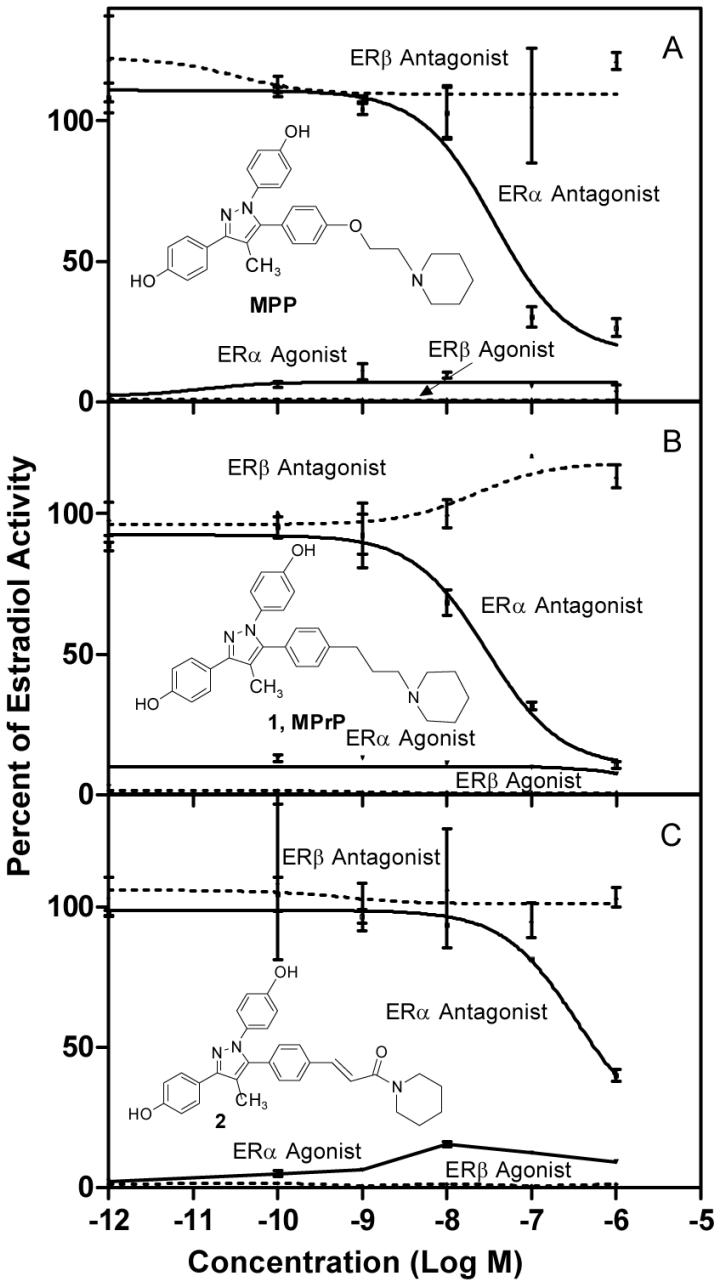


Figure 1.

Transcription activation through ERα (solid lines) and ERβ (dotted lines) of compounds **MPP**, **1** (**MPrP**), and **2**. HEC-1 cells were transfected with expression plasmids for ERα or ERβ and the estrogen responsive gene 2xERE-pS2-Luc and were incubated with the indicated ligand for 24 h. Antagonist assays were done in the presence of 1 nM estradiol (E_2). Values are the mean (± SD) of two or more experiments, expressed as a percent of the activity of ERα and ERβ with 1 nM E_2, which is set at 100%. IC_{50} values from the antagonist profiles: **MPP**, ERα 80 nM, compound **1** (**MPrP**), ERα 20 nM, and compound **2**, ERα 1 μM.