

Impact of Physicochemical and Structural Properties on the Pharmacokinetics of a Series of α_1 -adrenoceptor antagonists

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<p>Running Title Page:</p> <p>The pharmacokinetics of a series of $\alpha 1_L$-adrenoceptor antagonists</p> <p>Corresponding Author:</p> <p>Alison Betts</p> <p>Department of Pharmacokinetics, Dynamics and Metabolism</p> <p>Pfizer Global Research and Development</p> <p>Ramsgate Road,</p> <p>Sandwich,</p> <p>Kent, CT13 9NJ</p> <p>UK.</p> <p>Tel. +44(0)1304 642542</p> <p>Fax. +44(0)1304 651817</p> <p>Email: alison.betts@pfizer.com</p> <p>Number of text pages: 46</p> <p>Number of tables: 6</p> <p>Number of figures: 9</p> <p>Number of references: 40</p> <p>Words in abstract: 230</p> <p>Words in introduction: 775</p> <p>Words in discussion: 1921</p>	<p>List of abbreviations:</p> <p>ADME Absorption, distribution, metabolism and excretion</p> <p>AUC_{0-T} Area under the plasma concentration-time curve</p> <p>BLQ Below limit of quantitation</p> <p>BPH Benign prostatic hyperplasia</p> <p>Cl'int Intrinsic clearance</p> <p>clogP Calculated log P</p> <p>Cmax Maximum concentration</p> <p>Fu Fraction unbound</p> <p>GI Gastrointestinal</p> <p>HBA Hydrogen bond acceptor</p> <p>HBD Hydrogen bond donor</p> <p>HPLC High performance liquid chromatography</p> <p>IPRL Isolated perfused rat liver</p> <p>kel Terminal elimination rate constant</p> <p>logD_{7.4} Log of octanol/water partition coefficient at pH 7.4</p> <p>MS Mass spectrometry</p> <p>MW Molecular weight</p> <p>P-gp P-glycoprotein</p> <p>t_{1/2} Terminal elimination half-life</p> <p>Tmax Time to maximum concentration</p> <p>v/v volume per volume</p> <p>w/v weight per volume</p>
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Abstract

A rational drug discovery process was initiated to design a potent and prostate selective α_{1L} -adrenoceptor antagonist with pharmacokinetic properties suitable for once a day administration following oral dosing, for the treatment of BPH. Two series of compounds based on a quinoline or quinazoline template were identified with appropriate pharmacology. A series of high molecular weight cations with high hydrogen bonding potential had extensive in vivo clearance, despite demonstrating metabolic stability. Studies in the isolated perfused rat liver and fresh rat hepatocytes indicated that active transport protein-mediated hepatobiliary elimination is an efficient clearance process for these compounds. A reduction in molecular weight and hydrogen-bonding potential resulted in a second series of compounds with in vivo hepatic clearance predictable from in vitro metabolic clearance. Initially lipophilicity was reduced within this second series to reduce metabolic clearance and increase elimination half-life. However, this strategy also resulted in a concomitant reduction in volume of distribution and a negligible effect on prolonging half-life. An alternative strategy was to increase the intrinsic metabolic stability of the molecule by careful structural modifications whilst maintaining lipophilicity. Replacement of the metabolically vulnerable morpholine sidechain resulted in identification of UK-338,003, which fulfilled the objectives of the discovery programme with suitable pharmacology (human prostate α_{1L} pA₂ of 9.2 with 25 fold selectivity over rat aorta α_{1D}) and sufficiently long elimination half-life in human volunteers (11-17 hour) for once a day administration.

Introduction

α 1-antagonists are well preceded in the effective treatment of both hypertension and benign prostatic hyperplasia (BPH); e.g. doxazosin (Fulton et al., 1995), terazosin (Jonler et al., 1994) and tamsulosin (Wilde and McTavish, 1996). However, because many of the α 1-antagonists clinically used in the treatment of BPH were originally developed for hypertension, they are non-selective for α 1-adrenoceptor subtypes. These non-selective α 1-antagonists have similar efficacy and are generally well tolerated in BPH patients, but some patients experience dizziness and postural hypotension which may be minimized by dose titration. It has been postulated that a drug that blocks the α 1_L-adrenoceptor subtype would specifically target the prostate and thus have a better ratio of efficacy to safety, with fewer cardiovascular side effects than currently available non-selective α 1-adrenoceptor antagonists.

The aim of the drug discovery programme was to identify a novel and potent α 1_L-adrenoceptor antagonist that was functionally selective for the prostate gland over the cardiovascular system, and thus have the potential to be effective in the treatment of BPH with fewer dose limiting side effects. To assess potency for the prostate gland, functional antagonist potency of project compounds at human α 1_L-adrenoceptors was determined against noradrenaline- mediated contractions of an isolated human prostate preparation.

To assess selectivity over the cardiovascular system, antagonist potency of project compounds versus the vascular α 1_B- and α 1_D- adrenoceptors was considered. Antagonist potency against noradrenaline-induced contraction of rat spleen and rat aorta was determined as a functional index of activity at α 1_B- and α 1_D-adrenoceptors

respectively. The compounds evaluated in the drug discovery programme achieved selectivity versus α_{1B} -adrenoceptors relatively easily and therefore structure activity relationships focused on selectivity of α_{1L} -adrenoceptors in human prostate versus α_{1D} -adrenoceptors in rat aorta.

Terazosin has essentially balanced prostate and blood pressure effects in the clinic (Kirby, 1998). In vitro, terazosin has a pA_2 value of 9.8 versus α_{1L} -adrenoceptors in human prostate and a pA_2 value of 10.0 versus α_{1D} -adrenoceptors in rat aorta (in-house data) and thus is not selective for prostate over blood pressure effects. The objectives of the drug discovery programme were to provide symptomatic relief for BPH equivalent to established agents such as terazosin, with at least a 10 fold improvement in cardiovascular side effects. To achieve this, only compounds with pA_2 values of greater than 8.3 versus α_{1L} -adrenoceptors in human prostate were considered to be sufficiently potent to meet project objectives. Since current α_1 -adrenoceptor antagonists exhibit little or no selectivity in vitro for the cloned α_1 -adrenoceptor subtypes and no prostate selectivity in the clinic (Kirby et al., 2000) there was no clinical marker to provide guidance for in vitro-in vivo correlations of selectivity. Therefore a strategy based around the knowledge of antagonist pharmacology was employed to design a compound which lacked dose limiting cardiovascular side effects. From antagonist pharmacology it was assumed that 75% receptor occupancy is required for efficacy of α_{1L} -adrenoceptor antagonists in vivo for BPH (Smith, 1997). To provide selectivity over the cardiovascular system, a value of 10 fold selectivity was chosen vs. α_{1D} -adrenoceptors as this would represent a receptor occupancy of much less than 50%, thus diminishing the risk of α_{1D} pharmacology and cardiovascular side effects.

Two structurally distinct series of compounds, based on a quinoline or quinazoline central template, were identified and shown to be potent and prostate selective α_1 -adrenoceptor antagonists (Collis, 1997; Fox, 1998). In addition to possessing the desired pharmacology, the aim of the drug discovery programme was to identify a drug candidate with pharmacokinetic properties suitable for once a day administration to human following oral dosing. Design of a successful drug candidate incorporates both good pharmacological activity and appropriate pharmacokinetic properties and the study of absorption, distribution, metabolism and excretion (ADME) properties and pharmacokinetics is now well established in the drug discovery phase (Smith et al., 1996).

In addition to the requirement for drug efficacy, optimal pharmacokinetic properties ensure that a drug works in clinical practice when lack of patient compliance has to be taken into consideration. This aspect of drug behavior is termed 'forgiveness' (Urquhart, 1998) and reflects the actual duration of effect after administration.

In order to be suitable for oral administration it was ideal to design a drug with high oral bioavailability. To achieve the desired once daily dosing regimen, a compound was required with sufficient half-life to drive efficacy and minimize variability in concentration by reducing peak to trough ratio. A long half-life would also enable 'forgiveness'.

This paper will discuss the balance that needed to be struck between pharmacodynamics and pharmacokinetics in order to select a compound for development with suitable overall properties. In addition, this paper will discuss the differences in ADME properties between the two series of compounds in the context of their physicochemical properties.

Materials and Methods

Materials

The α 1-adrenoceptor antagonists studied in series 1 and 2 were synthesized by the Discovery Chemistry Department, Pfizer Global Research and Development (Sandwich, UK). The chemical purity of the compounds was determined using a range of methods including mass spectrometry, NMR and microanalysis. Chemical purity of all compounds was >95% (Collis, 1997; Fox, 1998). All other chemicals and reagents, unless stated otherwise, were obtained from Sigma-Aldrich Research (St. Louis, MO) and were of the highest grade available.

Lipophilicity determination

Distribution of the α 1-adrenoceptor antagonists between octanol and 0.1M potassium phosphate buffer, pH7.4, was determined by the method of Stopher and McClean (Stopher and McClean, 1990). Approximately 2mg of compound was dissolved in 2ml of octanol (octan-1-ol, specially pure; BDH, Poole, UK) and mixed with 2ml of 0.1M potassium phosphate buffer, pH 7.4 on a rotary mixer at 30rpm for 60minutes. Following centrifugation the two phases were separated and 10 μ l of a 1:10 dilution of the octanol and 100 μ l of buffer were directly injected onto the HPLC systems described for the analysis of plasma samples. The distribution co-efficient ($\text{LogD}_{7.4}$) was calculated from the log of the ratio of the concentration of compound in octanol to the concentration of compound in buffer. Duplicate determinations were carried out for each compound. Calculated log P values (clogP) were calculated using the Medchem computer programme (version 3.55; Biobyte corp., Claremont, CA).

pK_a determination

The ionization constant (pK_a) values of the α_1 -adrenoceptor antagonists were determined by potentiometric titration in water/ methanol using GlpKa apparatus (Sirius Analytical Instruments, Forrest Row, UK). All titrations were conducted under argon to exclude atmospheric CO₂ and at 25°C. Six separate semi-aqueous solutions in methanol/distilled water of compound and 0.15% KCl were acidified with 0.1M HCl to pH 2. The solutions were then titrated with standardized KOH to pH 12. Bjerrum difference plots were deduced from each titration curve and used to calculate approximate values of the apparent ionization constant in the mixed solvent. These approximate values were used in a weighted non-linear least squares procedure to refine the pK_a values by including previously determined values of citric acid as unrefined constituents. The refined values were extrapolated to zero co-solvent by the Yasuda- Shedlovsky procedure (Gobry V, 2000).

Plasma protein binding determination

Protein binding of the α_1 -adrenoceptor antagonists in plasma was determined in vitro by equilibrium dialysis. Samples of rat, dog or human plasma (1ml, n=3-5) containing compound at 1 μ g/ml were dialyzed (Spectrapor-1 dialysis membrane 6000-8000 mw cut-off; Spectrum, Laguna Hills, USA) against isotonic Krebs-Ringer buffer (1ml, pH 7.4) for 4h at 37°C in a rotating dialyser (Dianorm, NBS Biologicals, Huntingdon, UK). After dialysis, the compounds were extracted and the concentrations of drug in plasma and buffer were determined by HPLC analysis, as described for the analysis of plasma samples.

Plasma protein binding values were determined using the following equation:

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$$\text{Plasma protein binding (\%)} = 100 - \frac{(\text{buffer concentration})}{\text{plasma concentration}} \times 100$$

In vitro metabolism studies in liver microsomes

Human, rat and dog liver microsomal incubations were carried out at 1 μ M substrate concentration and 0.5 μ M cytochrome P450 concentration. The final incubation volume was 400 μ l for the human incubations and 1.2ml for the rat and dog incubations.

Microsomes were purchased from BD Biosciences (Franklin Lakes, NJ) and stored at –80°C. The concentrations of protein and cytochrome P450 were determined by standard methods (Lowry et al., 1951; Omura and Sato, 1964). The incubation volume was made up of 50mM potassium phosphate pH 7.4, and 5mM MgCl₂. The reducing equivalents required by cytochrome P450 were provided by NADPH which was generated in situ from NADP (1mM) using an isocitric acid (5mM) and isocitrate dehydrogenase (1unitml⁻¹) system. All the components were pre-incubated at 37°C, except NADP which was added at time 0 to start the reaction. Aliquots of the human liver microsomal incubation (25 μ l) and the rat and dog liver microsomal incubation (100 μ l) were removed over a 60 minute period and immersed in ice-cold acetonitrile (50 μ l for the human microsomes and 200 μ l for the animal microsomes) containing internal standard (0.2 μ g/ml eletriptan or fluconazole) to terminate the reaction. The resulting samples were centrifuged at 1700 g for 30 minutes.

For the human liver microsomes, 10 μ l of sample was analyzed by a specific LC-MS-MS method. Samples were injected onto an HPLC system consisting of a cartridge column (Optilynx cartridge C18, 15 x 2.1mm, porous 40 μ M) and an isocratic Jasco pump operating at 2ml/min. The eluting mobile phase was 90% methanol, 10% water, 2mM ammonium acetate and 0.03% formic acid.

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For the animal liver microsomes, 80 μ l was analyzed by a specific LC-MS-MS method. Samples were injected onto a gradient HPLC system comprising a Chromolith Speed ROD column (50 x 4.6mm) eluted by a gradient of 10 to 90% methanol in water containing 2mM ammonium acetate and 0.03% formic acid, over 3 minutes at a flow rate of 1ml/min.

Detection was by multiple reaction monitoring using a Sciex API2000 or API4000 triple quadrupole mass spectrometer (Perkin Elmer, Sciex, Ontario, Canada) in a positive atmospheric pressure chemical ionization mode.

Microsomal binding determination

The extent of binding of the α 1-adrenoceptor antagonists in Series 2 (compounds 1-7) to dog and human microsomes was determined in vitro by equilibrium dialysis using a 96-well micro-equilibrium Teflon dialysis device (HTDialysis, LLC, USA). Regenerated cellulose dialysis membrane strips with a molecular weight cut off of 12,000-14,000Da (HTDialysis, LLC, USA) were used.

Dog and human microsomal incubations were prepared as previously for in vitro metabolism studies, but without the addition of compound or NADP. The incubations were left to stand at room temperature for 12 hours before addition of compound at 1 μ M. Aliquots of microsomal incubation (150 μ l) were added to one side of the dialysis device and dialyzed against 50mM phosphate buffer (pH7.4, 150 μ l) by placement of the dialysis block on an oscillating platform for 4hrs at 37°C. After this period, microsome samples (100 μ l) and buffer samples (100 μ l) were removed from the apparatus and diluted with the opposite phase (100 μ l of control dialyzed microsome incubation or buffer). Compound and internal standard were extracted from the samples basified with 0.2M sodium borate buffer, pH9.0 (1ml) into *tert*.-butyl methyl ether (2ml). The

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samples were mixed by inversion for 10 min, centrifuged and the ether removed. The ether extract was evaporated to dryness under nitrogen at 37°C and dissolved in 200µl of 10:90 methanol/ water containing 2mM ammonium acetate and 0.03% formic acid. Samples (180µl) were analyzed by a specific LC-MS-MS system as described for the dog liver microsome in vitro metabolism studies previously.

Microsomal binding values were determined using the following equation:

$$\text{Microsome binding (\%)} = 100 - \left(\frac{\text{buffer compound:IS ratio}}{\text{microsome compound: IS ratio}} \times 100 \right)$$

Calculation of unbound intrinsic clearance in vitro

Using the assumption that a substrate concentration of 1µM is below the apparent Km, disappearance half-life values were then calculated from the negative slope of a plot of the natural log of the substrate: internal standard peak area ratio versus time. This was scaled to determine the clearance expected in vivo using the following equation (Obach et al., 1997):

$$Cl'_{int} = \frac{0.693 \cdot \text{liver weight}}{\text{In vitro } t_{1/2} \cdot \text{liver in incubation} \cdot fu(\text{inc})}$$

Where in vitro t_{1/2} is in min, liver weight is in g/kg of body weight and liver in incubation refers to the g of liver/ml in the incubation, resulting in units of ml/min/kg for Cl' _{int} . The “liver in incubation” value was calculated from the amount of microsomal protein in the incubation and a scale-up factor from microsomal protein to g of liver. Fu (inc) is the unbound fraction in the incubation which is determined from the microsomal binding value. Thus the Cl' _{int} calculated is based on free concentrations in the incubation.

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The following values were taken from the literature:

Human: 325 pmol P450/mg microsomal protein (Iwatsubo et al., 1997); 33 mg microsomal protein/ g liver (Wilson et al., 2003); 20 g liver/kg bodyweight (Obach, 1997).

Dog: 520 pmol P450/mg microsomal protein (Sakamoto et al., 1995; Eguchi et al., 1996); 77.9 mg microsomal protein/ g liver (Naritomi et al., 2001); 32 g liver/kg bodyweight (Davies and Morris, 1993).

Hepatocyte transport assay

Rat hepatocytes were isolated using a modified two step collagenase perfusion (Seglen, 1993) from livers of male Sprague-Dawley rats. Fresh hepatocytes (1×10^6 cells/ml) were incubated with UK-191,005 (0.2 – 100 μ M) in Krebs-Henseleit buffer at 4°C and 37°C for 10 min. 1 ml aliquots were immediately placed on ice and hepatocytes separated by low speed centrifugation (50g at 4°C). Hepatocytes were then washed twice with 1ml ice-cold buffer. The hepatocytes were then pelleted by centrifugation at 50g, 200 μ l acetonitrile was added and the samples were stored frozen until analysis. At the time of analysis, 25ng of internal standard (in 25 μ l of methanol) was added to each sample followed by 100 μ l Milli-Q water. Samples were vortex mixed and centrifuged at 4°C for 1 hour in a 96-well block. Samples were analyzed by turbulent flow chromatography/tandem mass spectrometry with multiple reaction monitoring as described in detail previously (Chassaing, 2001). Initial experiments were completed to assess the linearity of UK-191,005 uptake with time and uptake was found to be linear for 10 min.

Animal experimentation

All studies involving animals were carried out in accordance with the requirements of UK national legislation and conducted under necessary authorities. At all stages, consideration was given to experiment refinement, reduction in animal numbers and replacement with in vitro techniques.

Isolated Perfused Rat Liver (IPRL) preparation

Livers of male Sprague Dawley rats (250-300g) were perfused in situ as described previously (Lennard et al., 1983) with some modifications. Experiments were performed using the recirculation mode with a flow rate of 15ml/min. The perfusion medium (150 ml) consisted of Krebs-Henseleit bicarbonate buffer, bovine serum albumin (2.6%, w/v) and washed human erythrocytes (13%, w/v), adjusted to pH7.4 and oxygenated with 95% O₂/5% CO₂. Following a 5min equilibration period, taurocholate (1 µmol/min) was infused into the reservoir of the IPRL. After a further 10min, test α 1-adrenoceptor antagonist (1 mg dose; 1 ml added of a 1 mg/ml solution) was added to the reservoir. Perfusate samples (1 ml) were taken from the reservoir at regular intervals out to 90 min post dosing and centrifuged (3000 rpm, 10 min), the supernatant was collected and stored frozen at -20°C until assay. Bile was collected at approximately 15 min intervals and sample volumes recorded. Concentrations of drug in perfusate and bile were determined by HPLC analysis, as described for the analysis of plasma samples.

Intravenous and oral pharmacokinetics in the rat

UK-191,005 (Series 1) was administered intravenously at 3mg/kg to male Sprague-Dawley rats. UK-294,315 and UK-338,003 (Series 2) were administered intravenously

at 0.5mg/kg and orally at 4mg/kg and 1mg/kg respectively to male Sprague-Dawley rats. Intravenous doses were prepared by dissolving the compounds in 0.1M HCl, which was diluted with saline and back-titrated to pH 7.4 with 0.1M NaOH. Oral doses were prepared in the same way but used purified water instead of saline. Male Sprague-Dawley rats (~250g, Charles River, Manston, UK) were surgically prepared with an indwelling jugular vein cannula at least 2 days prior to administration of dose. Before and during the intravenous and oral studies, rats had free access to food (rat diet pellets) and water.

Rats (n=4/ compound and n=2/route) received intravenous doses of each compound via the caudal vein (0.5mg/kg, 1ml/kg) or oral doses of UK-294,315 (4mg/kg, 2ml/kg) and UK-338,003 (1mg/kg, 2ml/kg) by gavage tube. Blood samples (175µl) were collected from the jugular vein catheter at specific time-points and the cannula flushed with heparinised saline after each sample. All blood samples were collected into heparinised tubes and plasma was prepared by centrifugation. Plasma was stored frozen at -20°C until analysis.

Intravenous and oral pharmacokinetics in the dog

Intravenous doses of the α 1-adrenoceptor antagonists studied were freshly prepared by dissolving in 0.1M HCl, which was diluted with saline and back-titrated to pH7.4 with 0.1M NaOH. Oral doses were prepared in the same way, but used purified water instead of saline. Dose levels were selected based on pharmacological activity. Intravenous doses administered were 0.3mg/kg for UK-338,003 (Series 2) and 0.5mg/kg for all other α 1-adrenoceptor antagonists studied. Oral doses administered were 0.5mg/kg for compounds 2, 5 and 6, 0.6mg/kg for UK-338,003, 2.0mg/kg for compounds 3 and 4 and 4mg/kg for UK-294,315.

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Male or female beagle dogs (12-16kg) from the colony at Pfizer Global Research and Development were used for these studies. At least 2 animals received each of the 8 compounds. Animals were fasted overnight prior to administration of either intravenous or oral doses and for 7 hours following dosing.

Intravenous doses were administered into the left saphenous vein as an infusion at 1ml/min for a duration of 15 minutes via a temporary indwelling catheter. Dogs were restrained in canvas slings from just before dosing to 7h after dosing, at which point they were returned to their pens. Blood samples (5mls) were taken from the right saphenous vein via a temporary indwelling catheter for the first 7 hours and then subsequently by vena puncture of the cephalic vein. Blood samples were collected into heparinised tubes and plasma was prepared by centrifugation. Plasma was stored frozen at -20°C until analysis.

Seven days after intravenous administration, the dogs were given an oral dose of the same compound by gavage. Again dogs were restrained in canvas slings for the first 7 hours after oral dosing and then returned to their pens. Plasma samples were prepared and stored as above. When not in slings, the dogs had free access to food (at regular feeding times) and water throughout the study.

Administration to human volunteers

The pharmacokinetics of UK-294,315 and UK-338,003 from Series 2 were determined in healthy human volunteers in 2 separate studies. The human pharmacokinetic studies were conducted according to the Association of the British Pharmaceutical Industry (ABPI) guidelines and to the revised Declaration of Helsinki (Hong Kong, 1989). The clinical study protocols (single blind placebo controlled single escalating oral doses) were approved by a local Ethics Review Committee, and the studies were carried out at

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the Kent and Canterbury Hospital (Canterbury, UK). Written consent was obtained from 24 healthy male volunteers (aged 18 to 45 years, weighing 60-100kg) who took part in each study.

For UK-294,315, the pharmacokinetic study was divided into 2 cohorts of 12 subjects. Cohort A was a 3-way crossover study investigating 1mg, 3mg and 10mg active compound, incorporating placebo substitution. Cohort B was a 4-way crossover study investigating 30mg, 60mg and 100mg active compound and placebo.

For UK-338,003, the pharmacokinetic study was divided into 3 cohorts of 8 subjects. Doses were administered in an escalating fashion in cohort 1 (0.25mg, 0.75mg and 1.5mg active compound plus placebo) and cohort 2 (0.75mg, 1.0mg and 1.25mg active compound plus placebo). Dosing in cohort 3 was fully randomized (0.1mg, 0.5mg and 1.25mg active compound plus placebo).

In each pharmacokinetic study the subjects were fasted for 12 hours before dosing and for 4 hours following dosing. UK-294,315 was administered as a solution in water (250ml) and UK-338,003 was administered as a solution in 0.01M hydrochloric acid. There was a minimum of 7 days between each dose. Blood samples (6-7ml) were collected in heparinised tubes up to 24-48 hours post dose for UK-294,315 and up to 96 hours post dose for UK-338,003. Samples were centrifuged (~1500g at 4°C for 10mins) within 60 minutes of sample collection and plasma was removed and stored in screw capped polypropylene tubes at approximately -20°C.

Analysis of rat, dog and human plasma samples

Concentrations of the α 1-adrenoceptor antagonists in rat, dog or human plasma samples were determined using the following procedures:

Series 1 α 1-adrenoceptor antagonists and Compound 2 (Series 2)

Compound and internal standard were extracted from rat or dog plasma (1ml) basified with 0.2M sodium borate buffer, pH9.0 (1ml) into *tert.*-butyl methyl ether (6ml). The samples were mixed by inversion for 10 min, centrifuged and the ether was removed. The ether extract was evaporated to dryness under nitrogen at 37°C and dissolved in 100 μ l mobile phase for injection onto the HPLC column. The mobile phase comprised of acetonitrile/ 10mM potassium dihydrogen phosphate 10mM octane sulphonic acid pH3.0 (40/60 by vol) and the column was reverse phase basic 25cm x 4.6mm (Hichrom, Reading, UK). Detection was by UV absorbance at 254nm.

UK-294,315 (Series 2)

A solution of internal standard was added to aliquots of rat, dog or human plasma sample (0.1-1ml), followed by addition of 3ml borate buffer (pH 10, Fischer Scientific, Loughborough, UK). The sample mixture was added to Chem. Elut cartridges (3ml, Varian Ltd., Walton-on-Thames, UK), and allowed to soak in for 3min. Samples were eluted into polypropylene tapered tubes using 6ml ethyl acetate, and the eluent was evaporated to dryness under nitrogen at 37°C. Samples were reconstituted in 500 μ l of 10% (v/v) acetonitrile in water (containing 0.1% trifluoroacetic acid) and aliquots (300 μ l) were injected onto the HPLC system. The HPLC consisted of a column switching system comprising an ion exchange (SCX) cartridge, and two reverse phase base deactivated columns. The sample was injected onto the SCX cartridge (S5SCX-10C5, 1cm x 4.6mm, HiChrom Ltd., Reading, UK) in a mobile phase of 10% (v/v) acetonitrile in water (containing 0.1% trifluoroacetic acid), at a flow rate of 1ml/min. After 3min, the SCX cartridge was back flushed onto a C₁₈ RPB cartridge (HiRPB-10C5, 1cm x 4.6mm, HiChrom Ltd., Reading, UK) using a mobile phase of 30% (v/v)

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acetonitrile, 23% (v/v) methanol in water (containing 0.1% trifluoroacetic acid), at a flow rate of 0.5ml/min. The eluent from this cartridge was diverted to waste for 0.45min, and then switched onto the third column (C₁₈ RPB, 25cm x 4.6mm, HiRPB-250A, HiChrom Ltd., Reading, UK) for 1.5min. UK-294,315 was eluted from the third column using a mobile phase of 30% (v/v) acetonitrile, 23% (v/v) methanol in water (containing 0.1% trifluoroacetic acid), at a flow rate of 0.5ml/min. Detection was by fluorescence (λ_{ex} 248nm, λ_{em} 388nm). UK-294,315 had a retention time of approximately 7min. The limit of detection was 0.1ng/ml from 1ml of plasma.

Compounds 3 and 4 (Series 2)

Compound and internal standard were extracted from dog plasma (1ml) basified with 0.2M sodium borate buffer, pH9.0 (1ml) into tert.-butyl methyl ether (6ml). The samples were mixed by inversion for 10 min, centrifuged and the ether was removed. 100 μ l of 0.1M orthophosphoric acid was added to the ether and again the samples were mixed for 10mins and centrifuged. The ether was removed and the orthophosphoric acid injected onto HPLC. The mobile phase comprised of acetonitrile/ 0.1% trifluoroacetic acid (14/86 by vol for compound 3 and 20/80 by vol for compound 4) and the column was reverse phase basic 10cm x 4.6mm (Hichrom, Reading, UK). Detection was by UV absorbance at 254nm. The approximate retention time was 5 minutes for compounds 3 and 4.

Compound 5 (Series 2)

The concentrations of compound 5 were determined in dog plasma using a liquid-liquid extraction method followed by LC-MS-MS quantification. Compound and internal standard were extracted from dog plasma (1ml) basified with 0.1M NaOH (1ml) into

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tert.-butyl methyl ether (4ml). The samples were mixed by inversion for 10 min, centrifuged and the ether was removed. The ether extract was evaporated to dryness under nitrogen at 37°C and dissolved in 200µl 2mM ammonium acetate buffer, pH4.0, in methanol/ water (30:70 v/v). Samples (180µl) were injected onto the HPLC column (Hypersil C18, 50 x 4.6mm) with a mobile phase consisting of 2mM ammonium acetate, pH 4.0 in methanol/ water (90:10 v/v) at 1ml/min. Detection was by multiple reaction monitoring for the transitions m/z 483 to 396 using a Sciex APIIII+ triple quadrupole mass spectrometer (Perkin Elmer, Sciex, Ontario, Canada) in a positive atmospheric chemical ionization mode.

Compound 6 (Series 2)

Compound and internal standard were extracted from dog plasma (1ml) by solid phase extraction using C18 cartridges (Bond Elut, 100mg/1ml), which had been pre-treated with methanol (1ml) and water (1ml). The cartridges were washed sequentially with water (1ml) and 20% acetonitrile (1ml). The extracts were eluted with methanol, which was evaporated to dryness under nitrogen at 37°C and dissolved in 100µl of mobile phase for injection onto the HPLC column. The mobile phase comprised of acetonitrile/ 0.1% trifluoroacetic acid (13/87 by vol) and the column was reverse phase basic 10cm x 4.6mm (Hichrom, Reading, UK). Detection was by UV absorbance at 254nm. The approximate retention time for compound 6 was 4 minutes.

UK-338,003 (Series 2)

Compound and internal standard were extracted from rat or dog plasma (0.1ml -1ml aliquots respectively) by solid phase extraction using PH cartridges (Bond Elut, 100mg/1ml), which had been pre-treated with methanol (1ml) and water (1ml). The

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cartridges were washed sequentially with water (1ml) and 40% methanol (1ml). The extracts were eluted with methanol, which was evaporated to dryness under nitrogen at 37°C and dissolved in 100µl of mobile phase for injection on to the HPLC column. The mobile phase comprised of acetonitrile/ 10mM potassium dihydrogen phosphate, 10mM octane sulphonic acid pH3.0 (34/66 by vol) and the column was Kromasil 100-5C8 15cm x 3.2mm (Hichrom, Reading, UK). Detection was by fluorescence at excitation wavelength 340nm and emission wavelength 410nm. The approximate retention time for UK-338,003 was 5 minutes.

The method for analyzing UK-338,003 in human plasma used PH solid phase extraction followed by column switched trace enrichment HPLC with fluorescence detection. Plasma samples (1ml) were mixed with internal standard and buffered with 0.75ml pH7 0.05M phosphate buffer and applied to previously conditioned PH solid phase extraction sorbent packed in 96 well format (50mg sorbent per well). The sorbent was washed with water and UK-338,003 and internal standard selectively eluted with 2 x 0.25ml propan-2-ol into a 2ml 96 well block. The eluent was evaporated under nitrogen at 37°C and redissolved in 0.2ml of water/ acetonitrile/ tri-fluoroacetic acid (80/20/0.1). Extracted samples were centrifuged and 0.15ml injected onto an HPLC connected to a 1cm SAS trace enrichment column. A second HPLC using a mobile phase of acetonitrile/ water/trifluoroacetic acid (72/28/0.1) was used to wash the compounds off of the trace enrichment column and through a HiRPB column to a fluorescence detector. Detection wavelengths were set at 330nm (excitation) and 394nm (emission). The approximate retention time for UK-338,003 was 7.6 minutes.

Internal Standards

The dimethyl quinoline version of UK-191,005 was used as the internal standard for UK-191,005 (Series 1). For all other compounds UK-191,005 or the dimethyl quinoline version of UK-191,005 were used as internal standards. In each case the internal standards used were more lipophilic than parent compounds and not expected to be metabolites.

Pharmacokinetic analysis of data

Standard algorithms were used to perform the pharmacokinetic analysis of the data. C_{max} and T_{max} values were obtained directly from the recorded data. The terminal phase rate constant (k_{el}) was determined by linear regression of the log plasma concentration-time profile. The terminal elimination half-life ($t_{1/2}$) was calculated from $0.693/k_{el}$. Area under the plasma concentration –time curve (AUC_{0-T}) was calculated using the linear trapezoidal rule, and extrapolated to infinity ($AUC_{0-\infty}$) using k_{el} . Clearance (Cl) was calculated from intravenous data using the relationship dose/ $AUC_{0-\infty}$. Volume of distribution was calculated using the relationship Cl/ k_{el} . Oral bioavailability (F) was calculated from the ratio of $AUC_{0-\infty}$ values following oral and intravenous doses, after normalizing for dose. 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 100ml/min/kg in the rat and 50ml/min/kg in the dog. Based on the bioavailability observed and the estimate of hepatic extraction, the extent of absorption (A) was estimated ($A = F/(1-E)$). This method assumes that clearance is solely hepatic, which is supported by observations of low renal clearance (data not reported).

Free Drug Considerations

Only free drug in plasma is in equilibrium with free drug in the tissues and the relative equilibria between free and protein bound drug in both plasma and tissues will affect the extent of tissue distribution. In the same way, only free drug is available for clearance by metabolism and excretion (Wilkinson, 1983; Smith et al., 1996). It is therefore appropriate to correct clearance and volume of distribution for free fraction before considering the impact of physicochemical properties on these processes. Therefore unbound intrinsic clearance and unbound volume of distribution values were considered. Unbound volume of distribution and clearance values were calculated by dividing the values of total volume of distribution and clearance obtained from intravenous pharmacokinetic studies, by the free fraction of the compound in the plasma of that species.

Results

Physicochemical properties of the α 1-adrenoceptor antagonists

The structures and physicochemical properties of the α 1-adrenoceptor antagonists in series 1 and 2 are presented in Figures 1 and 2 and in Table 1. Physicochemical properties were determined by experimental methods ($\log D_{7.4}$ and pKa) or by computational analysis (molecular weight, clogP and number of HBDs and HBAs). All compounds are lipophilic and basic in nature with molecular weight values ranging from 416-609 Da. The higher the pKa, the larger the difference between clogP and $\log D_{7.4}$. The compounds containing a quinoline ring structure were more basic than the quinazoline containing compounds and therefore have the largest discrepancy between clogP and $\log D_{7.4}$. All compounds have the potential for hydrogen bonding with 2-3 hydrogen bond donor and 7-10 hydrogen bond acceptor groups per molecule. The compounds in series 1 had a higher molecular weight and $\log D_{7.4}$ than the compounds in series 2.

Isolated perfused rat liver (IPRL), in vitro metabolism and rat hepatocyte transport data for series 1 α 1-adrenoceptor antagonists

The IPRL data for the compounds in series 1 are summarized in Table 2 and Figure 3. UK-191,005 and analogues showed high hepatic extraction ratios in the IPRL (>0.7) with the majority of the dose excreted unchanged in the bile ($>90\%$). These data are consistent with the high plasma clearance observed in vivo for UK-191,005 in the rat. UK-191005 is taken up rapidly into the liver (Figure 3) with a liver to perfusate ratio of 11:1 after 5 min postdose.

Studies in rat liver microsomes showed that the compounds in series 1 are all slowly metabolized with disappearance half-life values of >120 min (Table 2). This suggests

that metabolic clearance is negligible for these compounds and correlates with IPRL data which demonstrates high biliary excretion of unchanged compound rather than metabolism as the principal route of clearance.

In agreement with the rapid hepatic uptake observed in IPRL experiments was the demonstration that UK-191,005 underwent temperature dependant, saturable active uptake in isolated fresh rat hepatocytes (Figure 4) with a K_m of $6.5 \pm 0.9 \mu\text{M}$ and V_{\max} of $481 \pm 37.5 \text{ pmol/min}/10^6 \text{ cells}$.

Pharmacokinetics of UK-191,005 in rat and dog

The pharmacokinetic parameters estimated for UK-191,005 (series 1) following intravenous administration to rat and dog are summarized in Table 3. Following intravenous administration to male rats, UK-191,005 exhibited a plasma clearance of 135 ml/min/kg which is greater than liver blood flow in the rat (Boxenbaum, 1980). The volume of distribution was 9.3 L/kg and the resulting elimination half-life was 1.4 h. In the dog, the intravenous clearance of UK-191,005 was 47 ml/min/kg, which approximates to liver blood flow in this species (Boxenbaum, 1980). The volume of distribution was 10 L/kg and plasma concentrations declined with an apparent half-life of 2.4 h.

Following oral administration of UK-191,005 to both rat and dog, drug levels were below the limit of quantitation. This suggests very low bioavailability and is in keeping with the high systemic clearance observed in both species and complete first pass extraction by the liver.

Choice of animal species for pharmacokinetic studies of α 1-adrenoceptor antagonists in series 2

Whilst the rat is typically the species used for initial pharmacokinetic evaluation of a compound, the clearance of UK-294,315 (series 2) and similar analogues were limited by blood flow in the rat. This resulted in short half-life values in the rat and low bioavailability due to complete first pass effect at the liver. Therefore the rat could not be used to rank compounds as unbound clearance was always limited by liver blood flow and bioavailability could not be observed.

In vitro and in vivo studies have shown that UK-294,315 and UK-338,003 are metabolized almost exclusively by CYP3A (data not reported). The high clearance values observed in the rat may occur due to the activity of CYP2C (Smith, 1991), which has substrate specificity that overlaps with CYP3A in this species.

In general the pharmacokinetics of substrates of CYP3A appear similar in dog and human (Smith, 1991). In addition, studies in vitro in hepatic microsomes showed that the rate of metabolism of these compounds was similar in dog and human (Fig 6). Dog was also the species used in in vivo pharmacology studies. For these reasons dog was chosen as the most appropriate species to evaluate the pharmacokinetics of the compounds in series 2 and most likely to be representative of the pharmacokinetics of the α 1_L-antagonists in human.

In vitro metabolism of series 2 α 1-adrenoceptor antagonists in dog and human liver microsomes

The disappearance half-life values for series 2 compounds in dog and human liver microsomes at 1 μ M substrate concentration and 0.5 μ M P450 concentration are shown in Table 4. No disappearance was detected in the absence of NADP. The extent of

binding in the incubation was also determined. In general, the most lipophilic and basic compounds have the highest microsomal binding. In addition, binding is greater in the human liver microsomal incubation than the dog, which is consistent with a higher protein concentration (1.5 mg/ml protein concentration in the human and 1.0 mg/ml protein concentration in the dog microsomal incubations). The disappearance half-life values and unbound fraction in the incubation were used to scale up to unbound intrinsic clearance in ml/min/kg. In dog, unbound intrinsic clearance values ranged from 23 to 162 ml/min/kg. Assuming phase 1 mediated metabolism is the major route of clearance for compounds in series 2, these values could be used to predict unbound intrinsic clearance in the dog. In human, unbound intrinsic clearance values ranged from 12 to 154 ml/min/kg. The values of unbound intrinsic clearance in dog and human were in general within 2-fold of each other, indicating a similar rate of metabolism in dog and human liver microsomes for these compounds (Figure 6).

Pharmacokinetics of series 2 α 1-adrenoceptor antagonists in dog, rat and human

Pharmacokinetic data following intravenous administration of 7 α 1-adrenoceptor antagonists in series 2 to the dog are shown in Table 5.

UK-294,315 (compound 1) is a quinoline with a homopiperazinoyl morpholine 2-substituent (Figure 2). Following intravenous administration to the dog it had a plasma clearance of 15 ml/min/kg, which is approximately one third of liver blood flow (Boxenbaum, 1980), and a volume of distribution of 4.8 L/kg. These values contributed to an elimination half-life of 6.3 h in the dog.

Compound 2 is similar in structure to UK-294,315. The clearance of compound 2 following intravenous administration to the dog was lower at 9 ml/min/kg. However,

the volume of distribution was also lower at 2.8 L/kg and thus compound 2 had a shorter half-life of 3.6 h.

Compounds 3 and 4 are quinazoline compounds with homopiperazinoyl morpholine 2-sidechains (Figure 2). They had higher plasma clearance values than the quinoline compounds of 23 and 20 ml/min/kg respectively, and similar volumes of distribution of 4.3 and 2.2 L/kg respectively, following intravenous administration to the dog. Therefore compounds 3 and 4 had short elimination half-life values in the dog of 2.3 and 1.6 h respectively.

Compound 5 is a quinoline compound with a homopiperazinoyl morpholine 2-sidechain (Figure 2). This compound had a low plasma clearance of 5.9 ml/min/kg following intravenous administration to the dog. However, the resulting elimination half-life was short at 1.9 h due to a drop in the volume of distribution to 1 L/kg. A higher volume of distribution of 5.1 L/kg was measured for the quinazoline compound 6, but its high plasma clearance of 40 ml/min/kg resulted in a short half-life of 1.5 h.

The desired pharmacokinetics were obtained with UK-338,003 (compound 7) which is a quinazoline compound with a methane sulphonamide tetrahydroisoquinolyl 2-sidechain. This compound had a low plasma clearance of 5.6 ml/min/kg and a volume of distribution of 4.1 L/kg following intravenous administration to the dog. UK-338,003 therefore had a long elimination half-life in the dog of 9.2 h.

Following oral administration to the dog, the majority of compounds in series 2 had bioavailability values consistent with complete absorption from the GI tract. The only notable exception to this was compound 2 which had an oral absorption of approximately 30% (Table 5).

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The pharmacokinetics of 2 compounds in series 2 were determined in rat and in human as well as in the dog and these data are summarized in Table 6. Following intravenous administration to the rat, UK-294,315 (compound 1) exhibited a short half-life of 1.0 h. This was due to a high systemic clearance of 91 ml/min/kg and a relatively high volume of distribution of 5.6 L/kg. Oral bioavailability in the rat was 11%, which is in keeping with the high systemic clearance in this species (approximately 90% liver blood flow) resulting in extensive first pass metabolism.

Following intravenous administration of UK-338,003 (compound 7) to the rat, plasma clearance was lower than UK-294,315 at 22 ml/min/kg. However, volume of distribution was also lower at 1.7 L/kg and as a result the elimination half-life of UK-338,003 in the rat was the same as UK-294,315 at 1.0 h. The oral bioavailability of UK-338,003 was 42% in the rat, which is consistent with an oral absorption of 72%.

In humans, across an oral dose range of 1-100 mg, UK-294,315 (compound 1) exhibited a mean elimination half-life of 11.6 ± 1.4 h. AUC_{0-T} increased proportionally with dose from 1-100 mg, peak plasma concentration (C_{max}) increased superproportionally up to a dose of 50 mg, with a 220 fold increase in C_{max} for a 50 fold increase in dose. Between 50 and 100 mg C_{max} increased in a dose proportional manner. T_{max} was variable, decreasing from 3.8 h at 1 mg to a plateau of 0.6 ± 0.2 h for all doses above, and including 30 mg.

UK-338,003 (compound 7) was administered to man as oral doses of 0.25 - 1.5 mg. UK-338,003 was rapidly absorbed following administration in solution with peak concentrations occurring 1-2 h after dosing, and had a mean estimated oral bioavailability of 73%. C_{max} and AUC increased approximately proportionally with dose. The terminal plasma elimination half-life was 11- 17 h.

Plasma protein binding

The plasma protein binding of UK-191,005 in series 1 was determined in rat, dog and human plasma at 1 $\mu\text{g/ml}$ ($n=3$) by equilibrium dialysis. The plasma protein binding values were 95, 90 and 97% in rat, dog and human plasma respectively.

The plasma protein binding of the compounds in series 2 was determined in dog plasma at 1 $\mu\text{g/ml}$ ($n=3$) by equilibrium dialysis. The protein binding values of compounds 1-7 in dog plasma were 96, 88, 78, 44, 78, 55 and 79% respectively.

Protein binding determinations were also performed in rat and human plasma for UK-294,315 (compound 1) and UK-338,003 (compound 7) at 1 $\mu\text{g/ml}$ ($n=3$). UK-294,315 had a plasma protein binding value of 92% in rat and 94% in human plasma. Rat and human plasma protein binding of UK-338,003 was 93%.

Discussion

This paper discusses the pharmacokinetic considerations in the design of an α_1 -adrenoceptor antagonist for the treatment of BPH. In doing so, it highlights the balance required between pharmacokinetics and pharmacodynamics in order to design a drug with optimum characteristics. The primary focus is the impact of physicochemical properties and structural modifications on pharmacokinetics and in particular clearance, which is a major determinant of dose size and elimination half-life, which in turn drives dosing frequency. Hepatic clearance was shown to be the major determinant of overall clearance for these compounds and hepatic extraction can be considered as a three step process involving hepatic uptake, metabolism and biliary clearance.

The first series of compounds investigated was a series of lipophilic bases, with large 2- and 4- substituents on a core quinazoline or quinoline ring template (Figure 1). These compounds are exemplified by UK-191,005, which is a weak base with a $\log D_{7.4}$ of 4, high MW (609) and a high propensity for hydrogen bonding (Table 1). The compounds were potent α_1 -adrenoceptor antagonists, with selectivity over the receptors in the cardiovascular system. Also, in vitro studies in rat, dog and human liver microsomes indicated that the compounds were metabolically stable (Table 2). As a consequence these compounds represented an attractive series for further study from both a pharmacological and pharmacokinetic perspective.

However, in rat and dog pharmacokinetic studies UK-191,005 was found to have high hepatic clearance approximating to liver blood flow (Table 3). As a result, UK-191,005 was not orally bioavailable in both species. Further studies were completed in

the IPRL in order to try and understand the mechanism of high hepatic extraction. In the IPRL, UK-191,005 and analogues showed high hepatic extraction ratios (>0.7) and levels of unchanged drug excreted in the bile were high ($>90\%$) (Table 2). These data agree with the high clearance observed for UK-191005 in the rat. UK-191005 was taken up rapidly into the liver with a liver to perfusate ratio of 11:1 after 5mins. Uptake of drugs from the blood into the hepatocyte can occur via passive diffusion or via active uptake across the sinusoidal membrane. The physicochemical properties of this series of compounds (high MW cations with high hydrogen bonding potential) suggest that passive diffusion across membranes is relatively slow and the rapid uptake of UK-191,005 into the liver is therefore more likely to be an active process, facilitated by carrier proteins on the sinusoidal membrane of the hepatocyte. A wide variety of active carrier systems are known to be present at the sinusoidal membrane facilitating the uptake of a diverse range of drugs (Hagenbuch, 1997; Meijer, 1997). The involvement of active transport processes in the hepatic uptake of UK-191,005 was confirmed in isolated rat hepatocytes at 4°C and 37°C where uptake of UK-191,005 was found to be temperature dependant and saturable (K_m 6.5 μ M; Figure 4). Therefore, active transport protein-mediated uptake in rat hepatocytes is likely to be a key determinant for entry of UK-191,005 into the liver.

For this series of compounds, rapid hepatic uptake was followed by extensive biliary excretion of unchanged drug with negligible metabolic clearance. UK-191,005 was shown to be a substrate for apical transport proteins in Caco-2 cells (data not shown) and such extensive biliary excretion is likely to be an active process mediated by canalicular (apical) transport proteins. As a consequence, this series of compounds had inadequate pharmacokinetics for further progression despite the attractive pharmacological properties of this series.

Compounds with high MW and high hydrogen bonding potential appear to be particularly susceptible to high hepatic extraction and biliary excretion (Ayrton and Morgan, 2001). Our studies showed that replacement of the large 2- and 4-substituents on the core quinoline or quinazoline template resulted in compounds with lower hepatic extraction ratios (<0.3) in the IPRL and low levels excreted in the bile. As a result, the project focus shifted to investigation of lower molecular weight (<510) quinoline or quinazoline compounds, exemplified by series 2 (Figure 2) in which metabolism is the predominant clearance route.

Indeed a significant difference between series 1 and 2 is that the rate of in vitro metabolism of the compounds in series 2 in liver microsomes translates to in vivo metabolic clearance. Unbound intrinsic clearance values for seven compounds in dog range from 23 to 536ml/min/kg. In general these values can be predicted within 2-fold from unbound intrinsic clearance values scaled from microsomal half-life in dog liver microsomes (Fig. 7). This relationship is in agreement with similar analyses in rat and human reported in the literature (Obach, 1997; Austin et al., 2002). However, due to the assumptions made in such predictions it is not unusual to find outliers. Here two compounds (UK-294,315 and compound 6) have a much higher unbound intrinsic clearance in vivo in dog compared to that predicted in vitro from dog liver microsome data. A possible explanation is that these compounds are subject to phase II metabolism or a mechanism of non-metabolic clearance. Dog liver has a high efficiency/ capacity for glucuronidation (Soars et al., 2001) which may account for the high unbound intrinsic clearance observed for compound 6 in the dog. UK-294,315 has been demonstrated to be a substrate for the efflux transporter P-glycoprotein (Harrison et al., 2004) which may contribute to its clearance in the dog, either by

clearance into the bile or via direct secretion across the gut wall (Ayrton and Morgan, 2001).

The quinazoline compounds within series 2 had higher unbound intrinsic clearance values than the quinoline analogues, reflecting their increased lipophilicity. For example comparison of the quinoline UK-294,315 (Compound 1) with its direct quinazoline counterpart, shows that the quinazoline compound has approximately five fold higher rate of metabolism in human liver microsomes (Figure 8). The quinazoline analogue is less basic than the quinoline compound (pKa values of 6.7 and 8.5 respectively), which results in an approximately 1 log unit higher logD_{7.4} and thus a higher propensity for metabolism.

Optimizing Half-Life via Modifying Physicochemical Properties.

Within series 2, the quinoline compound UK-294,315 exhibited a long half-life and high oral bioavailability in the dog. This compound was progressed to phase 1 studies in human volunteers where it exhibited a mean elimination half-life of 11.6 h (Harrison et al., 2004). However, the potency and selectivity of UK-294,315 for the prostate versus the cardiovascular system was not optimal giving rise to an inadequate therapeutic window. Therefore, project effort focused on the compounds containing a quinazoline ring system which were more potent antagonists of the human α_{1L} -adrenoceptors found in the prostate, with greater selectivity over the cardiovascular system.

However, as discussed, the quinazoline compounds were more lipophilic than the quinolines and therefore were predisposed to a higher metabolic clearance. Within the quinazoline series the challenge was therefore to design a compound with sufficient pharmacokinetic half-life. In order to balance pharmacodynamic and pharmacokinetic

requirements the initial strategy was to reduce $\log D_{7.4}$ in the quinazoline series of compounds in order to lower metabolic clearance and increase half-life.

To reduce lipophilicity within the quinazoline series, the 5'-phenyl analogues of the quinoline compounds 1 and 2 were replaced with a 6' or 5'-pyridine in the quinazoline compounds 3 and 4. Compound 3 has moderate lipophilicity ($\log D_{7.4}$ 2.1). This lipophilicity results in an unbound volume of distribution of 20L/kg in the dog, but also a high unbound intrinsic clearance of 200ml/min/kg. The elimination half-life of compound 3 is therefore short (2.3 h). Compound 4 is less lipophilic ($\log D_{7.4}$ 1.5) and this results in a decrease in unbound intrinsic clearance to 60ml/min/kg. However, the elimination half-life is still short (1.6h) and this is due to a drop in unbound volume of distribution to 4L/kg. This net effect of reducing unbound clearance and unbound volume of distribution after lowering $\log D_{7.4}$ was also observed in the quinoline series, e.g. compound 5, resulting in equally short half-life values (Table 5). The approach of increasing volume of distribution, and thus half-life, by increasing basicity has been shown to be successful e.g. amlodipine (Stopher et al., 1988). However, this approach was discounted with the quinazoline series due to deleterious effects on potency.

Optimizing Half-Life via Structural Changes.

To improve the half-life of the quinazoline series it was necessary to design compounds with less metabolic lability for a given lipophilicity. This can be achieved by removing or altering the functionality associated with metabolism, whilst maintaining lipophilicity in order to increase volume of distribution. It was necessary to do this in a low MW series in order to avoid clearance via active hepatic uptake as

encountered in series 1 and to maintain complete absorption in order to obtain high bioavailability. Steric and electron stabilization of a function to oxidative attack by cytochrome P450 is well preceded in the literature (van De Waterbeemd et al., 2001). For example, the metabolically vulnerable methoxyethyl substituent of metoprolol is replaced by a cyclopropyl group in bextaxolol which is much more stable to hydrogen abstraction (Manoury et al., 1987).

The 2' homopiperazinoyl morpholine sidechain of compounds 1 to 5 was found to be the major site of metabolism. Metabolism studies in liver microsomes have shown that this is susceptible to hydroxylation and ring opening (Fig. 9) which is consistent with other piperazine and morpholine containing compounds (Jauch et al., 1990; Cocchiara et al., 1991; Denissen et al., 1994). Efforts were made to replace this moiety with a less metabolically vulnerable 2' sidechain in order to decrease metabolic clearance.

Initially the homopiperazinoyl morpholine was replaced with a tetrahydronaphthyridine group (compound 6) which had moderate unbound intrinsic clearance from in vitro metabolism studies in dog liver microsomes. However, this was not reflected in the pharmacokinetics as compound 6 has a high unbound intrinsic clearance in the dog of 450ml/min/kg and a short half-life of 1.5hours. As discussed previously, this may be because compound 6 is subject to phase 2 metabolism or a non-metabolic route of clearance in vivo.

UK-338,003 (Compound 7) has a methanesulphonamido tetrahydroisoquinolyl side chain. This compound is weakly basic (pKa 6.7) and is lipophilic (logD_{7.4} 3.0) and

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thus is typical of the quinazoline series of compounds. The high $\log D_{7.4}$ results in an unbound volume of distribution of 21L/kg and the more metabolically stable 2' sidechain facilitates a low unbound intrinsic clearance of 29ml/min/kg (Fig 5). As a result UK-338,003 has a long elimination half-life of 9.2h in the dog. In addition it has an oral bioavailability of 89% in the dog which is consistent with complete transcellular absorption from the GI tract.

UK-338,003 fulfilled the objectives of the discovery programme as it had suitable pharmacology (human prostate α_{1L} pA_2 of 9.2 with 25 fold selectivity over rat aorta α_{1D}). In addition pharmacokinetic studies in the dog predicted a long elimination half-life and high oral bioavailability in human. UK-338,003 was progressed to phase 1 studies in young healthy volunteers, to assess pharmacokinetics and safety. Single doses of UK-338,003 in solution were administered at oral doses of up to 1.5mg. As predicted from the pharmacokinetics in dog, UK-338,003 was rapidly absorbed with peak concentrations occurring 1-2 h after dosing and a mean bioavailability of 73%. The terminal plasma half-life was 11-17 h and was superior to the prototype quinoline compound UK-294,315 (Harrison et al., 2004) and suitable for once a day administration to human (Table 6).

In conclusion, these data demonstrate the complex effect on pharmacokinetics and pharmacodynamics of change in structure or physicochemical properties of compounds. Understanding these relationships is essential to drug discovery so that drugs can be designed with optimum characteristics. In the programme described here ultimately a compound was identified with suitable pharmacokinetic-pharmacodynamic properties for progression to clinical development.

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Footnotes

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Figure Legends

Figure 1: Structures of α 1-adrenoceptor antagonists in Series 1.

Figure 2: Structures of α 1-adrenoceptor antagonists in Series 2.

Figure 3: Comparison of perfusate, liver and bile concentrations for UK-191,005 in the IPRL.

Figure 4: Uptake of UK-191,005 in fresh, isolated rat hepatocytes at 4°C and 37°C.

Figure 5: Relationship between lipophilicity and unbound intrinsic clearance in the dog for the α 1L-antagonists in series 2 (compounds 1-7).

Figure 6: Relationship between unbound intrinsic clearance in vitro in dog and human liver microsomes for the α 1L-antagonists in series 2 (compounds 1-7). The solid line represents the line of unity and the area between the dashed lines represents an area within 2-fold.

Figure 7: Relationship between predicted unbound intrinsic clearance from dog liver microsomal data and actual unbound intrinsic clearance in vivo for the α 1L-antagonists in series 2 (compounds 1-7). The solid line represents the line of unity and the area between the dashed lines represents an area within 2-fold. The identity of outlier compounds is indicated.

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Figure 8: Relationship between $\log D_{7.4}$ and rates of metabolism in human liver microsomes for a quinoline compound and its quinazoline counterpart.

Figure 9: Routes of metabolism of the homo-piperazine morpholine 2-substituent in vitro in human and dog liver microsomes.

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Table 1: Physicochemical properties of the α 1-adrenoceptor antagonists

Compound	Molecular Weight (Da)	clogP	logD _{7.4}	pKa	HBD	HBA
Series 1						
UK-191,005	609	4.4	4.0	6.2	2	10
Series 2						
UK-294,315	510	4.5	1.8	8.5	2	7.3
2	492	4.3	1.8	8.5	2	7.3
3	464	3.1	2.1	6.6	2	8.7
4	494	2.9	1.5	6.9	2	9.7
5	483	2.3	1.1	7.5	2	8.5
6	416	2.0	2.1	6.5	2	8.9
UK-338,003	507	2.7	3.0	6.7	3	9.8

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Table 2: IPRL and metabolism data for α 1-adrenoceptor antagonists in series 1

Compound	IPRL Data		Rat Liver Microsomes
	Hepatic E.R.	% of Dose in Bile	Half Life (min)
UK-191,005	0.7	90	>120
(2)	0.7	100	>120
(3)	0.75	90	>120
(4)	0.8	90	>120

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Table 3: Mean Pharmacokinetic parameters of UK-191,005 in the rat and dog

Compound	UK-191005	
	Rat	Dog
n	5	2
Intravenous Dose (mg/kg)	3	0.5
Elimination half-Life (h)	1.4 ± 0.3	2.4
Plasma clearance (ml/min/kg)	135 ± 2	47
Unbound plasma clearance (ml/min/kg)	2700	470
Volume of distribution (l/kg)	9.3 ± 3.9	10
Unbound volume of distribution (l/kg)	186	100

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Table 4: *In vitro* metabolism of the α 1-adrenoceptor antagonists in series 2

Compound	Human Liver Microsomes			Dog Liver Microsomes		
	Half-life (min)	Fu inc	Cl int un (ml/min/kg)	Half-life (min)	Fu inc	Cl int un (ml/min/kg)
UK-294,15	6	0.331	154	30	0.602	100
2	9	0.414	82	25	0.59	122
3	5	0.637	96	14	0.791	162
4	9	1	34	46	0.939	41
5	19	0.745	22	80	0.962	23
6	31	0.823	12	36	1	50
UK-338,003	41	0.564	13	36	0.602	83

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Table 5: Mean Pharmacokinetic parameters of the α 1-adrenoceptor antagonists in series 2 following single intravenous and oral administration to the dog

Compound	UK- 294,315	2	3	4	5	6	UK- 338,003
<i>n</i>	4	2	2	2	2	2	2
Intravenous dose (mg/kg)	0.5	0.5	0.5	0.5	0.5	0.5	0.3
Elimination half-life (h)	6.3 (\pm 1.9)	3.6	2.3	1.6	1.9	1.5	9.2
Plasma clearance (ml/min/kg)	15 (\pm 6.9)	8.8	23	20	5.9	40	5.6
Unbound intrinsic plasma clearance (ml/min/kg)	536	88	200	60	23	450	29
Volume of distribution (l/kg)	4.8 (\pm 1.5)	2.8	4.3	2.2	1.0	5.1	4.4
Unbound volume of distribution (l/kg)	96	23	20	4.0	4.5	11	21
Oral dose (mg/kg)	4.0	0.5	2.0	2.0	0.5	0.5	0.6
Oral bioavailability (%)	71 (\pm 4.1)	24	35	63	76	28	79
Oral absorption (%)	100	29	65	100	86	100	89

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Table 6: Mean Pharmacokinetic parameters of UK-294,315 and UK-338,003 following single intravenous administration to rat and single oral administration to rat and human

Parameter	UK-294,315		UK-338,003	
	Rat (n=2)	Human (n=10)	Rat (n=2)	Human (n=5)
Intravenous dose (mg/kg)	0.5	n/a ^a	0.5	n/a ^a
Elimination half-life (h)	1.0	n/a ^a	1.0	n/a ^a
Plasma clearance (ml/min/kg)	91	n/a ^a	22	n/a ^a
Unbound intrinsic plasma clearance (ml/min/kg)	61250	n/a ^a	40	n/a ^a
Volume of distribution (l/kg)	5.6	n/a ^a	1.7	n/a ^a
Unbound volume of distribution (l/kg)	70	n/a ^a	24	n/a ^a
Oral dose (mg/kg)	4	0.86 ^b	1.0	0.02 ^c
Cmax (ng/ml)	14	63 (41)	105	7.5 (1.56)
Tmax (h)	4	0.7 (0.42)	0.5	1.3 (0.27)
Elimination Half-Life (h)	2.8	12 (2.4)	2.9	11.4 (2.14)
Oral bioavailability (%)	11	n/a	42	n/a

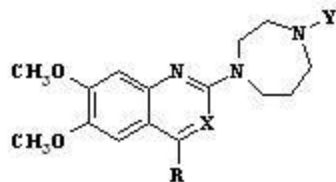
DMD#15180

^a *Intravenous data not available*

^b *Administered as 60mg in solution*

^c *Administered as 0.5mg in solution*

Figure 1



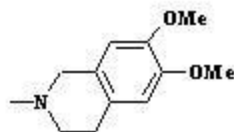
Physicochemical properties

MW = 510-615

log $D_{7.4}$ = 2.8-4

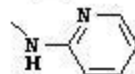
pKa = 6.2-8.5

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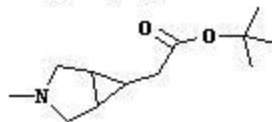


UK-191,005

(2): X=N; R =



(3): X=N; R =



(4): X=C; R =

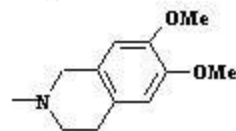
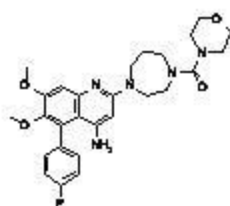
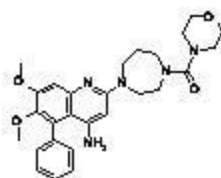


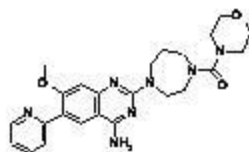
Figure 2



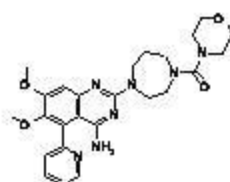
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(Compound 1)



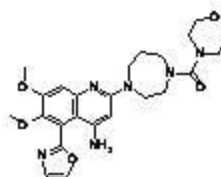
Compound 2



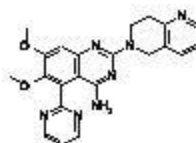
Compound 3



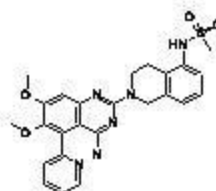
Compound 4



Compound 5



Compound 6



UK-338,003
(Compound 7)

Physicochemical properties

MW = 416-510

log $D_{7.4}$ = 1.1-3.0

pKa = 6.5-8.5

Figure 3

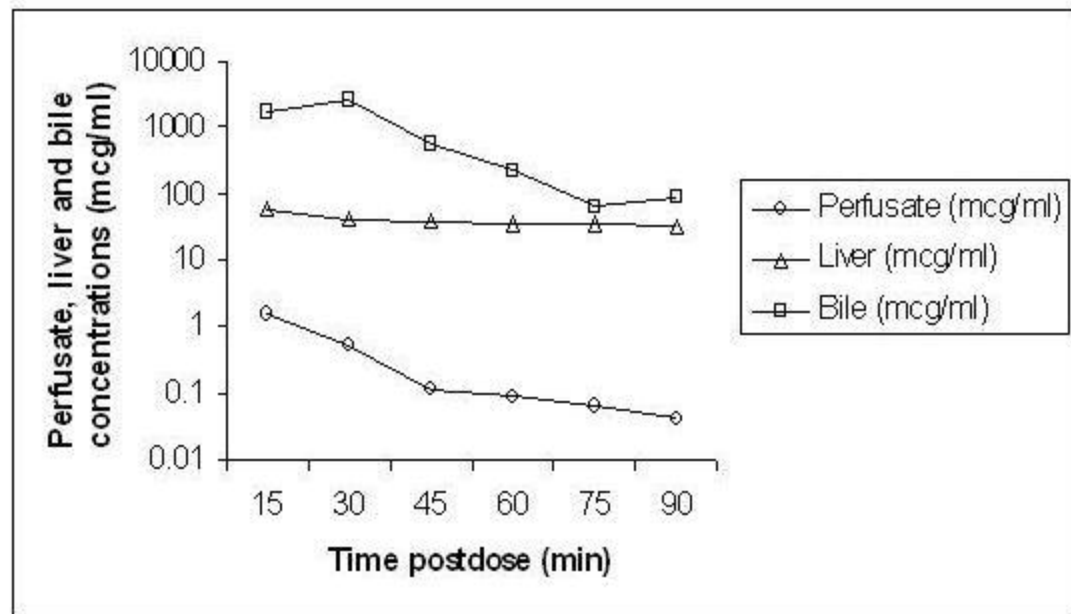


Figure 4

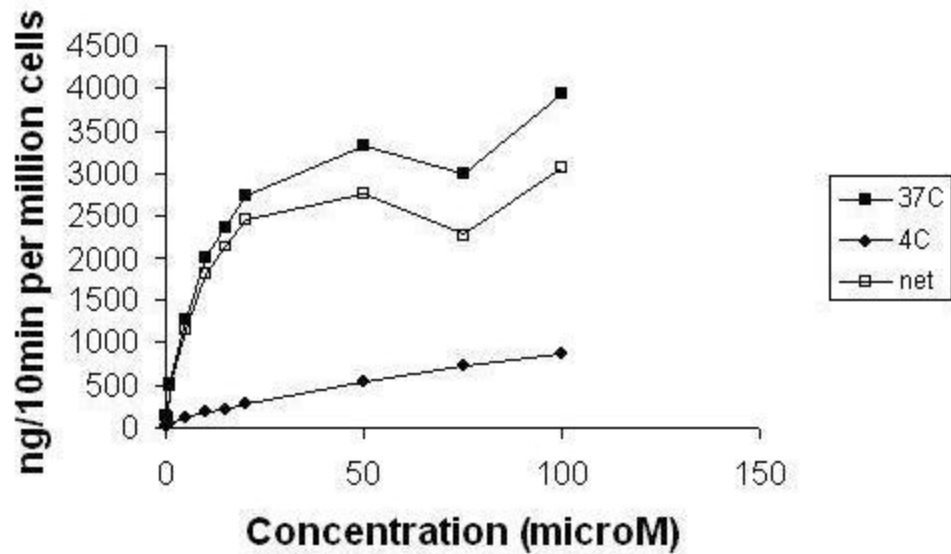


Figure 5

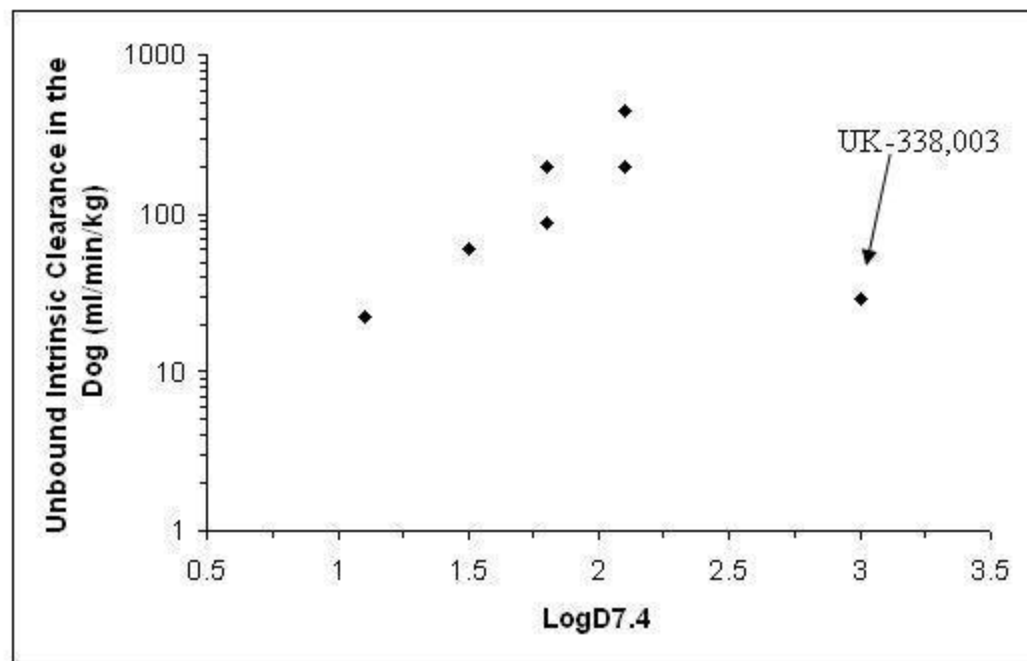


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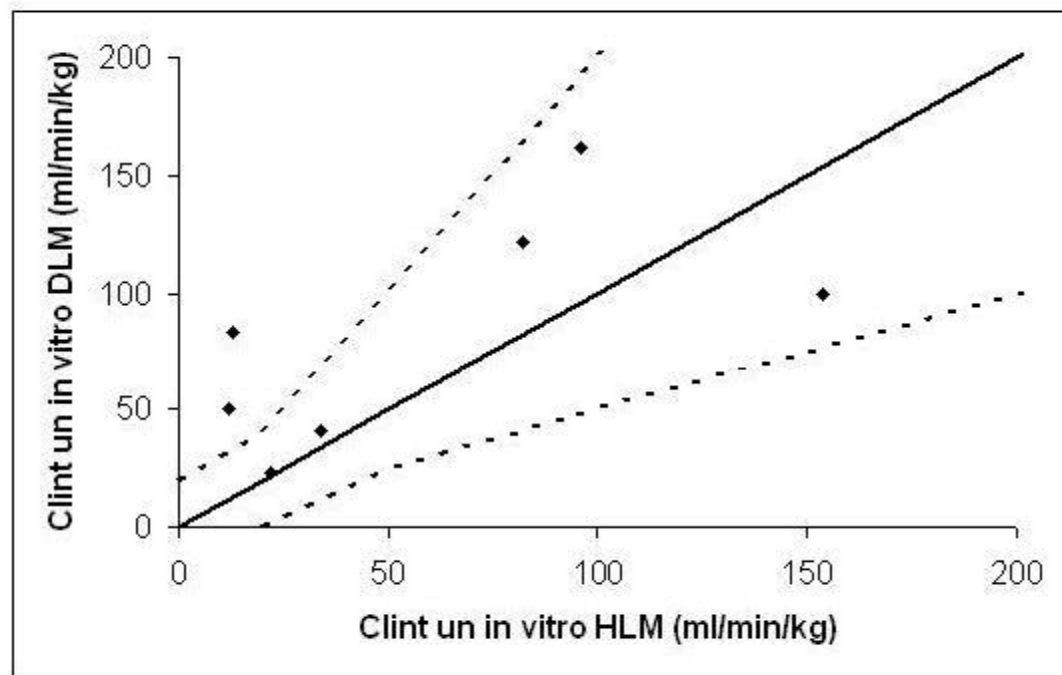


Figure 7

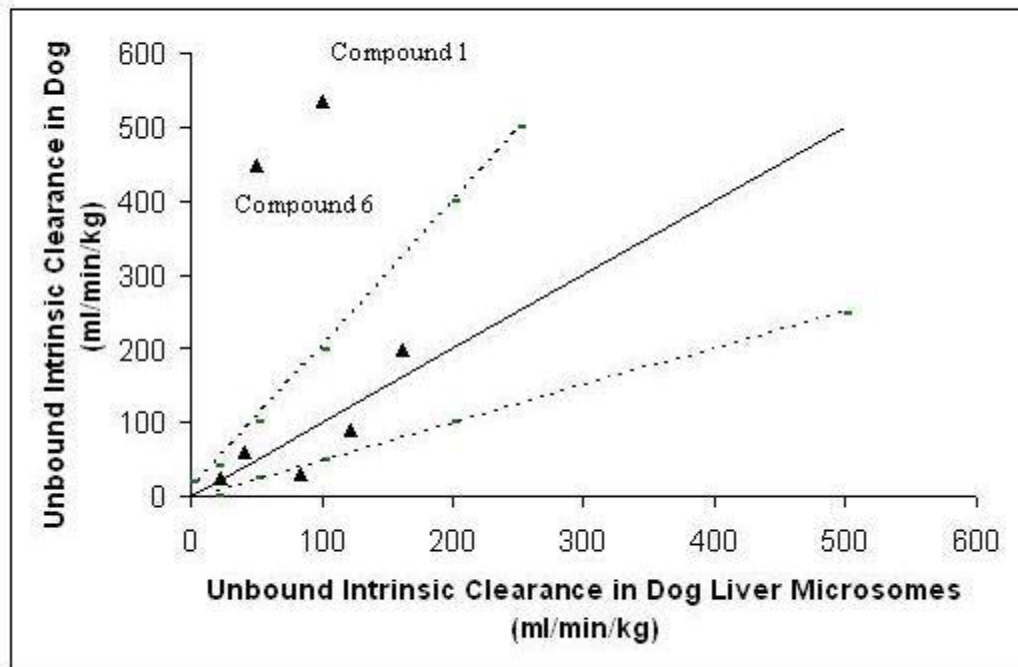
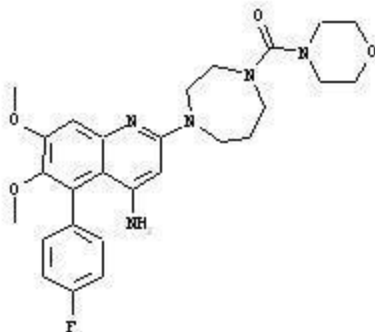


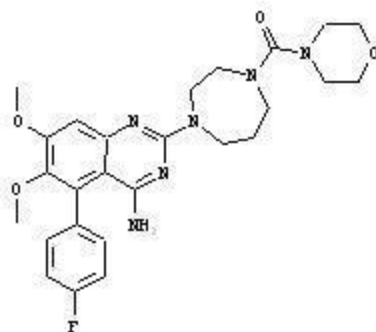
Figure 8

Quinoline



Log $D_{7.4}$ 1.8
In vitro half-life (min) 26

Quinazoline



3.0
 5

Figure 9

