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SAR and Species/Stereo-Selective Metabolism of the Sorbitol Dehydrogenase Inhibitor, CP-470,711

Margaret Y. Chu-Moyer,* William E. Ballinger, David A. Beebe, James B. Coutcher, Wesley W. Day, Jiancheng Li, Peter J. Oates and R. Matthew Weekly

Cardiovascular and Metabolic Diseases, Pfizer Global Research and Development, Groton Laboratories MS8220-3095, Eastern Point Road, Groton, CT 06340, USA

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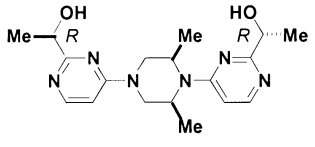
Abstract—SAR studies on the stereoisomers of CP-470,711 suggested that in vivo epimerization was taking place in rats. Further metabolism studies revealed that no epimerization was occurring in dogs, and that no epimerization was expected in humans. A mechanism for the in vivo epimerization is proposed involving an oxidation–reduction pathway of the secondary benzylic alcohol, in contrast to an acid/base-promoted epimerization of the same center during chemical synthesis.

Diabetes mellitus afflicts approximately 135 million people worldwide, with an estimated diabetic population of approximately 16 million in the United States alone. With its complications, diabetes is the sixth-leading cause of death by disease. These hyperglycemia-related diabetic complications include neuropathy, nephropathy, retinopathy and cardiovascular disease which can lead to lower-limb amputations, end-stage renal failure, loss of vision and myocardial infarction, respectively.[1,2]

Recently, we reported the synthesis and SAR leading up to the identification of CP-470,711 (**1**), a potent inhibitor of sorbitol dehydrogenase (SDH), the second enzyme in the polyol pathway.[3] The contribution to nerve complications from the redox imbalance (↑ NADH/NAD^+ ratio) caused by increased flux through this step in the pathway in the diabetic state was shown earlier with a prototype inhibitor.[4]



1: CP-470,711

*Corresponding author. Fax: +1-860-715-9628; e-mail: margaret_y_chu-moyer@groton.pfizer.com

CP-470,711 incorporates two chiral hydroxyethyl side chains of the (*R*)-configuration on each of its pyrimidine rings. Eariler SAR of molecules in this class showed that pyrimidines bearing only one (*R*)-hydroxyethyl side chain showed potent in vitro activity, while compounds containing only one (*S*)-hydroxyethyl side chain possessed lower activity.[5] Because of the fact that CP-470,711 bears two hydroxyethyl side chains, its (*R*,*S*)-, (*S*,*R*)-, and (*S*,*S*)-isomers were prepared to further understand this SAR (Scheme 1).

Previously, we had shown that the condensation of mono-benzyl protected piperazine **2** with reactive pyrimidines, such as triflate **3**, resulted in mixtures of **4*R***/**4*S***.[3] In the particular case when refluxing CH_3CN was employed, a 9:1 mixture was obtained. These enantiomers are easily separated by chiral HPLC, and the required (*R*)- or (*S*)-isomer can be carried on to subsequent steps. In the event, removal of the benzyl protecting group from **4*R*** or **4*S*** under transfer hydrogenolysis conditions provided **5*R*** and **5*S***, respectively. Subsequent condensation with the appropriate chloropyrimidine,[6] **6*R*** or **6*S***, and cleavage of the butyrate protecting groups, provided the target compounds, **1** and, **7**–**9**, in >95% ee by chiral HPLC.[7]

In vitro evaluation[3] of these analogues against both human and rat SDH showed that those compounds which contained at least one pyrimidine bearing the (*R*)-ethanol side chain (**1**, **7** and **8**) had similar activities.

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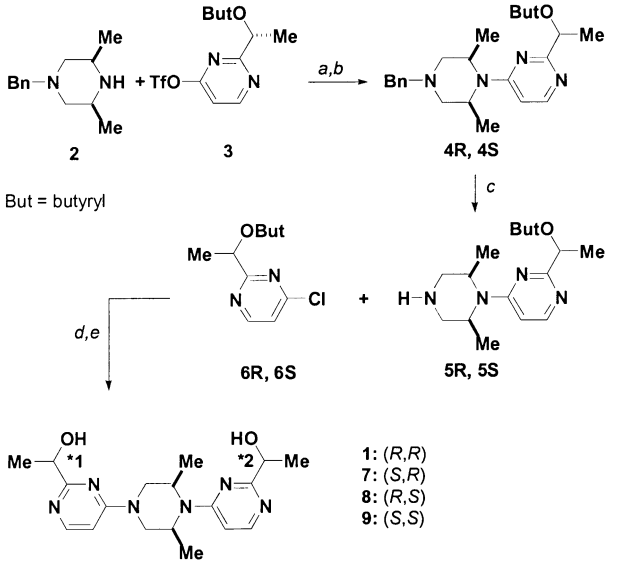


Scheme 1. (a) CH_3CN, reflux, 15 h; (b) Chiracel AD, 90:10 hexane/*i*-PrOH + 1% diethylamine; (c) 10% Pd/C, HCO_2NH_4, HCl, MeOH, reflux, 30 min; (d) Et_3N, *i*-PrOH, reflux, 18 h; (e) $LiOH{\cdot}H_2O$, 3:1 $MeOH/H_2O$, rt, 2 h.

However, the compound bearing two (*S*)-ethanol side chains (**9**) had ~30- to 40-fold lower activity, consistent with experience in our pyrimidine SDI series[3,5] (Table 1). Surprisingly, when these compounds were assayed in both the acute and chronic in vivo diabetic rat models,[3] all the derivatives had similar activity, *including* the (*S*,*S*)-isomer **9**. This result suggested that in vivo conversion of the isomers was occurring, possibly through an oxidation/reduction mechanism. Therefore, several new analogues were synthesized to evaluate this proposed hypothesis.

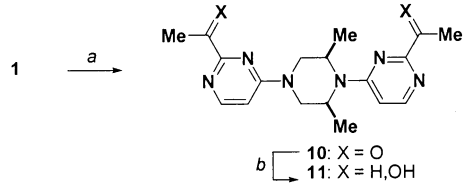
Diketone **10** was prepared via bis-oxidation of **1** under the action of MnO_2 in refluxing dichloroethane (Scheme 2). Bis-reduction of the diketone with $NaBH_4$ then provided **11**, the 1:1:1:1 mixture of all four isomers.

The two mono-ketone isomers were synthesized as follows (Scheme 3). (±)-Hydroxyethyl-pyrimidone **12**[6] was

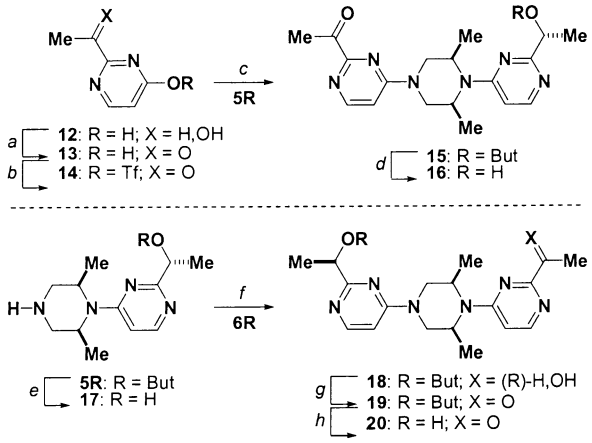
Table 1. Comparison of in vitro and in vivo activity for compounds **1**, **7**–**9**, **10**, **11**, **16** and **20**

Compd	*h*-SDH[a] IC_{50} (nM)	*r*-SDH[b] IC_{50} (nM)	Acute assay[c] ED_{90} (mg/kg/d)	Chronic assay[d] % norm. @ 1 mg/kg
1	10	17	0.2	90
7	33	29	0.4	81
8	19	27	0.8	81
9	831	640	0.7	84
10	212	227	0.9	92
11	28	8	0.6	92
16	19	18	0.2	94
20	6	5	0.5	77

[a]Inhibition of human recombinant sorbitol dehydrogenase.
[b]Inhibition of recombinant rat sorbitol dehydrogenase.
[c]Prevention of sciatic nerve fructose accumulation in streptozotocin (STZ) diabetic rats (*tid* dosing, 4-h post STZ).
[d]Reversal of sciatic nerve fructose accumulation in STZ rats (*qd* dosing, 5-days post STZ).



Scheme 2. (a) MnO_2, DCE, reflux, 7 h; (b) $NaBH_4$, MeOH, rt, 2 h.



Scheme 3. (a) MnO_2, DCE, reflux, 18 h; (b) Tf_2O, pyr, CH_2Cl_2, 0 °C, 15 min; (c) Et_3N, THF, rt, 45 min; (d) 12 N HCl/MeOH, rt, 18 h; (e) $LiOH{\cdot}H_2O$, 4:1 $MeOH/H_2O$, rt, 1 h; (f) Et_3N, *i*-PrOH, reflux, 15 h; (g) MnO_2, DCE, reflux, 19 h; (h) K_2CO_3, MeOH, rt, 4 h.

oxidized to ketone **13** and subsequently converted to triflate **14** under standard conditions. Coupling with piperazine **5*R*** provided **15**, which upon deprotection of the butyrate group under acidic conditions gave mono-ketone **16**. The isomeric mono-ketone was prepared via first removal of the butyrate ester from compound **5*R*** to provide free alcohol **17** followed by reaction with chloropyrimidine **6*R***. The resulting differentially protected alcohol **18** was oxidized to butyrate mono-protected ketone **19**. The butyrate protecting group was then subsequently cleaved under basic conditions to give the desired compound **20**.

As expected in vitro, the diketone **10** is less potent by ~10- to 20-fold; the mixture of four isomers, compound **11**, shows good activity which is consistent with 3/4 of the material being active; and the mono-ketones **16** and **20**, which each contain a pyrimidine bearing the (*R*)-ethanol side chain, possess potent in vitro activity against both human and rat SDH. However, once again, all have similar activities in vivo suggesting that these analogues are also possible intermediates in the biological interconversion of **1** in the biological milieu of the rat.

In order to confirm the above pharmacological findings, HPLC analysis of rat plasma samples was performed. An achiral method[8] was utilized to determine the composition of the mixture [e.g., presence or absence of di-alcohol (**11**), mono-ketone (**16** and **20**) and di-ketone (**10**)] and a chiral method[9] was used to determine the ratio of the di-alcohol stereoisomers (**1**, **7**, **8** and **9**).

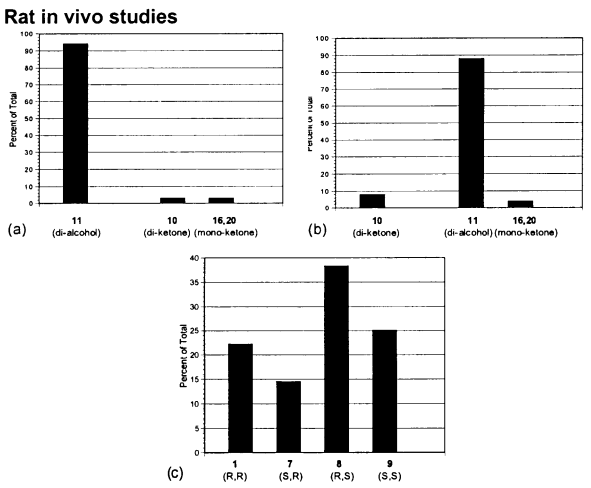


Figure 1. Rat drug metabolism studies. Data were obtained at 1 h post-dose for all experiments. (a) Achiral metabolism of compound **1**; (b) Achiral metabolism of compound **10**; (c) distribution of the di-alcohol stereoisomers **1**, **7**, **8** and **9** after dosing with compound **1**.

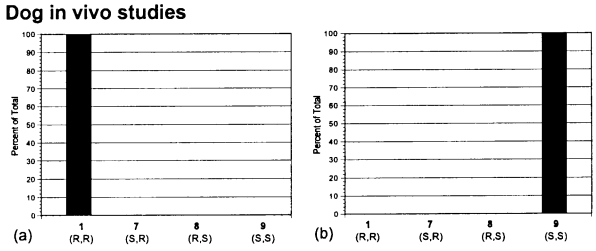


Figure 2. Dog drug metabolism studies. Data were obtained at 1 h post-dose for all experiments. (a) Distribution of the di-alcohol stereoisomers after dosing with compound **1**; (b) distribution of the di-alcohol stereoisomers after dosing with compound **10**.

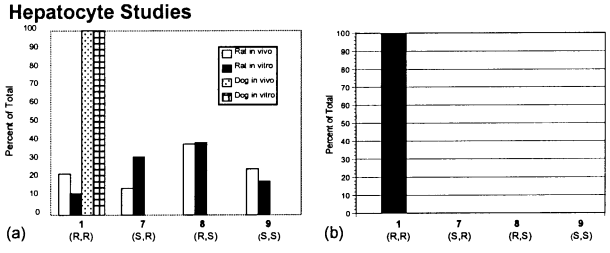


Figure 3. (a) Comparison of in vivo rat/dog chiral metabolism of compound **1** with in vitro chiral metabolism in rat/dog hepatocytes; (b) in vitro chiral metabolism of compound **1** in human hepatocytes.

In the event, when rats were dosed with **1** (20 mg/kg, po), achiral HPLC showed mainly the presence of di-alcohol **11** with a very small amount of di-ketone **10** and mono-ketones **16** and, **20**, suggesting that oxidation and reduction could be occurring to some extent (Fig. 1a). When rats were dosed with di-ketone **10** (5 mg/kg, po), di-alcohol **11** was again the major species in plasma with a small amount of di-ketone **10** and mono-ketones **16** and **20** present (Fig. 1b). Analysis of the chiral composition of the di-alcohol from the former experiment revealed that all four isomers, **1**, **7**, **8** and **9**, were present in nearly equal quantities (Fig. 1c). These results strongly suggest the intermediacy of a transient ketone species that is non-selectively reduced to give a statistical mixture of di-alcohols.

A comparable study in dogs revealed that no apparent epimerization was taking place upon oral administration (30 mg/kg) of compound **1** (Fig. 2a). As well, there was no evidence of formation of any mono-ketone or di-ketone intermediates. There are two possible explanations for apparent *lack* of epimerization of **1** in dog: (1) the alcohols are not oxidized by the dog or (2) the alcohols are indeed oxidized by the dog, but stereospecific reduction back to the (*R*)-alcohol takes place to result in no net stereochemical change. In order to further study the mechanism, di-ketone **10** was given orally to dogs (10 mg/kg). Interestingly, the di-ketone was indeed reduced to the di-alcohol, but chiral HPLC analysis revealed that it was completely of the (*S*,*S*)-configuration (Fig. 2b). These data suggest that the lack of epimerization seen in the dog is due to the inability of the dog to oxidize the alcohol to the ketone, for if this did occur, only compound with (*S*,*S*)-stereochemistry would be detected.

As it became apparent that the oxido-reductive metabolism of compound **1** was conspicuously different in the rat versus the dog, we were interested in determining which of these species would be a predictor for metabolism of **1** in humans. In order to do so, a relevant in vitro system was required. After extensive screening for marker cells, only rat and dog hepatocytes were found to mimic the metabolic pattern observed in the rat and dog in vivo studies. That is, whereas metabolic turnover of the chiral hydroxyethyl group was observed in the rat hepatocytes,[10] no turnover was seen with the dog hepatocytes[11] (Fig. 3a). Human hepatocyte experiments revealed that, like dog hepatocyte experiments, no metabolism of the hydroxyethyl group was taking place (Fig. 3b). To the extent that the metabolism of **1** in humans would be entirely mediated by hepatocytes, it is expected that results in humans would mirror the findings in dogs.

During the synthesis of the sorbitol dehydrogenase inhibitor CP-470,711 (**1**), epimerization of the secondary alcohol stereocenter was observed. Efforts to further study this reaction as well as to characterize the isomers arising from the two chiral centers present in **1** led to the synthesis of **7**, **8** and **9**. Although in vitro activity was consistent with known SAR, the in vivo results suggested that these compounds were undergoing epimerization through an oxidation–reduction mechanism. Various redox isomers of **1** were also synthesized and pharmacological data on these analogues supported the above finding.

Direct analysis of rat plasma revealed that in vivo chiral metabolism of **1** was indeed taking place, very likely through the intermediacy of ketone analogues **10**, **16** and **20**. However, in dogs, this metabolism was not observed. Further mechanistic studies showed that dogs did not oxidize the alcohol to the ketone and therefore, 'isomerization' of the alcohol stereocenter did not occur. In vitro hepatocyte studies reflected the results in rat and dog, thus suggesting that metabolism of **1** in humans would be akin to that observed in dog.

References and Notes

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7. We gratefully acknowledge Stephen M. Chesnut and Stephen M. Brown (PGRD Analytical Chemistry) for developing the chiral HPLC assay and evaluating these compounds according to the following conditions: Chiralpak AS (250×4.6 mm), 85:15 hexane/EtOH, $T = 40\,^{\circ}C$, 1.0 mL/min, UV detection at 254 nM.
8. Serum samples (300 μL) were collected at selected time points and combined with an internal standard. This mixture was diluted with distilled H_2O (1 mL), extracted with EtOAc (5 mL), evaporated to dryness and reconstituted in mobile phase (150 μL). These extracts (120 μL) were analyzed by reverse-phase HPLC using a Kromasil C4 column (250×4.6 mm) and elution with a 1:1 MeOH/H_2O mobile phase containing 10 mL Pic-B7/liter solution. The flow rate was set at 1.0 mL/min and UV detection at 237 nM was used.
9. Serum samples (300 μL) were collected at selected time points and combined with an internal standard. This mixture was diluted with distilled H_2O (1 mL), extracted with EtOAc (5 mL), evaporated to dryness and reconstituted in mobile phase (150 μL). These extracts (120 μL) were analyzed by normal-phase chiral HPLC using a Chiralpak AS column (250×4.6 mm) and elution with a 1:1 EtOH/hexane mobile phase. The flow rate was set at 1.0 mL/min and UV detection at 254 nM was used.
10. Compound **1** (6.7 μM) was incubated with rat hepatocytes for 2 h. Samples were prepared and assayed as described in ref 9.
11. Compound **1** (6.7 μM) was incubated with dog hepatocytes for 4 h. Samples were prepared and assayed as described in ref 9.