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An Azepanone-Based Inhibitor of Human Cathepsin K with Improved Oral Bioavailability in the Rat and the Monkey

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One critical component in the progression of a promising chemical series to clinical studies is the optimization of pharmacokinetic parameters in preclinical species. Before the widespread utilization of mass spectrometry as an analytical method, the acquisition of pharmacokinetic data was regarded as a luxury. Today it is viewed not only as commonplace but also as a pivotal driving force in the pharmaceutical industry's goal of reducing both lead compound attrition and cycle times to the clinic and subsequently the market. The rapid progression of lead compounds may be slowed or indeed terminated altogether by pharmacokinetic parameters which differ radically between preclinical species. A case in point has emerged with azepanone **3** (Figure 1), which possesses many of the desired biological properties that allow it to be considered for clinical development as an inhibitor of cathepsin K with potential for reversal of bone loss and as a treatment of osteoporosis.[1] Although the oral bioavailability of **3** is acceptable in the rat, it is poor in the monkey. As a consequence of the relatively poor inhibition of cathepsin K by our inhibitors in nonprimates such as the rat,[2] the monkey emerged as a critical species for preclinical evaluations of both efficacy and toxicity. Although alternate parenteral modes of administration, such as subcutaneous or intramuscular, could be used in preclinical animal studies, such studies are not as well received during candidate evaluation as they would be if the desired (oral) route of administration were viable. In addition, the failure to achieve good oral availability in more than one preclinical species is generally deemed to bode poorly for the prediction of oral bioavailability and ultimately efficacy in humans.

The underlying mechanisms of variable oral bioavailability between species are not well understood. However, it is most often related to interspecies differences in physiology such as hepatic blood flow as well as molecular differences such as substrate susceptibilities for the enzymes involved in first-pass clearance (i.e., efflux transporters and metabolizing enzymes) rather than species specific lipid bilayer permeation rates.[3−5] Recognition of the species specific enzyme systems responsible for the first-pass clearance of compound classes

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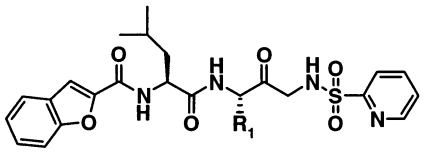
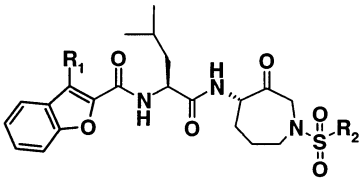


1; R = H
2; R = CH_3



3; R_1 = H, R_2 = 2-pyridyl
4; R_1 = CH_3, R_2 = 2-pyridyl
5; R_1 = H, R_2 = 2-pyridyl N-oxide
6; R_1 = CH_3, R_2 = 2-pyridyl N-oxide

Figure 1. Cathepsin K inhibitors.

can serve as the principal guide in the design of subsequent analogues for chemical synthesis and further in vivo evaluation.[6] In this communication, we describe in detail studies involving the chemical modification of azepanone **3** and the delineation of the putative mechanisms involved in limiting its oral bioavailability in the monkey. These studies have led to the identification of azepanone **6** which possesses improved oral bioavailability in both the rat and the monkey.

Azepanone **3** has been characterized as a potent, configurationally stable, inhibitor of cathepsin K with a K_i of 0.16 nM.[7] It is also a potent inhibitor of osteoclast-mediated bone resorption in an in vitro cell-based assay of bone resorption[8] with an IC_{50} of 70 nM. Pharmacokinetic analysis in the rat revealed **3** to be 42% orally bioavailable when administered as a solution. It has a plasma clearance of 49.2 mL min^{-1} kg^{-1} and a volume of distribution of approximately twice the total body water volume in the rat. Like the previously described acyclic cathepsin K inhibitors **1** and **2**, azepanone **3** demonstrates good membrane permeability through rat distal colon tissue but, unlike acyclic inhibitors **1** and **2**,[9] is not subject to P-glycoprotein-mediated recycling in human Caco-2 cells. We have speculated that the introduction of a conformational constraint (seven-member ring) has served the dual purposes of favoring the bioactive conformation for optimal cathepsin K binding while simultaneously disfavoring those conformations which would result in increased affinity for P-glycoprotein transporters and ultimately recycling.[10] Although encouraging, the bioavailability of **3** in the rat was not optimal, and further studies aimed at understanding the factors limiting rat oral bioavailability were pursued to optimize this parameter. Incubation of **3** (10 μM) with rat hepatic microsomes (0.5 mg/mL) followed by LC−MS analysis revealed the presence of two mono-oxygenated metabolites, one oxygenation occurring on the pyridine ring and the other on the benzofuran moiety. The structures of the exact regioisomers for this oxygenation were not assigned. A profound effect on the rate of in vitro metabolism of **3** in rat hepatic microsomes fortified with NADPH and UDPGA was observed upon treatment with ketoconazole, a known inhibitor of both cytochrome P450-mediated oxidation and P-glycoprotein-mediated recycling [Cli = 12.9 mL min^{-1} (g of liver)$^{-1}$ for **3** and Cli = 1.37 mL min^{-1} (g of liver)$^{-1}$ for **3** with ketoconazole].[11] These data suggest that one or more cytochromes could be primarily responsible for metabolism of **3** in the rat. Additional in vivo experiments in rats further supported this conclusion. Intraduodenal admin-

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Table 1. Pharmacokinetic Profiles of Cathepsin K Inhibitors

compound	species	$T_{1/2}$ (min)	Cli (mL min^{-1} kg^{-1})	V_{dss} (L/kg)	oral *F* (%)
1[a]	rat	27	33.1	0.65	3.3
2	rat	33.2	32.5	0.71	3.2
3	rat	29.8	49.2	1.86	42.1
3	monkey	36.0	15.0	1.05	4.8
4	rat	36.6	53.9	3.49	1.9
6	rat	22.7	35.2	4.46	66.3
6	monkey	31.1	11.0	0.35	23.4

[a] Pharmacokinetic parameters of **1** were determined in a four-component cassette dosing format.

istration of azepanone **3** (2 mg/kg) with ketoconazole (10 mg/kg) resulted in a portal vein dose normalized area under the plasma concentration versus time curve (DNAUC) approximately 2.7-fold higher than that of the control group treated with **3** alone. On the other hand, the systemic DNAUC of **3** in the ketoconazole group was 4.35-fold higher than in the control group. By comparison of the systemic and the portal vein DNAUC, hepatic extraction of **3** was approximately 34.3% for the ketoconazole-treated group and approximately 63% for the control group.[12] In total, these data suggest that the bioavailability of **3** in the rat is at least partially limited by cytochrome-mediated metabolism and/or P-glycoprotein recycling in the upper intestine and/or liver. These barriers to systemic exposure have singly, or in concert, resulted in suboptimal absorption of **3** followed by moderate hepatic extraction and/or metabolism in the liver of rats. Pharmacokinetic evaluation in the monkey showed **3** to have an oral bioavailability of approximately 4.8% (Table 1). The in vitro metabolic stability of **3** in monkey hepatocytes was poor as indicated by the high rate of clearance [Cli = 7.08 mL min^{-1} (g of liver)$^{-1}$]. In monkey hepatic microsomes (fortified with NADPH and UDPGA), **3** was rapidly cleared with a Cli of 37.8 mL min^{-1} (g of liver)$^{-1}$. Additionally, monkey intestinal microsomes isolated from the duodenum, jejunum, and ileum were also shown to metabolize **3** rapidly. Oral treatment in the monkey showed **3** to have both low portal vein and systemic DNAUCs of 5.42 and 8.48 kg min^{-1} L^{-1}, respectively. As a whole, these data suggest that the bioavailability of **3** in the monkey is limited by its rapid metabolism in both intestinal membranes and the liver. On the basis of these studies, several analogues were designed to eliminate formation of putative oxidative metabolites. Initial speculations guided the synthesis of an analogue that might be less susceptible to oxidation of the benzofuran carboxamide. Introduction of the 3-methylbenzofuran carboxamide as in analogue **4** did not affect inhibitor potency (cathepsin K $K_{i,app}$ = 0.27 nM) but did result in a substantially lower oral bioavailability (1.9%) in the rat relative to that of **3** (Table 1). In this instance, analogue **4** possessed artificial membrane permeability comparable to that of **3** (6.12×10^{-5} and 5.46×10^{-5} cm/s, respectively) as measured by diffusion rates across an artificial cholesterol−phospholipid membrane.[13] Additionally, analogue **4** was more rapidly cleared in rat hepatic microsomes [26.4 mL min^{-1} (g of liver)$^{-1}$] than **3**. We postulate that despite the potential blockade of aromatic ring oxidation, the methyl group of the benzofuran moiety may have introduced a new potential metabolic liability resulting in rapid metabolism and clearance of the parent molecule.

An alternative hypothesis for slowing oxidative metabolism was based on knowledge that one of the principal, well-established metabolites of pyridine ring oxidation by cytochrome P450 enzymes is pyridine *N*-oxide.[14] This led to the preparation of analogue **5**, which incorporates the 2-pyridine *N*-oxide moiety. Azepanone **5** is a potent inhibitor of cathepsin K with a $K_{i,app}$ of 0.13 nM and is active in the human osteoclast resorption assay with an IC_{50} of 30 nM. When administered orally in the monkey, **5** exhibited low levels of systemic exposure as determined by the systemic DNAUC. Analogue **5** gave a portal vein DNAUC similar to that of **3** (5.36 and 5.42 kg min^{-1} L^{-1}, respectively), suggesting that both analogues had similar intestinal membrane permeability but that the *N*-oxide **5** was more highly cleared by the liver, thereby limiting systemic exposure in the monkey.

In view of these results, an unexpected impact on oral bioavailability in both the rat and the monkey was seen with compound **6**. This compound arises from a combination of the 3-methylbenzofuran moiety of **4** and the pyridine *N*-oxide of **5**. In vitro compound **6** inhibits cathepsin K with a K_i of 0.11 nM, and it is a potent inhibitor of human osteoclast-mediated bone resorption with an IC_{50} of 30 nM. Pharmacokinetic studies in the rat and the monkey showed **6** to have oral bioavailabilities of 66.3 and 23.4%, respectively. When

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Table 2. Physicochemical Properties and Oral Bioavailabilities

compound	MW (g/mol)	*c* log *P*	H-bond donor	H-bond acceptor	no. of rotatable bonds	polar surface area	% *F* rat	% *F* monkey
1	486.5	2.88	3	6	11	147.47	3.3	–
2	500.6	3.19	2	6	11	147.47	3.2	–
3	526.6	3.08	2	6	8	138.68	42.1	4.8
4	540.6	3.23	2	6	8	138.68	1.9	–
5	542.6	2.22	2	6	8	157.66	–	–
6	556.6	2.38	2	7	8	157.66	66.3	23

administered orally in the monkey, analogue **6** had a portal vein DNAUC approximately 2-fold higher than that of **3** (10.34 and 5.42, respectively) and a 5-fold greater systemic DNAUC (43.44 and 8.48, respectively). On the basis of these data, the increased oral bioavailability of **6** over that of **3** in the monkey is likely due to a combination of greater intestinal absorption and slower hepatic clearance. In vitro experiments aimed at explaining this surprising result revealed that the metabolic stability of **6** in monkey hepatic microsomes was 2.2-fold higher than that of **3** [Cli = 37.8 mL min^{-1} (g of liver)$^{-1}$ for **3** vs 17.9 mL min^{-1} (g of liver)$^{-1}$ for **6**]. Additionally, the rate of metabolic clearance of **6** in rat hepatic microsomes was approximately 1.7-fold slower than that of analogue **3** [Cli = 20.1 mL min^{-1} (g of liver)$^{-1}$ for **3** vs 12.0 mL min^{-1} (g of liver)$^{-1}$ for **6**]. It is likely that this combination of increased metabolic stability and the lower rate of clearance accounts for at least a portion of the greater oral bioavailability of **6** over **3** in both the monkey and the rat. It is noteworthy that the metabolic stability of **6** in human microsomes is equivalent to that seen in the monkey, offering the possibility that the monkey may be a good predictor of oral bioavailability in humans for analogue **6**.

On the basis of the in vitro potencies and the promising pharmacokinetic profiles of analogue **6**, it was evaluated in the medically ovariectomized (OVX) monkey model of osteoporosis[15,16] to look for suppression of the urinary N-terminal telopeptide (NTx) as well as serum C-terminal telopeptide (CTx) bone biomarkers.[17] Upon oral treatment in the monkey (3 mg/kg), a peak systemic concentration of **6** of 2 μM was observed 1.5 h postdosing. Urinary NTx levels dropped 32% over the first 24 h, and serum CTx levels dropped 32% at the 1.5 h time point relative to vehicle controls.[18] These data suggest that an orally administered inhibitor of cathepsin K may indeed have therapeutic potential in bone loss diseases such as osteoporosis.

Clearly, active barriers, including oxidative enzymes and/or transporters, are limiting the oral bioavailability of **3**. We have slowed the rates of those pathways by structural modifications designed to block oxidative metabolism, thereby improving the oral bioavailability in two important preclinical species. As rational as the approach was intended to be, serendipity proved to be critical in achieving the desired outcome. It appears in retrospect that the attempt to block benzofuran oxidation with methyl substitution may have succeeded but in turn introduced a new oxidative pathway or increased its affinity for transporters. At the same time, blockade of further oxidative metabolism of the pyridine was ineffective at increasing oral bioavailability on its own. Yet the overall positive effect of simultaneously introducing both changes is effective in indirectly blocking metabolism and/or intestinal apical recycling of **4**.

In Table 2, we list several molecular properties for the compounds discussed here that have been generally postulated to be useful predictors of good oral bioavailability.[19,20] It is apparent from the lack of any clear correlation that none of these properties could have served to guide the success achieved here. Indeed, these predictive "rules" are not meant to be rigidly applied principles but rather guides for optimizing the probability for success. It is clear that an understanding of the complex mechanisms that limit oral bioavailability must guide analogue design. However, until these processes are more fully understood, analogue design must also be coupled with the thorough exploration that is characteristic of a classical iterative medicinal chemistry and pharmacokinetic screening approach. This ensures the potential for discovery independent of rationale or preconception.

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