Design principles for orally bioavailable drugs

Manuel A. Navia and Pravin R. Chaturvedi

Compound design is the hallmark of modern pharmaceutical research and development. Unfortunately, comprehensive and reliable guidelines for the introduction of favorable bioavailability properties into designed compounds remain elusive. Here, the authors discuss the limited set of design principles that address the problem of bioavailability, based on a retrospective analysis of orally bioavailable drugs. The roles of partition coefficient, molecular weight, carrier-mediated transport and conformational flexibility are evaluated. These properties are discussed as guiding principles only, and cannot be considered all-encompassing determinants of oral bioavailability.

The oral bioavailability of a drug is dependent on solubility, permeability and transport characteristics across biological barriers, chemical stability in the gastrointestinal tract and various clearance mechanisms. Optimal performance with respect to all of these processes may involve contradictory physicochemical properties, the simultaneous incorporation of which in a given molecule may be difficult to achieve. The purpose of this review is to explore the concept of bioavailability and the role of different physicochemical properties contributing to the oral availability of drugs.

Estimation of oral bioavailability

Oral bioavailability (F) is defined as the fraction of the ingested dose of a drug that is available to the systemic circulation following oral administration. The absorptive and metabolic processes involved in bioavailability can be estimated as the product of the true 'fraction absorbed' (f) and the fraction escaping the 'first-pass' effect (f_a)[1]. The latter represents the product of the fractions escaping the gastrointestinal tract, liver and lung, which may be individually estimated as f_g, f_h and f_i, respectively. Hence, a general representation of oral bioavailability would be:

$$F = f \cdot f_a \quad (1)$$

or

$$F = f \cdot f_g \cdot f_h \cdot f_i \quad (2)$$

An alternative estimate of bioavailability may be obtained as the ratio of the systemic clearance (Cl_s) to the 'apparent oral clearance' (Cl_o), as follows:

$$F = Cl_s / Cl_o \quad (3)$$

Determination of oral bioavailability parameters

Several *in vitro* approaches are available for the estimation of the absorption potential – which can allow the estimation of 'f' – of a drug that crosses the intestine. These approaches include the everted gut sac model, intestinal brush border vesicles, and transport across Caco-2 cell line monolayers[2]. Other methods, including absorption into intestinal rings and single-pass intestinal perfusion *in situ*, can provide an estimate of the overall intestinal absorption[3,4]. For peptides and peptide analogs in particular, *in vitro* microsomal metabolism and *in situ* single-pass or recirculating intestine, liver and kidney models can be used to demarcate the absorption, metabolism and clearance potential of a compound. A discussion of various techniques used to estimate absorption and metabolism of drugs is beyond the scope of this review, but excellent articles addressing such models are available in the literature[5–7].

For most drugs, absorption is mediated at least in part by passive diffusion, a process whose physicochemical

Manuel A. Navia* and **Pravin R. Chaturvedi**, Vertex Pharmaceuticals Inc., 40 Allston Street, Cambridge, MA 02139-4211, USA. *tel: +1 617 576 3111, fax: +1 617 576 2109, e-mail: navia@vpharm.com

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determinants include a compound's lipophilicity, intrinsic aqueous solubility, surface charge and molecular weight (M_r)[7]. Drug absorption involves transcellular and paracellular transport mechanisms, as well as carrier-mediated processes, as exemplified by absorption across the gastrointestinal tract of small peptide and peptidomimetic compounds *via* the recently described proton-coupled oligopeptide transporter[8].

Similarly, the clearance of drugs is also determined by specific physicochemical requisites. In addition, organ-dependent physiological determinants including organ blood flow, intrinsic metabolism and excretion *via* active transport mechanisms, such as the bile acid transporter in the liver, also play a role in drug clearance[9,10]. It should be noted that clearance is a disposition parameter that is not amenable to modification unless the structure of the molecule itself is changed. In other words, even though drug delivery strategies may be useful in altering the absorption potential of a drug, to alter a drug's clearance behavior, a new analog must be designed.

Complex and ambitious models have been proposed[11–14] to predict oral bioavailability from the *in vitro*, *in situ* and *in vivo* experimental data that are available. Predictive 'rules' derived from such models would be immensely useful, for example, in guiding the structure-based drug design of novel, *in vivo* efficacious drugs[15–17]. Unfortunately, the available data have failed to support predicted bioavailabilities in general across molecular classes. Within narrowly defined classes, however, one can always analyze structure–activity relationships determining the oral bioavailability retrospectively.

Role of partition coefficient (log *P*)

One physicochemical parameter that has been long accepted in the estimation and/or prediction of absorptive potential of drugs has been the logarithm of the octanol: water (or other lipid:water) partition coefficient (log *P*)[7,18]. No general rules have been inferred that are applicable across the vastly diverse drug molecule scaffolds, although each congeneric series for a drug backbone usually demonstrates its own optimal log *P*. From Table 1, it can be argued that log *P* values between 0 and 3 constitute an optimal window for drug absorption (Figure 1). Values that are too high (>6) or too low (<–3) can been associated with poor transport characteristics[19]. The low absorption observed for compounds with a high log *P* value can be attributed to the poor aqueous solubility of these compounds and their preferential residence in lipophilic regions. In turn, the suboptimal lipophilicity of the very polar compounds precludes their ability to penetrate membrane barriers.

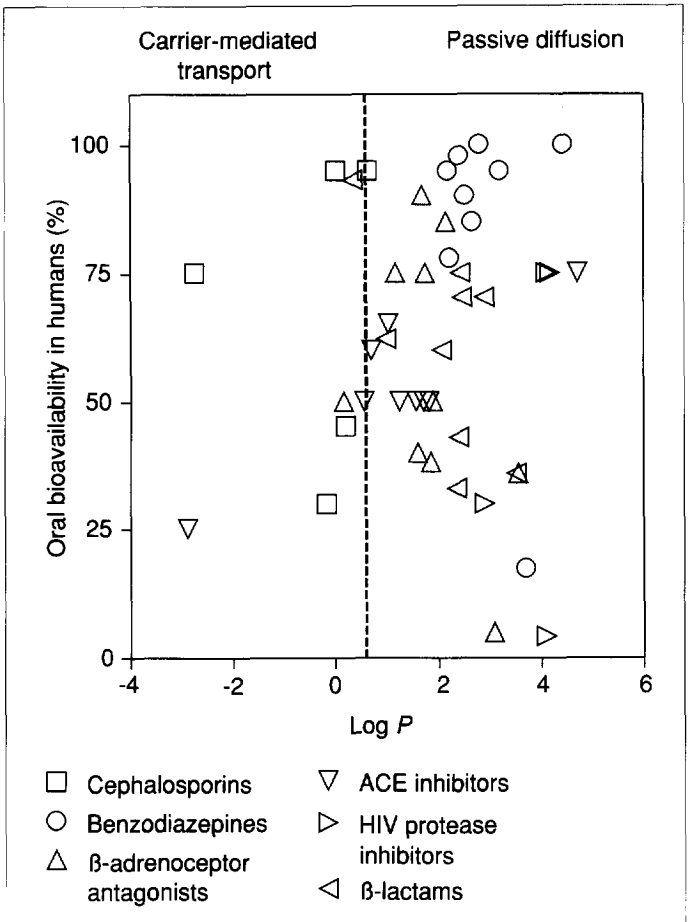


Figure 1. *Oral bioavailability of compounds listed in Table 1* vs. *the corresponding distribution of log* P *values. The dashed line (at log* P ~ *0.5) approximately defines those regions of the distribution where carrier-mediated* vs. *passive transport processes predominate.*

The parabolic relationship of log *P* to tissue transport is usually a retrospective exercise[20], and very few examples are available in the literature where log *P* estimates *a priori* have yielded predictable drug transport properties across the gastrointestinal tract. As a general rule, within a congeneric series, drug absorption usually increases rapidly as lipophilicity rises, and is maintained at a plateau for a few units of log *P*, after which there may be a steady decrease. Although a higher log *P* value tends to favor absorption across the gastrointestinal tract wall, it also tends to render compounds more susceptible to metabolism and/or biliary clearance. As such, in the design of new analogs in a congeneric series, addressing the problems of clearance should take priority over optimizing lipophilicity, given that clearance is a disposition parameter that is not amenable to modification.

For peptidomimetic drug absorption, the relationship to log *P* is tenuous at best. One hypothesis for the lack of passive absorption of peptidyl compounds is that the energy required to break water–peptide hydrogen bonds (in order for the molecule to enter the cell membrane) is not offset by the increasing lipophilicity of the molecule[21]. However, one should note that peptidyl compounds have a high clearance potential, which may also lead to poor bioavailability. As stated earlier, one must be careful to dissect absorption *vs.* clearance parameters *in situ*, prior to any attempts to improve the oral bioavailability of peptidomimetic drugs by lipophilicity optimization alone (e.g. Ref. 22).

Role of molecular weight

One common factor that has long been thought to favor compound performance with respect to oral bioavailability is relatively low M_r[7,22]. This broadly-held belief can be objectively quantified through an analysis of the M_r distribution of all marketed drugs[16] (Figure 2a). The M_r distribution is seen to be roughly Gaussian in character in the range of 150 to 550 (x_o = 341; σ = 100), reflecting our ability, *de facto*, to meet the conflicting physicochemical requisites for drug bioavailability. This empirical observation can be categorized further through an examination of the M_r distribution of marketed β-adrenoceptor antagonists and benzodiazepines (Figure 2c), the ACE inhibitors and all of the antihypertensives as a class (Figure 2d; Table 1a–d).

The increased absorption of compounds of low M_r can be explained, in part, on the basis of passive diffusion principles[7]. A suitable log *P* and a low M_r (<550; Figure 2a), appear to be the two physicochemical parameters that best determine oral absorption potential by passive processes. In addition, a low M_r also mitigates the risk of high clearance because such molecules are less likely to be substrates for hepatic clearance mediated *via* metabolism and/or biliary excretion. Based on Equation 3, such a reduction in clearance potential will also favor an increase in oral bioavailability.

In general, the pathways for oral drug absorption include passage through aqueous pores, lipid membranes, bulk flow or electrochemical potential gradients. Small hydrophilic molecules, in particular, are purported to be absorbed rapidly through aqueous pores. Indeed, studies with rat jejunum have indicated a pore diameter of 8 Å, which precludes hydrophilic molecules of M_r greater than 180 from being absorbed *via* this mechanism[23]. The design of peptide-like small molecules with, for instance, four amino acids yields, by default, M_r values of 500 to 700, an approximate molecular surface area of about 350 Å and a diameter of 10–12 Å. For compounds with a molecular diameter of this size or greater, transport through aqueous pores and/or paracellular transport through the tight junctions of the epithelial lining of the gastrointestinal tract is usually precluded[22].

Drugs of higher molecular weight

Figure 2a also exhibits a tail of marketed drugs extending to higher M_r values beyond the Gaussian distribution. Surprisingly, these compounds have overcome some of the problems associated with larger size that might have resulted in reduced diffusivity and solubility. The acceptable bioavailability of these compounds cannot be explained by a simple extrapolation of the clearance and/or absorption parameterized from the behavior of drugs of lower M_r. An understanding of the basis for this exceptional behavior (Figure 2b) would be of considerable interest because, for certain therapeutically important targets, including the enzymes renin (*vs.* hypertension) and HIV protease (*vs.* AIDS), agents with higher M_r values seem to be needed for the *in vitro* potency levels that are a prerequisite for *in vivo* efficacy.

Figure 2e shows that the M_r distribution of the aspartyl protease inhibitors is shifted away from the Gaussian population that encompasses the bulk of drugs currently marketed. Significantly, as shown in Table 2, these compounds with high M_r have not succeeded in reaching the market as readily as others with lower M_r (e.g. ACE inhibitors). The anomalous bioavailability of the marketed drugs in the high M_r tail of the distribution shown in Figure 2a may result in part from conformational flexibility, as we suggest below for cyclosporin A (CsA), an orally bioavailable cyclic undecapeptide with a M_r of 1,200 (Table 1g). However, such behavior might also be attributable to the formation of micellar or colloidal solutions in the gastrointestinal tract, or to the availability of carrier-mediated transport systems (see below).

Carrier-mediated transport in intestinal absorption and clearance

Carrier-mediated transport mechanisms can be found at the various biological barriers between the site of absorption of a drug and its appearance in the systemic circulation. Carrier-mediated transport can also drastically change the hepatic or renal clearance of a compound, as well as its absorption through the gastrointestinal tract. The marketed ACE inhibitors, for example, have been designed as prodrugs

Table 1. Summary of pharmacokinetic parameters for

(a) ACE inhibitors

Compound	Log *P*	Aqueous solubility[b]	pK_a	M_r	C_{max} (μg/mL)	Urinary excretion (%)	Protein binding (%)	Half-life (h)	Oral availability (%)	Dose (μg/day)
Benazepril HCl	1.74	–[c]	–	461.0	–	<1	96	1.0–3.0	50	10–40
Captopril	1.02	1–10	3.7; 9.8	217.3	–	50	30	2.0–3.0	65	25–150
Cilazapril	0.55	1–10	–	417.5	–	50	30	2.0	50	2.5 – 5
Enalapril maleate	0.71	30–100	3.04	492.5	–	60	55	11.0	60	5–20
Fosinopril sodium	–	–	–	585.7	–	45	95	4.0	36	10–40
Lisinopril	–2.86	10	1.70	441.5	–	>90	10	12.6	25	5–40
Perindopril	1.26	–	–	368.5	–	50	20	8.0	50	4–16
Quinapril HCl	1.84	–	–	475.0	–	50	35	2.5	50	10–80
Ramipril	1.59	–	–	416.5	–	50	65	4.5	50	5–20
Zofenopril calcium	4.72	–	–	897.2	–	<10	88	5.5	>70	30–60

(b) Benzodiazepines

Compound	Log *P*	Aqueous solubility[b]	pK_a	M_r	C_{max} (μg/mL)	Urinary excretion (%)	Protein binding (%)	Half-life (h)	Oral availability (%)	Dose (mg/day)
Alprazolam	3.20	>10,000	2.40	308.8	–	20	70	12–15	>90	1–4
Clonazepam	2.41	–	1.50; 10.5	315.7	–	<1	86	20.0–40.0	98	20
Clobazam	2.65	–	–	300.7	–	<1	90	24	>80	20 –30
Clozapine	–	–	–	326.8	–	<1	95	12	50	200–450
Diazepam	2.80	1,000–10,000	3.30	284.7	–	<1	98	24 –48	100	6–30
Flurazepam	4.45	<1	1.90; 8.20	424.4	–	<1	97	74	100	15–30
Lorazepam	2.51	>10,000	1.30; 11.5	321.2	–	<1	85	10 –20	90	1–10
Midazolam	3.70	–	6.20	362.2	–	<1	96	2–7	15–20	7.5–15
Nitrazepam	2.25	>10,000	3.20; 10.8	281.3	–	<1	87	24	78	5–20
Oxazepam	2.24	>10,000	1.70; 11.6	286.7	–	<1	98	6–20	>90	60–120
Temazepam	2.19	>10,000	1.60	300.7	–	<1	96	8–15	>70	10–30

(c) β-lactam antibiotics

Compound	Log *P*	Aqueous solubility[b]	pK_a	M_r	C_{max} (μg/mL)	Urinary excretion (%)	Protein binding (%)	Half-life (h)	Oral availability (%)	Dose (mg/day)
Amoxycillin	0.33	<1	2.4; 7.4; 9.6	365.4	5.0	60	20	1.0–1.5	93	0.75–1.5
Ampicillin	1.00	1–10	2.5; 7.3	349.4	2.0–6.0	60–80	20	1.0–1.5	62	1.0–4.0
Cloxacillin	2.43	100–1,000	2.70	435.9	7.0–14.0	75	95	0.5–1.0	43	2.0
Dicloxacillin sodium	2.91	1–10	2.70	510.3	10.0–18.0	60	97	0.5–1.0	50–85	0.5–1.0
Flucloxacilin	2.48	1	2.70	453.9	5.0–15.0	50–90	95	1.0	50–85	1.0
Nafcillin sodium	3.53	1–10	2.70	454.5	5.0–8.0	27	90	0.5–1.0	36	1.0–4.0
Oxacillin sodium	2.38	1–10	2.80	441.4	3.0–6.0	40–50	93	0.5–1.0	33	2.0–6.0
Phenoxy methyl penicillin	2.09	1,000–10,000	2.70	350.4	3.0–5.0	50–90	80	0.5–1.0	60	1.0–2.0
Piv-ampicillin	2.44	–	7.0	463.6	–	70	–	2.0–3.0	>70	1.0–2.0
Piv-mecillinam	–	–	8.9	439.6	5.0	45	–	2.0	>70	0.4–2.4

several orally bioavailable drug and drug candidate classes[a]

(d) β-adrenoceptor antagonists

Compound	Log *P*	Aqueous solubility[b]	pK_a	M_r	C_{max} (μg/mL)	Urinary excretion (%)	Protein binding (%)	Half-life (h)	Oral availability (%)	Dose (mg/day)
Acebutolol HCl	1.61	–	9.20	372.9	1.3–1.8	40	26	3.0–4.0	40	0.4
Alprenolol HCl	3.10	–	9.65	285.8	–	<1	85	3.0	<10	0.2–0.8
Atenolol	0.16	30–100	9.60	266.3	2.0	85	<5	6.0–7.0	50	0.05–0.1
Betaxolol HCl	2.17	1–10		343.9	0.04	15	50	16.0–20.0	80–90	0.02–0.04
Bisoprolol fumarate	1.69	–		767.0	0.05	50	30	13	90	0.005–0.02
Carteolol HCl	1.17	–		328.8	–	60	25	5.0–6.0	>70	0.003–0.01
Labetolol HCl	–	60	7.40	364.9	–	<5	50	5.5	20	0.4–0.8
Metoprolol tartrate	1.88	<1	9.70	684.8	0.02–0.34	10	13	3.0–7.0	38	0.1–0.4
Pindolol	1.75	>10,000	8.80; 9.70	248.3	–	54	50	3.0–4.0	75	0.01–0.02
Propranolol HCl	3.56	20	9.45	295.8	–	<1	93	3.0–6.0	36	0.16–0.32
Sotalol HCl	–	–	8.30; 9.80	308.8	–	75	<10	10.0–15.0	>80	0.16–0.6
Timolol maleate	1.91	15	9.21	432.5	–	15	10	4.0	50	0.01–0.04

(e) Cephalosporins

Compound	Log *P*	Aqueous solubility[b]	pK_a	M_r	C_{max} (μg/mL)	Urinary excretion (%)	Protein binding (%)	Half-life (h)	Oral availability (%)	Dose (mg/day)
Cefaclor	−2.71	–		385.8	6–13	85	25	0.5–1.0	>70	0.75–1.5
Cefadroxil	−2.73	100–1,000		381.4	16–30	90	20	1.5	>70	1.0–2.0
Cefixime	0.19	<1		507.5	2–5	20	65	3–4	40–50	0.2–0.4
Cefuroxime	−0.16	<1	2.50	424.4	–	96	50	1.1	Erratic	0.5–1.0
Cephalexin	0.65	100	2.5; 5.2; 7.3	365.4	18	>80	15	1.0	>90	1.0–2.0
Cephradine	0.03	100–1,000	2.50; 7.30	349.4	9–24	>90	15	1.0	>90	1.0–2.0

(f) HIV protease inhibitors

Compound	Log *P*	Aqueous solubility[b]	pK_a	M_r	C_{max} (μg/mL)	Urinary excretion (%)	Protein binding (%)	Half-life (h)	Oral availability (%)	Dose (mg/day)
Saquinavir	4.10	–	–	671.0	0.23	<10	90	13.2	4	1.8–7.2
Indinavir	2.92	1–10	5.90; 3.70	614.0	3.07	10–30?	56	1.0–2.0	20–40	2.4
Ritonavir	–	>10,000	–	771.0	10.0	<10	99	3.0–4.0	65	1.2
Nelfinavir	4.10	100–1,000	6.0; 11.06	586.0	3.22	–	90	3.0–4.0	40–70	1.5
VX–478	4.20	1,000–10,000	1.97	506.0	9.11	<1	93	8.0–9.5	75	2.4d

(g) Immunosuppressants

Compound	Log *P*	Aqueous solubility[b]	pK_a	M_r	C_{max} (μg/mL)	Urinary excretion (%)	Protein binding (%)	Half-life (h)	Oral availability (%)	Dose (mg/day)
Cyclosporin A	3.00	>10,000	–	1203	0.001–0.003	6	90	10–27	30	1.5–2.0
Tacrolimus (FK506)	5.58	>10,000	–	804	0.002	<1	77	3.5–40	25	0.01–0.04

[a]Data taken from Martindale[53], Goodman and Gilman[54], Venkataramanan *et al.*[55] and references cited therein. Supplementary data taken from the CMC-3D (release 94.1) and MDDR-3D (release 95.2) electronic databases (MDL Information Systems Inc., San Leandro, CA, USA)
[b]Aqueous solubility is defined as the parts of water required to dissolve one part of the commercially available drug substance
[c]Dashed entries indicate that no data were available
[d]Possible clinical dose based upon a pharmacokinetic model

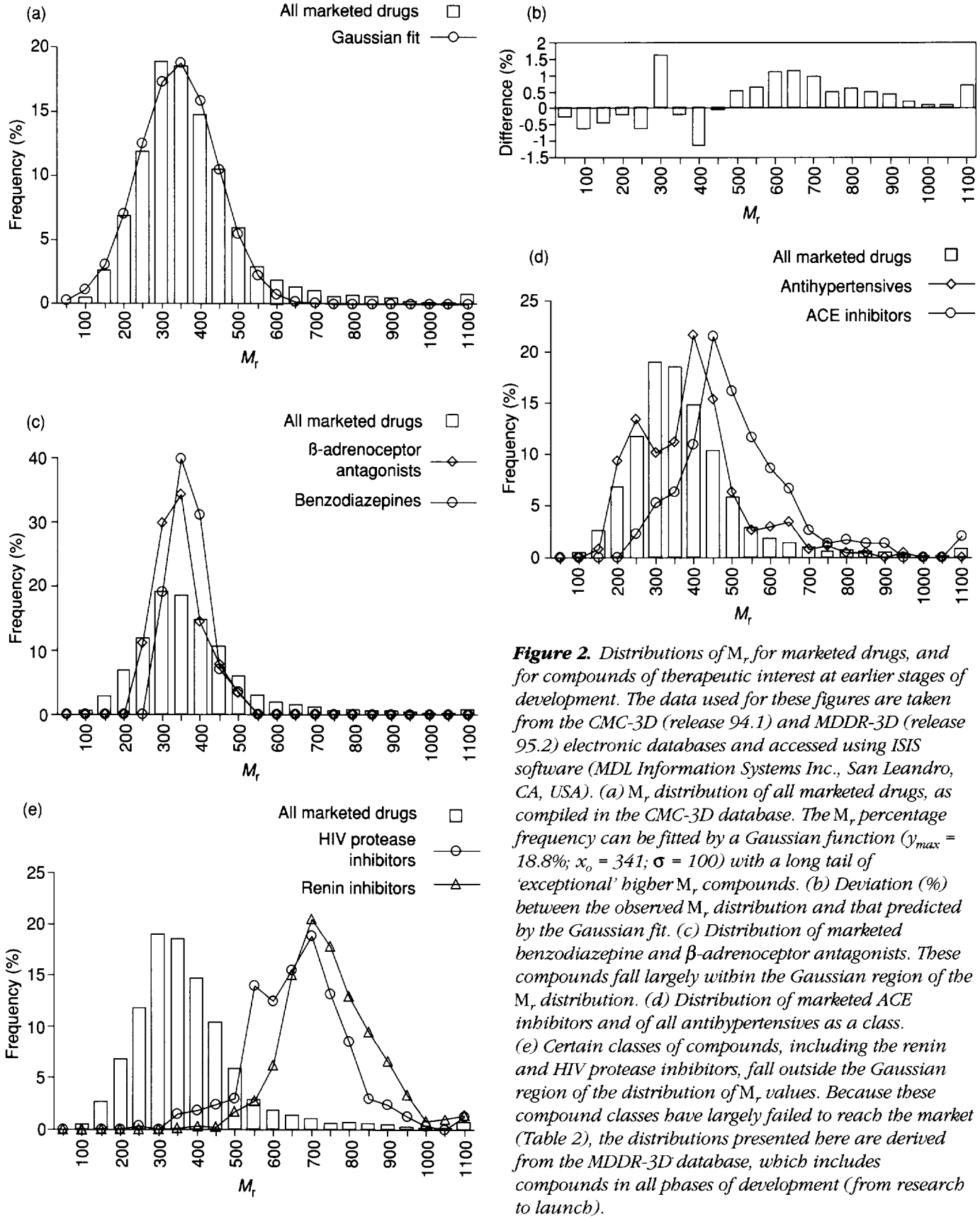


Figure 2. *Distributions of M_r for marketed drugs, and for compounds of therapeutic interest at earlier stages of development. The data used for these figures are taken from the CMC-3D (release 94.1) and MDDR-3D (release 95.2) electronic databases and accessed using ISIS software (MDL Information Systems Inc., San Leandro, CA, USA). (a) M_r distribution of all marketed drugs, as compiled in the CMC-3D database. The M_r percentage frequency can be fitted by a Gaussian function (y_{max} = 18.8%; x_o = 341; σ = 100) with a long tail of 'exceptional' higher M_r compounds. (b) Deviation (%) between the observed M_r distribution and that predicted by the Gaussian fit. (c) Distribution of marketed benzodiazepine and β-adrenoceptor antagonists. These compounds fall largely within the Gaussian region of the M_r distribution. (d) Distribution of marketed ACE inhibitors and of all antihypertensives as a class. (e) Certain classes of compounds, including the renin and HIV protease inhibitors, fall outside the Gaussian region of the distribution of M_r values. Because these compound classes have largely failed to reach the market (Table 2), the distributions presented here are derived from the MDDR-3D database, which includes compounds in all phases of development (from research to launch).*

Table 2. Comparative developmental status of lead compounds in three therapeutic classes

Inhibitors	Total	Launched	Clinical trials	Preclinical	Research
ACE	314	11 (3.62%)	12 (3.95%)	15 (4.93%)	276 (87.50%)
HIV protease	351	3 (0.91%)	6 (1.81%)[a]	19 (5.74%)	323 (91.54%)
Renin	976	0	11 (1.13%)	35 (3.59%)	930 (95.28%)

[a]Data taken from analysis of the MMDR-3D electronic database (MDL Information Systems Inc., San Leandro, CA, USA), which lists 57,000 compounds of therapeutic relevance reported in a wide range of information sources, including publications, meeting abstracts, patents and company communications. This complex and comprehensive compilation cannot be completely up to date at any one time; its value lies in highlighting strong trends rather than quantification of the current status in the therapeutic areas

of their active moieties (with some exceptions) to mimic dipeptides and tripeptides, in order to exploit their active transport mechanisms across the gastrointestinal tract[24,25]. Other examples of actively transported compounds include the oral cephalosporins and other β-lactam antibiotics[5,26,27].

Most compounds with good oral bioavailability possess a log *P* value of 0–3 (see Figure 1), but some compounds with negative log *P* values are also highly bioavailable; the hydrophilic character of such compounds suggests the involvement of carrier-mediated transport for these molecules. Other factors may need to be taken into account to explain the evident bioavailability of drugs at the negative extremes of log *P* in Figure 1, which include the ACE inhibitor lisinopril, for example (log $P = -2.86$). The structure (Figure 3a) and conformational flexibility of lisinopril (as compared with enalaprilat; Figure 3b) may also play a role in its oral bioavailability (see below).

In general, the role of carrier-mediated transport in clearance phenomena is not well understood. Ziegler and coworkers[28] have shown that the liver is not involved in the clearance of larger peptides (>12 amino acids), although it efficiently removes peptides of eight or fewer amino acids. It appears that a multispecific transporter plays a role in the removal of hydrophobic peptides of lower M_r (including the aspartyl protease inhibitors), bile acids and cholephile dyes[29,30]. Oral absorption considerations lead generally to the design of smaller peptidomimetic drugs, which may thus be excellent substrates for this transporter system[29]. Hence, in the design of such peptide analogs, one should evaluate the hepatic clearance of peptidomimetic drugs before conducting an optimization of the other physicochemical properties of a given compound.

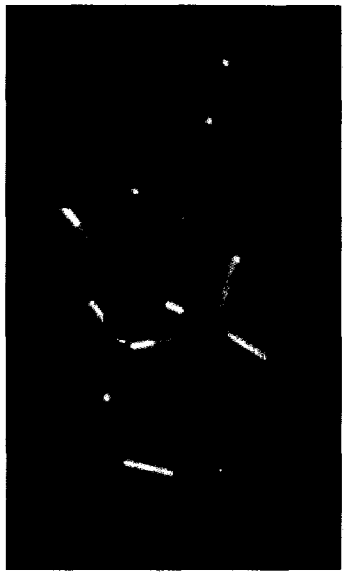
It is also generally recognized that the presence of an ionic charge is important for carrier-mediated transport[5,6]. Compounds that are neutral at physiological pH, or which exist in a zwitterionic state at pH 7.4, tend to be transported primarily by passive processes. This is evident from the literature relating to renal clearance of ACE inhibitors. Enalaprilat (Figure 3b), for example, which has a net negative charge at physiological pH, is excreted by the kidney both through filtration (passive), and through secretion by the organic anion transport pump (active)[31]. Lisinopril, on the other hand (Figure 3a), which exists as a zwitterion at physiological pH, appears to be passively excreted by the kidneys[31].

The net charge of a compound also plays a role in its protein binding. Because the free fraction of a drug is generally excreted by passive processes for drugs with restrictive clearance[32], protein binding may also alter the observed renal as well as hepatic clearance of drugs. It is of interest to note that compounds of low M_r, with negligible protein binding, are cleared rapidly by the kidneys by glomerular filtration.

Although carrier-mediated processes can influence the overall bioavailability of a given compound (favorably for absorption, or adversely for clearance), a systematic exploitation of the phenomenon is not always straightforward, or even possible. In particular, these mechanisms do not explain the anomalous oral bioavailability of many of the larger drugs that populate the tail of the M_r distributions shown in Figure 2a, including the marketed macrolide immunosuppressants CsA and tacrolimus (FK506) with M_r values of 1,200 and 800 respectively (Table 1g).

Conformational structure and molecular flexibility in drug transport

Through serendipity, solution NMR structural data are available on the conformation in both nonpolar and aqueous/polar solvents for the immunosuppressants CsA (Figure 4), FK506 (Figure 5) and their analogs, which have anomalously



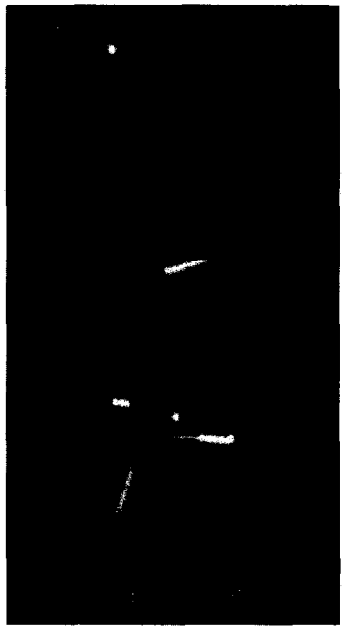
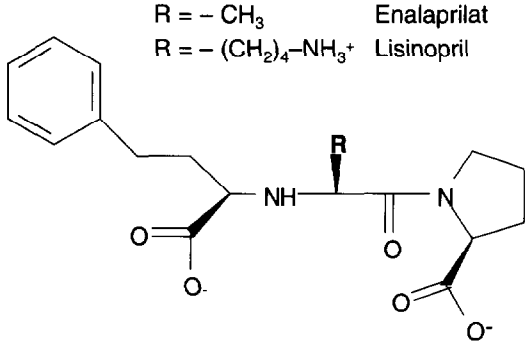


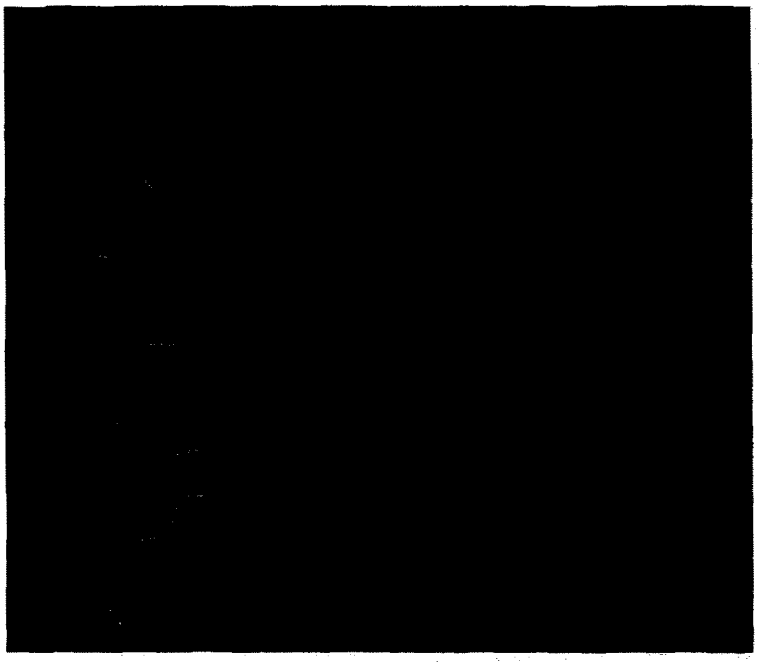
Figure 3. *ACE inhibitors lisinopril and enalaprilat. The model for the conformation of lisinopril is based on the geometrically-optimized conformation reported by Swaan* et al.[56]; *neither X-ray nor NMR structure determinations of lisinopril have been reported. The X-ray structure of enalaprilat is as reported by the Merck group*[57]. *The chemical structures of lisinopril and enalaprilat show a common framework; enalapril is a prodrug ester of enalaprilat*[31], *which is better absorbed. The conformation of lisinopril was minimized* in vacuo *using the program CHARMm, as implemented in Quanta (version 4.1) (Molecular Simulations, Inc., Burlington, MA, USA). As discussed in the text, lisinopril represents the anomalously negative (log* P = *–2.86) ACE inhibitor data point in Figure 1. The intramolecular interactions between charged groups in the* in vacuo *minimized structure of the compound are consistent with expectations for the conformation of the molecule in a nonpolar solvent, and suggest how the compound might unfold its conformation in an aqueous environment.*



high bioavailability (Table 1g, Figure 2a). Because of the poor aqueous solubility of these compounds, nonpolar conformations (in deuterated chloroform) were the first to be described[33–35]. Subsequently, CsA and FK506 were shown to adopt alternate conformations when bound to their cognate immunophilins cyclophilin and FKBP12, respectively[36–40], leading to the suggestion that protein binding induced the observed change[41,42]. In response to these observations, extraordinary efforts were mounted to synthesize and solve the solution structures of water-soluble analogs of CsA[43] and FK506[44]. These efforts ultimately demonstrated that the bound conformation of both immunosuppressants pre-existed in aqueous/polar solvents, clarifying our understanding of the mode of action of these compounds.

This experimentally determined ability to undergo a change in conformation in response to the polarity of the immediate environment, as shown in Figures 4 and 5, can be invoked – in a different context – to rationalize the anomalous oral bioavailability (relative to their M_r) of both CsA and FK506 (Table 1g). Wiley and Rich[45] have proposed the term 'hydrophobic collapse' to describe the presumed conformational transition of the thrombin inhibitor argatroban[46] that takes place on its binding to that enzyme[47]. Analogously, the term 'hydrophilic collapse' is suggested here to describe the conformational transition seen for CsA (Figure 4) and FK506 (Figure 5) in a nonpolar environment (e.g. in crossing a membrane barrier). This usage seems appropriate, given that the cyclophilin-bound conformation of CsA is believed to pre-exist in aqueous solution. In a hydrophilic collapse, the induced conformational transition would lead to an internalization of interactions (hydrogen bonds in this instance, as seen in Figure 4) that would be otherwise satisfied by solvent molecules in a polar environment.

In this context, if the conformation (and physicochemical properties) of a compound can change in response to the polarity of its environment, then behavior of the compound with respect to the conflicting requisites for absorption and transport *vs.* those involved in the mitigation of clearance can become quite complex, possibly favoring (or disfavoring, depending on circumstances presently beyond our knowledge) the overall bioavailability characteristics. As a consequence, many of those properties that are routinely parameterized for their presumed predictive potential



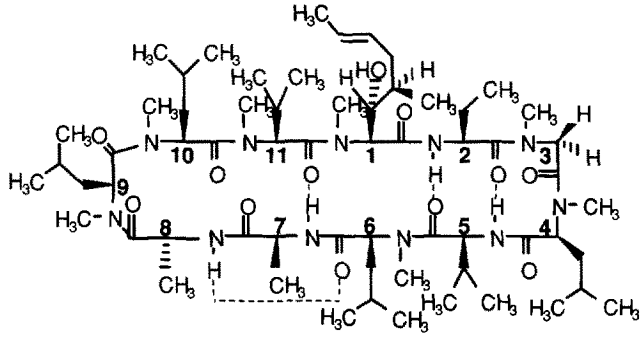


Figure 4. *Structures of the immunosuppressant drug, cyclosporin A (CsA), as determined experimentally in nonpolar and polar solvents. The chemical structure of CsA shows the intramolecular hydrogen bonds observed in the nonpolar conformation. The poor aqueous solubility of CsA led to an initial determination of its solution structure by NMR methods in chloroform, a nonpolar solvent. Subsequently, the NMR structure of a water-soluble CsA analog established the existence of an alternate conformation for the drug in that solvent – a conformation that was also found in the ligand complex with its cognate immunophilin protein, cyclophilin (Ref. 49 and references therein). Taken together, these experimental results suggest that CsA can undergo a conformational rearrangement in response to its solvent environment – a rearrangement that alters, in effect, the physicochemical character of the molecule. The red and blue asterisks in the figure show, for example, the individual carbonyl and amide moieties that are solvent-exposed in the polar conformation but are internally hydrogen-bonded in the nonpolar structure. A similar change in conformation in response to solvent is also observed for the macrolide immunosuppressant FK506 (Table 1) (Refs 44 and 49, and references therein).*

would be, at best, meaningless for molecules such as CsA and FK506, and, at worst, confounding. The same may be true for compounds with lower M_r, such as lisinopril, its presumed ability to undergo a hydrophilic collapse (Figure 3a), as compared with enalaprilat (Figure 3b), may rationalize its overall favorable bioavailability characteristics, in spite of its extreme log P value (Table 1a). In that regard, it should be noted that the interpretation of experimental log P measurements, as well as the calculation of theoretical log P, all depend tacitly on an assumption of constancy in structure, conformation and physicochemical character from solvent to solvent – an assumption that is evidently invalid for CsA (Figure 4), lisinopril (Figure 3a) and FK506 (Figure 5).

Unfortunately, it is impossible to estimate with any confidence how large a fraction of chemical leads can undergo conformational transitions such as those observed for CsA and FK506 – even within the limited number of 'exceptional' high M_r compounds in the distribution of marketed drugs in Figure 2a. Approaches using molecular dynamics, for example, were unable to sample the experimentally observed conformational changes in CsA and FK506 in any computational simulation of reasonable length[48]. In turn, the determination of the three-dimensional structures of CsA and FK506 in their free state[33,34,43,44,49], when bound to their cognate immunophilin receptors[36–40,49], and in complex with their ultimate intracellular target, calcineurin[50], may have been uniquely driven by the relative attractiveness of a structure-based drug design approach when compared with the synthetic inaccessibility of those molecules[51]. For other molecules and molecular classes, comparable incentives leading to the application of advanced (and expensive) X-ray and NMR structural methods do not appear to be in place.

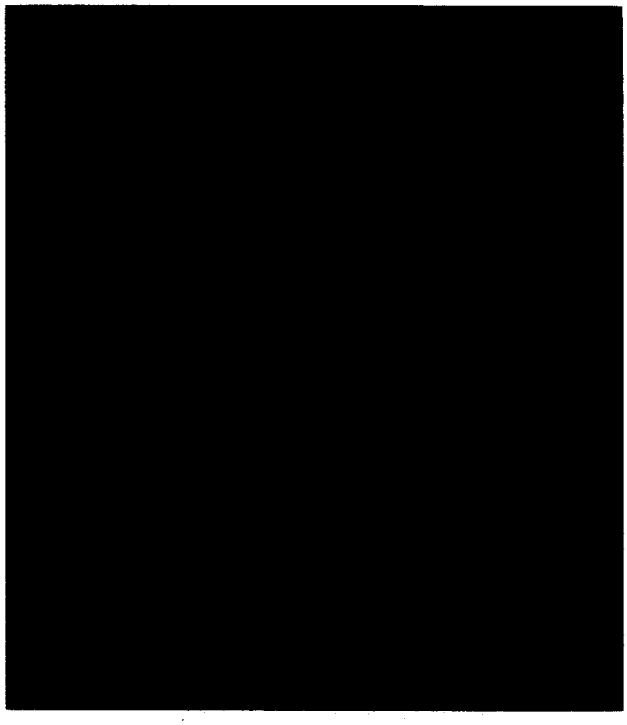


Figure 5. *Structure of the immunosuppressant tacrolimus (FK506) determined by solution NMR methods in polar (green) and nonpolar (violet) solvents. As with cyclosporin A (Figure 4), the structure of FK506 is seen to undergo a conformational change in response to its solvent environment (Refs 44 and 49, and references therein).*

Solvent-dependent conformational transitions might also be used to rationalize the improved intestinal uptake and oral bioavailability characteristics of a drug such as CsA when formulated in olive oil (as it is marketed). A hydrophobic medium will, of course, facilitate the solubilization of hydrophobic CsA, but it might also promote the overall oral bioavailability of the molecule by 'priming' the ensemble of ingested CsA molecules in the direction of the nonpolar conformation seen in Figure 4. That conformation might be expected to enable the compound to diffuse more readily through the membrane barriers of the gastrointestinal tract[5–7], allowing a greater proportion of ingested CsA to be absorbed prior to excretion, when compared to free drug.

Conclusion

Efforts at a systematic and predictive parameterization of the behavior of marketed drugs have been problematic. This is unfortunate, because the establishment of bioavailability 'rules', in conjunction with modern techniques, such as structure-based drug design[15,51], might lead to a more direct and predictable pathway to drug discovery (see, for example, Refs 16, 17 and 52). The importance of addressing the clearance of new chemical entities early in the drug discovery program in order to expedite the design of an orally bioavailable candidate is stressed here. Once an analog with reasonable clearance is obtained, subsequent modifications in the physicochemical determinants of drug absorption can be manipulated. As a general rule, compounds with a low M_r and an appropriate log P tend to favor oral bioavailability of drugs. However, caution is required in the broad application of these principles, because compounds of low M_r can be excreted rapidly (as can those of high M_r), precluding good oral bioavailability. We have also indicated above that compounds such as CsA, which have long been recognized as 'exceptional' in their bioavailability characteristics relative to M_r (Figure 2a), may achieve the desired behavior through altered conformation in response to the polarity of the environment.

The goals of this review have been to stimulate the synthesis of compounds with optimal physicochemical properties and/or a focused, comprehensive investigation of the physicochemical and conformational behavior of a number of drugs of higher M_r with anomalously high bioavailability. Only to the extent that the various phenomena contributing to the overall bioavailability of a drug can be deconvolved, can it be possible to generate predictive models for the design of novel bioavailable drugs of higher M_r that might accelerate the process of drug discovery and development.

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REFERENCES

1 Pond, S.M. and Tozer, T.N. (1984) *Clin. Pharmacokin.* 9, 1–25
2 Kararli, T.T. *et al.* (1994) *Int. J. Pharm.* 102, 177–184
3 Kim, J.S. *et al.* (1994) *J. Pharm. Sci.* 83, 1350–1356
4 Pang, K.S. *et al.* (1986) *Drug Metab. Dispos.* 14, 102–111
5 Humphrey, M.J. and Ringrose, P.S. (1986) *Drug Metab. Rev.* 17, 283–310
6 Amidon, G.L. and Lee, H.J. (1994) *Annu. Rev. Pharmacol. Toxicol.* 34, 321–341
7 Leahy, D.E., Lynch, J. and Taylor, D.C. (1989) in *Novel Drug Delivery and Its Therapeutic Applications* (Prescott, L.F. and Nimmo, W.S., eds), pp. 33–44, John Wiley & Sons
8 Fei, Y-J. *et al.* (1994) *Nature* 368, 563–566
9 Rowland, M., Benet, L.Z. and Graham, G.G. (1973) *J. Pharmacokin. Biopharm.* 1, 123–136

10 Gibaldi, M. and Perrier, D. (1982) *Pharmacokinetics*, Marcel Dekker
11 Amidon, G.L., Sinko, P.J. and Fleisher, D. (1988) *Pharm. Res.* 5, 651–654
12 Sinko, P.J., Leesman, G.D. and Amidon, G.L. (1991) *Pharm. Res.* 8, 979–988
13 Oh, D., Curl, R.L. and Amidon, G.L. (1993) *Pharm. Res.* 10, 264–270
14 Sinko, P.J., Leesman, G.D. and Amidon, G.L. (1993) *Pharm. Res.* 10, 271–275
15 Navia, M.A. and Murcko, M.A. (1992) *Curr. Opin. Struct. Biol.* 2, 202–210
16 Kim, E.E. *et al.* (1995) *J. Am. Chem. Soc.* 117, 1181–1182
17 Navia, M.A., Sato, V.L. and Tung, R.D. (1995) *International Antiviral News* 3, 143–145
18 Walter, A. and Gutknecht, J. (1986) *J. Membr. Biol.* 90, 207–217
19 Stella, V.J. and Charman, W.N.A. (1989) in *Novel Drug Delivery and Its Therapeutic Applications* (Prescott, L.F. and Nimmo, W.S., eds), pp. 57–68, John Wiley & Sons
20 Hamilton, H.W. *et al.* (1995) *J. Med. Chem.* 38, 1446–1455
21 Conradi, R.A. *et al.* (1991) *Pharm. Res.* 8, 1453–1460
22 Barlow, D. and Satoh, T. (1994) *J. Controlled Release* 29, 283–291
23 Knoefel, P.K. (1972) in *Absorption, Distribution, Transformation and Excretion of Drugs* (Knoefel, P.K. *et al.*, eds), pp. 39–55, C.C. Thomas
24 Friedman, D.I. and Amidon, G.L. (1989) *Pharm. Res.* 6, 1043–1047
25 Friedman, D.I. and Amidon, G.L. (1989) *J. Pharm. Pharmacol.* 78, 995–998
26 Nakashima, E. *et al.* (1984) *J. Pharm. Dyn.* 7, 452–464
27 Sinko, P.J. and Amidon, G.L. (1989) *J. Pharm. Sci.* 78, 723–727
28 Ziegler, K. *et al.* (1985) *Biochim. Biophys. Acta* 845, 86–93
29 Hunter, E.B. *et al.* (1990) *Hepatology* 12, 76–82
30 Gores, G.J. *et al.* (1989) *Am. J. Physiol.* 250, G350–G356
31 Lin, J.H. *et al.* (1988) *Drug Metab. Dispos.* 16, 392–396
32 Wilkinson, G.R. and Shand, D.G. (1975) *Clin. Pharmacol. Ther.* 18, 377–390
33 Loosli, H. *et al.* (1985) *Helv. Chim. Acta* 68, 682–704
34 Mierke, D.F. *et al.* (1991) *Helv. Chim. Acta* 74, 1027–1047
35 Tanaka, H. *et al.* (1987) *J. Am. Chem. Soc.* 109, 5031–5033
36 Theriault, Y. *et al.* (1993) *Nature* 361, 88–91
37 Pflugl, G. *et al.* (1993) *Nature* 361, 91–94
38 Van Duyne, G.D. *et al.* (1991) *Science* 252, 839–842
39 Itoh, S. *et al.* (1995) *Bioorg. Med. Chem. Lett.* 5, 1983–1988
40 Itoh, S. and Navia, M.A. (1995) *Protein Sci.* 4, 2261–2268
41 Wuthrich, K. *et al.* (1991) *Science* 254, 953–954
42 Jorgensen, W.L. (1991) *Science* 254, 954–955
43 Wenger, R.M. *et al.* (1994) *FEBS Lett.* 340, 255–259
44 Petros, A.M. *et al.* (1993) *J. Am. Chem. Soc.* 115, 9920–9924
45 Wiley, R.A. and Rich, D.A. (1993) *Med. Res. Rev.* 13, 327–384
46 Matsuzaki, T. *et al.* (1989) *J. Biochem. (Tokyo)* 105, 949–952
47 Banner, D.W. and Hadvary, P. (1991) *J. Biol. Chem.* 266, 20085–20093
48 Lautz, J. *et al.* (1987) *J. Comput.-Aided Mol. Design* 1, 219–241
49 Braun, W. *et al.* (1995) *FASEB J.* 9, 63–72
50 Griffith, J.P. *et al.* (1995) *Cell* 82, 507–522
51 Navia, M.A. and Peattie, D.A. (1993) *Trends Pharmacol. Sci.* 14, 189–195 and *Immunol. Today* 14, 296–302
52 Baldwin, J.J. *et al.* (1989) *J. Med. Chem.* 32, 2510–2513
53 Reynolds, J.E.F. (1993) *Martindale: The Extra Pharmacopeia, 30th Edition*, The Pharmaceutical Press
54 Gilman, A.G. *et al.* (1990) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th Edition, McGraw-Hill
55 Venkataramanan *et al.* (1995) *Clin. Pharmacokinet.* 29, 404–430
56 Swaan, P.W., Stehouwer, M.C. and Tukker, J.J. (1995) *Biochim. Biophys. Acta* 1236, 31–38
57 Wyvratt, M.J. *et al.* (1984) *J. Org. Chem.* 49, 2816–2819

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