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Lipophilicity in PK design: methyl, ethyl, futile

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Abstract

Lipophilicity, often expressed as distribution coefficients (log D) in octanol/water, is an important physicochemical parameter influencing processes such as oral absorption, brain uptake and various pharmacokinetic (PK) properties. Increasing log D values increases oral absorption, plasma protein binding and volume of distribution. However, more lipophilic compounds also become more vulnerable to P450 metabolism, leading to higher clearance. Molecular size and hydrogen bonding capacity are two other properties often considered as important for membrane permeation and pharmacokinetics. Interrelationships among these physicochemical properties are discussed. Increasing size (molecular weight) often gives higher potency, but inevitably also leads to either higher lipophilicity, and hence poorer dissolution/solubility, or to more hydrogen bonding capacity, which limits oral absorption. Differences in optimal properties between gastrointestinal absorption and uptake into the brain are addressed. Special attention is given to the desired lipophilicity of CNS drugs. In examples using β-blockers, Ca channel antagonists and peptidic renin inhibitors we will demonstrate how potency and pharmacokinetic properties need to be balanced.

Introduction

Lipophilicity often expressed by octanol/water distribution, either calculated or measured, has been widely used in quantitative structure-activity relationship (QSAR) studies. Lipophilicity appears to be a key property in drug disposition [1]. Although lipophilicity and hydrophobicity are often used as synonyms, it seems worthwhile to remember the IUPAC definitions at this point [2]:

Hydrophobicity is the association of non-polar groups or molecules in an aqueous environment which arises from the tendency of water to exclude non-polar molecules

Lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by its distribution behaviour in a biphasic system, either liquid-liquid (e.g. partition coefficient in 1-octanol/water) or solid-liquid (retention on reversed-phase high-performance liquid chromatography (*RP-HPLC*) or thin-layer chromatography (*TLC*) system).

Hydrophobicity is used to describe molecular surface properties and is associated with dissolution/solubility of a compound. As a rule of thumb solubility is inversely correlated with lipophilicity. Thus increasing lipophilicity leads eventually to solubility problems.

In terms of pharmacokinetics, much of the behaviour reflects to affinity of the molecule for a lipophilic environment [3]. Lipophilicity is thus used in relation to partitioning or distribution processes, such as absorption, cell membrane permeation, membrane and protein binding.

The assumptions that can be made for many compounds and targets is that there is a direct relationship between *in vitro* potency and the free drug plasma concentration required for efficacy. The free drug

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concentration is dependent on dose size, fraction absorbed (bioavailable) and free drug clearance. The latter term is predictable from physicochemical and structural considerations, or from *in vitro* experimentation. Building on previous experiences [4], we have used log D as the major physicochemical parameter to illustrate how physicochemistry relates to free drug pharmacokinetics and metabolism.

In more recent years it became clear that molecular size and hydrogen bonding capacity of a drug molecule are the major components of lipophilicity. In the following sections we will discus the role of each in the disposition of drugs. Building on this understanding we will illustrate how many of the processes are interconnected and lead to a lack of progress towards better drugs and also how a synthetic programme is doomed by not starting in the required physicochemical property space.

Oral absorption

For most drugs the first step to enter the body is absorption from the gastro-intestinal tract. Current understanding is that looking at a membrane we need to discriminate between a physicochemical and a biochemical barrier. Permeation via the trans- and paracellular pathway are largely biophysical processes controlled by the physicochemical properties of a drug. Most prominent are lipophilicity, charge, hydrogen bonding, and size of the compound. In addition it becomes increasingly clear that various transporter proteins such as P-glycoprotein and metabolising enzymes, particularly CYP3A4, may have a considerable impact on oral absorption [5].

An example of the influence of log D on human intestinal absorption is seen in Figure 1. Log D values >0 are often correlated to almost or complete absorption. It is expected that at higher log D values solubility-limited absorption is observed. Figure 1 does not reflect this. By it's very nature erratic or poor absorption data is not reported. Much of the data is on marketed drugs, which have reasonable physicochemical properties or are formulated to overcome this problem. It is also difficult to recognise a sigmoidal relationship as is often suggested. It should be remembered that human absorption data is biased by unquantified contributions on absorption of active or efflux transporters and of gut wall metabolism.

Larger compounds, here defined as those with MW > 500, may display less than complete absorption (see below for further discussion). The MW 500 limit is now known as one of the rule-of-5 criteria, which states that poor absorption is likely when MW > 500, CLOGP > 5, there are more than 5 H-bond donors, and the sum of N's and O's is over 10. Substrates for active transporters and natural products or mimics of these may be exceptions to this rule [6].

Log P or log D can be written as a linear combination of at least two more fundamental properties [10], one related to the size of a molecule and the second to its capacity to form intermolecular hydrogen bonds. Obviously this latter property is conformation-dependent. Polar surface area has been suggested as an easily assessable descriptor for H-bonding [11]. Others proposed to use the dynamic polar surface area to account for conformational freedom [12]. In most of the reported absorption or permeation versus H-bonding plots a clear sigmoidal relationship has been observed [13–15]. Figure 2 is a further illustration of a absorption vs H-bonding relationship using the compounds in [9] and absorption data from [16]. However, in this case it is observed that the descending part covers a relatively wide range of H-bonding characteristics. Possibly different subsets of compounds can be defined, but more data are needed to test such a hypothesis. The compounds with a MW below 200 are nearly on a straight line and a steep descent is observed. Some of the larger compounds are better absorbed than expected from their size and H-bonding. This includes a compound such as methotrexate, known to be actively transported.

Both hydrogen bonding and lipophilicity also play an important role in theoretical calculations to predict human oral absorption or Caco-2 cell permeability [13, 17, 18].

Limitations of the rule-of-5

The rule-of-5 properties are not independent. Increasing MW often gives better potency due to more unspecific binding. However, increasing a compound's size can by realised by adding more C or halogen atoms, leading to higher CLOGP, or by adding more hetero atoms, leading to higher hydrogen bonding capacity. Higher CLOGP, or lipophilicity, may result in poorer solubility. Higher H-bonding may result in less membrane permeability. Optimising on log D alone may be insufficient as well. Since log D is a combination of size and hydrogen bonding, combinations of high MW and extensive H-bonding capacity are possible to yield a log D and solubility in the desired range. However,

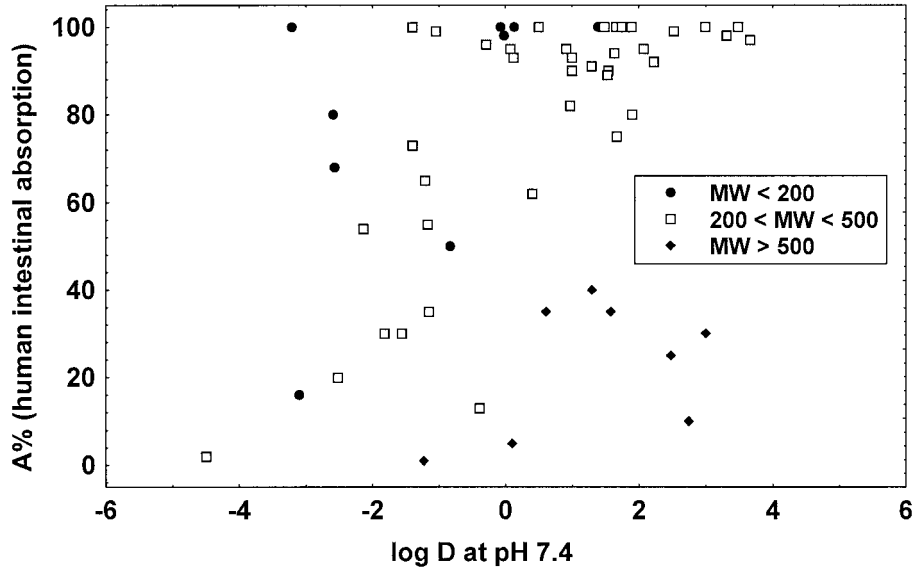

Figure 1. Influence of log D on human oral absorption [7–9, 16].

oral absorption of such compounds will nevertheless be limited because of the high H-bonding capacity. The balance between these properties begins to define the physicochemical space for success.

Targeting the brain

The influence of log P/log D on brain uptake for CNS agents has been widely studied and is well-documented [19, 20]. Most CNS drugs are relatively small in size, MW around 350. Octanol/water distribution coefficients typically are in the range 0–4, also reflected by a well-known rule-of-thumb that optimal CNS drugs have a log D of ca. 2 (see Figure 3). Based on a comparison of CNS versus non-CNS drugs it was concluded that the physicochemical constraints on compounds targeted to the brain are slightly more restrictive than for oral absorption. MW should be below 450, while for GI absorption a limit of 500 has been suggested (see Oral Absorption). The total polar surface area, a measure for hydrogen bonding capacity, should be below 90 $Å^2$, while for GI absorption this limit can be somewhat higher [8, 20]. Using surface activity measurements it was established that compounds with a cross-sectional area >80 $Å^2$ are unlikely to cross the blood-brain barrier [21].

Octanol/water partition coefficients do not always correlate well with brain uptake [19]. Therefore solvent systems such alkane/water have been suggested to reflect to very apolar nature of the BBB. Partitioning systems using cyclohexane (log P) approach have been cited as better mode systems than octanol [22]. In a classic paper it was demonstrated that the difference between octanol/water and alkane/water partition coefficients, $\Delta \log P$, gives satisfactory correlations with BBB crossing [22]. $\Delta \log P$ was later identified as encoding mainly for H-bonding capability. However, measuring many log P values is still quite tedious and in the case of alkane/water prown to (solubility-limited) errors. Therefore various theoretical approaches to predict BBB partitioning have been explored [23–25].

Much of the data produced has actually studied the partitioning of drugs into whole brain from blood or plasma. These studies have shown a need for compounds with low H-bond potential and relatively high lipophilicity. Whole brain partitioning actually represents partitioning into the lipid of the brain, and not actually access to drug receptors. For instance, desipramine partitions into brain and is distributed unevenly [36]. The distribution corresponds to lipid content of the brain regions and not to specific desipramine binding sites. For receptors such as 7-TM's ECF (extracellular fluid) concentrations determine activity. CSF (cerobrospinal fluid) concentrations can be taken as a reasonable guide of ECF concentrations. The apparent dramatic differences in brain distribution described for total brain collapse to a small ratio when free (unbound) concentration

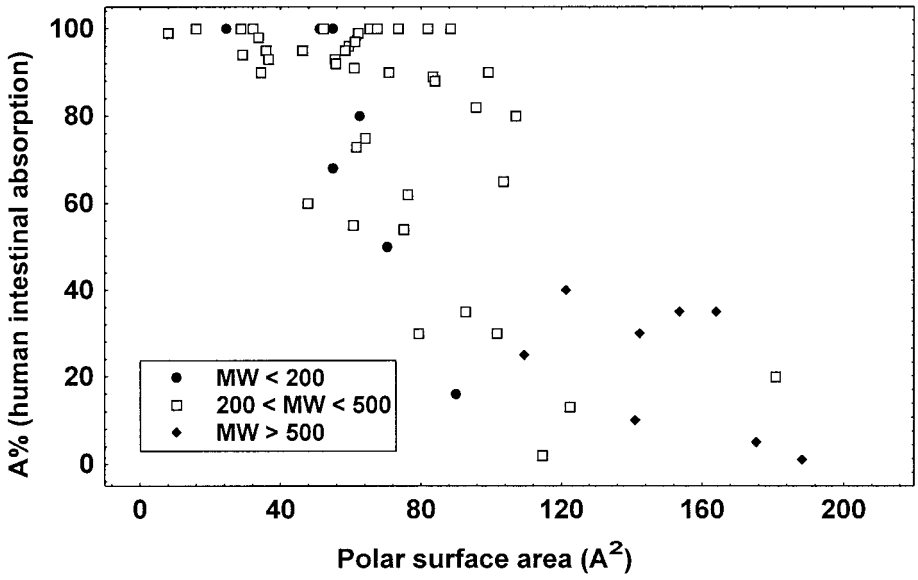

Figure 2. Relationship between human intestinal absorption and polar surface area as a measure for hydrogen bonding capability.

of drug in plasma in compared to CSF concentration. Whole brain/blood partitioning reflects nothing but an inert partitioning process of drug into lipid material. The lack of information conveyed by total brain concentration is indicated by studies on KA-672 [39] a lipophilic benzo-pyranone acetylcholinesterase inhibitor. The compound achieved total brain concentrations 0.39 μM at a dose of 1 mg/kg. This is equivalent to the IC_{50} determined *in vitro* (0.36 μM). Doses up to 10 mg/kg were without pharmacological effect. Analysis of CSF indicated concentrations of the compound were below 0.01 μM, readily explaining the lack of activity. These low concentrations are due presumably from high (unbound) free drug clearance and resultant low concentrations of free drug in the plasma (and CSF).

Free unbound drug partitioning actually reflects the drug reaching the receptor and pharmacological effect. Unless active transport systems are invoked the maximum CSF to plasma partition coefficient is 1. This should be contrasted to the 10- or 100-fold affinity of total brain compared to blood or plasma. The minimum partitioning based on a limited data set appears to be 0.1. Figure 4 compares lipophilicity (log D) to a series of diverse compounds that illustrate the limited range of partitioning. It should be noted that the term log D is not a perfect descriptor and some of the measures which incorporate size and hydrogen bonding may be better. Clearly though, the CNS is more permeable than imagined, allowing drugs like sulpiride (compound 3) to be used for CNS applications.

The CNS penetration of the hydrophilic sulpiride is compared in Figure 5 to the more lipophilic D2 antagonists, remoxipride, haloperidol and thioridazine. The figure contrasts receptor occupancy calculated from the free (unbound) concentration of drug in plasma compared to that measured by PET scan. Clearly the lipophilic compounds are in close equilibria between the plasma and the ECF. Sulpiride requires much greater free (unbound) concentrations present in the plasma.

Sulpiride's need for a higher free (unbound) plasma concentration stems directly from the compound's poorer permeability characteristics. Figure 6 shows how these concentrations relate to CSF concentrations (about 4-fold lower) and how these correlate with receptor occupancy and pharmacological activity.

Hydrophilic compounds may offer advantages in many applications despite the problems of lower membrane permeability and lower potency. The compounds have low intrinsic clearance and low affinity for metabolising enzymes and a resultant lack of drug interactions.

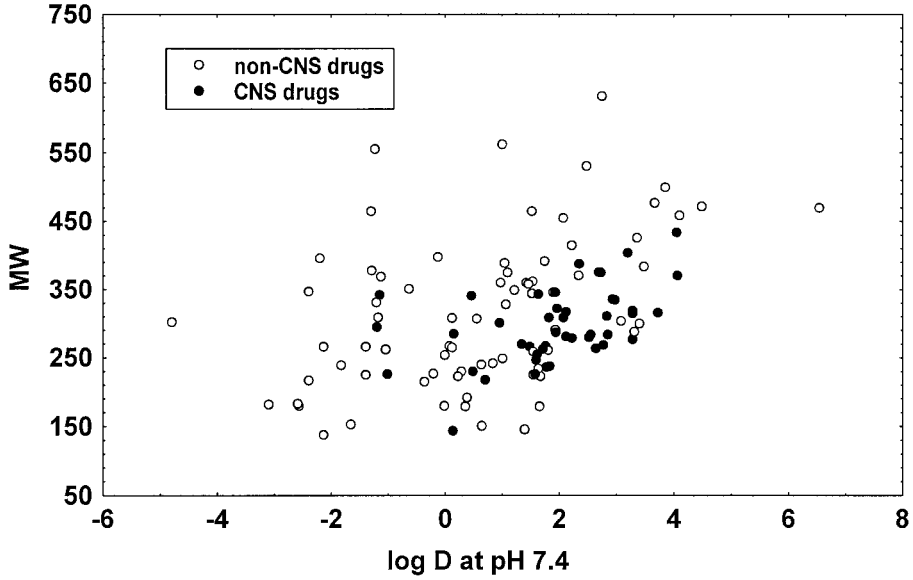

Figure 3. Log D against molecular weight for a series of CNS and non-CNS drugs [20].

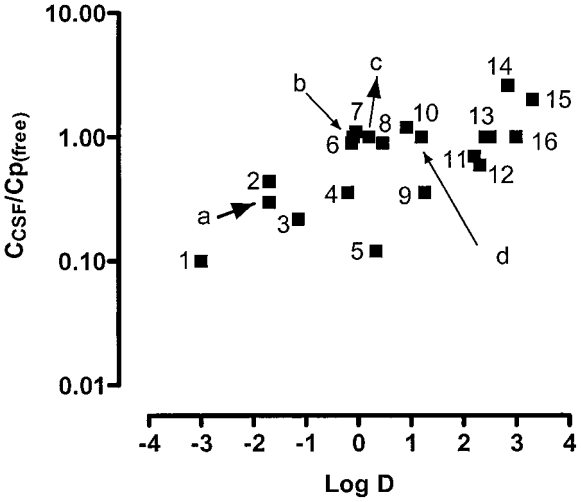

Figure 4. CSF concentration/free (unbound) plasma concentration ratios compared to log D for neutral and basic drugs ritropirronium (1), atenolol (2), sulpiride (3), morphine (4), cimetidine (5), metoprolol (6), atropine (7), tacrine (8), digoxin (9), propranolol (10), carbamazepine (11), ondansetron (12), diazepam (13), imipramine (14), digitonin (15), chlorpromazine (16), and acidic drugs, salicylic acid (a), ketoprofen (b), oxyphenbutazone (c) and indomethacin (d).

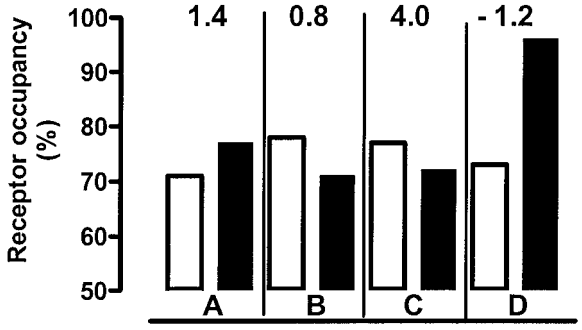

Figure 5. Receptor occupancy calculated from the free (unbound) concentation of drug in plasma compared to that measured by PET scan for remoxipride (A), haloperidol (B), thioridazine (C), sulpiride (D). Log D values are shown above each compound.

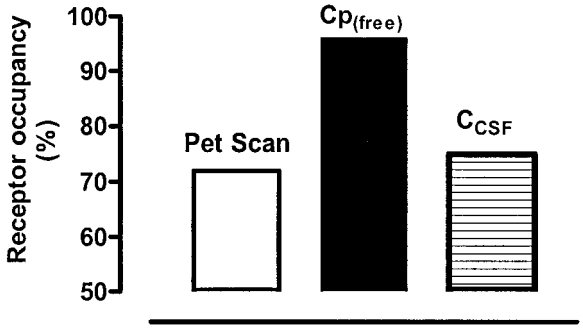

Figure 6. Receptor occupancy calculated from the free (unbound) concentration of drug in plasma, the concentration in CSF compared to that measured by PET scan for sulpiride.

Influence of lipophilicity on pharmacokinetic parameters

The increase in free volume of distribution (V_{du}) with increasing lipophilicity (see Figure 7) largely reflects association with plasma proteins (albumin) for acidic compounds and association with tissues and

lipoproteins for neutral and lipid compounds. Basic compounds bind mainly to albumin and partially to α_1-acid glycoprotein. With increasing lipophilicity probably also unspecific binding to red blood cells, leukocytes and platelets increases. The high association for acidic compounds with albumin is due to both ion-pair and hydrophobic interactions. Similar reasons apply for the affinity of basic compounds for tissues. Here the ion-pair and hydrophobic interactions are due to the head and tail groups of phospholipids present in cell membranes. Similar to the prediction of intrinsic metabolic clearance, such associations can be studied with *in vitro* systems such as plasma protein and cell membrane binding.

Figure 7 shows the results when unbound volume of distribution (volume of distribution at steady state corrected for plasma protein binding) is plotted against log D values. The earlier [4] observed trend in which basic and acidic compounds have higher values than neutral compounds remains clearly visible.

As explained above, in the free drug concept we are interested in unbound drug clearance and volume. Plasma protein binding permits estimates of these properties although it is important to stress it is free drug clearance that determines free drug concentrations at steady state. Figure 8 shows that within a class of different compounds (acid, base, etc.) an approximately sigmoidal relationship exists between protein binding and log D. The curve for basic and neutral compounds practically overlap and are at higher log D values than the curve for acids. Acidic compounds have high plasma protein binding for log D values above -1, while for basic compounds high protein binding can be expected for log D > 2. The four 'acids' on the right-hand side of the neutrals & base curve are the barbiturates phenobarbital, hexobarbital and pentobarbital, and the derivative primidone. We believe these compounds have a very delocalised negative charge and practically behave as a neutral compound.

Examples of design using physicochemistry

β-*Adrenoceptor antagonists*

Although it can be criticised on statistical grounds due to the numerical range of pharmacokinetic parameters compared to the large range of protein binding/lipophilicity measurements, the use of unbound (free) pharmacokinetic data is an important method of probing the behaviour of a series of compounds. This technique is particularly important when *in vitro* potency, rate of metabolism, plasma clearance and dose and duration are being explored and compared. These are the properties that describe drug-like behaviour. A compelling example of the interplay between these factors is provided by β-adrenergic antagonists which have been the subject of detailed investigations [28–30]. The marketed compounds have an in vitro potency as measured by pA_2 values ranging from 6.5 to 8.8 (atenolol and betaxolol), respectively. Despite the magnitude of these differences the daily dose of most of these agents, is in the range of 25–100 mg.

Yamada et al. [28] have illustrated how the pharmacokinetics of the β-adrenergic antagonists combined with daily dose and affinity yield the same degree of receptor occupancy (80%) and hence efficacy. Another feature of this range of compounds is their lipophilicity, as measured by log $D_{7.4}$ or log P spans four log units. Since lipophilicity is a key parameter in determining affinity for receptors (potency), enzymes (clearance), tissues and membranes (volume of distribution) and permeability (tubular reabsorption), it is illuminating to examine the various trends in these parameters changes in lipophilicity invoke.

Figure 9 illustrates the structures of ten clinically used β-adrenoceptor antagonists. All contain the same aryloxy-propan-2-ol amino unit. The aryl unit shows different substituent positions, the para position substituents giving cardio-selectivity (β_1/β_2). Amino substitution is either iso-propyl or tertiary butyl except for carvedilol, which combines α- and β-adrenoceptor antagonism.

Figure 10 illustrates their potency against the β_1 receptor as a pA_2 value. The potency shows a marked correlation with lipophilicity, in that the pA_2 value indicates a 300 fold increase in potency with a 10 000 fold increase in lipophilicity (D or P). Such changes can be simply rationalised considering the features of the natural agonists (e.g. adrenaline) and the key binding interactions which the aryloxy-propan-2- of amino unit fulfils. Any increase in potency is likely to come from more non-specific lipophilic interactions.

Figure 11 shows the relationship between unbound volume of distribution and lipophilicity. The major component of volume of distribution is the affinity for the membranes of cells. This affinity is derived from two physiochemical properties: the compound's intrinsic lipophilicity and its basicity. Membranes are comprised of phospholipids with charged head groups, some acidic, and lipid tails organised as a bilayer

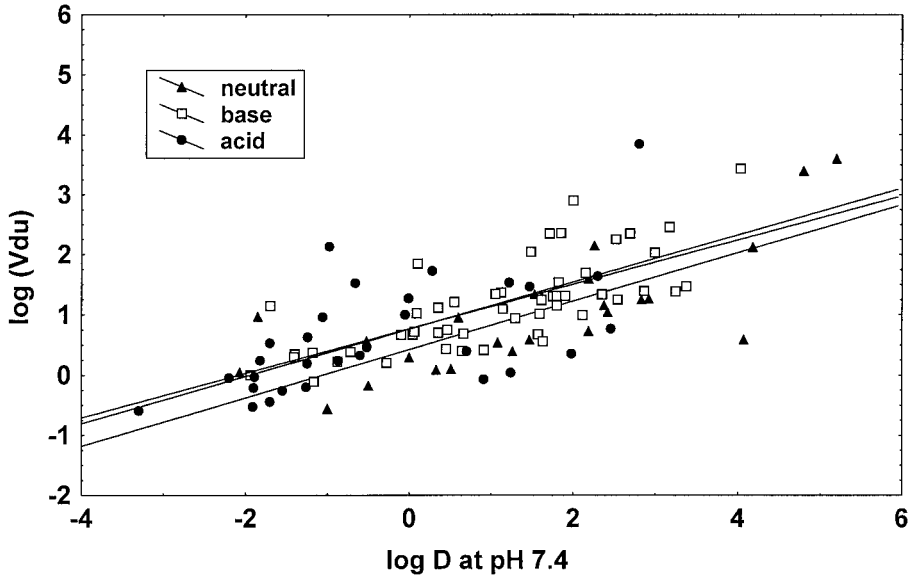

Figure 7. Effect of log D on unbound volume of distribution for different types of compounds.

to form a lipid interior. Lipophilicity determines the compound's ability to interact with the lipid core of the membrane, whilst the basic nitrogen group allows ion pair interactions with the ionised head groups. Since the compounds all possess a similar basic centre (pKa ca. 9.4) then lipophilicity is the predominant driving force in determining volume of distribution. The strong correlation been unbound (free) volume and lipophilicity is in keeping with these interactions.

Figure 12 contrasts the intrinsic unbound clearance and lipophilicity with unbound renal clearance with lipophilicity for these agents. Unbound renal clearance is approximately constant over the range illustrated, however, probably declines with compounds of higher lipophilicity. The amount excreted in urine declines dramatically with lipophilicity due to the increased importance of metabolic clearance, making calculations unreliable. Intrinsic unbound clearance Cli(u) measures the metabolism of the compounds and is related to total clearance Cl by the equation:

$$\mathrm{Cl} = \mathrm{Q}\frac{[\mathrm{Cli(u)fu}]}{[\mathrm{Cli(u)fu} + \mathrm{Q}]} \tag{1}$$

where Q is liver blood flow and fu is the fraction (free) unbound in blood or plasma. Cli(u) is also directly related to the affinity (Km) and metabolic rate (V_{max}) of the enzyme system(s) responsible for clearance (metabolism).

$$\mathrm{Cli(u)} \sim \frac{V_{max}}{\mathrm{Km}} \tag{2}$$

Where the clearance of these β-adrenocepor antagonists is via metabolism it is the cytochrome P450 family of enzymes which are responsible for this. Cytochrome P450s metabolise lipophilic compounds into molecules which can ultimately renally cleared. Whilst there a vast number of these enzymes, there are probably only three which are key in human drug metabolism namely CYP2C9, CYP2D6 and CYP3A4. The substrate specificity of these enzymes has been described previously [1]. Given the important role these enzymes have in detoxification and hence protection, it is perhaps not surprising that different enzymes have evolved to metabolise lipophilic molecules of acidic or basic nature. Briefly, CYP2C9 is mainly concerned with the metabolism of lipophilic compounds that are either acidic or have a high degree of hydrogen bonding capacity. CYP2D6 typically metabolises lipophilic bases where the basic nitrogen atom is a fixed distance from a site of metabolism. CYP3A4 metabolises lipophilic bases, neutral compounds, and some acids.

With cytochrome P-450 enzymes it is not surprising that Km shows an inverse relationship to lipophilicity since the binding of the substrate to the enzyme relies largely on hydrophobic forces [31, 32]. This is illustrated even for CYP2D6, an enzyme which also interacts by ion pair interactions [31–33] and is a key enzyme in the clearance of several of the β-adrenocepor antagonists. Ferrari et al. [34] have shown a close correlation between lipophilicity and Ki values

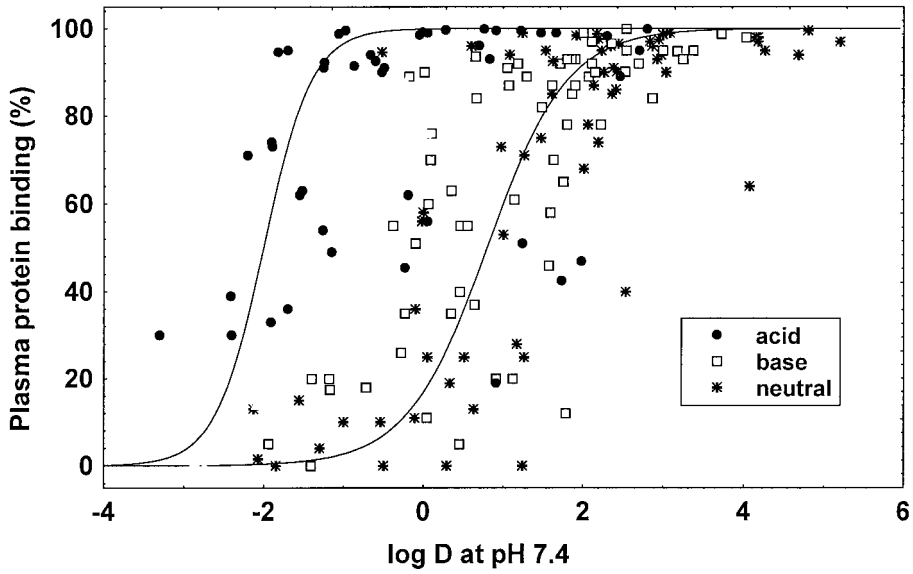

Figure 8. Effect of log D on plasma protein binding for different categories of compounds.

for a range of these compounds against the CYP2D6 isoenzyme. This can be explored further if actual rate of metabolism is considered. Figure 13 illustrates the rate of metabolism obtained from disappearance half-life estimations *in vitro* using human liver microsomes for several β-adrenoceptor antagonists. There is in general a good relationship with lipophilicity but compound 6 (betaxolol) appears to be more stable. This is emphasised in the insert plot showing a linear rate scale.

CYP2D6 is a high affinity, low capacity enzyme so under these conditions it is possible that the rate of metabolism is governed more by the Km than the V_{max}. Since the pKa is the same the ion pair interaction between these compounds and CYP2D6 is constant and hence can be ignored. Protein modelling of this enzyme has highlighted a number of residues which are able to make 'lipophilic' interactions (π-π interactions).

However, rate of metabolism is a function of chemical lability and the ability to enter and leave the active site of the enzyme. Clearly actual functionality is important here, rather than just the lipophilicity of the substituent. Comparison can be made between compound 4 (metoprolol) and compound 6 (betaxolol). Metoprolol (4) has an ethoxy-methyl para substituent that renders the compound liable to metabolism at both the benzylic and methyl position. In contrast betoxolol (6) has an ethyoxy-methyl cyclopropyl substituent that is sufficiently bulky to prevent efficient binding of the compound to the CYP2D6 active site [33]. These differences are reflected in the Cli(u) values of metoprolol and betaxolol of 35 and 10 ml/min/kg, respectively, despite betaxolol being 3–4 fold more lipophilic.

Whilst these differences are very important, the overall trend is that much of the chemistry around the basic aryloxy-propan-2-ol amino unit has the effect of changing potency and key pharmacokinetic properties in a compensating manner. This is also illustrated by the pharmacokinetic half-life, which controls frequency of administrations. Half-life is related to elimination rate constant by the equation

$$t_{1/2} = \frac{0.693}{\text{kel}} \tag{3}$$

where

$$\text{kel} = \frac{\text{Cl}}{\text{Vd}} \text{or} \frac{\text{Clu}}{\text{Vdu}}. \tag{4}$$

As shown above, increasing lipophilicity raises both Vdu and Cli(u) effectively cancelling out any changes in half-life. Moreover the daily dose size is governed by the unbound steady state concentration of unbound drug and potency. Cli(u) mostly determines the steady state concentration of orally administered drug. Both Cli(u) and potency increase with lipophilicity again effectively cancelling each other out. These trends are shown in detail for two of the compounds atenolol (1) and propranolol (9) in Table 1.

These inter-relationships illustrate why it is bad practice to optimise on only one property, such as *in*

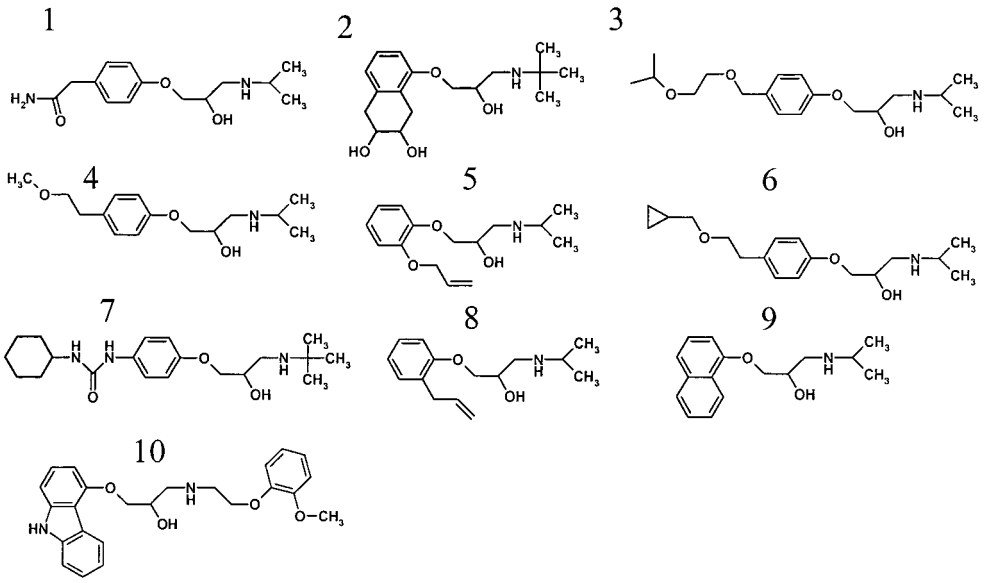

Figure 9. Structures of atenolol (1), nadolol (2), bisoprolol (3), metoprolol (4), oxprenolol (5), betaxolol (6), talinolol (7), alprenolol (8), propranolol (9), carvedilol (10).

Table 1. Comparison of physicochemical, pharmacological and pharmacokinetic properties of atenolol and propranolol.

	log $D_{7.4}$	Affinity (pA_2)	Absorption (%)	Renal Cli(u) (ml/min/kg)	Metabolic Cli(u) (ml/min/kg)	Vol(u) L/kg	Half-life (h)	Dose (mg)
Atenolol	−1.7	6.5	50	2	–	1	3–5	50–100
Propranolol	1.2	8.3	100	–	470	50	3–5	30–90

vitro potency. Rational design, such as moving from the cardio-selective, moderately potent (pA_2, 7.8) but metabolically labile metoprolol, to the metabolically stable betaxolol are highly productive. The advantages of metabolic stability are emphasised because the three extra methylene units of the replacement function give the expected increase in lipophilicity and resultant increased in potency ($pA_2 = 8.8$) and volume (Vd(u) = 5–6 L/kg). The reduction in clearance and increase in volume of distribution result in an extension of half-life from 3 to around 16 hours.

Whilst the systemic pharmacokinetics and even intrinsic potency can be understood, in terms of lipophilicity, the passage through the membranes of the gastro-intestinal tract are harder to rationalise. Absorption across the gastrointestinal tract normally involves

(i) the dissolution of the drug into the aqueous contents of the gastrointestinal tract, followed by

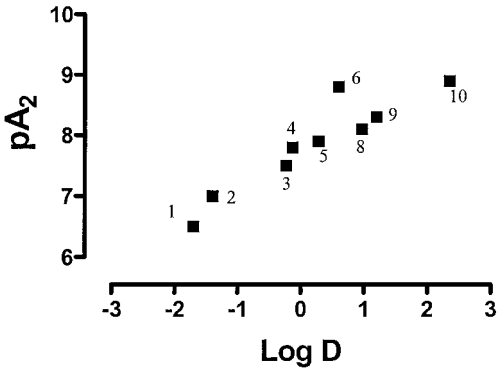

Figure 10. Correlation of lipophilicity (log $D_{7.4}$) with *in vitro* potency (pA_2). The numbers refer to compounds in Figure 9.

(ii) the diffusion of the molecule through the apical and basolateral membranes of the cells lining the gastrointestinal tract (transcellular absorption).

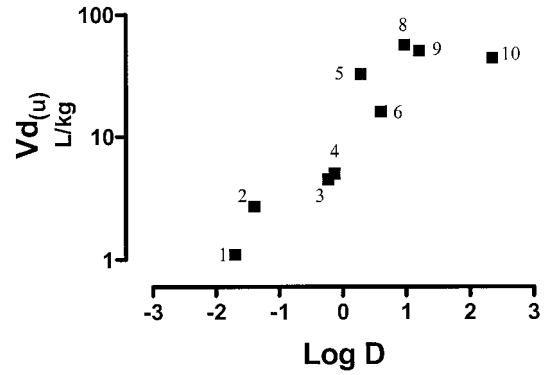

Figure 11. Correlation of lipophilicity (log $D_{7.4}$) with unbound (free) volume of distribution ($Vd_{(u)}$). For compound numbering see Figure 9.

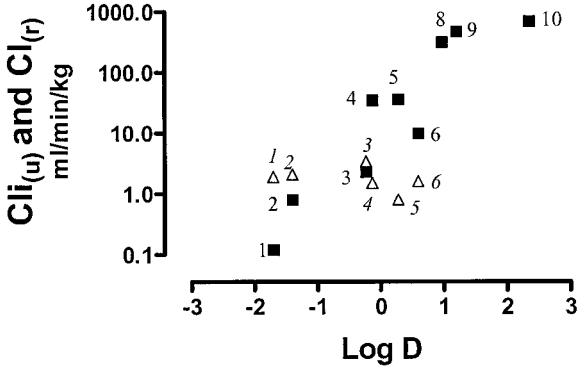

Figure 12. Correlation of lipophilicity (log $D_{7.4}$) with unbound (free) hepatic intrinsic clearance ($Cli_{(u)}$, filled squares) and unbound (free) renal clearance ($Cl_{(r)}$ open triangles). For compound numbering see Figure 9.

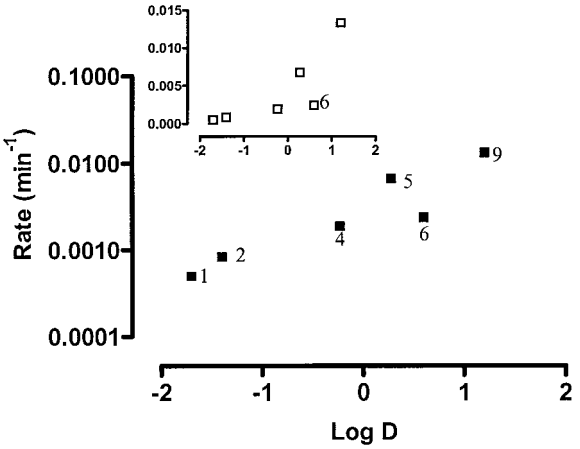

Figure 13. Plot of elimination rate constant for the *in vitro* metabolism of selected β-adrenoceptor antagonists against lipophilicity.

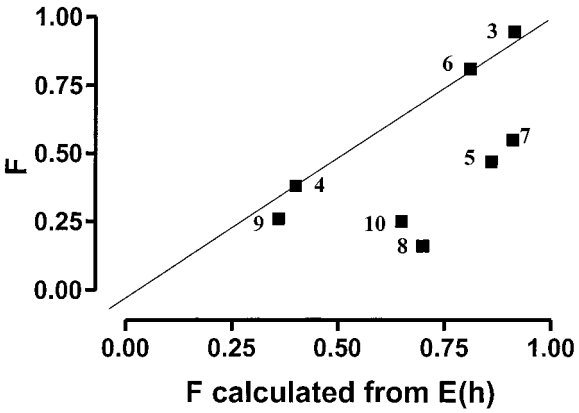

Figure 14. Comparison between expected bioavailability (F calculated from E(h)) and actual bioavailability.(F) Compounds 10, 8, 5 and 7 (see Figure 9) show evidence for gut wall first pass metabolism.

To traverse this route, the compound has to have sufficient hydrophilicity to dissolve in the aqueous phase and sufficient lipophilicity to 'dissolve' within the lipid core of the cell's membranes. The aqueous solubilities of the β-adrenoceptor antagonists are all high in their medicinal form either as free base or suitable salt. Atenolol (free base) has an aqueous solubility of 10 mg/ml and propranolol.HCl has a solubility of 50 mg/ml.

If a molecule has sufficient lipophilicity, the large surface area of the microvilli means that absorption is rapid. Tight junctions formed by the proteins that act as the junction between cells, offer an aqueous channel (pore) for paracellular absorption. These pores are estimated to be a size of 3 to 10 Å and represent only 0.01% of the total absorptive surface area. The β-adrenoceptor antagonists being small molecules can make efficient use of this route. Thus atenolol and nadolol are absorbed 56% and 34% in man, respectively. The eight more lipophilic compounds are able to cross the gastro-intestinal tract by the transcellular route but may encounter the effects of P-gp and CYP450 enzymes. P-gp works as an efflux pump and prevents entry of compounds into the blood stream. The effects of CYP450 enzymes present in the gut may be enhanced by a 'recirculation' effect of P-gp efflux and reabsorption. It is likely that compounds with relatively lower rates of diffusion across the gastro-intestinal tract will show the effects of P-gp and CYP450 metabolism the greatest. These effects are gut wall extraction in that absorption is not 100%, despite the compound having the physicochemical properties to cross membranes. Figure 14 shows the lipophilic

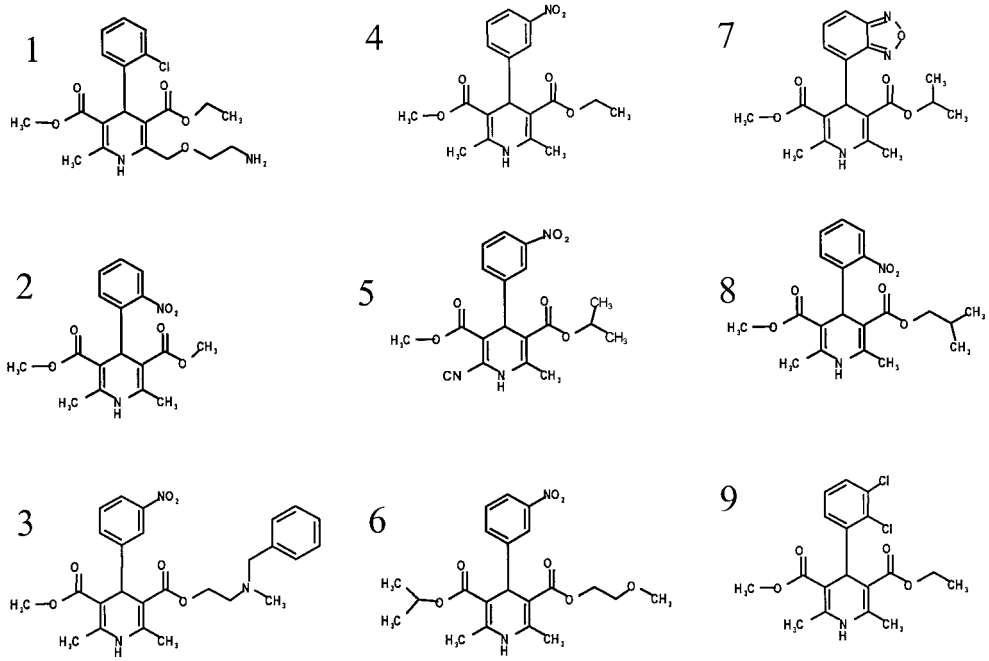

Figure 15. Structures of calcium channel antagonists: amlodipine (1), nifedipine (2), nicardipine (3), nitrendipine (4), nilvaldipine (5), nimodipine (6), isradipine (7), nisoldipine (8), felodipine (9).

compounds and contrasts the bioavailability expected from good absorption properties and first pass extraction by the liver (F calculated from E(h), and E(h) calculated from the unbound intrinsic clearance fu.Cli and liver blood flow Q as E(h) = fuCli/(Q + fu.Cli)) and the real measured bioavailability (F).

Compounds on the line of identity are 100% absorbed, as would be expected for the lipophilic compounds, whilst compounds below the line show gut extraction. Most noticeable are compounds such as talinolol (compound 7) which has low metabolic lability (low liver extraction) but considerably reduced bioavailability. Since the same CYP450 enzymes are largely present in the gut, this points to a large role for P-gp. Indeed talinolol has been shown to be an excellent P-gp substrate [35]. Clearly no simple relationships exist although it is noteworthy that para-substitution is tolerated when the chain is simple alkoxy derivatives (compounds 3, 9, 4), but not when it is a urea (compound 7). Gut extraction lowers bioavailability and hence increases dose size. It also may be a major factor in non-linear pharmacokinetics due to the high concentrations that occur locally during drug absorption and the likelihood of some degree of saturation occurring over the clinical dose range.

Calcium channel antagonists

The dihydropyridine calcium channel antagonists (Figure 15) also show strong trends between lipophilicity and unbound intrinsic clearance Cli(u) and unbound volume Vd(u) (Figures 16 and 17). The relationship for Cli(u) and lipophilicity can be rationalised by oxidation of the dihydropyridine ring, via an electron abstraction process, being a common primary step. This oxidation is conducted by CYP3A4 an enzyme whose interactions are primarily lipophilic.

The relationship with volume of distribution and lipophilicity reflects the interaction with the predominantly neutral molecules and the lipid portion of biological membranes. Thus there is a high dependence on lipophilicity. Also the volume/lipophilicity ratio is lower than the basic β-adrenoceptor antagonists, referred to above, due to the absence of the ion-pair interaction.

In general most of the dihydropyridines have a relatively short half-life (around 2–5 hours). This is due to the moderate volumes of distribution and high intrinsic clearance, and their close relationship with lipophilicity. Most compounds therefore require frequent inconvenient dosage regimens. Amlodipine (compound 1) incorporates a basic centre and the additional interactions with phospholipid (see above) gives

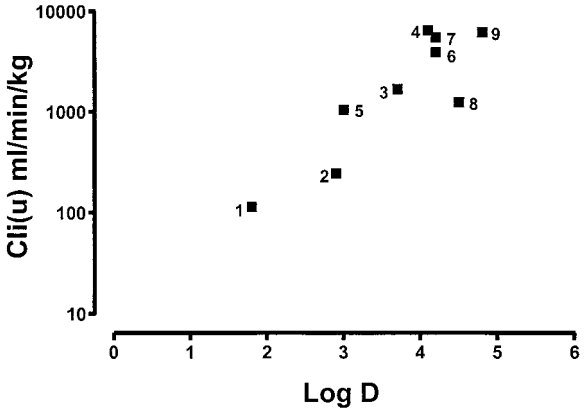

Figure 16. Correlation of lipophilicity (log $D_{7.4}$) with unbound (free) hepatic intrinsic clearance ($Cli_{(u)}$). For compound numbering see Figure 15.

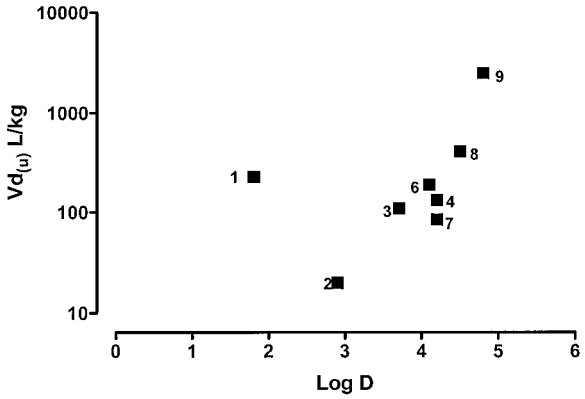

Figure 17. Correlation of lipophilicity (log $D_{7.4}$) with unbound (free) volume of distribution ($Vd_{(u)}$). Compounds 2–9 are neutral at physiological pH whereas amlodipine, compound 1, is basic and ionised. For compound numbering see Figure 15.

the compound a larger volume of distribution than expected for neutral dihydropyridines. The combination of this volume increase, plus its low unbound intrinsic clearance gives amlodipine a long half-life (around 40 hours).

All the calcium channel antagonists are lipophilic and absorbed by the transcellular pathway. The relatively high values for lipophilicity and the neutral form for most of the compounds renders the water solubility as low, with typical values of less than 0.001 mg/ml. This low solubility is offset somewhat by the potency and low oral dose requirements for the compound. Special formulations like soft-gelatin capsules have also been required, however, to offset the potential erratic absorption that the low solubity/erratic dissolution causes. The basic centre of amlodipine allows a highly soluble salt form (besylate) to be produced (2 mg/ml) that eliminates any need for special formulations.

Like the β-adrenoceptor antagonists, the calcium channel antagonists also show gut extraction to a variable extent. CYP3A4 is the enzyme responsible for hepatic metabolism [Cli(u)] and is the main oxidative enzyme present in the gut. Figure 18 compares Cli(u) (ease of CYP3A4 metabolism) with gut extraction E(g.i.) (= 1 - % absorbed). In general compounds of low Cli(u) show low E(g.i.) and compounds of high Cli(u) show high E(g.i.).

However, nitrendipine (4) and nimodipine (6) show low E(g.i.) with very high Cli(u). No obvious structural features distinguish these compounds and like the β-adrenoceptor antagonists the need for deeper understanding is obvious.

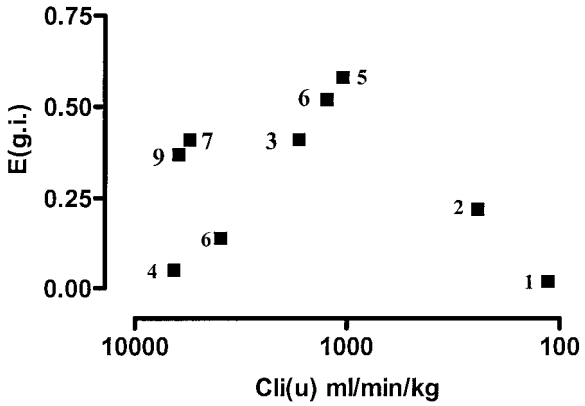

Figure 18. Comparison of ease of metabolism by CYP3A4 (Cli(u)) with hepatic extraction (E(g.i.). For compound numbering see Figure 15.

Renin inhibitors

Many companies have tried to develop peptidic renin inhibitors. These are rather large compounds with MW in the range 500–1000. A first concern in peptidic structures is to prevent proteolytic cleavage. A second hurdle is intrinsic oral absorption. It was observed that higher lipophilicity and lower MW give better absorption, while low lipophilicity and low MW result in less bile excretion [36–38]. In general the compounds had low solubility.

The introduction of solubilising functional groups in order to improve bioavailability was a major focus in many programmes. Overall the exercise appeared to be admirable in terms of objective but in retrospect had very limited prospects of success. The initial overall size of the compounds, as well as the total hydrogen bonding capacity, was far outside the rule-

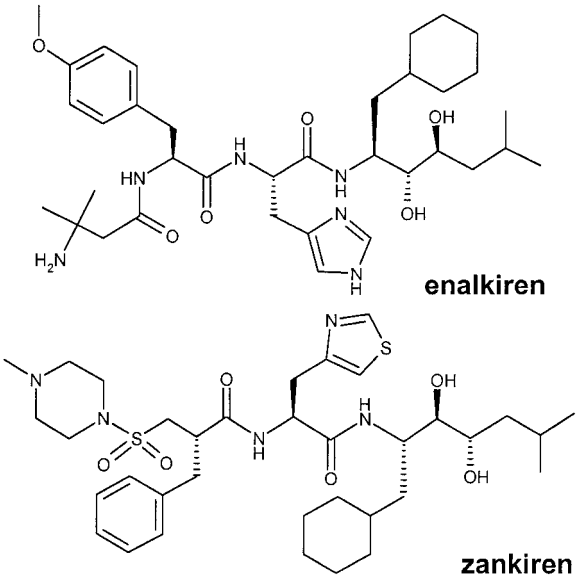

Figure 19. Examples of peptidic renin inhibitor enalkiren (MW 657) and zankiren (MW 706).

of-5 limits. Adding solubilising functionality moves the compounds even futher outside desireable physicochemical space. An example is enalkiren, which was designed to overcome poor solubility, thought to be one of the major reasons for poor bioavailability [38]. The solubility was enhanced by introduction of a basic center at the N-terminus. Nevertheless, the bioavailability was less than 2% in dogs and monkeys, and was confirmed in humans. Zankiren was claimed to have oral bioavailability, but was not developed beyond Phase II due to 'priority changes'.

Within the HIV area similar restrictions on molecules has not prevented development and marketing despite unfavourable properties. Here medical need has outweighed all the disadvantages of suboptimal doseage regimens. Drugs with these properties often lead medical breakthroughs but are rapidly superceeded by drugs with optimum properties and regimens.

Conclusions and outlook

Interrelationships among various physicochemical properties and their role in oral absorption, distribution to the brain and their influence on various pharmacokinetic properties are reasonably well understood. Potency can often be increased by making a compound more lipophilic. However, poorer dissolution/solubility as well as higher clearance may be the price to pay.

Based on this knowledge it seems inadvisable to devote much medicinal chemistry and drug development work on chemical classes not meeting the physicochemical criteria required for oral absorption and appropriate pharmacokinetics.

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