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BDDCS, the Rule of 5 and Drugability

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Abstract

The rule of 5 methodology appears to be as useful today in defining drugability as when it was proposed, but recognizing that the database that we used includes only drugs that successfully reached the market. We do not view additional criteria necessary nor did we find significant deficiencies in the four Rule of 5 criteria originally proposed by Lipinski and coworkers. BDDCS builds upon the Rule of 5 and can quite successfully predict drug disposition characteristics for drugs both meeting and not meeting Rule of 5 criteria. More recent expansions of classification systems have been proposed and do provide useful qualitative and quantitative predictions for clearance relationships. However, the broad range of applicability of BDDCS beyond just clearance predictions gives a great deal of further usefulness for the combined Rule of 5/BDDCS system.

Graphical abstract

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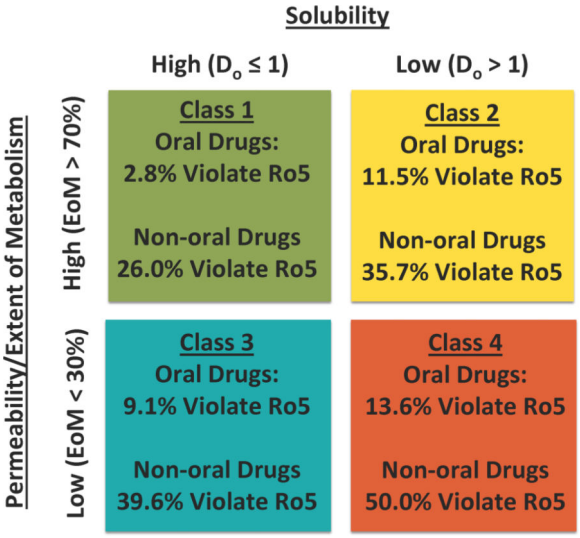

Keywords

BCS; BDDCS; Biliary elimination; Drug-likeness; Metabolism; Renal elimination; Rule of 5

1. Introduction

In their 2005 introduction of the Biopharmaceutics Drug Disposition Classification System (BDDCS) Wu and Benet [1] wrote, “New molecular entities (NMEs) today are frequently large-molecular-weight, lipophilic, poorly-water soluble compounds that most often fall into BCS Class 2. Lipinski et al. [2] pointed out that leads obtained through high-throughput screening (HTS) tend to have higher molecular weights and greater lipophilicity than leads in the pre-HTS era. Lipinski’s Rule of 5 was developed to set ‘drugability’ guidelines for NMEs [3]. In the drug discovery setting, the Rule of 5 predicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight is greater than 500, and the calculated Log P (CLog P) is greater than 5. However, Lipinski specifically states that the Rule of 5 only holds for compounds that are not substrates for active transporters [2, 3]. When the Rule of 5 was developed, information about drug transporters was very limited. We believe that almost all drugs are substrates for some transporter. Studies to date have not been able to show this because we are just beginning to gain the knowledge and tools that allow investigation of substrates for uptake transporters. In addition, unless a drug molecule can passively gain intracellular access, it is not possible to simply investigate whether the molecule is a substrate for efflux transporters.”

Now, more than ten years beyond that 2005 publication we do have much more information about the prevalence and relevance of transporters to drug disposition through the initial publication of the International Transporter Consortium [4] and many subsequent publications from this group and others. It is likely that all drugs are substrates for at least one transporter, but in this manuscript we discuss when transporters are likely to mediate a

clinically relevant response, such that a drug's in vivo disposition depends on and reflects the functionality of transporters.

Here, we evaluate whether the concepts that underlie BDDCS are equally applicable to traditional (within Rule of 5) drugs and also to the increasing number of compounds in development that sit outside the Rule of 5. Yet, academics such as us and many of the other contributors to this compilation are limited in our ability to evaluate the relevance of "beyond Rule-of-5" advances. We suggest this is due to two major factors. First, industrial scientists such as Lipinski and his colleagues have the distinct advantage over academic scientists in that they have access to information about a multitude of candidate drugs that were not successful in achieving regulatory approval for marketing, in addition to the approved drug products that serve as the data base for investigations by academic scientists. Even so, drug companies are limited to the unsuccessful candidates in their pipeline. Thus, in an effort to more fully understand reasons for attrition, four major pharmaceutical companies have evaluated their combined datasets including all candidates and approved drugs [5]. Second, the universal acceptance of the Rule of 5 (Ro5) principles by medicinal chemists and industrial firms may have markedly changed the number of compounds with two or more Ro5 violations being pursued in drug development. It should be noted that the above observations are applicable to chemically synthesized small molecule drugs (in contrast to natural products or chemical derivatives of natural products) that are intended for oral delivery, and are not likely to be relevant to injectable small molecule drugs, nor are they relevant to other therapeutic categories such as biologics or volatile anesthetics.

Soon after the Ro5 publication, Oprea [6] showed that Ro5 criteria do not serve to discriminate drugs from "non-drugs", i.e., approved drugs compared to molecules that are not likely to be therapeutically relevant. Over 90% of the compilation of chemical reagents known as the Available Chemicals Directory are also Ro5 compliant. This observation, however, does not negate the notion that the criteria embodied by the Ro5 can be used to narrow the properties that are useful for what could be termed the "therapeutically relevant pharmacokinetic space".

BDDCS was not developed as an alternative or even an extension of the Ro5. Rather the purpose of BDDCS is to predict drug disposition and potential drug-drug interactions with an emphasis on defining which drugs would be amenable to enzymatic-only and transporter-only disposition and drug-drug interactions, as well as where transporter enzyme interplay may be important. However, as detailed below, BDDCS applications have extended beyond these original intentions.

2. The BDDCS

2.1. Historical Development of BDDCS

The BDDCS was an outgrowth of the Biopharmaceutics Classification System (BCS), which developed from the seminal 1995 paper of Amidon et al. [7] that led to the FDA BCS Guidance in 2000 [8]. Wu and Benet recognized that the overwhelming majority of BCS Class 1 and Class 2 drugs were eliminated in man primarily via metabolic processes, while the overwhelming majority (41 of 42) of BCS Class 3 and Class 4 drugs classified at that

time were primarily eliminated in man unchanged in the urine and bile [1]. Recognizing that the BCS classification may be predictive of drug disposition, in addition to drug absorption, Wu and Benet evaluated enzymatic and transport characteristics of drug substrates with respect to the BCS classification. Our laboratory had published many papers related to metabolism and transporters and was the first to propose the potential and relevance of transporter-enzyme interplay [9]. We incorporated the many studies from our laboratory evaluating enzymes and transporters from molecular, *in vitro* animal, *in vivo* animal, and human studies leading to the BDDCS proposal [1].

It is important to recognize that the 22 predictions made by Wu and Benet with respect to the BDDCS system were based on observations, not theory, primarily from studies investigating the relevance of transporters, enzymes and transporter-enzyme interplay, particularly for immunosuppressives [10–15]. These studies served as the basis for the prediction of the relevance of transporters for the various BDDCS classes as depicted in Fig. 1 for orally dosed drugs [16].

For the 153 drugs initially classified in the BDDCS system by Wu and Benet [1], we were unable to identify any clinically relevant transporter effects in the gut or the liver for the BDDCS Class 1 drugs. This prediction from 2005 was not based on theoretical concepts, but rather findings that were familiar to us as biopharmaceutical scientists with knowledge of drug metabolism, transporter effects and pharmacodynamics. In that paper [1], Wu and Benet cautioned that “there will always be exceptions to the broad, general rules presented here”. Yet even when the BDDCS classification was expanded to more than 900 drugs [17] and most recently an additional 175 drugs were added [18], the predictions in Fig. 1 for the BDDCS Class 1 drugs holds remarkably well and we do not know of any class 1 drugs that require a dosage change as a result of transporter inhibition or induction. Recently, Varma et al. [19] have suggested that two statins, fluvastatin and cerivastatin, classified as BDDCS Class 1, do exhibit rate limited uptake into hepatocytes as a function of OATPs. But, their suggestion is not supported, and is in fact contradicted, by clinical data. Niemi and co-workers [20,21] report that OATP1B1 polymorphisms that have been shown to affect the pharmacokinetics of all of the BDDCS Classes 2, 3 and 4 statins, do not affect the pharmacokinetics of the BDDCS Class 1 statin, fluvastatin. Cerivastatin was removed from the market before any such evaluation was carried out. Varma et al. [19] have fallen into the trap noted in Fig. 1; BDDCS Class 1 compounds, which represent 37.5% of classified drugs [17, 18], can be shown to be substrates of transporters, but these transporter effects are clinically insignificant. The disposition of the remaining 62.5% of classified drugs may be modified by transporters.

Similarly, the predictions for transporter effects for the highly metabolized Class 2 drugs, as depicted in Fig. 1, were based on observations and studies in our laboratory. The prediction that efflux transporter effects predominate in the gut was primarily based on the work of Dr. Carolyn Cummins [12, 15, 22–25]. These studies showed that when an extensively metabolized drug is a substrate for an efflux transporter, absorption of parent drug as well as the extent of intestinal metabolism are modified by efflux transporters. In particular, inhibition of efflux transporters has been shown to decrease the extent of intestinal metabolism, since efflux inhibition prevents a recycling mechanism that allows the drug

multiple times to access a metabolizing enzyme. However, we were unable to identify any uptake transporter effects in the intestine for BDDCS Class 2 drugs. The recognition of the potential importance of both uptake and efflux transporters in the liver was based on the previously referenced studies of Drs. Chi-Yuan Wu and Yvonne Lau [13–15]. The prediction that BDDCS Class 3 and Class 4 drugs would require an uptake transporter in the intestine was based on the recognition that these poorly metabolized, poorly permeable drugs would require uptake transport to achieve a relatively rapid rate of absorption, but then once absorbed, these drugs could be substrates for efflux transporters in the intestine as well as the liver.

In general, now with the BDDCS Classification expanded to more than 1,100 drugs and active metabolites (primarily active compounds from prodrugs) [17,18], the predictions in Fig. 1 and the relationship between intestinal permeability and metabolism hold up quite well, such that we do not know of any drugs predicted to not have a clinically relevant transporter effect (37.5% of drugs) that exhibit a relevant transporter effect in the clinic, thus leading to the potential drug-drug interaction (DDI) predictability of BDDCS. That is, DDIs will only be metabolic in the intestine and the liver for the BDDCS Class 1 drugs. For the Class 2 drugs, DDIs can include metabolic, efflux transporter and efflux transporter-enzyme interplay in the intestine, as well as metabolic, uptake transporter, efflux transporter and transporter-enzyme interplay in the liver. The primary DDIs for BDDCS Class 3 and 4 drugs will be uptake transporters, efflux transporters and uptake-efflux transporter interplay. However, we always caution that a simple four category system won't predict every interaction. BDDCS doesn't propose that every drug in a class will be substrates or not substrates for uptake and efflux transporters, rather, BDDCS serves to prioritize which interactions should be investigated, and in what order.

2.2 The Differences between BDDCS and BCS

"The major differences between BDDCS and BCS relate to their purpose and the measurement for classification, as previously presented in Table 1 [26]. The purpose of BCS is to characterize drugs for which products of those drugs may be eligible for a biowaiver of *in vivo* bioequivalence studies. The purpose of BDDCS is to predict drug disposition and potential drug-drug interactions in the intestine and the liver and potentially the kidney and brain. Both BCS and BDDCS use solubility as one of the two classification criteria. The solubility parameter utilized, referred to as 'US FDA solubility', estimates the ability of a drug at its highest dose strength to completely dissolve in 250mL of water over a pH range of between 1 and 6.8 at 37°C" [8]. (Note: with the universal use of plagiarism detection software by journals today, there is a great deal of unjustified concern about self-plagiarism even when the text indicates that the sources are earlier publications of the authors. Thus, rather than reword the same text to which we are referring, we have just included many quotation marks even though the wording may not be an exact quote due to the inclusion of attributions and references.) The volume parameter (250 mL) estimates the volume of a glass of water, ideally reflecting the minimum volume available for dissolution of a drug product in the stomach and intestine, while the pH range reflects the pH range a drug may encounter in the stomach and intestine prior to absorption. "For a drug to be considered highly soluble in both classification systems, the drug from its highest strength regulatory

approved dosage form must go completely into solution at its lowest solubility over this pH range in 250mL of water. As we have recently noted, US FDA solubility is a property of the drug in a formulation and is not an intrinsic property of the active pharmaceutical ingredient itself" [27]. There is no inherent accuracy to the 250ml value chosen for the FDA criterion. However, it seems to usefully differentiate Class 1 and Class 2 drugs in terms of their transporter susceptibility and thus we continued the BCS solubility criterion into BDDCS. "The second classification parameter and where the two systems differ, is related to intestinal permeability. In BDDCS, predictions are based on intestinal permeability rate, which was found to be related to the extent of drug metabolism. In BCS, biowaivers are based on the extent of intestinal absorption, which in a number of cases does not correlate with intestinal permeability rate" [8].

2.3 The Scientific Basis for BDDCS

As noted above, the BDDCS classification and the predictions from the 2005 paper [1] were based on observations and knowledge of pharmacokinetics and pharmacodynamics for drugs on the market. The recognition of the correlation between intestinal permeability rate and the extent of metabolism preceded an explanation for these findings. One might ask, Why should jejunal intestinal permeability rate in humans, the measurement from the initial human BCS studies, predict the extent of metabolism? and, Why should a kinetic measure, permeability rate, predict a thermodynamic outcome, extent of metabolism? We hypothesize now that high permeability rate compounds are readily reabsorbed through the kidney lumen and from the bile facilitating multiple accesses to the metabolic enzymes. This would be particularly important for metabolically eliminated drugs with a low hepatic clearance (e.g., diazepam and letrozole). The explanation for letrozole, previously presented [26], is repeated here, "For example, consider the BCS/BDDCS Class 1 drug letrozole. This completely oral available drug is primarily eliminated by CYP3A4 and CYP2A6 enzymatic processes with less than 4% of the dose excreted unchanged in the urine. Letrozole is only 60% bound to plasma proteins and thus it might be expected, based on glomerular filtration rate and fraction unbound, that renal clearance should approach 48mL/min. Yet, the total clearance of letrozole is only 45mL/min with less than 4% excreted unchanged. Thus, this high-permeability rate compound is reabsorbed in the kidney tubules (and possibly from the bile) with the major route of elimination being metabolic processes. The rationale for the correlation between intestinal permeability rate and the extent of metabolism appears to be based on the fact that high permeability rate compounds are reabsorbed from potential unchanged drug excretion routes in the body and thus can only be eliminated through metabolism."

One may also ask, "Why should solubility play such a prominent role in differentiating the effect of transporters between BDDCS Class 1 and Class 2 drugs?" As noted above, both BCS and BDDCS use solubility as one of the two classification criteria and that US FDA solubility is a property of the drug in a formulation and is not an intrinsic property of the actual pharmaceutical ingredient itself [27]. Similarly, Varma et al. [19] note that "Solubility is a fundamental principle for oral absorption as only drug in solution has the ability to permeate across enterocytes…however, it is not directly related to drug clearance." We emphasize again that the predictions in Fig. 1 concerning the importance of transporters for

BDDCS Class 2 drugs, versus BDDCS Class 1 drugs, were based on observation, not theory. As an explanation, we very recently noted [28] “For Class 1 compounds, which are highly soluble, highly permeable and extensively metabolized, the passive permeability at concentrations unrestricted by solubility appears to overwhelm any potential transporter effects. In this situation, Class 1 compounds may be substrates of transporters in cellular systems, but transporter effects will be clinically insignificant in the gut, liver or brain. For example verapamil was shown to be a substrate of Pglycoprotein (P-gp) in the MDCK-MDR1 cellular system, but it exhibits no clinically significant P-gp transporter effects in the intestine, liver and brain.” It has been suggested that given its high permeability, verapamil overloads the ATP-dependent transporter capacity of P-gp, effectively acting as a P-gp inhibitor [29]. Our latest thinking is that solubility is a characteristic of a drug substance that subsumes a number of individual characteristics that we and others have not yet been able to identify or quantify that are determinants of drug disposition. Our latest analyses suggest that a 100mg (or very slightly poorer, 50mg) in 250ml water over the pH range 1–6.8 adequately predicts BDDCS class, independent of highest approved dose strength. And that this pH range is important, so we would not reclassify acids that only fail the solubility criterion at pH 1, or suggest that a drug may be a different BDDCS class at a lower dosage. We emphasize again our belief that there is nothing magical about the 250ml volume and that for an NME, of course, it could be prohibitive to test 100mg. It is the ratio that appears defining. Thus, high solubility compounds would exhibit full solubility over the pH range 1–6.8 of ≥ 0.4mg/ml, while poor solubility compounds would exhibit solubilities < 0.4mg/ml.

3. BDDCS and The Rule of 5

3.1 Marketed Drugs’ Characteristics

“To facilitate use of the BDDCS system for making predictions for marketed drugs, in 2011 we compiled the BDDCS classification for 927 drugs, which included 30 active metabolites, primarily the active species from prodrugs” [17]. More recently, Hosey et al. [18] incorporated an additional 175 drugs into the system and amended the classification of 11 drugs from the previous compilation. Our analysis here evaluates the more than 1100 drugs compilation as amended, where we have excluded 14 Class 0 drugs (those drugs where the extent of metabolism and BDDCS class would change dependent upon urine pH), 31 active metabolites (when the active metabolite is not also marketed in a regulatory approved dosage form) and 11 drugs for which we could not confirm an approval history. Thus the Ro5-BDDCS analysis here was carried out on 1064 parent drugs, of which 852 drugs are administered orally. In our initial compilation [17] “where the lowest measured solubility was found in the literature, this value was reported. Measured values are also reported for the percentage excreted unchanged in the urine, LogP and LogD 7.4, when available” [17]. For the more than 1100 compounds in both compilations [17,18] we “determined the *in silico* parameters for predicted Log solubility in water (both dependent and independent of pH), calculated LogP, polar surface area, and the number of hydrogen bond acceptors and hydrogen bond donors for the active moiety, thereby allowing comparison between *in silico* and experimentally measured values. When comparing the *in silico* parameters across the four classes, the most distinct difference was noted between Class 2 and Class 3 compounds [17]. However, CLogP and *in silico* solubility parameters for the Class 1 drugs were found to

have intermediate values between those for Class 2 and Class 3 drugs, and not very different from the values for Class 4 drugs. We emphasized this failure of the *in silico* parameters to efficiently predict whether a drug could be Class 1, believed by many to be the most desirable because of high solubility and high permeability, versus Class 4 drugs that are low solubility and low permeability."

3.2. Analysis of BDDCS Compounds Using Ro5

Since the 2011 compilation [17] included the four Ro5 criteria for each of the parent drugs, and it was easy for us to determine the Ro5 criteria for the additional compilation [18], it is possible to evaluate these marketed drugs in terms of the Lipinski criteria. In Table 2 we list the results for the 852 drugs administered orally to achieve systemic concentrations and the 212 drugs not administered orally to achieve systemic concentrations (N = 1064 compounds as detailed above). As noted in Table 2, most compounds that exhibit 2 or more Ro5 violations are natural products or natural product derivatives (certainly true for non-oral drugs). Strictly speaking, Ro5 criteria are not applicable to those compounds, but they were included in this evaluation for the sake of completeness. Ro5 violations were determined for molecular weight > 500, CLogP >5.0, sum of nitrogen and oxygen (N, O) atoms > 10 and hydrogen bond donors > 5. For the 322 orally administered Class 1 compounds, 35 exhibited a single Ro5 violation, five (bromocriptine mesylate, dabigitran etexilate, fosinapril, olmesartan medoxomil [all four essentially prodrugs] and reserpine) exhibited two Ro5 violations, and four drugs (acarbose, cyanocobalamin, everolimus and ivermectin) exhibited three Ro5 violations. As noted above, Lipinski's Ro5 was developed to set "drugability" guidelines from an oral bioavailability perspective [6] for small molecules [3] and suggested that when a compound exhibited two or more Ro5 violations the compound would exhibit poor solubility and/or poor permeability. Thus, 9 of the 322 approved BDDCS Class 1 orally administered drugs (2.8%) would have been predicted to exhibit poor drugability. As noted above, when the Ro5 was developed, Lipinski specifically stated that the Ro5 only holds for compounds that are not substrates for active transporters [2,3] and little information was available concerning drug transporters at that time. As noted in Fig. 1, transporter effects are minimal in the gut and liver and clinically insignificant for BDDCS Class 1 compounds. Thus, it is not surprising, that the Ro5 performs so well for class 1 compounds, at least for drugs on the market. Furthermore, 6 of the 9 Ro5 violation drugs, bromocriptine (an ergot alkaloid derivative), reserpine (a rauwolfia alkaloid), acarbose and everolimus (both microbial products), ivermectin (derived from a fungi product) and cyanocobalamin (vitamin B12) are natural products or derivatives, and are specifically excluded from the Ro5 criteria. Only the three remaining prodrugs are chemically synthesized small molecule oral BDDCS Class 1 violators.

However, even for Class 2 through 4 BDDCS compounds, the Ro5 performs quite well for orally administered drugs, with only a slightly better predictability for the highly soluble Class 3 drugs versus the poorly soluble Class 2 and Class 4 BDDCS drugs. Thus, even when transporters may mediate the absorption of compounds (for some class 2 drugs and presumably all class 3 and 4 drugs), the Rule of 5 can predict oral absorption. Just because a drug is a substrate for a transporter does not mean that it will fall outside of the Rule of 5, rather, a drug that falls outside of the Rule of 5 and is orally available is assumed to be a

substrate for a transporter. Highly soluble, highly permeable predicted to be metabolized Class 1 drugs appear to require adherence to Rule of 5 to be absorbed, while classes 2–4 may "break" the Rule of 5 and still be absorbed. However, orally administered compounds, even those that are substrates for transporters, rarely break Rule of 5. This is in marked contrast to Ro5 violations of two or more for all of the non-oral drugs, independent of BDDCS class, as noted in Table 2. This is logical, since many of these drugs are formulated for non-oral delivery because of difficulties of membrane permeability and solubility.

3.3. The Rule of Five: Cross- and Temporal Validation

Subtracting the 73 non-oral drugs exhibiting 2 or 3 Ro5 violations from the total 212 in Table 2, suggests that 139 (65.6%) of the non-oral drugs should have been orally available according to the Ro5. Of the 139, 118 compounds are high solubility (i.e., BDDCS class 1 or class 3), which suggests the possibility that some of these compounds could have been orally formulated if the sponsor had chosen to do so. An additional 67 (7.9%) of the 852 orally formulated drugs (Table 2) violate 2 or 3 of the Ro5 criteria; however, since most of these are natural products or natural product derivatives, Ro5 criteria would have not been applicable. This "cross-validation" analysis, far from diminishing the importance of the Rule of Five, substantiates the observation that, in drug discovery, no rule is absolute.

To temporally validate the Rule of Five, we evaluated Ro5 parameters for orally dosed drugs approved before and after (661 and 191, respectively) the first Ro5 paper was published (December 1997) as presented in Table 3. For the 191 orally dosed drugs approved after 1997 almost three times (2.8 fold) as many violated 2 or more Ro5 parameters as for the 661 orally dosed drugs approved before the Ro5 publications (for all classes 5.6% before and 15.7% after). This more than doubling was found for both the Class 1 and Class 2 drugs, although as previously noted in Table 2, the Class 1 drugs, those for which clinically relevant transporter effects are not expected, exhibited the fewest violations for metabolized compounds. In contrast to the 2.8 fold differentiation seen with orally dosed drugs, approved non-orally dosed drugs in all classes exhibited only a slightly greater percentage of Ro5 violations (all classes 32.4% before and 43.6% after). We add the caution previously raised that we only have data available for drugs that successfully received regulatory approval, and not the full range of new molecular entities examined by the industry.

3.4. Is There A Need to Update Ro5 Criteria?

The results for the two or more Ro5 violations for the 852 orally dosed drugs as presented in Table 2 suggest that the originally proposed criteria function quite well, especially for the BDDCS Class 1 drugs where little clinical relevance for transporters is observed. A few outlier categorizations can be seen. The first, for large molecular weight cyclic peptide immunosuppressants (i.e., cyclosporine, sirolimus, tacrolimus and everolimus) has been well recognized. Macrolide antibiotics (e.g. clarithromycin, erythromycin and roxithromycin) are also observed to show higher violation numbers. Once again, we note that the cyclic peptide immunosuppressants and the macrolide antibiotics are (for the most part) natural products and, as such, are not subject to Ro5 criteria. There is also a strong tendency for HIV protease inhibitors (e.g., atazanavir, lopinavir, nelfinavir and tipranavir) and tyrosine kinase inhibitors (e.g. lapatinib and nilotinib) to show this variance. Very recently, Leeson reported in this

journal [30] that 14 of 19 (74%) HIV protease inhibitors and HCV drugs exhibited 2 or more Ro5 violations. We concur with the extent reported by Leeson. Among 12 HIV protease inhibitors we find that 7 exhibit two Ro5 violations, one (atazanavir) exhibit three violations and the remaining four exhibit one Ro5 violation. We include 9 HCV drugs in our listing, 8 of which exhibit 2 or 3 violations. Thus, 16 of the 21 (76%) HIV protease inhibitors and HCV drugs exhibit two or more Ro5 violations. This prevalence of HIV protease inhibitors and HCV drugs in the listing of drugs approved after the Ro5 publications reflects in part the increased percentages noted in Table 3.

It is obvious from the listings of the 661 oral drugs that came to the market prior to the publication of the Lipinski Ro5 papers [2, 3] that the criteria proposed did an outstanding job of characterizing oral drugability, especially considering that a large fraction of the Ro5 violators are natural products or natural product derivatives. This is true in spite of the fact that other definitions of the Ro5 criteria may be employed. For example, with respect to number of H-bond acceptors, is the ester oxygen an acceptor in C(=O)OR? What about the nitrogen in an amide, is that an acceptor? In essence, no to both questions, but because there is an occasional X-ray where this can be observed, there could be some disagreement. Furthermore, using the actual publication date of the Ro5 papers as the before/after divisor, does not consider that many approved drugs after the Ro5 publications could not have been influenced by Ro5. We only use this arbitrary time point to note that significantly more Ro5 violations are observed after the publication. In other words, the Ro5 papers did an excellent job of subsuming the characteristics inherent in good drugability at the time they were published. Subsequently, less emphasis on these Ro5 criteria is observed, yet still showing good drugability characteristics. We have already noted above the various classes of drugs exhibiting marked Ro5 relations and also note that the increased prevalence of prodrugs has been incorporated in drug discovery and development to overcome these Ro5 criteria.

There appears to be no differentiation in metabolic pathways and clearance patterns when small molecules enter into the beyond Ro5 chemical space. In addition, first pass gut metabolism appears to be of equal importance for oral drugs in all of the categories listed in Table 2, where metabolism is significant. Thus, we believe Ro5 criteria function as well today in defining drugability from an ADMET criterion as they did when first proposed by Lipinski and coworkers. As stated above, there is no competition between Ro5 and BDDCS; the purpose of BDDCS is to define and predict the disposition characteristics of NMEs whether they meet or violate Ro5 criteria.

4. Use of BDDCS to Predict NME Drug Disposition Characteristics

4.1 Metabolism versus Excretion of Unchanged Drug in the Urine and Bile as the Major Elimination Route for an NME in Humans

The major, but simple, discovery from the BDDCS was the recognition that the jejunal intestinal permeability rate could differentiate metabolism versus excretion of unchanged drug as the primary route of elimination of an NME in humans [1]. It was then shown that *in vitro* permeability rate measures of an NME in cellular systems and even in non-biological membranes such as PAMPA would allow this prediction to be made before the NME had ever been dosed to animals or humans. Hosey and Benet [31] showed that depending on the

system studied, the best differentiation between metabolized (BDDCS class 1 and 2) versus biliary and renal eliminated drugs (BDDCS class 3 and 4) would be obtained using labetalol as the reference compound when *in vitro* permeability rates were measured in the Caco-2 system, zidovudine when *in vitro* permeability rates were measured in the MDCK cell line and theophylline when the permeability rates were determined in the PAMPA cell line.

The performance measures for these reference compounds in the various permeability systems are presented in Table 4, taken from Hosey and Benet [31]. Sensitivity is a measure of the fraction of the drugs eliminated primarily by metabolism (BDDCS class 1 or 2) that are correctly predicted in the three systems. Specificity is a measure of the fraction of the drugs eliminated unchanged in the urine and bile (BDDCS class 3 or 4) that are correctly predicted by the three systems. Positive predictive value is a measure of the fraction of drugs that the three systems predict to be eliminated primarily by metabolism (by a high *in vitro* permeability rate) that are, in fact, eliminated by this mechanism while negative predictive value is a measure of the fraction of the drugs predicted to be eliminated primarily by biliary and renal excretion (by a low *in vitro* permeability rate) that are, in fact, eliminated by these processes.

However, measures of permeability in cellular and artificial membrane systems require experimental studies and it would be beneficial if such predictions could be based on *in silico* calculations. Table 5 from Hosey and Benet [31] provides a comparison between *in vitro* models and *in silico* predictions. Column 2 compares the ability to predict extensive metabolism versus elimination of unchanged drug in the three model systems, all exhibiting receiver operating curve areas greater than 0.9 versus evaluation using three *in silico* permeability rate models: ADMET Predictor MDCK [32], ADMET Predictor P_{eff} [32] and VolSurf+ Caco-2 [33]. In our opinion, at present these three *in silico* predictors of elimination by metabolism versus elimination as unchanged drug are not sufficiently accurate.

If we can compare the success of the Ro5 *in silico* parameters to predict drugability of approved orally administered drugs in Table 2 with the receiver operating curve measures in column 2 of Table 5 to differentiate metabolism versus non metabolism as the major route of elimination, one could suggest that the *in silico* drugability predictability is better. However, as we noted above, we only have the ability to evaluate drugability success for drugs that have been approved, i.e., by definition successful. As early as 2000, we showed that over 82% of the non-drug (chemically filtered) subset of molecules from the Available Chemicals Directory (over 123,000 molecules) were Ro5 compliant [6]. As we wrote, “The ‘rule of 5’ test cannot be used to discriminate between ‘drugs’ and ‘nondrugs’. This inability is due to the lack of significant differences in the distribution of the four properties examined in this test, i.e. MW, CLOGP, H-bond donors and H-bond acceptors.” [6] Thus, Ro5 is not an intrinsic metric for drugability, and it is not recommended as a tool to “enrich” a set of chemicals with drug-like entities from a random set of molecules, except with respect to oral bioavailability potential.

4.2 Extension of BDDCS to Predict Specific Transporter Effects and Quantitative Clearance Estimates

Ro5 criteria are designed to estimate the upper bounds in defining the pharmacokinetic space for oral dosing. BDDCS expands upon the Ro5 criteria to qualitatively predict route of elimination, the relevance of transporters and enzymes and potential DDIs. However, neither the Ro5 nor BDDCS provide quantitative predictions.

Recently, Camenisch and coworkers [34–36] have proposed an Extended Clearance Concept Classification System (ECCCS) to identify the rate-determinant hepatic clearance step for an NME and then provide a quantitative prediction of *in vivo* hepatic clearance. Varma et al. [19] also proposed an Extended Clearance Classification System (ECCS) to predict clearance mechanisms early in drug discovery. Furthermore, Sugiyama and coworkers propose an *in silico* classification method designated as CPathPred to predict the major clearance pathways of drugs [37, 38]. All of the above methods provide valuable insight into predictions of hepatic clearance both in terms of pathways and some for quantitative estimations. In contrast, BDDCS is not limited to predicting hepatic clearance only (this is true for Ro5 also), but a number of the advances noted in these further classification systems [19, 34–38], can be incorporated into BDDCS. We note, however, that Ro5 was not intended, nor used, for predicting drug clearance.

All three of the groups referenced above recognize that high molecular weight (>400) acids and zwitterions exhibit rate limited hepatic clearance as a function of OATP uptake into the liver, that is specifically designated as a separate class by Varma et al. [19]. This appears to be true for high permeability rate compounds that are metabolized such as atorvastatin and glyburide (BDDCS Class 2 compounds) and low permeability compounds that are excreted unchanged in the bile like valsartan and cefoperazone (BDDCS Class 4 and 3 compounds, respectively), but as we noted earlier this appears not to be true for BDDCS Class 1 compounds such as fluvastatin.

Thus, we would add to our BDDCS recommendations that all Class 2, 3 and 4 compounds with molecular weights >400 be tested for hepatic uptake by OATPs as being the rate limiting step for elimination of these compounds whether they are primarily metabolized or excreted unchanged in the bile. We suggest all MW>400 BDDCS Class 2, 3 and 4 compounds (but possibly excluding cations) since in the supplemental material for the Varma et al. [19] paper the authors report that 26% are neutral compounds, 60% are acids, 9% are zwitterions and 5% are cations. We note that we list olmesartan medoxomil as a BDDCS Class 1 drug [17]. This is not an error in classification since BDDCS designations are based on the drug formula (moiety) in the approved drug product. Olmesartan medoxomil is an ester prodrug that is metabolized to the acid form, which is then rate limited in its elimination by hepatic OATP uptake. In BDDCS, olmesartan as the acid form would be listed as a metabolite.

4.3 Renal versus Biliary Elimination of Unchanged Drug as the Major Excretion Pathway

The extended clearance methodologies listed above [19, 34–38] propose further usage of physicochemical and *in silico* characteristics such as molecular weight, lipophilicity,

ionization state, and protein binding to further differentiate the major clearance mechanisms. We do not find these proposals to provide significant advantages over BDDCS. For example, we believe that ECCS [19] is too limited and has many more exceptions than BDDCS. For example, no drugs with MW>700 are considered, the system does not predict the importance of gut metabolism or disposition of prodrugs, ionization state is given more significance than justified and biliary excretion is not addressed except for drugs rate limited by hepatic uptake. We also find the list of compounds testing predictability in CPathPred [38] to be limited. As noted in Table 5, the *in vitro* model systems (determining permeability rate) perform quite well in differentiating extensive metabolism versus renal elimination and somewhat poorer, but acceptable in differentiating extensive metabolism versus biliary elimination, but are not successful at all in differentiating biliary versus renal elimination. Again, the *in silico* models (for permeability rate) are even less successful in differentiating metabolism from renal elimination and almost comparable to *in vitro* measures in differentiating metabolism and renal elimination from biliary elimination. Since transporters play a role in biliary elimination, we further suggest that these computed properties contribute little, if anything, in evaluating transporter effects.

We are concerned that the *in silico* methodologies being utilized have not adequately addressed biliary excretion of unchanged drug as an important disposition characteristic. Hosey et al. [39] has addressed this issue and proposed a classification scheme to differentiate renal and biliary elimination based on polarizability and predicted metabolic stability. We note that it is quite difficult to differentiate drug molecules that are primarily eliminated by metabolism from those that are excreted primarily via biliary excretion based only upon *in silico* characteristics. Here we present in Table 6, a listing of 20 drugs eliminated primarily by metabolism versus 20 drugs eliminated primarily via biliary excretion that cannot be differentiated based only on molecular weight, ionization state and CLogP. We believe that the proposed clearance concept methodologies [19, 34–38] need to include these 40 compounds in their validation tests. Reviewing the compounds utilized by Varma et al. [19] and by Toshimoto et al. [38] less than 10% of these drugs are included.

5. The BDDCS Extensions

5.1 Metabolism as a Biowaiver Criterion

Since the BDDCS was proposed in 2005 [1], a number of extensions, providing new insights and predictions have been proposed. The excellent correlation between the high extent of absorption and the extent of metabolism led a number of experts in the field to recommend the use of BDDCS in classifying the permeability of marketed drugs using measures of the extent of metabolism following systemic absorption as justifying how much of the drug was absorbed [40]. This proposal was accepted by the EMA in their 2010 Guideline [41] and more recently by the FDA [8]. Ro5 does not incorporate criteria relevant to metabolism.

5.2 Food Effects

BDDCS also has a food effect extension. The exposure and “bioavailability of many drugs are greatly affected by concomitant food intake. Many factors are believed to contribute to these food effects, including changes in gastric emptying time, bile flow, pH of the intestine,

splanchnic blood flow and gut wall metabolism. Different degrees of evidence support food effect on transporters", as described by Custodio et al. [42], with Class 1 drugs generally showing no effect of high fat meals on the extent of absorption, Class 2 drugs generally showing an increase in the extent of absorption, and Class 3 drugs generally exhibiting a decrease in the extent of absorption. However, these general trends are only accurate approximately for 70% of drugs evaluated, and do not preclude the need to test the effect of food on the extent of absorption in humans for the to-be-marketed final dosage form [8, 42].

5.3 Uremic Toxins and Changes in Metabolism with Renal Failure

BDDCS extensions have also led to explanations related to drug disposition. Changes in the elimination of drugs where disposition is overwhelmingly due to metabolism were observed in a number of studies in patients with renal failure. Previously, this had been thought to be primarily due to product inhibition, i.e., the formed metabolite competes with the parent drug for metabolism, or the "effects of uremic toxins as either potential inhibitors or down regulators of metabolic enzymes. However, these hypotheses could be tested *in vitro* and were shown not to occur in many cases. We recognized that previously unexplained effects of renal disease on hepatic metabolism could result from accumulation of substances, such as uremic toxins, in renal failure that modify hepatic uptake and efflux transporters" [43–45]. "This mechanism could explain why BDDCS Class 2 drugs could demonstrate changes in metabolism in renal failure, whereas this would not be observed for BDDCS Class 1 drugs when *in vitro* uremic toxins did not alter microsomal metabolism. Inhibition of hepatic uptake by uremic toxins could also increase the exposure of BDDCS Class 3 and 4 drugs", as we demonstrated with erythromycin [46].

5.4 Central Effects and Brain Efflux Transporters

BDDCS may also predict when central effects may or may not occur for new molecular entities. P-gp has the potential to modify brain concentrations and it has been hypothesized that for a drug to successfully penetrate and achieve pharmacodynamic effects in the brain, the drug should not be a P-gp substrate. However, we demonstrated that highly permeable-extensively metabolized, highly soluble (BDDCS Class 1) compounds can have a central effect at clinically approved doses, even if the drug is a good substrate for P-gp *in vitro*, regardless of whether the effect is desired or not [47]. We recently further addressed this issue examining the reliability of *in vitro* and *in vivo* methods for predicting the effect of P-gp on the delivery of antidepressants to the brain [28].

5.5 Flip-Flop Pharmacokinetics

As noted in Fig. 1, BDDCS Class 3 and 4 drugs require an uptake transporter in the intestine to achieve clinically meaningful systemic concentrations. For drugs with short disposition half-lives, the rate of this uptake process would be expected to be relatively slow. Thus, we suspected that drugs exhibiting flipflop kinetics (i.e., where the absorption half-life is longer than the elimination half-life) would predominantly be BDDCS Class 3 and 4 drugs, those requiring an uptake transporter, as we have recently shown [48].

5.6 Toxicity and Environmental Predictions

BDDCS extensions have begun to appear related to toxicity predictions and environmental implications. Broccatelli et al. [49] used the BDDCS to help consider for which drugs hERG voltage-gated potassium channel inhibition is likely to lead to Torsade de Pointes. Vuppalanchi et al. [50] and we [51] utilized BDDCS in evaluating drug induced liver injury, while our laboratory has also proposed the use of BDDCS to predict which anti-epileptic drugs will cause drug hypersensitivity reactions [52]. Finally, Daughton [53] has suggested that BDDCS could be used in attempts to decrease environmental exposure of active pharmaceutical ingredients.

6. Conclusions

The Ro5 methodology appears to be as useful today in defining therapeutically relevant pharmacokinetic drugability as when it was proposed, but recognizing that the database that we evaluated includes only drugs that successfully reached the market. As shown earlier, Ro5 fails to discriminate drugs from “non-drugs”. From our perspective, we do not view additional criteria to be necessary or find significant deficiencies in the four Ro5 criteria originally proposed by Lipinski and coworkers [2,3]. BDDCS builds upon the Ro5 criteria and can quite successfully predict drug disposition characteristics for drugs both meeting and not meeting Ro5 criteria. More recent expansions of classification systems have been proposed and do provide useful qualitative and quantitative predictions for clearance relationships. However, the broad range of applicability of BDDCS beyond just clearance predictions gives a great deal of further usefulness for this system.

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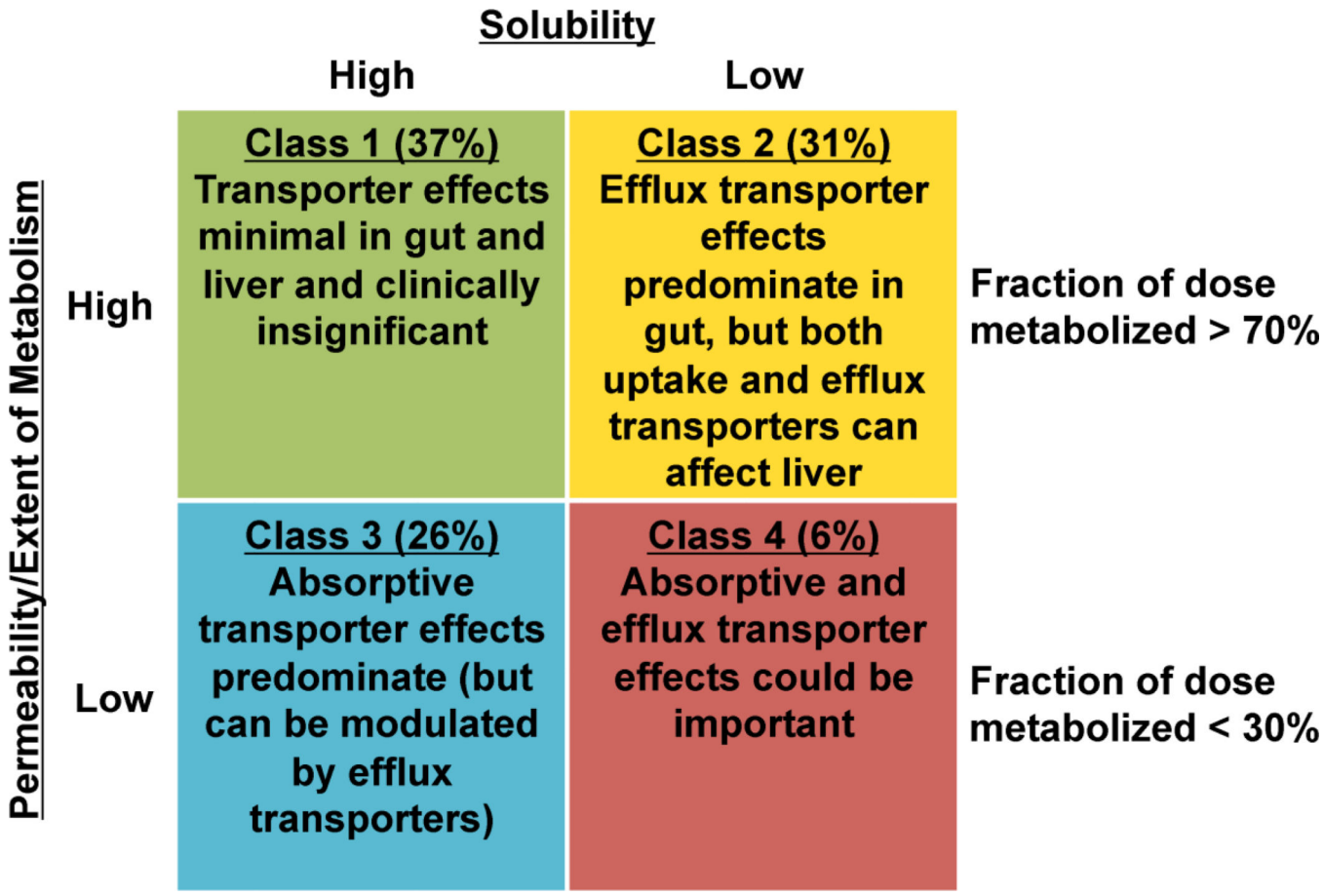

Figure 1.
BDDCS Classes for Orally Dosed Drugs, Percentage of Drugs in Each Class and Prediction of Transporter Effects [16]

Table 1

Major Differences Between BDDCS and BCS

BDDCS	BCS
Purpose	
Predicting drug disposition and drug-drug interactions in the intestine and liver	Facilitate biowaivers of *in vivo* bioequivalence studies
Criterion	
Predictions are based on intestinal permeability rate	Biowaivers are based on extent of intestinal absorption (permeability), which in a number of cases does not correlate with rate of jejunal permeability.

Table 2

Rule-of-5 Violations by BDDCS Class for 852 Orally Dosed Drugs and 212 Non-orally Dosed Drugs Compiled by Benet and co-workers [17, 18]

ORAL DRUGS						
		Number of Drugs with Ro5 violations			% of Drugs with 2 or 3 Violations	Comments with Respect to Natural Products (NPs)
BDDCS CLASS	TOTAL NUMBER	1	2	3		
1	322	35	5	4	2.8%	6 of 9 "2 or 3-rule violators" are NPs or NP derivatives
2	296	59	26	8	11.5%	3 of 8 “3-rule violators" are NPs or NP derivatives
3	175	13	10	6	9.1%	12 of 16 "2 or 3 rule-violators" are NPs or NP derivations
4	59	5	4	4	13.6%	3 of 8 "2 or 3-violators" are NPs
NON ORAL DRUGS						
1	77	6	2	18	26.0%	17 of 20 "2 or 3-violators" are NPs or NP derivatives
2	28	7	6	4	35.7%	7 of 10 "2 or 3-violators" are NPs or NP derivatives
3	101	18	25	15	39.6%	10 of 15 “3-rule violators" are NPs or NP derivatives
4	6	0	1	2	50.0%	All “2 or 3-rule violators" are NPs

Table 3

Rule-of-5 Violations for Oral and Non-orally Dosed Drugs Approved Before and After Publication of the Rule-of-5

	BEFORE					AFTER				
						Oral drugs				
		Number of Drugs with Ro5 Violations			% of Drugs with 2 or 3 Violations		Number of Drugs with Ro5 Violations			% of Drugs with 2 or 3 Violations
BDDCS Class	**Total**	**1**	**2**	**3**		**Total**	**1**	**2**	**3**	
1	271	27	3	3	2.2%	51	8	2	1	5.9%
2	203	44	13	4	7.9%	93	15	13	4	18.3%
3	141	11	7	5	8.5%	34	2	3	1	11.8%
4	46	4	2	0	4.3%	13	1	2	4	46.2%
All Classes	661	86	25	12	5.6%	191	26	20	10	15.7%
						Non Oral Drugs				
1	66	4	2	16	27.3%	11	0	0	2	18.2%
2	18	5	2	2	22.2%	10	2	4	2	60.0%
3	86	17	22	11	38.3%	15	1	3	4	46.7%
4	3	0	0	1	33.3%	3	0	1	1	66.7%
All Classes	173	26	26	30	32.4%	39	3	8	9	43.6%

Table 4

Performance Measures of the Best Reference Compounds for Experimental *in vitro* Permeability Rates to Differentiate Extensively and Poorly Metabolized Drugs [32]

	System/Reference Compound		
Performance Measure[a]	**Caco-2/Labetalol**	**MDCK/Zidovudine**	**PAMPA/Theophylline**
Sensitivity	0.83 ± 0.13	0.90 ± 0.09	0.88 ± 0.11
Specificity	0.87 ± 0.03	0.81 ± 0.13	0.81 ± 0.24
Positive Predictive Value	0.92 ± 0.02	0.90 ± 0.04	0.90 ± 0.12
Negative Predictive Value	0.77 ± 0.11	0.85 ± 0.14	0.81 ± 0.10

[a]Sensitivity represents the proportion of all extensively metabolized drugs correctly predicted by high permeability rate; Specificity represents the proportion of all poorly metabolized drugs correctly predicted by low permeability rate; Positive predictive value represents the proportion of high permeability rate drugs that are extensively metabolized; Positive predictive value represents the proportion of poor permeability rate drugs that are poorly metabolized

Table 5

Area Under the Receiver Operating Curve for Bootstrapped Sampling of Measured or Predicted Permeability Rate as a Predictor of Extensively Metabolized and Poorly Metabolized Drugs Eliminated Primarily as Unchanged Drug in Either the Bile or Urine. From Hosey and Benet [32].

	Extensive Metabolism vs			
***In vitro* Model**	**Elimination as unchanged drug (N)**	**Renal elimination of unchanged drug (N)**	**Biliary elimination of unchanged drug (N)**	**Biliary vs Renal**
Caco2	0.93 ± 0.07 (11)	0.90 ± 0.11 (11)	0.82 (1)	0.53 (1)
MDCK	0.91 ± 0.03 (5)	0.95 ± 0.02 (5)	0.89 (1)	0.53 (1)
PAMPA	0.93 ± 0.05 (6)	0.95 ± 0.04 (6)	---	0.71 (1)
***In silico* Model**				
AP MDCK	0.78 ± 0.03	0.82 ± 0.04	0.81 ± 0.05	0.56 ± 0.09
AP Peff	0.74 ± 0.03	0.76 ± 0.04	0.69 ± 0.07	0.58 ± 0.09
VS+ CACO2	0.82 ± 0.03	0.81 ± 0.07	0.87 ± 0.03	0.61 ± 0.10

(N) represents the number of datasets
AP: ADMET Predictor
VS+: VolSurf

Table 6

Drugs (20 each) Where the Major Route of Elimination is via Metabolism or via Biliary Excretion

Metabolism[a]	Biliary Excretion	Reference
Amlodipine	Aliskiren	[54]
Benazapril	Alvimopan	Product Information
Budesonide	Atovaquone	[55]
Carvedilol	Azithromycin	[56]
Clindamycin Hydrochloride Hydrate	Ceftriaxone	[57]
Darunavir	Celiprolol	[58]
Ezetimibe	Colchicine	[59]
Domperidone	Digitoxin	[60]
Fluvastatin Sodium	Eprosartan	[61]
Indinavir Sulfate	Erythromycin (Base)	[62]
Ramipril	Fexofenadine	[63–65], Product Information
Rifabutin	Irbesartan	[61]
Rifampin	Moxifloxacin Hydrochloride	[66]
Risperidone	Orlistat	[67]
Tamsulosin	Pirenzepine	[68]
Telithromycin	Rifaximin	[69, 70]
Terazosin	Rosuvastatin Calcium	[62]
Triamcinolone Acetonide	Roxithromycin	[71]
Vardenafil	Telmisartan	[61]
Verapamil Hydrochloride	Valsartan	[61]

[a]Documentation that metabolism is the major route of elimination for the 20 drugs can be found in the drug label and product information for each compound.