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Analogs of methyl-piperidinopyrazole (MPP): Antiestrogens with estrogen receptor α selective activity

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ABSTRACT

Methyl-piperidino-pyrazole (**MPP**), an estrogen receptor α (ER α)-selective antagonist we developed, has a basic side chain (BSC) attached to an ER α -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 **MPP**. This new analog retains the high ER α -selective binding affinity and antagonist potency of **MPP**.

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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, ER α and ER β , 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 ER α 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 ER α than on ER β .^{16,17} By attach-

ing a BSC onto members of the pyrazole triol family of nonsteroidal ER ligands, we obtained antagonist compounds that retained this affinity and potency preference for ER α .¹⁸ Of the seven BSC-pyrazole combinations that we investigated, the most selective was a methyl-piperidino-pyrazole, which we termed **MPP** (Scheme 1). In binding and transcription activation assays, **MPP** is very ER α selective, with its antagonistic activity on ER α being complete at concentrations at which it has neither agonist nor antagonist activity on ER β .¹⁸

While **MPP** appeared to be a complete ER α antagonist in cellbased assay systems and has been widely used by others to evaluate the role of ER α in various estrogen-responsive systems, it resembles a SERM. Therefore, it was not surprising that we (unpublished) and others¹⁹ 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 ER α 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 ER α 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



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109

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

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*-hydroxybenz aldehyde **4**, according to a modi-

fied 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)₂PdCl₂ 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



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



Figure 1. Transcription activation through ER α (solid lines) and ER β (dotted lines) of compounds **MPP**, **1** (**MPrP**), and **2**. HEC-1 cells were transfected with expression plasmids for ER α or ER β 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 (E₂). Values are the mean (±SD) of two or more experiments, expressed as a percent of the activity of ER α and ER β with 1 nM E₂, which is set at 100%. IC₅₀ values from the antagonist profiles: **MPP**, ER α 80 nM, compound **1** (**MPrP**), ER α 20 nM, and compound **2**, ER α 1 μ M.

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 ER α and ER β 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 ER α binding affinity (5.1%) slightly lower than that of **MPP** (12%), but because its ER β binding is further reduced from that of **MPP**, **MPrP** has a somewhat increased ER α binding selectivity (320-fold). The ER α 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 ER α and ER β 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; Fig. 1; IC₅₀ values are given in the legend).²⁷ All three compounds are ER α antagonists with no significant agonist or antagonist activity on ER β . The potency of analog **1** (**MPrP**) appears to be somewhat higher than that of **MPP** itself, and it also lacks the residual, low ER α 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 ER α -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 ER α of the parent ligand and should be a useful probe for the biological activity of ER α .

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