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Physicochemical Space for Optimum Oral Bioavailability: Contribution of Human Intestinal Absorption and First-Pass Elimination

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Oral bioavailability (*F*) is a product of fraction absorbed (Fa), fraction escaping gut-wall elimination (Fg), and fraction escaping hepatic elimination (Fh). In this study, using a database comprised of Fa, Fg, Fh, and *F* values for 309 drugs in humans, an analysis of the interrelation of physicochemical properties and the individual parameters was carried out in order to define the physicochemical space for optimum human oral bioavailability. Trend analysis clearly indicated molecular weight (MW), ionization state, lipophilicity, polar descriptors, and free rotatable bonds (RB) influence bioavailability. These trends were due to a combination of effects of the properties on Fa and first-pass elimination (Fg and Fh). Higher MW significantly impacted Fa, while Fg and Fh decreased with increasing lipophilicity. Parabolic trends were observed for bioavailability with polar descriptors. Interestingly, RB has a negative effect on all three parameters, leading to its pronounced effect on bioavailability. In conclusion, physicochemical properties influence bioavailability with typically opposing effects on Fa and first-pass elimination. This analysis may provide a rational judgment on the physicochemical space to optimize oral bioavailability.

Introduction

The quest for new chemical entities that will prove to be clinically useful drugs is challenging. In addition to the challenges posed with identifying suitable molecular targets and designing potent ligands for these targets, optimization of the dispositional profile is also needed.[1] Drugs must be able to be administered by a reasonable route, with convenience of the dosing regimen preferred (e.g., oral administration, once-per-day, etc.) to enhance patient compliance. They must also be able to distribute to the tissue where the target receptor resides. Thus, considerable research efforts have been made in the application of absorption, distribution, metabolism, excretion (ADME) sciences in drug design.[2−6] Within drug research programs, structure−activity relationships (SAR[a]) are derived for ADME properties, highlighting suboptimal features in new chemical scaffolds from which drug design efforts will be based. Using such an approach, much can be gleaned from SAR but these relationships will be only useful for the particular chemical scaffold being investigated.[7] A need for understanding overall trends between chemical properties and dispositional properties exists, as it would be useful to apply these attributes to individual chemical scaffolds.

Oral exposure is of paramount importance in the design of new drugs. However, there are multiple processes occurring which determine the exposure to a drug after oral administration, and each of these processes is driven by multiple molecular determinants. For example, the groundbreaking analysis by Lipinski and colleagues showed that particular physicochemical attributes are associated with high or low oral bioavailability.[8] Molecular weight >500, lipophilicity >5 (calculated LogP), total hydrogen bond acceptors >10, and total hydrogen bond donors >5 are all properties identified as those that would decrease the likelihood of good oral absorption. Similar observations were made by others in an effort to define descriptors that can provide a rationale for establishing qualitative, semiquantitative, and quantitative structure−absorption relationship (QSAR) models.[9−15] The dependence of human intestinal absorption on the readily accessible physicochemical properties like lipophilicity (cLogP), molecular size, hydrogen bonding capacity, polar surface area (PSA), and number of free rotatable bonds (RB) has been demonstrated.[9,13,14] Identification of these basic physicochemical properties as determinants is consistent with notions regarding the ability of small organic molecules to pass through lipid bilayer membranes. However, oral exposure is determined not only by absorption through membranes of the gastrointestinal tract but also by the extent to which organs just after absorption are able to extract these orally administered drugs. The most important organs causing first-pass extraction include the liver (which extracts by metabolism, uptake transport, and biliary secretion) and the intestine (which extracts mainly by metabolism).[16−20] Note that lung, heart, and blood are also possible first-pass extraction tissues, but are considerably less important in oral drug exposure. Thus, to better understand the relationship between fundamental physicochemical properties and oral exposure, not only must absorption be considered but hepatic and intestinal extraction must also be taken into account.

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[a] Abbreviation: CL_{renal}, renal clearance; CL_{total}, total body clearance; Fa, fraction absorbed; Fg, fraction escaping intestinal elimination; Fh, fraction escaping hepatic elimination; FPE, first-pass elimination; fu, plasma free fraction; HBA, hydrogen bond acceptors; HBD, hydrogen bond donors; MSA, molecular surface area; MW, molecular weight; PSA, polar surface area; QSAR, quantitative structure−activity relationships; RB, free rotatable bond; RPSA, relative polar surface area; SAR, structure−activity relationships.

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In this report, pharmacokinetic literature was extensively mined to identify a data set of over 300 compounds for which the three main individual determinants of oral bioavailability, (absorption (Fa), fraction escaping intestinal extraction (Fg), and fraction escaping hepatic extraction (Fh)) could be estimated for humans. These values were then used to determine relationships of these parameters to basic physicochemical properties. With these relationships available, they can be used in drug design to optimize one or more specific drivers for a given chemical series in the process of achieving optimum bioavailability.

Data Set Used in the Analysis. The fundamental concept underlying the analysis of human pharmacokinetic data after oral administration is that oral bioavailability is a function of intestinal absorption (Fa), intestinal metabolism (Eg = 1 − Fg), and hepatic extraction (Eh = 1 − Fh) which occur in series and hence are related as:

$$F = \mathrm{Fa} \times \mathrm{Fg} \times \mathrm{Fh}$$

Human pharmacokinetic studies in which Fa, Fg, and Fh are determined are extremely rare due to the need for invasive sampling techniques (e.g., sampling from the portal vein; patients in the anhepatic phase of liver transplant). Therefore, these values needed to be derived from multiple sources from the scientific literature for each drug. Starting with the 670 intravenous clearance values listed in Obach et al.,[21] renal clearance data was sought for each drug and the nonrenal clearance calculated from:

$$\mathrm{CL_{non\text{-}renal}} = \mathrm{CL_{total}} - \mathrm{CL_{renal}}$$

Renal clearance was derived from two possible sources:[22] (1) measurement of the amount of unchanged drug excreted in urine (A_{urine}) and the plasma drug exposure after any route of administration:

$$\mathrm{CL_{renal}} = \frac{A_{\mathrm{urine}}}{\mathrm{AUC}}$$

or from excretion studies in which the drug was administered intravenously:

$$\mathrm{CL_{renal}} = \mathrm{CL_{total}} \times f_{\mathrm{urine}}$$

in which f_{urine} is the fraction of the dose excreted in urine as unchanged drug. From the original 670 drugs, 524 remained that had these data available in the public domain.

A major assumption for these data is that the $\mathrm{CL_{nonrenal}}$ is all due to hepatic clearance. While the presence of drug metabolizing enzymes in extrahepatic tissues is unquestionable, their quantitative contribution to intravenous drug clearance relative to the liver is negligible. Thus, the value Fh, the fraction that passes through the liver unextracted, could be calculated from:

$$\mathrm{Fh} = 1 - \frac{\mathrm{CL_{non\text{-}renal}}}{Q_{\mathrm{h}}}$$

in which Q_{h} is hepatic blood flow, set at 23 mL/min/kg body weight[23−25] and blood to plasma ratio was assumed to be 1. Thirty-five drugs needed to be removed at this point because their $\mathrm{CL_{nonrenal}}$ values were greater than 23 mL/min/kg, indicating substantial extrahepatic clearance and invalidating application of the assumptions. It should be noted that the Fh values are independently estimated using $\mathrm{CL_{total}}$ data obtained exclusively from the intravenous pharmacokinetic studies, and thus the values are not confounded by the effects of slow and incomplete absorption.

For the drugs remaining, oral bioavailability was calculated using the aforementioned $\mathrm{CL_{total}}$ data and oral clearance ($\mathrm{CL_{po}}$) data derived from reports of oral pharmacokinetics. In some cases, these were from the same reports and in others, these needed to be taken from separate reports:

$$F = \frac{\mathrm{CL_{total}}}{\mathrm{CL_{po}}} = \frac{\mathrm{AUC_{po}}}{\mathrm{AUC_{iv}}} \times \frac{\mathrm{dose_{iv}}}{\mathrm{dose_{po}}}$$

For the 489 drugs remaining up to this point, 441 possessed oral pharmacokinetic data that permitted calculation of oral bioavailability.

The following methods were primarily used to collect values for fraction absorbed (Fa).[15] First, for some drugs, mass balance excretion studies in which radiolabeled drug was administered orally and parenterally, and radioactivity (reflecting total drug-related material) excreted in the urine was compared to estimate Fa:

$$\mathrm{Fa} = \frac{A_{\text{radioactivity excreted, urine, po}}}{A_{\text{radioactivity excreted, iv}}}$$

Second, calculation of the percentage of cumulative excretion of radioactive drug-related material in urine following oral administration was done for some compounds:

$$\mathrm{Fa} = \frac{A_{\text{radioactivity excreted, urine, po}}}{\mathrm{dose_{po}}}$$

or third, for some drugs the amount of unchanged drug in feces after oral administration was measured (assuming that all metabolites observed arise from absorbed material and not from gut microflora):

$$\mathrm{Fa} = 1 - \frac{A_{\text{excreted, feces}}}{\mathrm{dose_{po}}}$$

Using these three approaches for estimating Fa along with the values obtained from the compiled literature,[15,26] there remained 324 drugs for analysis.

From the oral bioavailability data, the fraction absorbed and the fraction evading hepatic extraction (determined as described), the fraction evading gut metabolism can be calculated:

$$\mathrm{Fg} = \frac{F}{\mathrm{Fa} \times \mathrm{Fh}}$$

Finally, after this treatment, 15 compounds were calculated to have Fg values greater than 1.05, indicating that they must have violated one or more of the requisite assumptions. These were removed to yield a final data set of 309 drugs. The Fg values thus obtained are in close agreement with the values reported elsewhere for small sets of drugs.[16,27] To our knowledge, the current data set represents the largest known systematic compilation for Fg values in humans.

The pharmacokinetic data were exclusively obtained from reports in which healthy young adult subjects were studied or patient populations in which health or physiological condition is not severely compromised with respect to total and renal clearance.[21,22] However, for some classes of compounds, for example anticancer and anti-HIV compounds, data were only available from patient populations and/or populations taking concomitant medications, and in these instances, the data were included. The pharmacokinetic

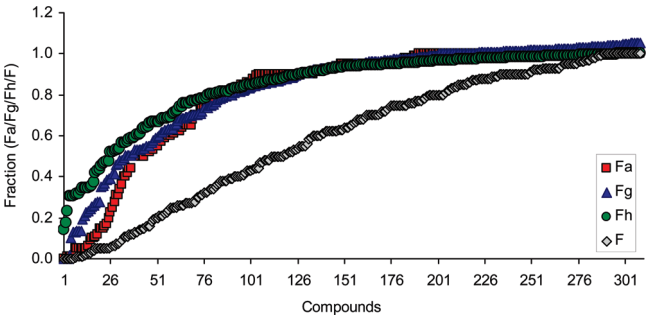

Figure 1. Distribution profiles of the parameters for the 309 compounds in the data set.

parameter values reported here are mean values and do not account for intersubject variability. Nevertheless, to our knowledge, this database available as Supporting Information data is the largest of its kind in the literature and may be used to provide insight into the relationship between structure and oral bioavailability and useful in building computational models.

Calculated Physicochemical Properties. Properties including molecular weight (MW), calculated *n*-octanol/water partition coefficient (cLogP, ACD), calculated *n*-octanol/water distribution coefficient ($cLogD_{pH6.5}$ and $cLogD_{pH7.4}$, ACD), molecular surface area (MSA, Pipeline Pilot), polar surface area (PSA Å^2, ACD), number of free rotatable bonds (RB) and number of hydrogen bond donors (HBD) and acceptors (HBA) were obtained using an in-house program. The relative polar surface area (% rel PSA = PSA/MSA × 100) and relative free rotatable bonds (% rel RB = RB/MW × 100) were also calculated.

Statistics. Standard statistical tests have been carried out to analyze the differences in the pharmacokinetic parameters and physicochemical properties of various data subsets. The parametric *t*-test (unpaired, 2-tailed, unequal variance) was employed to determine the significance. However, data was also analyzed by the nonparametric Mann−Whitney test (2-tailed), as the distributions of some properties are skewed away from normality.[22,28]

Results

Characteristics of the Data Set. The current data set suggested an even distribution of the bioavailability values (Figure 1). About 17% of compounds showed *F* less than 0.2 and 34% of compounds showed *F* more than 0.8. However, the vast majority of compounds showed Fa (71%), Fg (70%), and Fh (73%) more than 0.8. The distribution of the data set in physicochemical space is heterogeneous and thoroughly covered the range of conventional small molecule marketed drugs.[29] The molecular weight distribution in the data set ranged from 76 (hydroxyurea) to 1449 (vancomycin) with a median of 331 g/mol. The values for cLogP ranged from −8.6 (suramin) to 14.4 (cyclosporine), $cLogD_{pH7.4}$ ranged from −8.3 (risedronate) to 6.9 (amiodarone), and PSA ranged from 3.2 (selegiline) to 530 Å^2 (vancomycin). The median values for number of HBA, HBD, and RB were 4, 2, and 5, respectively. Furthermore, the current data set included acids (27%), bases (39%), neutrals (24%), and zwitterions (10%).

The compounds in the data set were grouped into six therapeutic categories.[22,29,30] In general, the physicochemical space of each therapeutic area was similar to the previous reports (data not shown).[22] Mean and median values of the

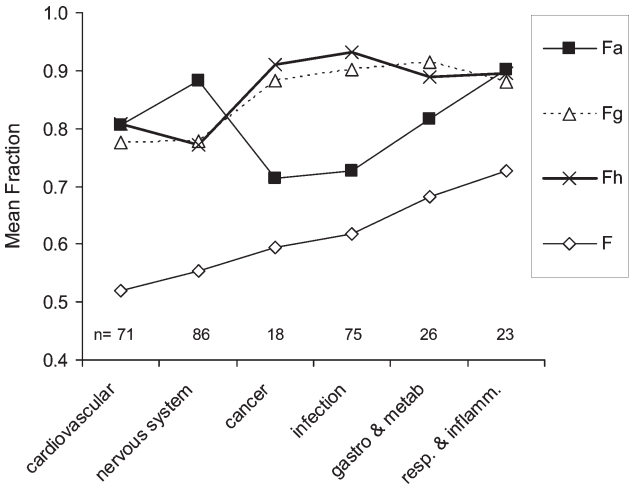

Figure 2. Plot of mean values of Fa, Fg, Fh, and *F* in each therapeutic area.

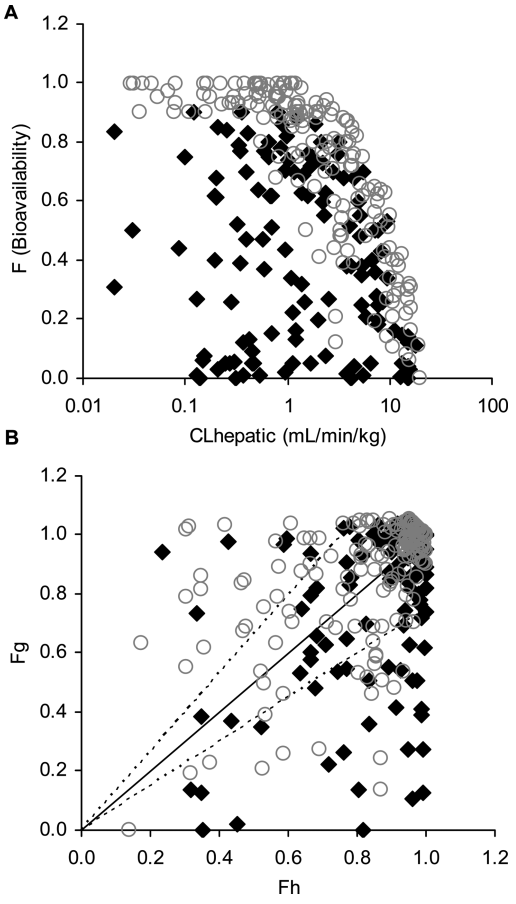

Figure 3. Dependence of oral bioavailability on hepatic clearance (A). The relationship between Fh and Fg (B). Data set represent subsets with Fa > 0.9 (open points) and Fa ≤ 0.9 (closed points).

bioavailability showed a rank-order of respiratory and inflammation > gastrointestinal and metabolic > infection > cancer > nervous system > cardiovascular (Figure 2). However, it is evident that in the case of cardiovascular drugs all three processes (Fa, Fg, and Fh) are limiting bioavailability, while in the case of nervous system compounds first-pass elimination (FPE; Fg and Fh) is the limiting process. In contrast, Fa was found to determine the bioavailability for compounds in cancer and infection therapeutic categories.

Table 1. Mean (Median) of Absorption Parameters and the Physicochemical Properties of the Compounds in Various Bioavailability Bins

F bins	*n*	*F*	Fa	Fg	Fh	mol wt	polar surface area [Å^2]	cLogP	cLogD pH6.5	cLogD pH7.4	free rotatable bonds	H-bonding acceptors	H-bonding donors	rule-of-five[a] [%]
<0.2	49	0.07	0.43	0.57	0.73	444	130	1.27	−0.13	0.11	6.8	6.2	3.7	20.4
		(0.06)	(0.20)	(0.54)	(0.87)	(398)	(103)	(1.80)	(0.87)	(1.11)	(7.0)	(5.0)	(2.0)	
0.2−0.8	146	0.52	0.82	0.81	0.80	358	83	2.09	0.18	0.39	5.6	4.4	2.2	9.6
		(0.52)	(0.90)	(0.85)	(0.85)	(330)	(78)	(2.12)	(0.34)	(0.49)	(5.0)	(4.0)	(2.0)	
≥0.8	114	0.92	0.97	0.99	0.95	317	78	1.37	0.29	0.24	4.16	3.8	2.0	0.9
		(0.92)	(1.00)	(1.00)	(0.97)	(317)	(75)	(1.62)	(0.31)	(0.32)	(4.0)	(3.0)	(2.0)	

[a] Rule-of-five: percentage of compounds failing to meet 2 or more rule-of-five criteria.

Clinical data clearly indicated that bioavailability is highly dependent on hepatic clearance (Figure 3A). Notably, for completely absorbed compounds (Fa > 0.9), the correlation of determination (r^2) of bioavailability with hepatic clearance is 0.74. We further explored the relationship between Fh and Fg in order to evaluate the use of the relatively easily accessible parameter Fh to predict the Fg in humans (Figure 3B).[24] These two parameters showed a relationship with about 72% of 309 compounds falling within 25% of unity slope. However, it should be noted that the data is skewed toward higher Fg and Fh values. For those compounds which are off the unity slope, it is interesting to note that there appears to be a predominance of compounds with lower absorption (Fa < 0.9) that have lower Fg values when compared to Fh, suggesting that these poorly absorbed compounds (low solubility and/or low permeability) are more prone to intestinal metabolism. The reverse is suggested for compounds with higher absorption (Fa > 0.9).

Relationship between Physicochemical Properties and the Bioavailability Parameters. Table 1 shows the trend of parameters and physicochemical properties of the compounds that are binned based on their oral bioavailability range. It should be noted that trends could not be easily observed with mean values for some physicochemical properties because of the scatter and overlap in the data (Figures 4−7). However, median values give a clear picture of this large data set. The current data set indicated that bioavailability is mainly limited by Fa. This is evident from the subset of compounds showing bioavailability less than 0.2, where mean and median values suggest the rank-order of limiting parameters as Fa > Fg > Fh (Table 1).

Ionization state analysis of the compounds in the database indicated that bases are relatively less bioavailable ($p < 0.005$, Mann−Whitney test), although they showed higher Fa than acids and neutrals, evidently due to higher FPE (Figure 4A). The mean and median values of bioavailability for acids, neutrals, and zwitterions are quite similar. Furthermore, compounds existing in these ionization states showed similarity in Fa, Fg, and Fh. The lower median Fa for acidic drugs was likely due to permeability limitations, as such compounds mainly exist in the ionized state at the small intestine pH and are least permeable through negatively charged lipid membranes.[31] The higher FPE (low Fg and Fh) of basic molecules can be attributed to their affinity for metabolic enzymes and their relatively lower protein binding.[10,21,32] No meaningful relationship for Fg and Fh with MW was evident with the data set (Figure 4B). However, median Fa for compounds with high MW dropped to 0.57 ($p < 0.005$). Overall, MW trends indicate that increasing the size of molecules above 400 g/mol will on average lead to a steady decline in bioavailability ($p < 0.005$), mainly due to the effect on Fa.

Lipophilicity (cLogP or $cLogD_{pH7.4}$) showed distinct but opposing trends for Fa and FPE (Fg and Fh), resulting in a parabolic relationship for bioavailability (Figure 5). Very hydrophilic compounds have drastically reduced intestinal absorption. $cLogD_{pH6.5}$ at mean gastrointestinal pH (pH 6.5) showed similar overall trend with Fa (data not shown), although compounds with pK_a values in the critical region may have different values from the $cLogD_{pH7.4}$. Mean and median values of Fg and Fh steadily declined as lipophilicity increase. The only exception to this trend was observed for very lipophilic compounds (amiodarone, drotaverine, montelukast, paricalcitol, and ritonavir). These compounds with $cLogD_{pH7.4}$ values more than 5 showed higher Fg and Fh.

In contrast to lipophilicity trends, polar descriptors (PSA, hydrogen bond count (HBD + HBA) and relative PSA) showed an inverse relationship with Fa, especially for compounds with PSA greater than 125 Å^2 ($p < 0.005$) and/or hydrogen bond count more than 9 ($p < 0.005$) (Figure 6). Mean and median values of Fg and Fh also showed trends with polar descriptors. An increase in Fg and Fh was evident moving through the PSA and hydrogen bond count bins in increasing order, noticeably for compounds with PSA less than 75 Å^2. For example, median Fh values associated with PSA bins of <25, 25−50, and 50−75 Å^2 are 0.65, 0.85, and 0.94 ($p < 0.005$), respectively. To eliminate the obvious relationship between polar descriptors (PSA and hydrogen bonding) and the molecular size, the data was also analyzed as a function of relative PSA (% of PSA/MSA) (Figure 6C). Similar trends were observed with the individual parameters as of PSA, confirming the influence of polar descriptors. For this data set, PSA correlated closely ($r^2 = 0.91$) with hydrogen bond count, suggesting that these physicochemical properties are closely associated and influence the involved processes to a similar extent.

Interestingly, the number of free rotatable bonds showed a negative relationship with all three processes leading to a dramatic effect on bioavailability (Figure 7A). The median *F* values associated with the RB bins of 0−3, 7−9, and >12 are 0.83, 0.50, and 0.27 ($p < 0.005$), respectively. Further analysis, after normalizing RB with MW (% rel RB), showed similar trends as that of RB with Fg and Fh parameters (Figure 7B), substantiating the negative impact of RB count on drug metabolism. However, no particular trend was noted between % rel RB bins and Fa, suggesting that the effect of RB on Fa is majorly associated with the molecular size (Figure 4). Overall, % rel RB showed significant effect ($p < 0.005$) on *F*, as was observed with RB count.

Physicochemical Profiles of Fa-Limited and FPE-Limited Compounds. Trend analysis suggested that intestinal absorption (Fa) and FPE (Fg and Fh) are associated with a reasonably distinct physicochemical space (Figures 4−6). To further

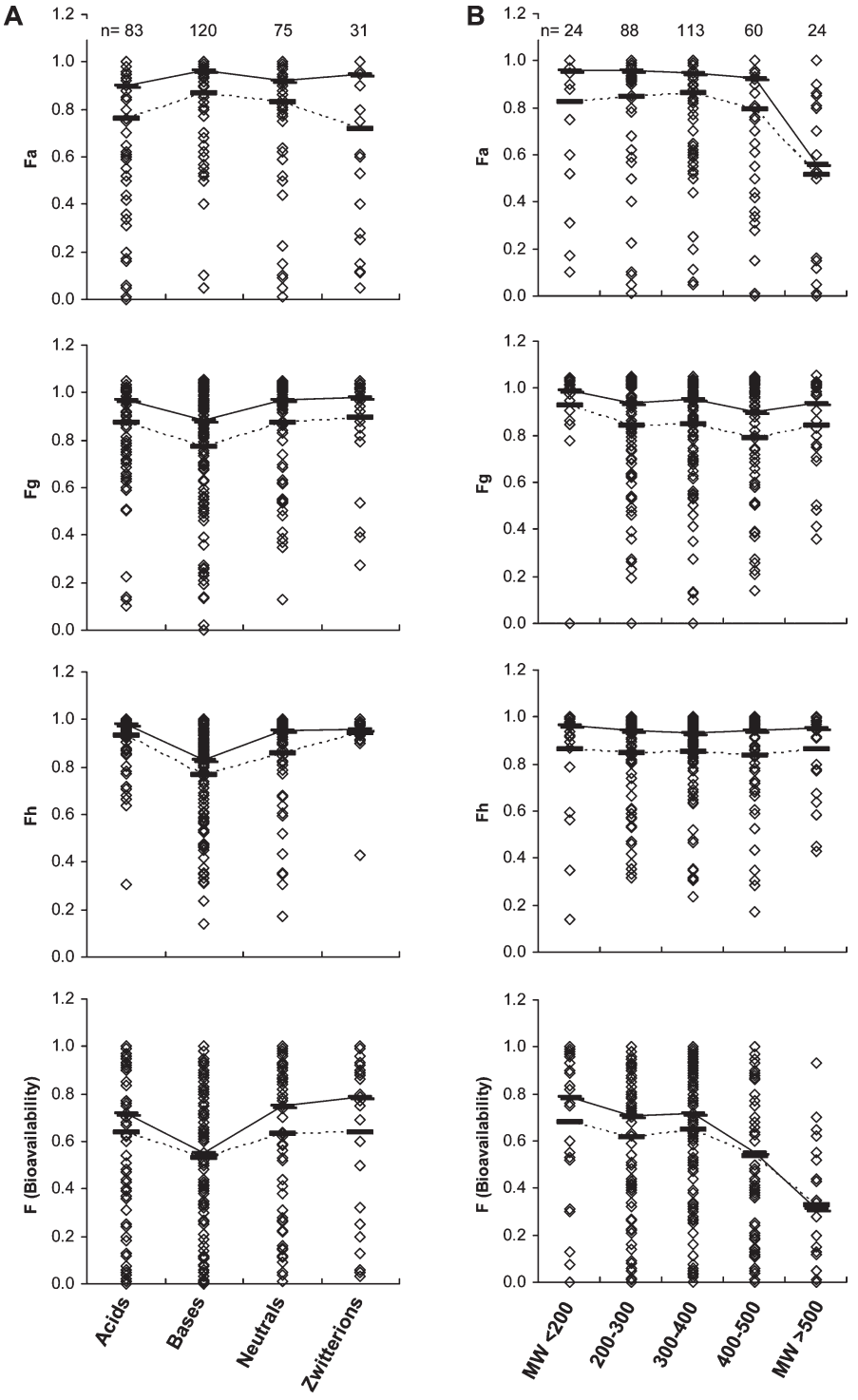

Figure 4. Relationship between bioavailability parameters and (A) ionization state and (B) molecular weight. Dotted and solid lines denote mean and median values, respectively. "*n*" is the number of compounds in each bin.

substantiate these differences, we identified 46 (15%) and 108 (35%) of the 309 compounds for which bioavailability is limited only due to intestinal absorption ($Fa < 0.8$ and $Fg \times Fh \geq 0.8$; Fa-limited) and due to significant FPE ($Fg \times Fh < 0.8$ and $Fa \geq 0.8$; FPE-limited), respectively. Cumulative fraction curves (Figure 8) revealed that certain physicochemical properties of the two subsets, Fa-limited and FPE-limited, differ from each other significantly. Clearly, Fa-limited compounds are very hydrophilic with high polarity, and hydrogen bond count was more frequent than they were for FPE-limited compounds. About 75% of the Fa-limited compounds have $cLogD_{pH\ 7.4} < 0$, while 80% of FPE-limited compounds have $cLogD_{pH\ 7.4} > 0$ (Figure 8C). Profiles for PSA and hydrogen bond count also showed statistically significant differences. However, RB count was found to be similar between the two subsets, which is consistent to the observation that RB showed similar trends with Fa, Fg, and Fh (Figure 7C). Overall, due to the observed distinct effects on the individual processes (intestinal absorption and FPE), lipophilicity, PSA, and hydrogen bond count tend to show parabolic relationships with oral bioavailability.

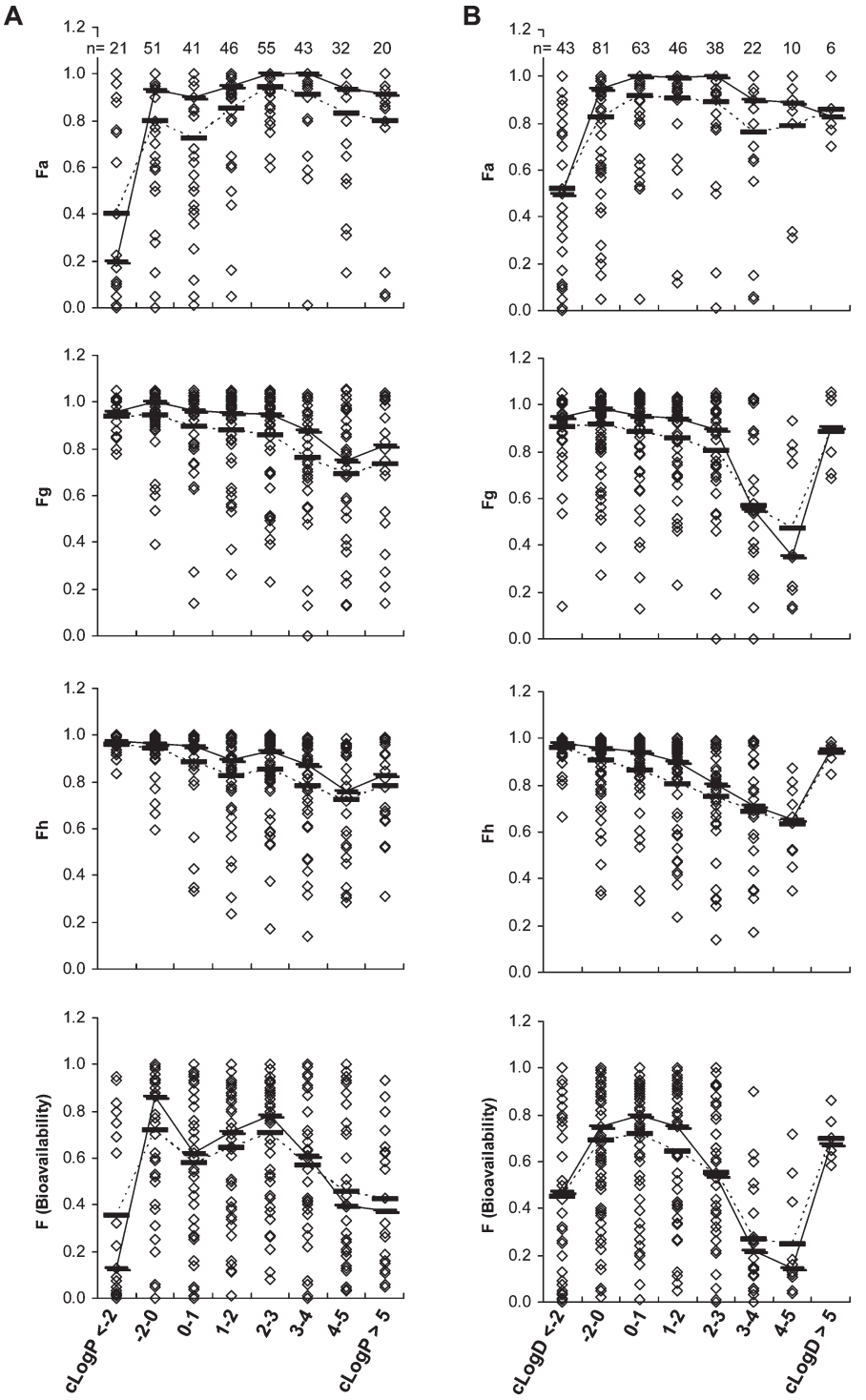

Figure 5. Relationship between bioavailability parameters and lipophilicity. (A) cLogP and (B) cLogD pH 7.4. Dotted and solid lines denote mean and median values, respectively. "*n*" is the number of compounds in each bin.

Influence of Rule-of-Five. Poor bioavailability is more likely when the compounds violate two or more of the following rules: (i) cLogP < 5, (ii) MW < 500, (iii) HBD < 5, and (iv) HBA < 10.[8] Using the current data set, we evaluated the relationships between number of violations and bioavailability and the individual processes. From Figure 9, it is evident that median bioavailability dropped considerably from 0.70 to 0.35 ($p < 0.005$) for the compound subsets with no violation and two violations, respectively. Compounds with three violations showed a further decline in median bioavailability (0.05). However, this relationship was observed only with Fa but not with Fg and Fh, suggesting that relationship of rule-of-five and bioavailability is associated mainly with intestinal absorption.

Discussion

Oral bioavailability is one of the most important determinants of the dosing regimen for drugs. The extent to which a drug fails to be absorbed or is removed by FPE before it can reach the tissue containing the pharmacological target receptor,

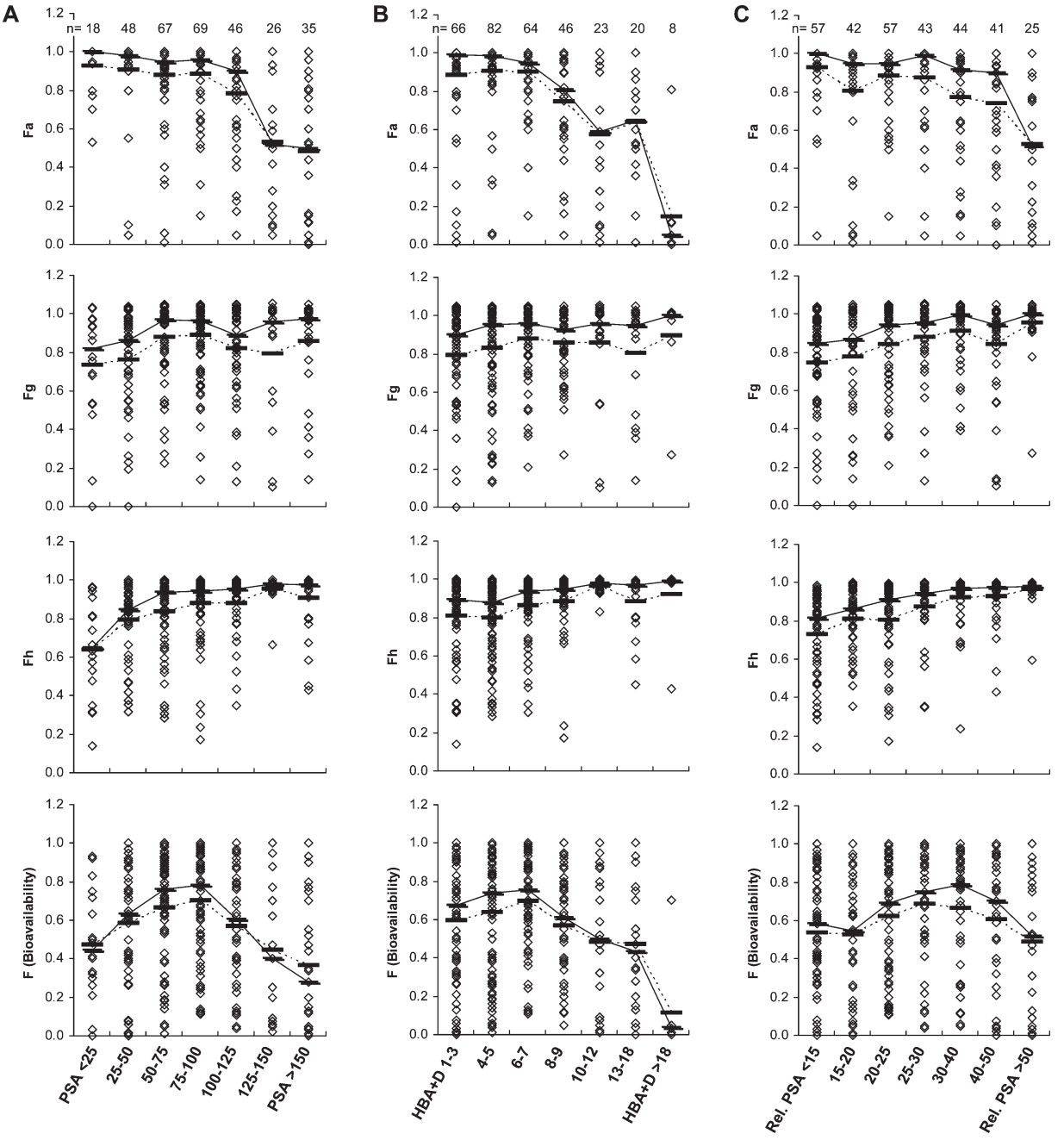

Figure 6. Relationship between bioavailability parameters and hydrogen bonding ability. (A) polar surface area, (B) hydrogen bonding acceptors and donors, and (C) relative polar surface area (% rel PSA = PSA/MSA × 100). Dotted and solid lines denote mean and median values, respectively. "*n*" is the number of compounds in each bin.

along with other determinants, will determine how large a dose must be administered. Thus, during the design phase of new drug research, considerable effort is expended to optimize oral bioavailability. It has been well established that physicochemical attributes contribute to oral bioavailability. The "rule-of-five" devised by Lipinski and co-workers[8] provided an important advance, with analysis of a large data set showing that compounds within certain physicochemical space tended to be more successful in clinical development than others. Further reports by Veber[14] and Yu[33] were generally consistent with this, and the number of rotatable bonds was also recognized as an important determinant. However, these analyses were primarily directed toward the absorption component of oral bioavailability.

In the present work, we have separated out the three important components of oral bioavailability: absorption, first-pass gut metabolism, and first-pass hepatic extraction. Regarding Fa, our observations are consistent with those previously described.[14,34−36] Fa decreases with increasing MW (especially above 500), polarity (cLogD < -2), polar surface area (> 125 Å^2), total H-bond donors and acceptors (> 9), and rotatable bonds (> 12). Such properties limit the capability of small organic molecules to traverse lipid membranes. It should be noted that only a small fraction of compounds in the data set are on the unfavorable side of these generalized cut-offs. For example, about 7% of the compounds in the data set are high MW (> 500) compounds. Nevertheless, only a few in this sizable human data set

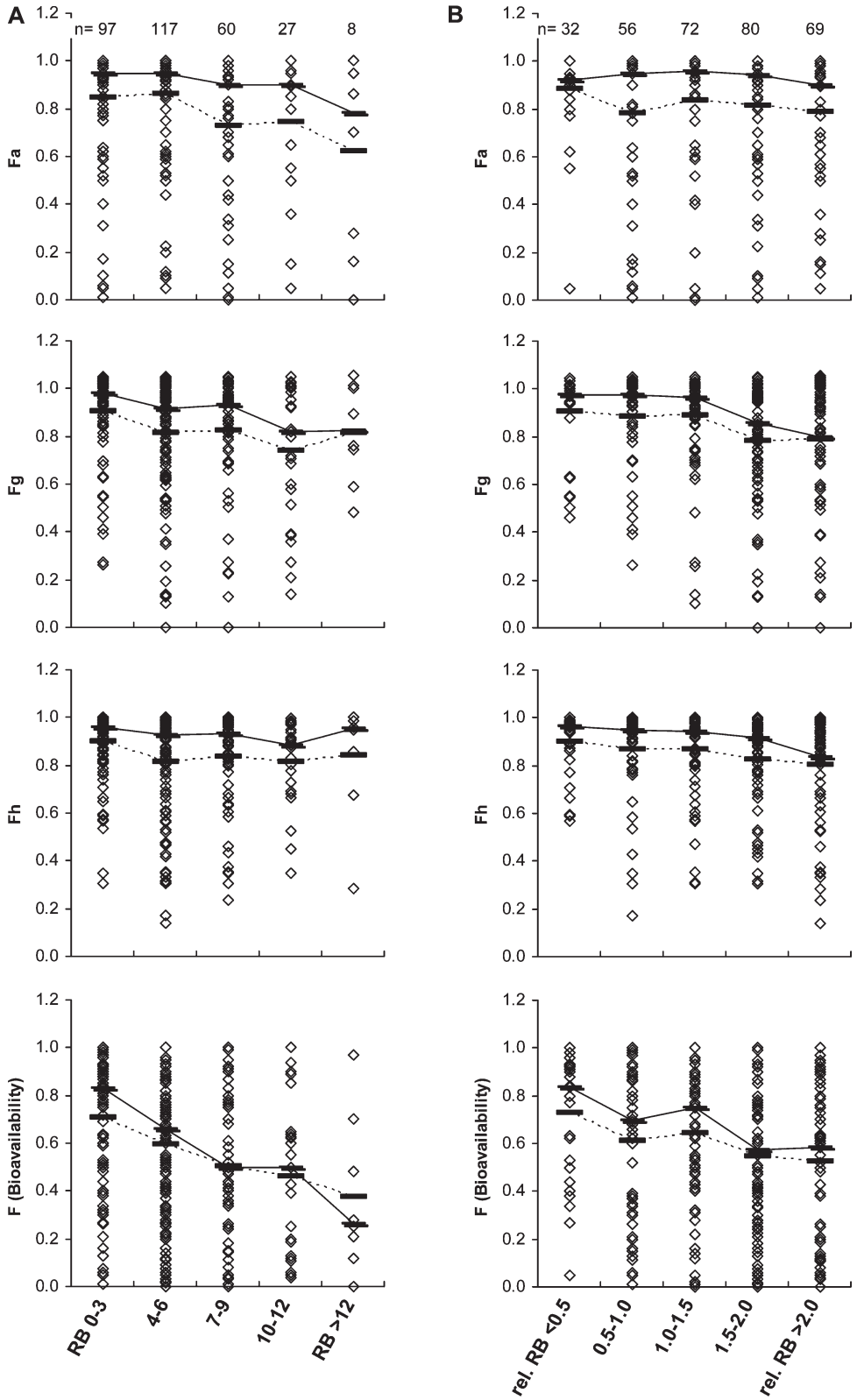

Figure 7. Relationship between bioavailability parameters and free rotatable bonds. (A) free rotatable bonds, and (B) relative free rotatable bonds (% rel RB = RB/MW × 100). Dotted and solid lines denote mean and median values, respectively. "*n*" is the number of compounds in each bin.

suggests that compounds in such unfavorable physicochemical space do not easily make it to clinical development. Intestinal absorption is a composite function of both solubility and permeability, where solubility is negatively related to lipophilicity. A bell-shaped relationship is often reported between lipophilicity and permeability with compounds in the LogP range of 1−3 considered to be highly permeable.[26] However, in the current analysis, we noted that high lipophilicity does not necessarily have a detrimental effect on Fa. On the other hand, the inverse relationships between hydrogen

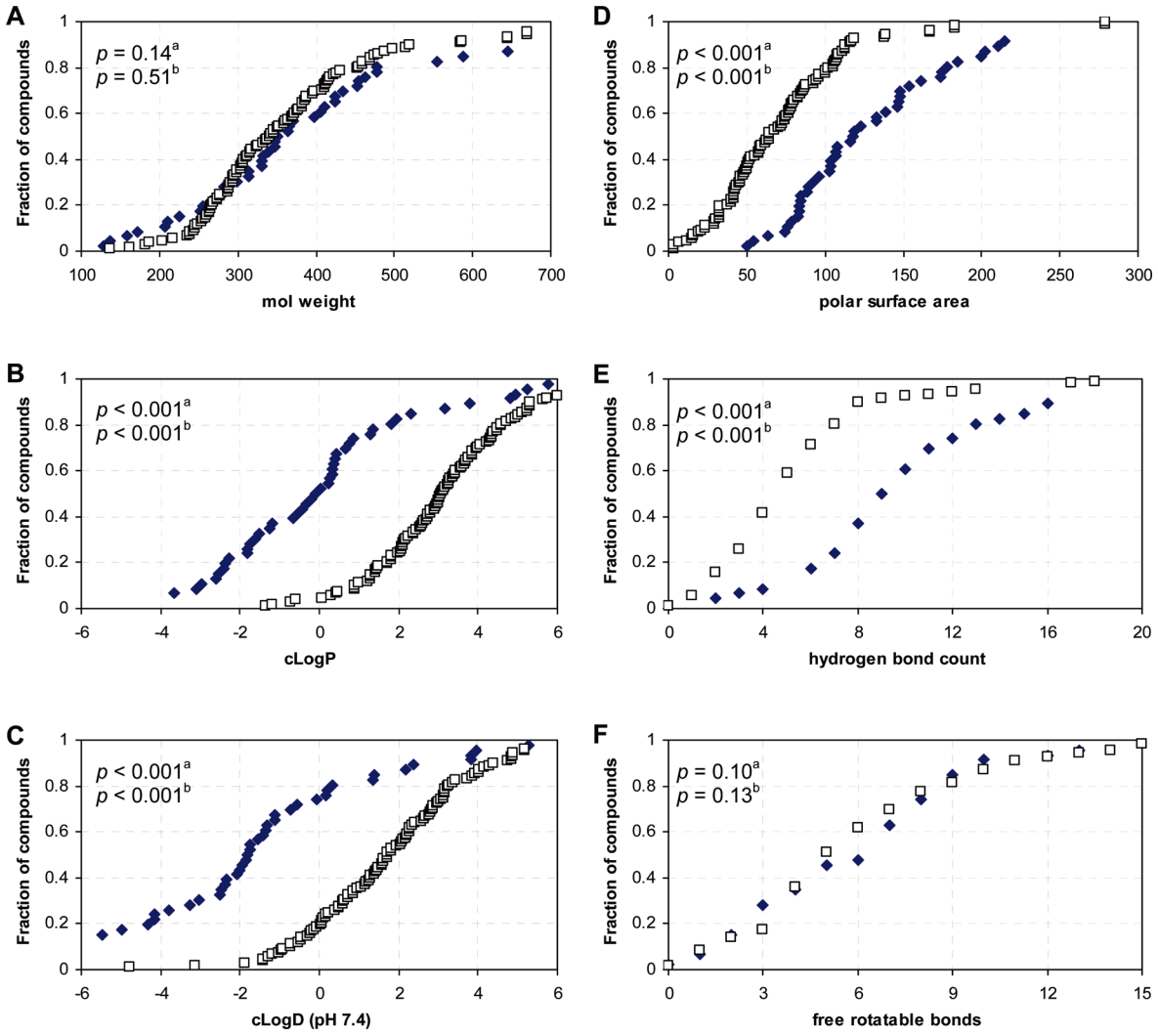

Figure 8. Differential physiochemical profiles of subsets of compounds with bioavailability limited by intestinal absorption (Fa-limited; Fa < 0.8 and Fg × Fh ≥ 0.8; solid points; $n = 50$) and compounds with bioavailability limited by FPE (FPE-limited; Fg × Fh < 0.8 and Fa ≥ 0.8; open points; $n = 108$). The x axes of the plots were limited for clarity. Superscript "a" denotes unpaired (two-tailed) t-test assuming unequal variance. Superscript "b" denotes Mann−Whitney (two-tailed, nonparametric) test.

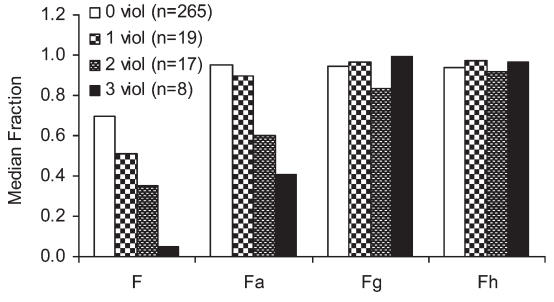

Figure 9. Relationship between number of violations of rule-of-five and bioavailability and individual processes. "n" is the number of compounds in each bin.

bonding descriptors and Fa substantiate the fundamental tenet that a higher value of PSA or hydrogen bond count is energetically unfavorable for the prerequisite molecule desolvation necessary for membrane permeability.[1,37,38]

Xenobiotic metabolism, in almost all cases, increases the polarity of foreign molecules so that membrane permeability is reduced and the material can be flushed from the body by excretion in urine and/or bile. Metabolism is the main mechanism by which Fg and Fh is reduced, albeit the importance of hepatic uptake as a clearance mechanism has been increasingly recognized. As such, the body is well equipped to prevent orally introduced foreign substances from gaining access to the systemic circulation. Among the physicochemical properties examined, Fg and Fh were most impacted by lipophilicity, with compounds having $cLogD_{pH7.4}$ values greater than 3 demonstrating higher gut and hepatic extraction. Charge type also appeared to have an impact, with cationic compounds tending to be more readily extracted than others.[21,39] These observations are consistent with knowledge of the substrate types acted upon by the major cytochrome (CYP) P450 enzymes in liver and intestine.[1,21]

Lipophilicity (cLogP and cLogD) and polar descriptors (PSA and hydrogen bond count) show significant differences between the Fa-limited and FPE-limited subsets (Figure 8), suggesting that the physicochemical properties that favor high Fa tend to also be associated with high rates of metabolism and hence low Fg and Fh. For example, enough lipophilicity is needed to ensure good membrane penetrability, but too much will cause high hepatic and potentially intestinal extraction due to metabolism. On the other hand, reducing the number

of rotatable bonds may enhance all three components of *F* (Figure 7A). It is anticipated that larger molecules typically have high RB, and therefore the observed relationship with RB may be partially or completely accounted to MW effects. Nevertheless, on the basis of the analysis of % rel RB (normalized with MW), we note that RB in a molecule has negative impact on first-pass elimination. Apparently, compact molecules constrained to fewer conformations may be more resistant to binding to drug metabolizing enzymes. However, influence of RB count on intestinal absorption was a contribution from molecular size, which is consistent with the observed molecular weight effect on Fa (Figure 4A).

One goal of this study is also to assess the importance of gut-wall metabolism and addresses a key question, "Are many compounds significantly extracted via intestinal first-pass?"[40] CYP3A4, the most abundant P450 present in human hepatocytes and intestinal enterocytes, is implicated in the metabolic elimination of many drugs.[18,41−45] It has also been proposed that drug interactions involving CYP3A inhibition and induction may be largely occurring at the level of the intestine.[16,46−49] Although, the average human intestinal content of CYP3A has been estimated to be only 70 nmol, versus the average hepatic content of about 5000 nmoles,[42,50] the current data set indicated that intestinal metabolism may contribute to FPE more than the hepatic metabolism for certain drugs. This could be a result of better access to the enzymes in the enterocytes, a function of transcellular flux and the large absorptive area, and/or due to reduced access to hepatic enzymes because of potential plasma protein binding.[51] Also, recent studies demonstrated that efflux transporters present on the apical membrane of enterocytes, in particular P-glycoprotein, can affect the intestinal metabolism by prolonging the enterocytic transit time and consequent exposure to CYP3A enzymes.[52,53] A significant overlap has also been identified between substrates and inhibitors of CYP3A4 and P-glycoprotein, suggesting that these two proteins may act complementarily in further limiting the Fg of CYP3A substrates. Finally, we note that roughly 30% of the compounds in the current data set showed Fg less than 0.8, underscoring the importance of considering intestinal metabolism in predicting bioavailability and dose projections in drug discovery and development settings.

The observations from this analysis reinforce the notion that attaining good oral bioavailability requires a careful balancing act among several physicochemical parameters. The trends observed here can aid the chemist in making a judgment on altering the molecular properties to achieve optimum bioavailability. However, it should be noted that the observations made in this report are general trends. Exceptions abound, as shown in the scatter in Figures 4−7, and the processes may not be described accurately by any single physicochemical descriptor. Furthermore, specific chemical modifications used in compound design can have profound impacts on oral bioavailability without having a high impact on physicochemical attributes. For example, small modifications can affect metabolism by blocking sites of oxidation or sterically hindering binding to enzymes. Nevertheless, it is important to bear in mind that while general trends between physicochemical properties and oral bioavailability are apparent, each chemical series will have specific structural elements that can impact dispositional properties, and in those cases, a greater emphasis on structural considerations will be needed to generate reliable structure−activity relationships for human bioavailability.

Supporting Information Available: Excel worksheet of 309 compound names, CAS numbers, data on human pharmacokinetic parameters, therapeutic area, and appropriate literature references. This material is available free of charge via the Internet at http://pubs.acs.org.

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