ABSTRACT:
A series of potent indole-containing endothelin antagonists were evaluated in rat pharmacokinetic studies as part of a rational drug design program. Early compounds in this series were found to show poor gastrointestinal absorption, limiting their utility as oral agents. Structural modifications and pharmacokinetic studies indicated that reducing the overall H-bonding potential, through a reduction in the number of H-bond donors and acceptors, could increase absorption of the molecules. There was a correlation between calculated H-bonding capacity and rate of permeability across Caco-2 monolayers for this series of compounds. Caco-2 permeability was also shown to be indicative of the estimated extent of absorption in rats. Balancing the requirements of absorption and systemic clearance lead to the selection of an alcohol-containing compound, compound 7a (single enantiomer of compound 7) that was moderately absorbed after oral administration and converted to an active acid metabolite, which itself was of low intrinsic clearance. Species differences were observed between the absorption of compound 7a in rat and dog and also in the extent of conversion to the acid metabolite. Absorption was estimated at 30% in rat and 100% in dog. Approximately 30% of the absorbed drug was converted to systemically available acid metabolite in rat, compared with only 3% in dog.

Endothelin-1 is a potent vasoconstrictor and growth factor released from the vascular endothelium. It has been implicated in the pathophysiology of a number of diseases (Miyauchi and Masaki, 1999) and as such represents an attractive drug target, particularly in the cardiovascular area (Kentsch and Otter, 1999). Selective and mixed endothelin A (ET_A) and endothelin B (ET_B) receptor antagonists have been investigated for the treatment of essential hypertension, chronic heart failure, pulmonary hypertension, and in the prevention of restenosis (Dupuis, 2000).

Based on chemical structure precedents (Williams et al., 1995), a rational drug discovery program was undertaken to provide a selective ET_A receptor antagonist (Rawson et al., 2001). Potency and selectivity were achieved in a series of indole-containing compounds; however, these were found to have poor oral bioavailability in rat pharmacokinetic studies. Based on additional studies examining the systemic pharmacokinetic properties of these agents and their physicochemical profiles, it became apparent that the poor oral pharmacokinetic profiles were primarily the result of poor permeation of the membranes of the gastrointestinal tract. In vitro and physicochemical evidence indicated that absorption was also likely to be limited in humans. The subsequent medicinal chemistry program and pharmacokinetic studies were therefore directed toward achieving improved oral absorption within this chemical series. Throughout the program, reference to physicochemical properties, in particular the H-bonding capacity of the molecules was used to improve the understanding of structural requirements for membrane permeation. Further pharmacokinetic studies were performed in both rat and dog to more fully profile the absorption properties of the eventual lead compound, compound 7a (single active enantiomer of compound 7). Pharmacological potency and selectivity were key reasons for focusing on the series of compounds discussed. However, for the purposes of this evaluation, potency was not a primary determinant of compounds that underwent pharmacokinetic testing and physicochemical profiling, hence potency values are not presented and only considered in broad terms.

The aims of the work described here were therefore 2-fold: first to provide physicochemical and pharmacokinetic guidance in the design of an oral ET_A antagonist; and second, to define the pharmacokinetic profile of the selected compound, compound 7a.

Materials and Methods
Chemicals. Compounds 1 to 16 (Fig. 1) were synthesized as part of a medicinal chemistry program at Pfizer Global Research and Development, Sandwich, UK. All compounds were initially synthesized as racemic mixtures. Compounds 6 and 7 were subsequently resolved into individual enantiomers by preparative chromatography and these single enantiomers subjected to further evaluation. Single enantiomers were then synthesized by inclusion of a chiral resolution step and these compounds were designated compound 6a [(S)-(+)-3-[1-(1,3-benzodioxol-5-yl)-2-[(2-methoxy-4-methylphenyl)sulfonylamido]-2-oxoethyl]-6-(carboxy)-1-methyl-1H-indole] and compound 7a [(S)-...
(+)-3-[1-(1,3-benzodioxol-5-yl)-2-[(2-methoxy-4-methylphenyl)sulfonamido]-2-oxoethyl]-6-(hydroxymethyl)-1-methyl-1H-indole].

Rat Pharmacokinetic Studies. Male Sprague-Dawley rats (250 g; Charles River, Manston, UK) were surgically prepared with jugular vein catheters at least 2 days before dose administration. For each compound (1–16) one animal received an intravenous dose (2 mg/kg) in a vehicle containing up to 20% dimethyl sulfoxide (DMSO) in saline and one animal received an oral dose (10 mg/kg) in a vehicle containing up to 20% DMSO in water. Dose volumes were approximately 1 ml/kg for intravenous doses and 2 ml/kg for oral doses. Blood samples (200 μl) were collected from the indwelling jugular vein catheter up to 24 h after dose administration and placed into heparinized tubes. These samples were centrifuged at 3000 rpm for 10 min and the plasma removed and stored frozen before analysis.

Further pharmacokinetic studies were undertaken with the single enantiomers compound 6a and compound 7a. Both compounds were administered to jugular vein-cannulated male rats by the intravenous route at a dose level of 2 mg/kg (n = 3 for each compound). Both compounds were dissolved in Cremophor (Cremophor EL, polyoxy15 castor oil; Ph. Eur., BASF, Ludwigshafen, Germany) and the dose volume was 0.8 ml/kg. Compound 7a was also administered by oral gavage to two male jugular vein-cannulated rats at a dose of 10 mg/kg. Blood samples (200 μl) were collected from the indwelling jugular vein catheter up to 24 h after dose administration and placed into heparinized tubes. These samples were centrifuged at 3000 rpm for 10 min and the plasma removed and stored frozen before analysis.

Three male rats (~350 g) were surgically prepared with catheters in the jugular vein and hepatic portal vein at least 2 days before compound administration. Compound 7a was administered at a dose level of 2 mg/kg into the portal vein cannula in a vehicle of DMSO/ethanol/tetraglycol/water (10:9:36:45, v/v) in a volume of 0.8 ml/kg. Blood samples were collected from the jugular vein catheterer up to 11 h postdose, as previously described.

Dog Pharmacokinetic Studies. Compound 7a and compound 6a were intravenously administered to two male and one female dog (Pfizer colony, 12–18 kg) at dose levels of 0.2 and 0.5 mg/kg, respectively. Compound 7a was dissolved (0.2 mg/ml) in a vehicle of 1% DMSO (v/v) in saline containing 10% (w/v) hydroxypropyl-β-cyclodextrin and administered by intravenous infusion over 15 min into a saphenous vein. Compound 6a (0.5 mg/ml) was prepared in the same vehicle and similarly administered. Compound 7a was orally administered by gavage to the same three dogs 7 days after the intravenous dose. The dose level was 2 mg/kg and the vehicle (10-ml total volume) was the same as that used for intravenous dosing but with 10% (v/v) DMSO. Blood samples (5 ml) were collected from temporary indwelling saphenous vein catheters or by venepuncture of the cephalic vein. The blood samples were transferred to lithium heparin tubes, mixed, and centrifuged. Plasma samples were transferred to glass vials and stored frozen before analysis. Urine samples (0–7 h) were collected from male dogs after intravenous administration by catheterization of the bladder and stored frozen before analysis.

Analysis of ET₄ Antagonists in Plasma Samples from Rats and Dogs. The concentrations of the ET₄ antagonists were determined in rat and dog plasma by a solid phase extraction method followed by HPLC with ultraviolet detection. The method involved addition of internal standard (1 μg of analog with suitable HPLC retention time) to 0.1 ml of rat plasma or 1.0 ml of dog plasma. The plasma samples were diluted with 1.0 ml of 0.1 M sodium citrate buffer, pH 4.5, and applied to solid phase extraction cartridges (C₁₈ Isolute; Analytichem International, Harbor City, CA) and the cartridge washed with a
Pharmacokinetic parameters of ET A antagonists 1 to 6 in rat

<table>
<thead>
<tr>
<th>Compound</th>
<th>t½ (h)</th>
<th>CL (ml/min/kg)</th>
<th>fu</th>
<th>CLu (ml/min/kg)</th>
<th>Vd (l/kg)</th>
<th>F</th>
<th>Estimated A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>1.8</td>
<td>0.004</td>
<td>450</td>
<td>0.2</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>12</td>
<td>0.003</td>
<td>4000</td>
<td>0.9</td>
<td>1.0</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>2.3</td>
<td>0.2</td>
<td>12</td>
<td>0.2</td>
<td>3.0</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>6.6</td>
<td>0.048</td>
<td>138</td>
<td>0.3</td>
<td>1.9</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>0.7</td>
<td>9.3</td>
<td>0.007</td>
<td>1329</td>
<td>0.6</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1.1</td>
<td>6.7</td>
<td>0.011</td>
<td>609</td>
<td>0.6</td>
<td>1.0</td>
<td>1</td>
</tr>
</tbody>
</table>

fu: fraction unbound in plasma; CLu: unbound systemic clearance; Vd: volume of distribution; F: bioavailability; A: Absorption.

Pharmacokinetic studies were performed in male rats after single intravenous (2 mg/kg, n = 1) and oral (10 mg/kg, n = 1) doses.

Further 1 ml of buffer. Analytes and internal standard were then eluted with 1 ml of methanol and evaporated to dryness under a stream of nitrogen at 37°C. The residues were reconstituted in 100 µl of methanol/water (1:1, v/v) and 80 µl injected onto the HPLC column (RPB 25 x 0.5 cm; Hichrom, Reading, UK). The column was eluted at 1 ml/min with a mobile phase of which the aqueous component comprised a solution of 10 mM sodium octane sulfonate, 20 mM sodium dihydrogen orthophosphate adjusted to pH 3 with concentrated hydrochloric acid. The aqueous phase was mixed with up to 60% acetonitrile (v/v) to elute the compounds within a retention time of 15 min. Detection was by ultraviolet absorption at a wavelength of 295 nm. The limits of quantitation were 50 to 100 ng/ml in rat plasma and 10 to 20 ng/ml in dog plasma. For compound 7a and compound 6a for which analysis of quality control samples provided values within 15% of the added value throughout calibration ranges of 50 to 10,000 ng/ml in rat plasma and 10 to 2,000 ng/ml in dog plasma. Dog and rat urine samples were analyzed by the same method with appropriate sample volumes.

Permeability across Caco-2 Cell Monolayers. The permeability of compounds 1 to 16 was assessed using Caco-2 cell monolayers prepared as previously described (Yee, 1997). Compound permeability was assessed at an initial concentration of 10 µM applied to the apical chamber of the apparatus. Determining the amount of compound present in the basolateral chamber after 3-h incubation at 37°C assessed the extent of permeation. All analyses were performed in duplicate. Quantification of compound was by modification of the method described previously for plasma.

Plasma Protein Binding Determinations. Samples of rat and dog plasma (1 ml) containing ETA antagonists at a concentration of 5 µg/ml were dialyzed (Spectrapor 1 dialysis membrane 6000–8000 molecular weight cut-off; Spectramed Medical Industries, Rancho Dominguez, CA) against isotonic Krebs-Ringer-bicarbonate buffer, pH 7.4 (1 ml), for 4 h at 37°C in a rotating dialyzer (Dianorm; NBS Biologicals, Huntingdon, UK). Determinations were performed on pooled samples of male rat plasma for each compound. After dialysis, the concentrations of compound in plasma and buffer were determined using solid phase extraction and HPLC analysis, as previously described. For compound 7a and compound 6a additional plasma protein binding determinations were made in control dog and human plasma.

Pharmacokinetic Analysis of Data. Terminal elimination rate constant (Kₑ) was determined by linear regression of the log plasma concentrations. The terminal elimination half-life (t½) was calculated from 0.693/Kₑ. The area under the plasma concentration time curve (AUC) was calculated to the last time point at which drug could be measured using the linear trapezoidal rule and extrapolated to infinity using Kₑ. Clearance (CL) was calculated by the relationship dose/AUC. The volume of distribution was calculated by the relationship CL/Kₑ. Oral bioavailability was calculated from the ratio of AUCs after oral and intravenous doses after normalizing for the dose. Unbound clearance was calculated by dividing the systemic clearance value after intravenous dosing (CL) by the free fraction measured in plasma. The extent of first-pass extraction (E) was estimated by reference to the well stirred model of hepatic clearance and assuming hepatic blood flow of 100 ml/min/kg in rat and 50 ml/min/kg in dog. Based on the bioavailability observed and the estimate of hepatic extraction the extent of absorption (A) was estimated (A = f(1 – E)). For compound 7a, which was orally dosed to rats and plasma sampled from the hepatic portal vein, the extent of absorption was calculated from the relationship CL multiplied by portal vein AUC. This assumes that no significant extraction occurs in passage across the gut wall (Kwon and Inskeep, 1996) and that the clearance is solely hepatic. These assumptions are supported by observations of low renal clearance and stability to oxidative metabolism in liver microsomal preparations.

Lipophilicity Determination. Distribution of the ETA antagonists (compounds 1–16) between octanol and 0.1 M sodium phosphate buffer, pH 7.4, was determined by the method of Stopher and McClean (1990). Approximately 0.1 mg of compound was dissolved in 1 ml of octanol (octan-1-ol, specially pure; BDH, Poole, UK) and mixed with 1 ml 0.1 M sodium phosphate buffer, pH 7.4, on a rotary mixer at 30 rpm for 60 min. After centrifugation the two phases were separated and duplicate 5-µl aliquots of each phase directly injected onto the HPLC system described previously. The distribution coefficient (Dₑ,a) was calculated from the ratio of the concentration of compound in octanol to the concentration of compound in buffer. Calculated log P values (C log P) were calculated using the Medchem computer program (version 3.55; Biobyte Corp., Claremont, CA).

Assessment of H-Bond Free Energy. For each of the ETA antagonists, the free energy-based hydrogen bond acceptor and donor factors, Ca and Cd, were computed with the HYBOT 5.0 program and database by using experimental data from 12,000 H-bonded complexes (Raevsky, 1997). The total H-bonding capability was determined from the sum of the Ca and Cd values to provide hydrogen-bond free energy (Cad) values, as previously described for the comparison of H-bonding capability and permeation (van de Waterbeemd et al., 1996). The number of H-bond acceptors (HBAs) was simply assessed from counting the total number of nitrogen and oxygen atoms in each molecule, whereas the total number of HBDs was obtained from the total number of —NH and —OH functions within each molecule (Lipinski et al., 1997).

Results

Pharmacokinetic Studies with ETA Antagonists 1 to 16 in Rat. Pharmacokinetic parameters for racemic ETA antagonists 1 to 6 (Fig. 1) obtained after single intravenous (2 mg/kg) and oral (10 mg/kg) doses (n = 1 per compound per route) to male jugular vein-cannulated rats are presented in Table 1. This table also includes the free fraction in plasma obtained from plasma protein binding experiments. The same parameters for racemic ETA antagonists 7 to 16 are presented in Table 2.

Physicochemical Parameters. Physicochemical parameters of ETA antagonists 1 to 16 obtained through experimental methods (Caco-2 permeability and log Dₑ,a) and by computational analysis (molecular weight, number of HBAs and HBDs, C log P, and Cad) are presented in Table 3.

Pharmacokinetics of Compound 6a and Compound 7a in Rat. After single intravenous doses of compound 6a (single enantiomer of ETA antagonist 6) to male jugular vein-cannulated rats (2 mg/kg, n = 3), mean values for plasma clearance, volume of distribution, and elimination half-life were 1.7 ml/min/kg, 0.16 l/kg, and 1.1 h, respectively. Compound 6a was observed as a circulating metabolite of compound 7a (single enantiomer of ETA antagonist 7) after oral and intravenous administration of this compound. The plasma concentration time profiles of compound 7a and compound 6a after single
intra venous (2 mg/kg, n = 3) and single oral (10 mg/kg, n = 2) administration of compound 7a are shown in Fig. 2. Mean pharmacokinetic parameters for the parent compound, 7a, after intravenous doses were plasma clearance of 20 ml/min/kg, volume of distribution of 1.9l/kg, and an elimination half-life of 1.1 h. The mean oral bioavailability of compound 7a (by comparison with dose-normalized AUC values) was 14%. Comparison with dose-normalized AUC values for compound 6a after oral administration of compound 7a and intravenous administration of compound 6a provided a value for the bioavailability of compound 6a from oral compound 7a of 4%. The pharmacokinetic parameters of compound 7a and compound 6a in male rats are summarized in Table 4. Compound 7a was not detectable in rat urine after intravenous dosing of either compound 7a or compound 6a.

### Pharmacokinetics of Compound 7a and Compound 6a in Dog

After single intravenous doses of compound 6a to dogs (0.5 mg/kg, n = 3), the mean elimination half-life was 0.5 h, due to a plasma clearance of 5.8 ml/min/kg and a volume of distribution of 0.3 l/kg. Urinary excretion of 6a (0–7 h) accounted for 2.8% of the administered dose (n = 2 male dogs). After single intravenous doses of compound 7a to dogs (0.2 mg/kg, n = 3), the mean elimination half-life was 2.0 h, due to a plasma clearance of 4.1 ml/min/kg and a volume of distribution of 0.8 l/kg. Compound 6a was not observed as a circulating metabolite after intravenous doses of compound 7a to dogs. Urinary excretion of parent compound (0–7 h) accounted for 2.8% of the administered dose (n = 2 male dogs). After single oral doses of compound 7a (2 mg/kg) to the same animals the mean Cmax and Tmax of parent compound.
compound were 3448 ng/ml and 0.83 h, respectively. Mean oral bioavailability was 100% by comparison with dose-normalized oral and intravenous AUC values. Low levels of compound 6a were detected after oral administration of compound 7a with mean $C_{\text{max}}$ and $T_{\text{max}}$ values of 60 ng/ml and 1.7 h, respectively. The dose-normalized AUC of compound 6a after oral administration of 7a compared with intravenous administration of 6a provided a bioavailability of 6a from oral administration of 7a of 3.2%. The pharmacokinetic parameters of compound 7a and compound 6a in dogs are summarized in Table 4.

Plasma Protein Binding and Unbound Clearance Estimates of Compound 7a and Compound 6a. The plasma protein binding of compound 7a in rat and dog plasma was determined at 20 μg/ml ($n = 2$) by equilibrium dialysis. The compound was highly bound with protein binding values of 99.6 and 99.0% in rat and dog plasma, respectively. Unbound clearance values of compound 7a in rat and dog were therefore 5000 and 410 ml/min/kg, respectively. The protein binding of compound 6a was determined at a concentration of 10 μg/ml ($n = 3$) and was found to be 99.0% in rat plasma and 90.7% in dog plasma. Unbound clearance values of compound 6a in rat and dog were therefore 180 and 62 ml/min/kg, respectively. Protein binding determinations were also performed in control human plasma for both compounds at the same concentrations as those studied in animals.

Human plasma protein binding of compound 7a and compound 6a was 99.3 and 99.7%, respectively.

Discussion

Structure Pharmacokinetic Relationships for ET$_\alpha$ Antagonists. ET$_\alpha$ antagonists 1 to 6 all show extremely low oral bioavailability (less than 3%) in the rat (Table 1). On the basis that systemic clearance after intravenous dosing of all of these compounds was less than 12 ml/min/kg, which is less than 20% liver blood flow (estimated at 100 ml/min/kg), high first-pass extraction would not be expected to account for this. In addition compounds showed negligible turnover in standard in vitro metabolism screens (method as in Walker et al., 1999) in rat liver microsomes (data not shown), indicating low metabolic turnover. This strongly suggests that the compounds are poorly absorbed across the membranes of the gastrointestinal tract. In terms of the broad physicochemical properties of the molecules, all have molecular weights in excess of 500, a property previously related to a propensity for poor absorption (Lipinski et al., 1997).

Poor membrane permeability has been ascribed as the cause for low oral bioavailability of various drugs and other molecules. In several specific cases this has been linked to excessive H-bonding capacity of the molecules (Burton et al., 1996; Chan and Stewart, 1996), resulting in the energy of desolvation required to allow the molecules to diffuse...
H-bonding capacity is expressed as Cad values, which represent the sum of absolute values of free energy H-bond factors, characterizing the total H-bond ability of each compound.

The main emphasis of the synthetic modifications was at position 6 of the indole ring (R1 in Fig. 1), which was amenable both in terms of chemical accessibility and had the potential to retain pharmacological activity. In general, these modifications sought to remove the strong H-bond donor functions present in the acid, amide, and imidazoline groups of compounds 1 to 6. This strategy resulted in markedly improved membrane permeability for compounds 7 to 16 as measured in the Caco-2 system. In these compounds, the 6-substituent on the indole ring was either a non-H-bond donor function or an alcohol function (compounds 7 and 14), which is a weaker H-bond donor than the equivalent acid, based on thermodynamic calculation (Raevsky et al., 1992). A convenient way of comparing the overall H-bond capacity of the various molecules was through calculation of the Cad values previously applied by van de Waterbeemd et al. (1996). This approach sums thermodynamic estimates for each constitutive H-bonding function within the molecule to provide an overall measure of the total H-bond capacity in much the same way as log P values are calculated. When the Cad values are compared with the membrane permeability in the Caco-2 system (Fig. 3), a sigmoidal relationship is observed. Compounds with Cad values above 15 show very poor membrane permeability, whereas compounds with Cad values below about 13 show moderate-to-good permeability. Compounds in the Cad value range of 13 to 15 show some limited membrane permeation, but permeability would be difficult to predict from this calculated value alone. This relationship between membrane permeability and calculated H-bond capacity is similar to that previously observed for a more diverse set of molecules where a cut-off Cad value for permeability of around 15 was also observed (van de Waterbeemd et al., 1996). When considering the nature of the R1 substituent, not surprisingly, poorest permeability is associated with ionic and strong H-bonding substituents (e.g., acid/amide) and greatest permeability with no H-bonding function. Intermediate permeability is observed with weaker H-bonding substituents (e.g., cyano/alcohol). Overall, the calculated values for H-bond capacity provide an indication of the potential for membrane permeability and permit visualization of the data. However, they are not entirely predictive, do not add greatly to anticipated trends and are likely to be species-dependent.

Permeability through Caco-2 monolayers has long been regarded as a model for gut absorption (Artursson et al., 1996). Estimation of the extent of absorption of the series of ET<sub>A</sub> antagonists has allowed a comparison of Caco-2 permeability and absorption in the rat as shown in Fig. 4. Permeability studies across the Caco-2 monolayer clearly identify ET<sub>A</sub> antagonists of good absorption and very poor absorption. However, compounds of more intermediate permeability in Caco-2 studies (1–2 × 10<sup>-6</sup> cm·s<sup>-1</sup>) show highly variable extents of absorption in rats (20–100%). There appears to be a very sharp cut-off in the Caco-2 system between <1 × 10<sup>-6</sup> cm·s<sup>-1</sup> and >1 × 10<sup>-6</sup> cm·s<sup>-1</sup> wherein compounds may be completely unabsorbed or totally absorbed. Thus, although the Caco-2 system provides a sieve that can remove compounds of very poor absorption potential, it is not sufficiently predictive to entirely remove the need for in vivo studies. In general the rat is considered a relatively good predictor of absorption in humans compared with other laboratory species (van de Waterbeemd et al., 2001). Within this series, minimal H-bonding at the 6-indole position is clearly optimal in terms of the absorption potential of a compound. However, consideration of pharmacological activity clearly reveals the tension between balancing the requirements of pharmacokinetics and potency. Potency of the compounds was assessed in binding assays with cloned human ET<sub>A</sub> expressed in Chinese hamster ovary cells (Williams et al., 1991). Although compounds with polar 6-indole substituents had IC<sub>50</sub> values in the 1 to 10 nM range, complete removal of H-bonding function in this position rendered the compounds markedly less potent (IC<sub>50</sub> of >50–100 nM). The two orders of magnitude range in potency is approximately equivalent to the loss in binding energy that may be expected from removal of a carboxylic acid involved in a drug-receptor interaction (Andrews et al., 1984). Hence, the requirements of potency and pharmacokinetics are to a great extent pulling in opposite directions. As previously observed,
this is a common tension in modern drug discovery, especially where the chemical starting point is close to the physicochemical boundaries of good absorption (Lipinski et al., 1997). Thus, in the current series, where molecular weight is generally above 500 and the number of H-bond donors and acceptors are close to the limits considered compatible with good absorption, there is an extremely fine balancing act between pharmacological activity and membrane permeability.

Although drug absorption showed correlation with both physicochemical measurements and in vitro assays, other pharmacokinetic parameters were generally less predictable. Attempts to assess potential rates of P450-mediated systemic clearance of this series of compounds by using in vitro metabolism systems indicated that all compounds had low-to-nondetectable metabolic turnover (data not shown). On the basis of this observation and previous observations with relatively high molecular weight acidic molecules (Gardner et al., 1995), it is likely that hepatobiliary transport plays a significant role in the clearance of these molecules. In such a case, in vivo pharmacokinetic studies represent the only straightforward option to assess clearance.

The volumes of distribution of compounds 1 to 6 are generally low (<0.9 l/kg), reflecting the acidic nature of the molecules (Smith et al., 1996). Low membrane permeability will also serve to trap the molecules within the circulatory system. In addition, with the exception of compound 3, all molecules have high plasma protein binding (>95%), which will also restrict their ability to permeate into tissues due to the high plasma protein affinity. Compounds 7 to 16 generally show higher values for volume of distribution (0.5–5.0 l/kg). This is despite similarly high levels of plasma protein binding and acidic character. It would therefore appear that the increased ability to permeate the gut wall is accompanied by a general increase in tissue distribution.

Compounds 7 to 16 all tend to show higher values for plasma clearance than compounds 1 to 6 with all but one compound having systemic clearance of greater than 20 ml/min/kg compared with <12 ml/min/kg for compounds 1 to 6. Hence, there is a tendency for higher clearance with reduced H-bonding capacity. However, other factors are also clearly important in determining clearance as demonstrated by the 6-fluoro compound (compound 10), which has complete absorption and low systemic clearance. In addition, an alternative clearance mechanism is present for at least compound 7 (which has the highest clearance value), which has been shown to undergo metabolism to the carboxylic acid (compound 6). A scatter plot of H-bond capacity versus systemic clearance demonstrates the lack of a clear relationship between these two parameters (Fig. 5). Compounds classified as having poor permeability all show low clearance; however, compounds of moderate-to-high permeability show a range of clearance values. It was not possible to fit a curve to these data. The lack of correlation between H-bond capacity and clearance is consistent with earlier observations for peptidic compounds (Karls et al., 1991), where again H-bond capacity influenced absorption but not clearance. No relationship was apparent between clearance and other physicochemical parameters.

When the pharmacokinetic data were combined with pharmacological activity, ETₐ antagonist 7 had a particularly attractive profile, especially when it was observed that this alcohol-containing compound was metabolized to the low-clearance compound 6 in vivo. On the basis of this encouraging profile obtained with the racemic compounds, single enantiomers of the alcohol and acid (compound 7a and compound 6a, respectively) were prepared for further, more detailed evaluation.

Pharmacokinetics of Compound 7a and Compound 6a. The pharmacokinetic studies in rats with the single enantiomer of racemic ETₐ antagonist 7 (compound 7a) provided a pharmacokinetic profile broadly similar to that observed for the racemate. However, the lower value obtained for plasma clearance (20 versus 72 ml/min/kg) and similar oral bioavailability (14 versus 16%) suggested the original estimate of absorption for the racemate (~55%) was too high. It is not known whether the different intravenous vehicles used for compounds 7 and 7a influenced the pharmacokinetics observed. Separate studies in which plasma concentrations of compound 7a were determined in the hepatic portal vein of nonjugular vein cannulated rats provided a measure of absorption of 22%, assuming no gut wall extraction (Kwon and Inskeep, 1996). Alternatively, the administration of compound into the hepatic portal vein allowed absorption to be estimated based on comparison of normalized systemic AUC values after oral and portal vein dosing and provided a value of 36%. Thus, in the rat the absorption of compound 7a would appear to be around 30%. In contrast, absorption of compound 7a in the dog was complete with 100% oral bioavailability observed. This is in keeping with comparisons of other compounds in rat and dog where dog has provided a higher extent of absorption than rat and where rat is the better predictor of human absorption (Beaumont et al., 2000; van de Waterbeemd et al., 2002).

Analysis of the acid, compound 6a, after oral doses of compound 7a confirmed that this was a significant circulating metabolite in the rat. Single oral doses of the alcohol provided sustained exposure to the acid for 12 h as shown in Fig. 2. Based on the premise that pharmacological activity is governed by free drug exposure, unbound clearance values will be a prime factor in the in vivo potency of different compounds. The lower unbound clearance of compound 6a compared with compound 7a (170 versus 5000 ml/min/kg), therefore, results in the acid being the major pharmacologically active species in the circulation of rat. Somewhat surprisingly only low levels of compound 6a were formed after oral doses to dogs. This metabolic profile is analogous to the situation observed for the angiotensin II receptor antagonist losartan. This compound is also a primary alcohol that undergoes oxidation to a carboxylic acid, which is the predominant active species in humans (Munafo et al., 1992). In common with compound 7a, losartan shows extensive formation of the acid metabolite in rat but not dog (Wong et al., 1991). In addition, the acid metabolite of losartan is formed in human but not monkey. The bioavailability of compound 6a after oral doses of compound 7a to male rats ranges from 4 to 11%. Because 30% of the administered oral dose of 7a is absorbed and 11% of the administered dose appears as 6a in the systemic circulation (noncannulated rat data), the overall conversion of 7a to 6a in the rat is approximately 30%. Clearly, this
provides a better method for delivering compound 6a to the systemic circulation than oral administration of the acid, which itself is very poorly absorbed (≈1% as racemate). The lower than expected bioavailability of the acid after portal vein administration of the alcohol to rat may reflect saturation due to the dose route or atypical metabolism as a consequence of the surgical procedure.

Compound 7a is not considered as a prodrug of compound 6a as such, because both compounds are present in the systemic circulation and both have pharmacological activity. The marked differences in the pharmacokinetic profile between dog and rat for the two compounds are intriguing. Although the rat is considered more predictive of human absorption than dog, the converse is often the case for metabolism. However, as demonstrated by losartan, the dog failed to be representative of the metabolic profile of this compound in the human. An interesting observation is the higher systemic clearance of compound 6a in dog compared with rat (5.8 versus 1.7 ml/min/kg), which is contrary to the usual trend for an allometric relationship between species for clearance of acidic molecules. However, when plasma protein binding is taken into consideration the much lower binding in dog reverses this trend with lower unbound clearance in dog compared with rat (62 versus 180 ml/min/kg), which is more in keeping with the expected allometric relationship. This provides clear evidence for protein binding limiting the clearance of this molecule. The possibility exists that the higher plasma protein binding in the rat could contribute to the increased systemic availability of compound 6a after hepatic metabolism of compound 7a by increasing the blood to liver partitioning. This raises further speculation as to the potential profile of compound 7a in human where plasma protein binding of compound 6a is higher than in rat.

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References