Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 9, pp. 1047-1050, 1996
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0960-894X/96 $15.00 + 0.00

S0960-894X(96)00165-5

BONE TARGETED DRUGS 2. SYNTHESIS OF ESTROGENS WITH HYDROXYAPATITE AFFINITY

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Abstract: The utility of the bone targeting 4-carboxy-3-hydroxy-1,2-pyrazole heterocycle was tested by the synthesis of hybrids with the non-steroidal estrogen hexestrol. Compounds **1** and **2** demonstrated significant hydroxyapatite affinity, while maintaining weak estrogenic activity in whole cell assays.
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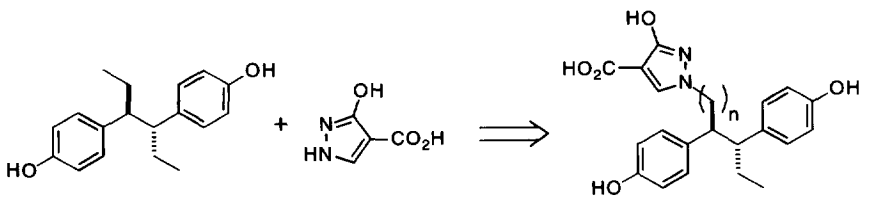
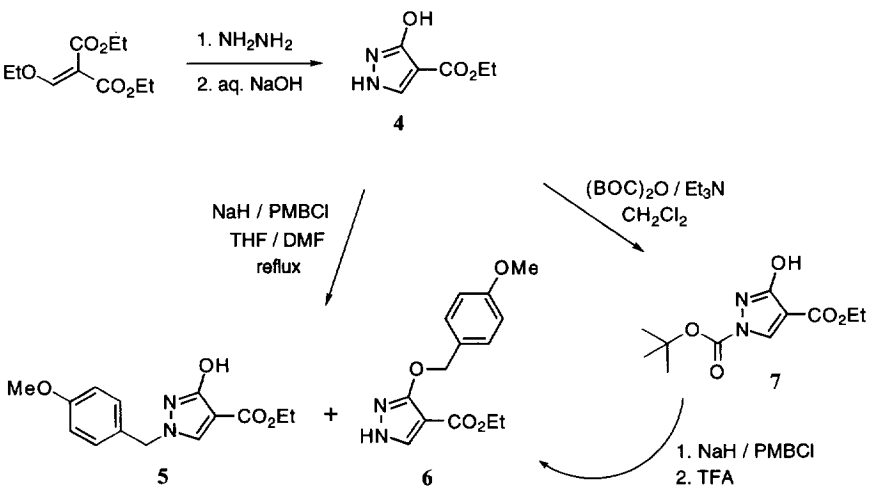
The disease of postmenopausal osteoporosis is caused by increased bone resorption following the loss of endogenous estrogens. Hormone replacement therapy with steroidal estrogens has been shown to prevent the onset of osteoporosis, however the observed increase in uterine cancer had limited its utility.[1] Notably, in vivo experiments have shown that estrogens infused directly into the bone of ovariectomized rats acted locally to inhibit bone loss and stimulate bone formation without systemic side effects.[2] Thus, estrogenic drugs that were physically targeted to bone may have a reduced incidence of side effects and an increased margin of safety. Several investigators have attempted to generate bone targeted prodrugs of estradiol; for example, the tetracycline pro-drug of estradiol benzoate has been reported to display hydroxyapatite (HA) affinity.[3] To explore the utility of the bone targeting 4-carboxy-3-hydroxy-1,2-pyrazole heterocycle,[4] we designed a series of hybrid estrogens (Scheme 1). Our approach differed from previous studies,[3] in that a metabolically inert linker was employed to tether the heterocycle to the estrogen. The resulting hybrid molecules demonstrated HA affinity and weak estrogenic activity in cell based assays in vitro.



Hexestrol **1-2**

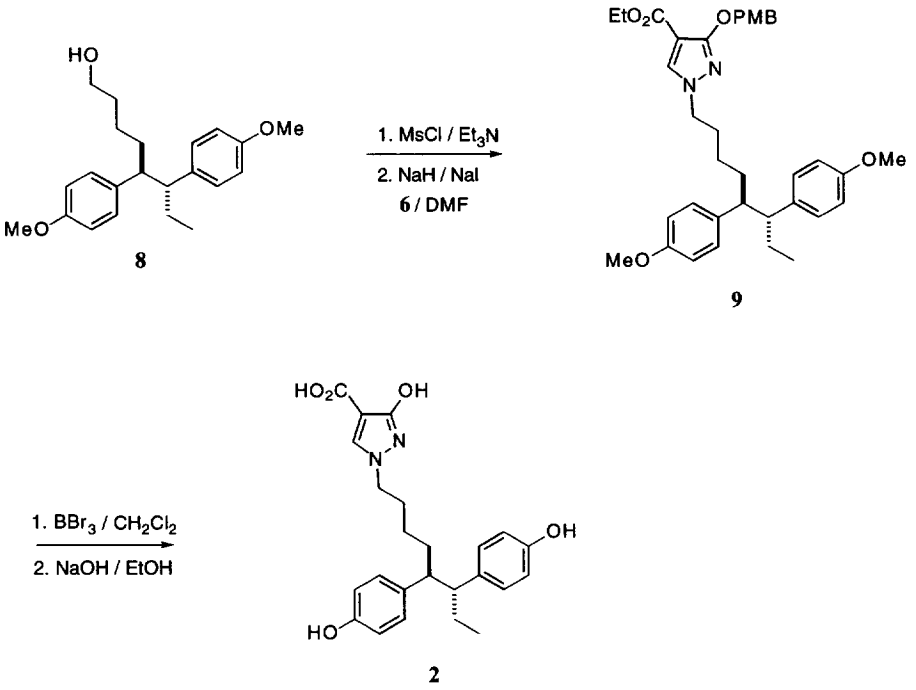
Scheme 1. Design of bone targeted estrogens

The unsubstituted pyrazole **4** was generated from the reaction of diethyl (ethoxymethylene)malonate with anhydrous hydrazine followed by base catalyzed cyclization (Scheme 1). An *O*-protected analog of **4** was required for the synthesis of the hybrid estrogens. Reaction of the sodium salt of **4** with *p*-methoxybenzyl bromide gave a mixture of the *N*- and O-protected compounds **5** and **6**. At best, by running the reaction at reflux in a 1:1 mixture of THF/DMF, a 1:1 mixture of **5** and **6** was isolated. The compounds were separated by a combination of flash chromatography and fractional crystallization.[5] Structural assignment was made by ^{1}H-NMR NOE experiments in d_6-DMSO. In compound **5**, irradiation of the benzyl methylene protons (δ 5.05) resulted in an enhancement of the signal for the pyrazole C-5 proton (δ 8.05). In compound **6**, irradiation of the corresponding methylene protons (δ 5.19) did not affect the signal for the C-5 proton (δ 8.20). In an alternate approach to the synthesis of **6**, reaction of **4** with BOC anhydride generated only the *N*-protected heterocycle **7**. Structural assignment and generation of the desired *O*-protected heterocycle was accomplished by conversion of **7** into **6** by benzylation and deprotection.



Scheme 1. Synthesis of functionalized pyrazoles

For the estrogenic core of the hybrid molecules we opted to use the hexestrol backbone. This non-steroidal estrogen has efficacy and potency comparable to steroidal estrogens, but offered improved ease of synthesis for the hybrid compounds. The general scheme is illustrated by the synthesis of compound **2** (Scheme 2). The functionalized hexestrol analog **8**[6] was converted to the mesylate and reacted with the sodium salt of heterocycle **6** in DMF. The structure of the resulting *N*-alkylated product **9** was confirmed by X-ray crystallography (data not shown). **9** was deprotected by sequential treatment with boron tribromide followed by sodium hydroxide. The hybrid estrogen **2** was isolated as white powder following recrystallization from ethyl acetate.[5] Compound **1** was synthesized by an analogous routes from its respective alcohol.[5,7]



Scheme 2. Synthesis of hybrid estrogens

The hybrid compounds were assayed for estrogen receptor binding affinity and for induction of alkaline phosphatase activity in Ishikawa cells[8,9] (Table 1). Compound **1** showed low affinity for the estrogen receptor and correspondingly weak estrogenic activity in cells. The homologated analog **2** showed increased receptor binding, although the relative affinity was only 0.1% of hexestrol. Comparable receptor affinities have been reported with other hybrid hexestrol analogs.[6] Compound **2** displayed micromolar estrogenic activity in Ishikawa cells, however the increase in potency was accompanied by a decrease in relative efficacy. The hybrid estrogens were also assayed for HA affinity. We were gratified to observe that both **1** and **2** demonstrated HA affinity comparable to tetracycline using the HA HPLC assay.[4] In a control experiment, the hybrid compound **3** was synthesized from 4-carboxy-1,2-pyrazole,[5,10] a heterocycle that was inactive in the HPLC assay. Hybrid compound **3** had the same in vitro biological activity as the related compound **2**, but as expected, lacked affinity for HA.

In summary, we have demonstrated the utility of the 4-carboxy-3-hydroxy-1,2-pyrazole bone targeting heterocycle by the synthesis of hybrid estrogens that bind to HA and retain weak estrogenic activity in vitro. This heterocycle has advantages over other bone targeting groups due its small size (c.f. tetracycline) and its ability to penetrate cells (c.f. aminobisphosphonates). It may find application in the generation of other novel bone targeted drugs.

Table 1. In vitro activity of hybrid estrogens

Compound	n	IC_{50} (μM)[a]	EC_{50} (μM)[b]	E_{max} (%)[c]	k' (pH 6.8)[d]
Hexestrol	—	0.002	0.0001	80	—
1	1	100	20	50	2.5
2	4	5.0	1.1	10	2.5
3	4	12	1.6	10	0

[a] Binding affinity for the rat estrogen receptor; — , not tested

[b] Dose at which 50% of agonist activity was observed in Ishikawa cells (reference 9),

[c] Relative efficacy, 10 nM estradiol = 100 %

[d] Hydroxyapatite affinity, determined by HPLC; k' = capacity factor (reference 4); — , not tested

Acknowledgements: The authors wish to thank Mr. Tom Ropchak and Dr. Bob Dougherty (Glaxo Wellcome) for conducting estrogen receptor binding assays, and Dr. John Katzenellenbogen (University of Illinois at Urbana-Champaign) for providing experimental details for the preparation of hexestrol analogs.

References and notes:

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5. Analytical data for selected compounds:
   1: m.p. 157-160 °C; Found: C, 64.5; H, 5.9; H, 7.1. $C_{21}H_{22}N_2O_5 \cdot 0.5H_2O$ requires: C, 64.4; H, 5.9; N, 7.2
   2: m.p. 159-163 °C; Found: C, 67.0; H, 6.7; H, 6.3. $C_{24}H_{28}N_2O_5 \cdot 0.5H_2O$ requires: C, 66.5; H, 6.7; N, 6.5
   3: m.p. 208-210 °C; Found: C, 72.1; H, 9.1; H, 6.6. $C_{24}H_{28}N_2O_4 \cdot C_{12}H_{23}N \cdot 0.5H_2O$ requires: C, 72.2; H, 8.8; N, 7.0
   5: m.p. 129-130 °C; Found: C, 60.9; H, 5.9; H, 10.2. $C_{14}H_{16}N_2O_4$ requires: C, 60.9; H, 5.8; N, 10.1
   6: m.p. 107-108 °C; Found: C, 60.7; H, 5.8; H, 10.1. $C_{14}H_{16}N_2O_4$ requires: C, 60.9; H, 5.8; N, 10.1
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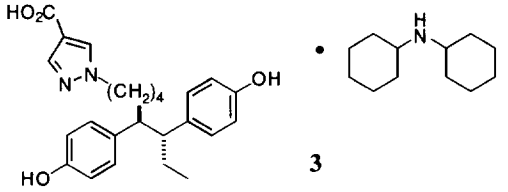
10. The control compound **3** was synthesized from reaction of the mesylate of alcohol **8** and the sodium salt of ethyl 4-pyrazole carboxylate, followed by the standard deprotection and purification as the dicyclohexylamine salt.



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(*Received in USA* 6 *March* 1996; *accepted* 5 *April* 1996)