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RELATIONSHIP BETWEEN STRUCTURE AND BIOAVAILABILITY IN A SERIES OF HYDROXAMATE BASED METALLOPROTEASE INHIBITORS[1]

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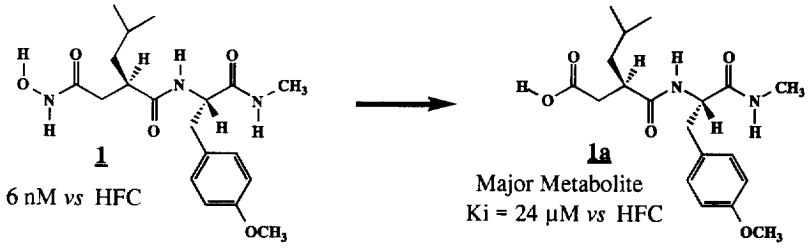
Abstract : Pharmacokinetic parameters for a series of C-terminally modified hydroxamate dipeptides were evaluated by *in vitro* and *in vivo* models. The presence of a tertiary base at the C-terminus significantly reduced biliary excretion and increased plasma half-life. Moreover, introduction of a thioether functionality produced a more favorable pharmacokinetic profile compared to the corresponding oxo- and aza-analogs.

One approach to arresting the progress of degenerative inflammatory disease is administration of highly specific inhibitors of the proteases responsible for the breakdown of cartilage. These endogenous endo-peptidases are collectively known as matrix-metalloproteases (MMPs, e.g. collagenase, stromelysin, etc.). To date, potent inhibitors of MMPs are peptides or peptide-like structures and there is growing evidence that even metabolically stable peptide-like molecules may be subject to rapid and extensive clearance by biliary excretion.[2] A variety of *in vitro, in situ* and *in vivo* models have been utilized to examine potential barriers which limit the bioavailability of hydroxamate-based MMP inhibitors. This report describes some structural features which influence the bioavailability of hydroxamate-based MMP inhibitors in the rat.

Hydroxamate **1** has been reported[3] to be a potent inhibitor of human fibroblast collagenase (HFC). Although **1** is stable in rat biological fluids [Table 1], its oral bioavailability in the rat is negligible. While *in situ* models reveal that the drug is absorbed across rat intestine by measuring the portal blood supply[4] (data not shown), no intact **1** escapes the liver. Low levels of **1** were detected in pre-hepatic circulation, but neither the parent hydroxamate **1**, nor its major metabolite, carboxylate **1a** [Figure 1], was detected in post-hepatic circulation. Analysis of bile revealed extensive excretion of unchanged drug **1** [Figure 2].

Figure 1.

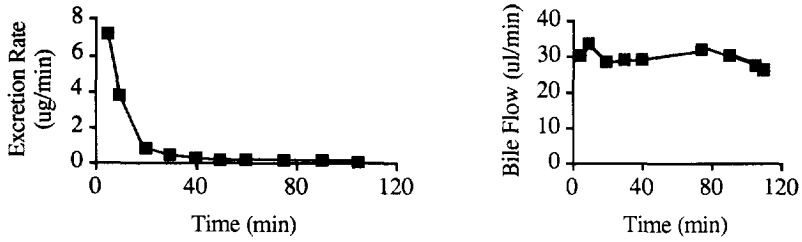


In fact, following *i.v.* administration of 5 mg/kg of **1** to rats with jugular and bile duct cannulae,[4] more

Table 1. *in vitro* Stability of Hydroxamate 1

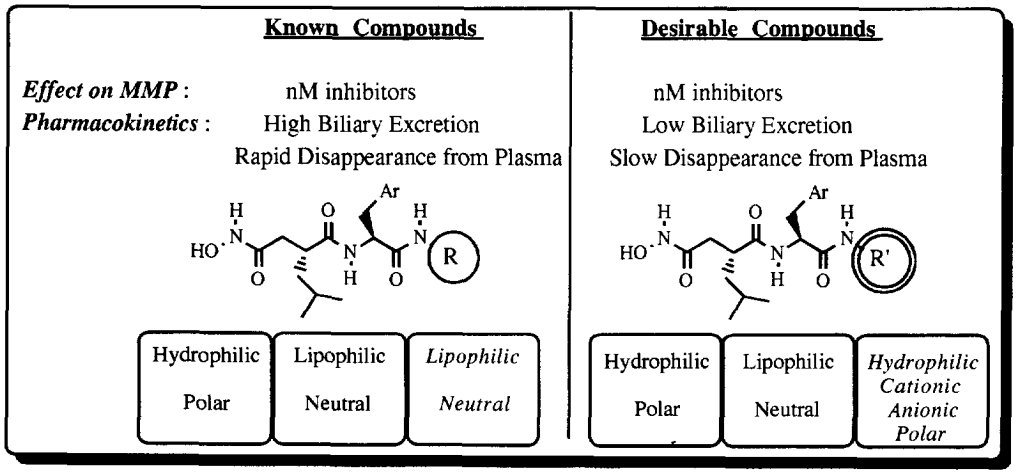
Matrix	Half-Life @ 37^{0}C
Buffer pH 3.1	Stable
Rat Intestinal Wash	Stable
Buffer pH 9.6	7.8 h
Rat Plasma	3.5 h
Rat Whole Blood	10.6 h
Human Plasma	17.0 h

Figure 2. Time Course for Biliary Excretion of Compound (1)



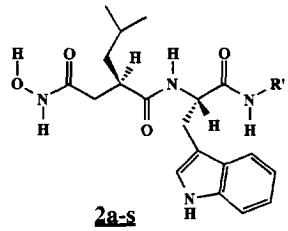
than 90% of **1** was excreted in the bile in one hour. Moreover, 33% of the total biliary excretion occurred within the first 5 minutes. The rapid elimination exhibited by hydroxamate **1** was hypothesized to be due to the overall high lipophilicity of this compound.

Figure 3. Hypothesis for Modification of C-Terminal Amides to Improve Pharmacokinetic Parameters.



In order to improve the pharmacokinetic properties of the inhibitor, the C-terminal amide portion of inhibitor **1** was chosen for modification. This portion was viewed as a site at which significant change in the overall physicochemical properties could be created while still maintaining nM enzyme inhibition. The hypothesis for employing this strategy is outlined in Figure 3.

It has been shown that the tryptophan analog, **2a** [Figure 4, R'=CH_3] is a 3-fold more potent inhibitor [Ki = 2 nM] of HFC than **1**. Neutral, polar, cationic and anionic functional groups have been incorporated into various C-terminal amides, **2b-s** [Table 2]. The use of tryptophan containing analogs allowed fluorescence detection of lower amounts of the compound in biological fluids. As shown in the Table 2, incorporation of a variety of functional groups provided analogs with comparable or greater activity against HFC vs. the reference compound **1**. These analogs were synthesized following previously reported methods.[5]



2a-s

Figure 4.

Table 2. Inhibition Data for Human Fibroblast Collagenase (HFC) [6]

Compounds	R'	Ki *vs* HFC (nM)
1		6
2a	$-CH_3$	2
2b	$-(CH_2)_4-COOH$	1
2c	$-(CH_2)_2-N(CH_3)_2$	12
2d	$-(CH_2)_3-N(CH_3)_2$	3
2e	$-(CH_2)_4-N(CH_3)_2$	3
2f	$-(CH_2)_2-O-CH_3$	5
2g	$-(CH_2)_2-S-CH_3$	5
2h	$-(CH_2)_2-S(O)-CH_3$	2
2i	$-(CH_2)_2-SO_2-CH_3$	3
2j	$-(CH_2)_2-SO_2-NH_2$	6
2k	$-(CH_2)_2-S-(CH_2)_2-N(CH_3)_2$	5
2l	$-(CH_2)_4-CO-C_6H_4-OCH_3$	2
2m	$-(CH_2)_4-O-CH_2-C_6H_5$	2
2n	$-(CH_2)_2-S-CH_2CH_3$	3
2p	$-(CH_2)_2-S-C_6H_5$	3
2q	$-(CH_2)_3-S-CH_3$	1
2r	$-CH_2-C_6H_4-CH_2-N(CH_3)_2$	4
2s	$-(CH_2)_2-S-(CH_2)_2-N$(1,4-morpholine)	4

Analogs incorporating tertiary amines, **2c-e**, improved plasma half-life ($t_{1/2}$) 2-3 fold over the reference compound **1**. More importantly these analogs have considerably reduced biliary excretion [Table 3]. However, the carboxylate analog **2b**, behaved like the methyl compound **1** and was essentially completely excreted in

bile. Moreover, tertiary amine analog **2r**, containing an aromatic spacer, also showed very high biliary excretion. As anticipated, the analogs **2l** and **2m**, containing the non-polar aromatic ketones and benzyl ether groups, respectively, were significantly excreted into bile. The aromatic ketone analog **2l**, showed the shortest plasma half-life.

In a comparable series of tertiary amine **2c**, ether **2f**, and thioether **2g**, surprisingly the thioether gave the longest plasma $t_{1/2}$ (32 min. vs. 21 min. & 19 min. for the amine and ether analogs, respectively) [Figure 5]. The longer plasma $t_{1/2}$ of **2g** was also reflected in the lower biliary excretion of the thioether compared to the ether **2f** [Figure 6]. It was confirmed that the result of the thioether **2g** was not due to enzymatic oxidation at sulfur, as authentic sulfoxides **2h**, and sulfone **2i**, gave different results. In addition, the identity of the parent thioether in plasma and bile was confirmed by HPLC by comparison with the corresponding sulfoxides and sulfone, **2h** and **2i**, respectively. The plasma half-life and biliary excretion results for analogs **2b-s** are summarized in Table 3.

Table 3. Pharmacokinetic Parameters [a] for HFC Inhibitors (2b-2s) with C-Terminal Modifications

Compounds	R'	Plasma $t_{1/2}$ (min.)	Biliary Excretion (% of total dose*)
1	$-CH_3$	9.0 ± 2.9	84.1 ± 7.3
2b	$-(CH_2)_4-COOH$	6.9 ± 2.4	108.0 ± 18.9
2c	$-(CH_2)_2-N(CH_3)_2$	21.0 ± 1.5	18.9 ± 3.7
2d	$-(CH_2)_3-N(CH_3)_2$	25.0 ± 4.2	18.1 ± 4.2
2e	$-(CH_2)_4-N(CH_3)_2$	26.6 ± 8.3	17.9 ± 8.0
2f	$-(CH_2)_2-O-CH_3$	19.0 ± 4.4	59.2 ± 11.8
2g	$-(CH_2)_2-S-CH_3$	32.4 ± 4.1	27.3 ± 4.3
2h	$-(CH_2)_2-S(O)-CH_3$	7.4 ± 0.7#	44.8 ± 10.1
2i	$-(CH_2)_2-SO_2-CH_3$	24.0 ± 3.1	67.2 ± 6.1
2j	$-(CH_2)_2-SO_2-NH_2$	17.6 ± 5.4	61.3 ± 5.7
2k	$-(CH_2)_2-S-(CH_2)_2-N(CH_3)_2$	43.7 ± 7.7	60.8 ± 11.0
2l	$-(CH_2)_4-CO-C_6H_4-OCH_3$	2.5 ± 0.6	74.5 ± 5.3
2m	$-(CH_2)_4-OCH_2C_6H_5$	27.0 ± 1.7	99.9 ± 15.3
2n	$-(CH_2)_2-S-CH_2CH_3$	37.6 ± 5.8	46.0 ± 4.2
2p	$-(CH_2)_2-S-C_6H_5$	33.3 ± 1.5	43.9 ± 8.0
2q	$-(CH_2)_3-S-CH_3$	37.0 ± 3.1	53.0 ± 2.0
2r	$-CH_2-C_6H_4-CH_2-N(CH_3)_2$	<MQL	80.7 ± 0.9
2s	$-(CH_2)_2-S-(CH_2)_2-N$(1,4-morpholine)	42.5 ± 6.8	30.8 ± 4.1

a Data shown for each compound are the Mean ± SEM for n =3. (see refrences 4 and 7 for details)
\# Mixture of diastereoisomers at sulfur; two peaks observed by HPLC, so value is questionable.
* All compounds were dosed (*i.v.* via jugular vein) at 5 mg/kg; except compounds 2e and 2r were dosed at 2.5 mg/kg

The analog **2k**, formed by insertion of a thioether functionality in the tertiary amine analog **2e**, resulted in an analog which was still potent and had the longest plasma $t_{1/2}$ (44 min.). A similar analog **2s**, containing a less basic amine group, gave a comparable plasma $t_{1/2}$; moreover, this analog had reduced biliary excretion

compared to the amine analog **2k**, [Table 3]. It was shown that introduction of a basic amine functionality markedly improves the pharmacokinetic properties of compounds in this series. In addition, the biliary excretion data for analogs **2k** and **2s** indicates that the pKa of the amines may be a significant variable which needs further evaluation.

Figure 5. Plasma Half-Life Data for Compounds 2c, 2f and 2g

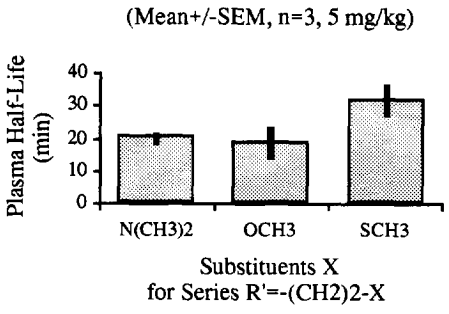
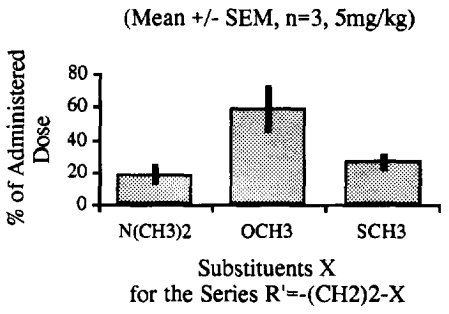


Figure 6. Percent of Total Dose Excreted in Bile for Compounds 2c, 2f and 2g in 2hr.

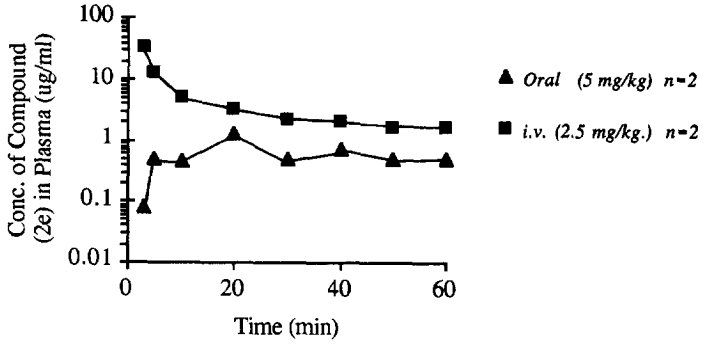


While methyl thioether analog, **2g**, was not absorbed across intestinal barrier, the corresponding ethyl thioether analog, **2n**, showed signs of overcoming the intestinal barriers. The thioether analog **2k**, containing a tertiary amine group, gave the highest portal blood concentration among the thioethers examined in the *in situ* model[7] [Table 4]. Even though **2n** and **2k** show significant absorption across the intestinal barrier *in situ*,

Table 4. Blood Levels of Thioether Analogs 2g, 2n and 2k in Portal Vein (*in situ* Model).[7]

Compounds	R'	dose (mg/kg)	Cmax. μg/ml (portal)
2g	-$(CH_2)_2$-S-CH_3	10	0
2n	-$(CH_2)_2$-S-CH_2CH_3	20	0.900
2k	-$(CH_2)_2$-S-$(CH_2)_2$-$N(CH_3)_2$	10	2.8

Figure 7. Plasma Concentration of Compound 2e Following Oral or IV Administration to Rats



these analogs do not show any detectable oral bioavailability.

The tertiary amine analog **2e** was administered intravenously (2.5 mg/kg) and orally (5 mg/kg) to rats cannulated in the jugular vein and the plasma samples were analyzed for parent drug by HPLC. The AUC_{0-60} values were used to calculate approximate oral bioavailability of analog **2e** [Figure 7]. This analog was found to be 8.5% orally bioavailable in rat.

In conclusion, the presence of a tertiary base at the C-terminus significantly reduced biliary excretion and increased plasma half-life. Introduction of a thioether functionality at the C-terminus produced a more favorable pharmacokinetic profile in this series of hydroxamate inhibitors. The rational design of C-terminally modified MMP inhibitors has led to analogs that are less susceptible to first pass loss by biliary excretion and the preliminary SAR appears to be useful for identifying MMP inhibitors with potential oral bioavailability.

Acknowledgment

The technical expertise of Cynthia Johnson and Nellie Seger is gratefully acknowledged for parmacokinetic parameter measurements.

References and Notes:

a. Current address - Gilead Science Research, 346 Lakeside Dr. Foster City, CA 94404.
b. Current address - Biomeasure Inc., 27 Maple Street, Milford, MA 01757.
c. Current address - Eastman Kodak Company, Rochester, NY 14650
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6. The activity of human gingival fibroblast collagenase was measured using 350 μM Pro-Leu-Gly-Leu-Trp-NHMe (PLALW-NHMe) in a 100 μl volume of 0.05 tricine, pH 7.5, 10mM $CaCl_2$, 200 mM NaCl, 0.02% NaN_3 in microtiter plates. Typical reaction conditions were 1 h reaction using 1 nM HFC. The reaction was stopped with 25 μl of 10 mM 1,10-phenanthroline and the product H-Leu-Trp-NHMe was determined fluorometrically with a Flow Titertek II plate reader, after 30 min reaction with 25 μl of 5.5 mg fluorescamine in dioxane. The Km for PLALW-NHMe is 140 mM for collagenase. Ki values were calculated from the expression $IC_{50} = V_i / V_o / (1-V_i / V_o)$ and $Ki = IC_{50} / (1+S / Km)$.
7. Bile flow was ascertained by gravimetric determination of samples collected in preweighed tubes. Blood was collected into heparinized microcentrifuge tubes and centrifuged to remove plasma. Plasma half-life, as measured by disappearance of parent compound, was monitored via isocratic reversed-phase (C-18) HPLC methodology with fluorescence detection. The individual HPLC methods were optimized for each compound in the various biological matrices. To obtain the metabolic rate constant and half-life, a linear regression of the natural logarithm of the peak areas as a function of time was obtained. The metabolic rate constant was recorded as the slope of the regression line and the half-life calculated as 0.693 multiplied by the reciprocal of the slope.Biliary excretion rate was estimated by summing the amount of drug-related material (parent and metabolites) recovered in the bile over the collection period and dividing by the total dose administered. For he purpose of this experiment, drug-related peaks (parent and metabolites) were defined as peaks appearing in the fluorescence chromatograms of bile from dosed animals that were not present in control bile samples collected from the same rats prior to drug administration. Since no formal metabolite isolation and identification procedures were undertaken, no correction factors for molecular weight normalization were applied.

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