Communications to the Editor

Orally Active Water-Soluble N,O-Acyl Transfer Products of a β,γ-Bishydroxyl Amide Containing Renin Inhibitor

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The search for orally active inhibitors of human renin for the treatment of hypertension in humans continues to challenge medicinal chemists.[1] One of the principal obstacles to the development of renin inhibitors is poor oral activity due in part to low aqueous solubility which compromises absorption.[2-4] In this report, we will show an example of two isomeric forms of a known renin inhibitor, acting as prodrugs, that maintain the oral activity of the parent and which exhibit greatly enhanced aqueous solubility over the entire range of physiological pH values. The functional groups involved in the isomerization of the parent compounds, a β-hydroxyl amide or a β,γ-bishydroxyl amide, are common Pl-Pl′ groups to most known renin inhibitors. Our results indicate that renin inhibitors with greater solubility may be designed from any active compound that contains these functional groups.

HIV protease, like renin, is an aspartyl protease. The aqueous solubility of potential HIV protease inhibitors is important for biological activity in vivo. Since most known HIV protease inhibitors contain either a β-hydroxyl amide or a β,γ-bishydroxyl amide,[5] the isomerization process described in this report may be used in attempts to design soluble prodrugs of HIV protease inhibitors.

Compound 1 is an orally active renin inhibitor with a long duration of action.[6] In performing accelerated decomposition studies on 1 for HPLC assay validation, the starting material was observed to convert to a major unknown compound when heated at 80 °C for 2 h in 50/50 1 N HCl/acetonitrile. A second major unknown appeared after 5 h of reaction time. These two compounds were isolated by preparative chromatography and identified by infrared, ^{1}H-NMR, and mass spectroscopy. The first product, compound 2, is derived from the N,O-acyl transfer of 1, presumably via a hydroxyoxazolidine intermediate (Scheme I). The migration of acyl groups from an N-acylamino alcohol to form an O-acyl analog under acidic conditions has previously been reported.[7-9] Transesterification of 2 results in the formation of compound 3. Compounds 2 and 3 in solution exist in an equilibrium which is readily observed at pH values under 3.5. At pH 6.0, compound 2 in aqueous solution reconverts rapidly (i.e., <2 min at room temperature) to 1. Compound 3 reconverts through 2 to 1 rapidly at pH 8.5. At this pH, the equilibrium concentration of 2 is too low to observe by HPLC. The newly formed esters are much more soluble than 1 in aqueous buffers over a full range of physiological pH values. When administered orally, 2 and 3 demonstrate comparable blood pressure lowering activity to 1 in salt-depleted normotensive monkeys.

Isolation of 2 and 3. Parent compound 1 was reacted for 3 h at 15 mg/mL in 100/1 chloroform/concentrated HCl at 37 °C in order to generate crude 2 (in equilibrium with 1 and 3). Preparative silica gel chromatography on a sample from this crude product resulted in a white solid, 2·HCl. A 24 mg/mL solution of 1 in 100/1 chloroform/concentrated HCl was reacted for 16 h at 45 °C in order to generate crude 3 (in equilibrium with 1 and 2). This product was purified by silica gel chromatography, giving 3·HCl.

Solution Half-Life Calculations (pH 7.4). The half-lives of reconversion for 1.0 mg/mL solutions of 2 and 3 in 50/50 0.10 M Na_2HPO_4 (pH 7.4)/acetonitrile at 37 °C were determined by HPLC assay. Similar half-life determinations were made on 1.0 mg/mL solutions of 2 and 3 is distilled water at room temperature. The experiment was concluded after 96 h. Table I summarizes the kinetic data for 2 and 3.

Aqueous Solubility. The HCl salts of 1-3 (as well as the free base form of 1) were suspended for 1 h on a rotating mixer at 100 mg/mL in water. The suspensions were filtered, and solution solubilities were determined by HPLC assay. Similar solubility determinations were made using 0.1 N HCl, 0.05 M Na_2HPO_4 (pH 6.0), and 0.05 M Na_2HPO_4 (pH 7.4). pH measurements of the filtered solutions were taken. Table II summarizes the aqueous solubility data found for 1-3.

Because of compound instability, solubility data for 2·HCl was not determined at pH 6.0 and 7.4.

Oral Efficacy. Compounds 1-3 were evaluated for oral efficacy in salt-depleted normotensive monkeys. Male cynomolgus monkeys weighing between 5.2 and 7.8 kg were placed on a low-sodium diet (Bio-Serv Inc., Frenchtown, NJ) 7-10 days prior to testing. Each monkey was then treated with furosemide (Lasix, INJ 5%, Hoechst-Roussel) at 2 mg/kg per day im for 4 consecutive days prior to testing.

Because of limited water solubility, 1 was prepared as a solution in a mixture of water, dimethylacetamide and Tween 80 (62.5:7.5:30). Compounds 2 and 3 were prepared in distilled water. The solutions were administered by oral gavage using a 16-French rectal-colon tube (Davol, Cranston, RI). Compound 1 was given in a volume of 2 mL/kg. Compounds 2 and 3 were given in a volume of 10 mL/kg. Monkeys were instrumented with vascular access ports (Norfolk Medical Products, Skokie, IL) for intraarterial blood pressure monitoring. Blood pressure was measured using a computer data acquisition system. Monkeys selected for these studies had been trained to rest quietly in a basic macaque restrainer (Primate Products, Woodside, CA).

Figure 1 illustrates the oral efficacy of compounds 2 and 3 compared with 1. All three compounds showed comparable reductions in blood pressure following oral administration of 10 mg/kg.

The above experiments demonstrate the potential for the use of N,O-acyl transfer products of known renin

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Scheme I. *N,O*-Acyl Transfer of 1

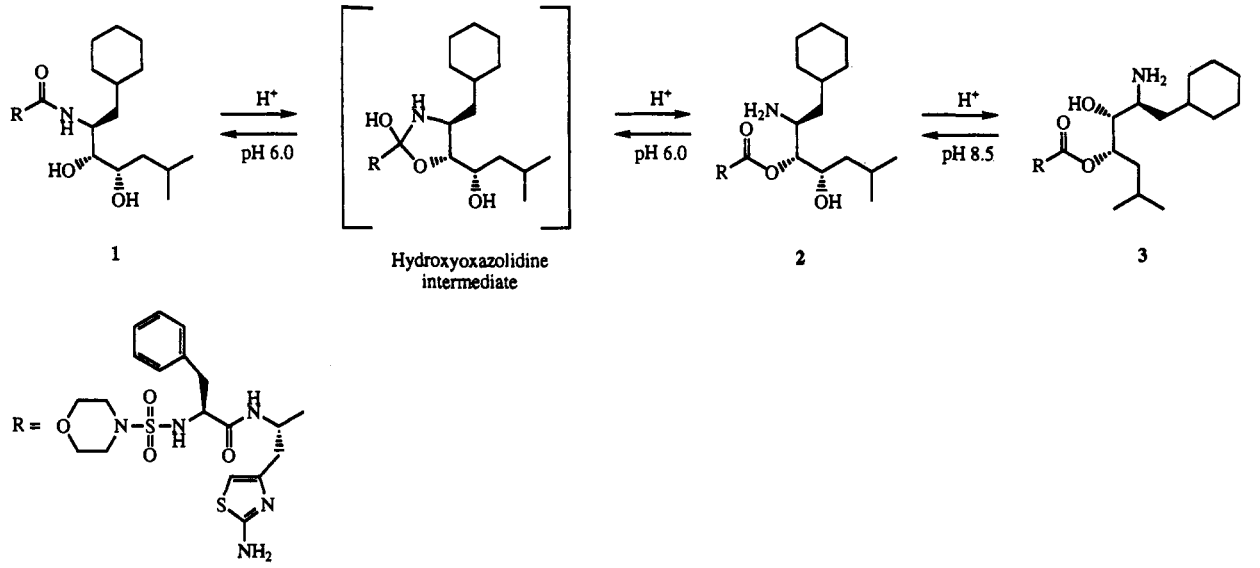

Table I. Kinetic Data for the Conversion of 2 and 3 to 1

compd	solvent	temp, °C	$t_{1/2}$
2	CH_3CN/buffer, pH 7.4	37	1 min
3	CH_3CN/buffer, pH 7.4	37	4.8 h
2	distilled water	RT	>96 h
3	distilled water	RT	>96 h

Table II. Aqueous Solubility

compd	solvent	solubility (mg/mL)	pH of filtered solution
1·HCl	water	0.020	4.8
1 (free base)	water	0.002	5.8
2·HCl	water	17.6	3.3
3·HCl	water	20.9	3.2
1·HCl	0.10 N HCl	6.6	1.6
2·HCl	0.10 N HCl	54.6	1.5
3·HCl	0.10 N HCl	71.3	1.7
1·HCl	pH 6.0 buffer[a]	0.001	6.0
3·HCl	pH 6.0 buffer[a]	1.94	6.0
1·HCl	pH 7.4 buffer[a]	0.001	7.4
3·HCl	pH 7.4 buffer[a]	0.300	7.4

[a] Suspended at 5.0 mg/mL.

inhibitors as soluble prodrugs. The primary amine moiety in compounds such as 2 and 3 allows for a much simplified formulation for parenteral administration. After 4 h in distilled water at room temperature, no reconversion of compounds 2 and 3 to parent 1 was detected by HPLC assay. The short half-life of 3 and the very short half-life of 2 in pH 7.4 solution at 37 °C (4.8 h and 1 min, respectively, Table I) suggest that the esters are likely converting to the active form (amide 1) in vivo. The conversion of 2 and 3 was demonstrated by detection of 1 in postdosing blood samples. Due to the reverse isomerization of 2 and 3 during an in vitro bioassay, accurate IC_{50} values for renin inhibition by 2 and 3 cannot be measured.

The HCl salts of both 2 and 3 are approximately 1000× more water soluble than the HCl salt of 1 (Table II). Compounds 2 and 3 are approximately 10× more soluble than 1 in 0.10 N HCl. 3 is approximately 2000× more soluble than 1 in pH 6.0 buffer and approximately 300× more soluble than 1 in pH 7.4 buffer. These data show a solubility advantage for *N,O*-acyl transfer products 2 and 3 versus parent compound 1 over the entire range of physiological pH values. Of nine β,γ-bishydroxyl amide renin inhibitors randomly selected from our research program, all exhibited characteristics of reversible isomerization via *N,O*-acyl transfer.[10] The phenomenon was observed by HPLC analyses of the compounds. Prodrugs of other aspartyl protease inhibitors (e.g., HIV protease inhibitors) with higher solubility may be designed from the *N,O*-acyl transfer isomers of active compounds that contain either a β-hydroxyl amide or a β,γ-bishydroxyl amide functional group.

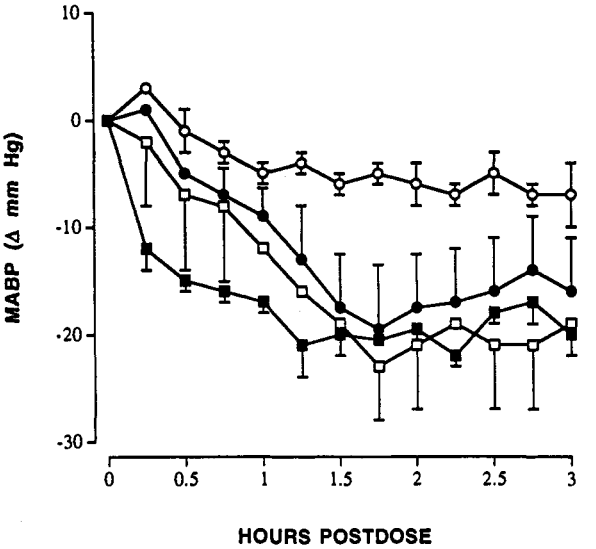

Figure 1. Comparative activity of compounds 1–3 in salt-depleted normotensive monkeys after an oral dose of 10 mg/kg. (●) 1 (N = 5); (■) 2 (N = 2); (□) 3 (N = 4); (○) vehicle* (N = 6). *The vehicle (2 mL/kg) consisted of 7.5% dimethylacetamide, 30% Tween 80, and 62.5% distilled water and was used in the administration of 1. Compounds 2 and 3 were administered in distilled water.

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Supplementary Material Available: NMR, IR, microanalytical, and mass spectral data for compounds 1–3, a detailed description of the isolation of 2 and 3, HPLC chromatograms of 1–3, and a detailed description of *N,O*-acyl transfer in related compounds (22 pages). Ordering information is given on any current masthead page.

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(10) Full details of the observed *N,O*-acyl transfer of other β-hydroxyl and β,γ-bishydroxyl amides can be obtained as supplementary material.