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BONE TARGETED DRUGS 1. IDENTIFICATION OF HETEROCYCLES WITH HYDROXYAPATITE AFFINITY

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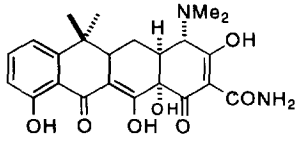
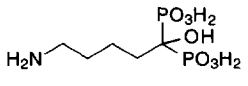
Abstract: A series of small heterocycles were identified with hydroxyapatite affinity comparable to tetracycline. From this series, the 4-carboxy-3-hydroxy-1,2-pyrazole was chosen for use in the synthesis of bone targeted drugs.

Bones are the weight bearing elements of the skeleton that undergo constant remodeling in response to applied compressive and torsional strain.[1] An imbalance in bone remodeling leads to disorders such as osteoporosis and Paget's disease, and is also associated with metastatic bone cancer.[1,2] The development of compounds targeted specifically to bone for the treatment of these diseases may lead to drugs with increased potency and selectivity compared to systemically active agents. For example, the potent inhibition of osteoclast activity by aminobisphosphonates in vivo is due in part to their localization on the surface of actively resorbing bone.[3] The major inorganic component of bone is hydroxyapatite (HA), a hydrated form of calcium phosphate. Compounds such as tetracycline or bisphosphonates (Figure 1), which localize in bone following systemic administration, have been shown to bind to hydroxyapatite with high affinity.[3] In order to identify compounds for use as "bone targeting" agents, we set out to identify small molecules with HA affinity. This report details the identification of a series of small heterocycles, related to the thiadiazole **1**, with an affinity for HA comparable to tetracycline.



Tetracycline



Aminobisphosphonate

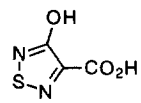


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Figure 1. Compounds with HA affinity

A HA HPLC column was used to rapidly screen compounds for their HA affinity.[4] Using a phosphate gradient, control compounds where shown to elute in an order consistent with their known HA affinity.[5] The data was expressed as a capacity factor k', where larger values represent increased HA affinity, and

$$k' = (t_r - t_o) / t_o \qquad t_r = \text{retention time}, t_o = \text{void volume}$$

Table 1. Hydroxyapatite binding affinity of heterocycles [a]

Compound	k' (pH 6.8)	k' (pH 8.8)	Δ k' [b]	pKa_2 [c]
Tetracycline	3.9	4.2	0.3	–
1	3.5	3.1	-0.4	6.98
2	0.1	1.0	0.9	10.12
3	2.9	3.0	0.1	8.90
4	2.9	0.4	-2.5	4.87
5	0.6	2.9	2.3	9.23
6	3.7	2.8	-0.9	6.61
7	2.9	3.1	0.2	9.49
8	2.9	3.2	0.3	–

[a] HPLC method; column, Bio-Gel HPHT 4.6 mm x 5 cm; program, 1 mM sodium phosphate for 4 min followed by a rising gradient to 500 mM sodium phosphate over 30 min; flow rate, 0.5 mL/min; n = 2
[b] k' (pH 8.8) - k' (pH 6.8)
[c] Second pKa, spectrophotometric determination

In a routine toxicological screen thiadiazole **1**[6] was found to accumulate in the skeleton of rats (6% of a single iv dose).[7] The k' for **1** was found to be comparable to tetracycline at pH 6.8 (Table 1). Preliminary experiments demonstrated that both the free hydroxyl and carboxylic acid were required for HA affinity (data not shown). To investigate the structural requirements for HA affinity, a series of heterocycles related to **1** were synthesized (**2-6**, Figure 2).

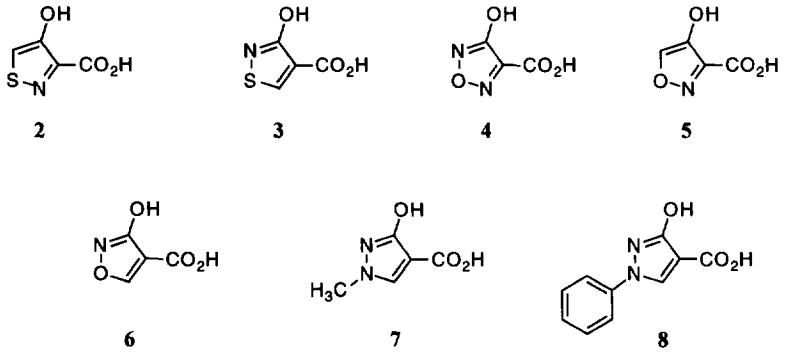
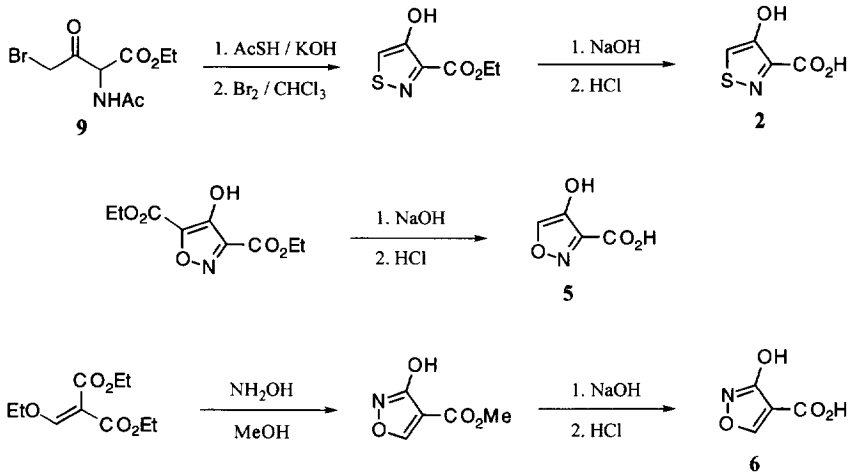


Figure 2. Analogs of thiadiazole **1**



Scheme 1. Synthesis of heterocycles **2**, **5** and **6**

Compounds **3**[8] and **4**[9] were prepared by literature methods. The synthesis of the heterocycles **2**, **5** and **6** is outlined in Scheme 1. Isothiazole **2** was prepared from bromoester **9**,[10] by alkylation with thioacetate, followed by cyclization with bromine, and saponification of the ester. Isoxazole **5** was prepared by saponification and acid catalyzed decarboxylation of 4-hydroxyisoxazole-3,5-dicarboxylic acid diethyl ester.[11] The isoxazole **6** was synthesized by condensation of diethyl (ethoxymethylene)malonate with hydroxlamine using a modification of the method of Bowden,[12] followed by saponification of the ester.

The HA affinity of heterocycles **2-6** is shown in Table 1. At pH 6.8, isothiazole **2** lacked HA affinity, however the isomeric isothiazole **3** showed an affinity close to that of the thiadiazole **1**. A similar trend was observed in the oxygen series; oxadiazole **4** showed good HA affinity, and the isomeric isoxazoles **5** and **6** showed poor and good HA affinity respectively. Examination of the SAR revealed that the heterocycles **1**, **3**, **4**, and **6**, which possessed a latent amide group, performed well in the HA binding assay. By contrast, heterocycles **2** and **5** which lacked this group did not bind well to HA at pH 6.8. The SAR could be explained by considering the tautomeric forms of the heterocycles (Figure 3). For a particular heterocycle, the equilibrium mixture would be affected by the structure and pH. Heterocycles **1**, **3**, **4**, and **6** would be expected to exist as a mixture of the keto/enol forms of the amide, with a contribution from the dianion depending on the pKa_2. The heterocycles **2** and **5** would be expected to exist primarily in the keto form[13] with little contribution from the dianion at pH 6.8. Thus, the enol or dianion forms of the heterocycles must be responsible for binding to HA. To further examine this phenomenon, the HA HPLC assay was repeated at pH 8.8 (Table 1). The increase in pH was expected to increase the proportion of the dianion forms of the heterocycles. A clear trend emerged from this study; heterocycles with $pKa_2 < 8.8$ had a lower k' at the higher pH. Notably, the compound **4** with the lowest pKa_2 showed the largest drop in k'. By contrast, heterocycles with $pKa_2 > 8.8$ had a higher k' at the higher pH, although the change was not proportionate to the pKa_2. From these results, it appeared that it was the enol form of the heterocycles that was required for high HA affinity. Those compounds that already exist in the enol form showed decreased affinity as the pH was raised and the

equilibrium was forced toward the dianion. It is reasonable to expect that the dianion would have reduced HA affinity as the energy for desolvation was increased. The increase in affinity of **2** and **5** at the higher pH can be explained if the keto form of the heterocycle must pass through the enol form in order to generate the dianion.

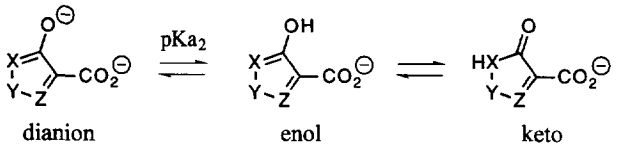


Figure 3. Heterocycle tautomers

With some understanding of the structural requirements for HA affinity, we set out to design a heterocycle to function as a bone targeting group. The 4-carboxy-3-hydroxy-1,2-pyrazole analog of **3** and **6** was chosen, since it contained a cyclic amide and would also allow facile functionalization at the central *N*-1 position (Figure 2). The model pyrazoles **7** and **8** were synthesized from diethyl (ethoxymethylene)malonate by reaction with the corresponding hydrazine[14] and saponification of the ester. Both pyrazoles demonstrated good HA affinity at pH 6.8 and 8.8, consistent with their structure and their relatively high pKa_2 (Table 1). Significantly, the HA affinity was unaffected by the nature of the *N*-1 substitutent.

In summary, 4-carboxy-3-hydroxy-1,2-pyrazoles have been identified as potential bone targeting heterocycles; they show good HA affinity independent of the *N*-1 substituent, and are monoanions at physiological pH which should aid cell penetration and intestinal absorption of hybrid drug molecules.

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References and Notes:

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