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Review

Oral Druggable Space beyond the Rule of 5: Insights from Drugs and Clinical Candidates

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The rule of 5 (Ro5) is a set of in silico guidelines applied to drug discovery to prioritize compounds with an increased likelihood of high oral absorption. It has been influential in reducing attrition due to poor pharmacokinetics over the last 15 years. However, strict reliance on the Ro5 may have resulted in lost opportunities, particularly for difficult targets. To identify opportunities for oral drug discovery beyond the Ro5 (bRo5), we have comprehensively analyzed drugs and clinical candidates with molecular weight (MW) > 500 Da. We conclude that oral drugs are found far bRo5 and properties such as intramolecular hydrogen bonding, macrocyclization, dosage, and formulations can be used to improve bRo5 bioavailability. Natural products and structure-based design, often from peptidic leads, are key sources for oral bRo5 drugs. These insights should help guide the design of oral drugs in bRo5 space, which is of particular interest for difficult targets.

In spite of huge advances in science and technology, research and development (R&D) costs per new molecular entity have increased dramatically in preceding decades (Bunnage, 2011; Kinch et al., 2014; Paul et al., 2010). This decline in efficiency has been attributed to a number of underlying problems that have affected the pharmaceutical industry during this time frame, as recently reviewed (Scannell et al., 2012). Hence, an increasing number of therapeutic indications are now managed with established drugs, driving R&D toward complex, hard-to-treat diseases. In addition, regulatory agencies are less tolerant to risk, in particular for diseases in which safe drugs already exist. This translates into more costly and lengthier clinical trials for broad indications due to the larger patient populations required, which has induced several companies to focus on narrow indications or diseases currently without therapeutics. Overestimation of the ability of new technologies to impact drug discovery and the trend for ever-increasing resources were also identified as contributing to declining R&D efficiency. As a potential consequence of some of these problems, the quality of target selection has been poor, contributing to low success rates in phase II due to lack of efficacy (Bunnage, 2011). While success rates related to target selection remain to be improved, compound attrition due to poor bioavailability and pharmacokinetics (PK) has undergone significant improvements. In 1991, attrition due to poor bioavailability and PK was responsible for 39% of compounds being halted in clinical studies (Kola and Landis, 2004). This led to an altered perception of the importance of investigating PK in early drug discovery and formulation of the rule of 5 (Ro5), also known as Lipinski's rules, for in silico assessment of PK (Lipinski et al., 1997). By 2000, attrition due to PK had already been reduced to 8%, whereas attrition due to toxicity had increased to 19% and the lack of efficacy remained relatively constant at 24% (30% in 1991) (Kola and Landis, 2004). An analysis of attrition in the last decade revealed similar rates and reasons for attrition as in 2000 (Hay et al., 2014). Despite this reduction of attrition originating from poor PK, a number of commentaries questioning the strict implementation of the Ro5 have emerged, arguing that this may have resulted in lost opportunities (Abad-Zapatero, 2007; Walters, 2012; Zhang and Wilkinson, 2007). In order to examine opportunities for discovery of oral and cell-permeable drugs beyond the Ro5 (bRo5), we have performed an extensive analysis of all known drugs and clinical candidates in this chemical space. Our analysis is focused on how oral bioavailability bRo5 is related to the physicochemical properties and in vitro cell permeability of compounds in this space. We have chosen not to include discussions of potency, or related metrics such as ligand efficiency (LE) (Hann and Keserü, 2012), to maintain focus on bRo5 PK within the context of this review, although it is worth mentioning that all compounds discussed here displayed sufficient potency to be taken into the clinic or to be marketed as drugs.

Oral Drug Delivery

Oral delivery has many advantages in comparison to other noninvasive delivery methods, such as buccal, nasal, and transdermal, when systemic exposure of a drug is desired. These include increased reliability of exposure, ability to deliver large variations in dosage, and potentially greater stability in storage of solid forms compared with liquids and suspensions (Smith, 2010). Intracellular targets also require cell permeability, which is expected for orally absorbed drugs. Oral drug delivery is also the patient preferred method of drug delivery; for instance, it is preferred by 54%–89% of oncology patients (Verbrugghe et al., 2013). In particular, for chronic diseases, quality of life for patients is increased dramatically compared with intravenous (i.v.) therapy due to the ability to noninvasively self-administer at home (O'Neill and Twelves, 2002). It should be pointed out that in oncology, where i.v. administration predominates, 70%–74% of patients would not compromise on efficacy for oral dosage (Liu et al., 1997). While this may be an extreme scenario due to the terminal nature of the illness, it can be expected that there is a tradeoff between convenience of delivery and efficacy in other

therapeutic areas. Systemic oral dosage, however, remains the preferred delivery method for the vast majority of drug therapies, and oral bioavailability represents a major hurdle in drug development (Thomas et al., 2006).

Absorption after Oral Dosage

Systemic oral dosage requires compound properties that allow for dissolution and stability in the gastrointestinal (GI) tract, including the acidic environment of the stomach (pH 1–2 in fasted state, 3–7 in fed state) and the close to neutral environment (pH 4.4–6.6) of the small intestine (Kerns and Di, 2008; Smith, 2010; Smith et al., 2006). The majority of compounds are absorbed in the first section of the small intestine, called duodenum, by specialized epithelial cells, enterocytes. Absorption can be separated into three categories, paracellular (between enterocytes), transcellular (passive diffusion through enterocytes), and active transport (utilizing transporter proteins) (Artursson et al., 2012; Smith, 2010). Paracellular absorption is limited to small hydrophilic compounds having molecular weight (MW) < 350 Da and a low lipophilicity (LogD(7.4) < 0), which can pass through the restrictions of tight junctions between enterocytes. Active uptake is also known to occur for only a small proportion of drugs (DeGorter et al., 2012; Giacomini et al., 2010; Sugano et al., 2010; Varma et al., 2010), requiring recognition by native transporter proteins. Hence, active uptake is usually limited to analogs of peptides, amino acid, lipids, and sugars.

Transcellular transport is the most common route for drug absorption and requires passive diffusion through the apical membrane, facing the GI tract, into the enterocyte, followed by diffusion across the cell and through the basolateral membrane into the blood. Enterocytes have a number of efflux transporters such as P-glycoprotein (Pgp) (Lin and Yamazaki, 2003) that actively transport compounds from the blood into the enterocyte or enterocyte into the GI tract (DeGorter et al., 2012; Giacomini et al., 2010). Throughout this absorptive process, enzymes in the GI tract, enterocyte, blood, and lymph may metabolize drugs to less active and less permeable species. Once in the blood, drugs travel via the portal vein to the liver and undergo hepatic metabolism before reaching the systemic circulatory system (Smith et al., 2006). The overall metabolism during absorption and the first liver passage is referred to as "first-pass metabolism"; hence, oral bioavailability (F%) constitutes the fraction of the oral dose that reaches the systemic circulation after absorption and first-pass metabolism as compared with i.v. administration.

Passive diffusion through a cell membrane is a key element of transcellular absorption. Compounds must be able to partition into the membrane after desolvation from the polar aqueous GI tract and then diffuse across the nonpolar membrane and resolvate upon exit at the other side. This process is difficult to study in vivo but can be assessed using in vitro model systems. The most widely used model is human colorectal carcinoma (Caco-2) cells grown in a layer that allows measurement of permeability (P_{app}) in the apical to basolateral (AB) direction (Artursson et al., 2012). Caco-2 cells also express a number of endogenous transporters, and measuring P_{app} in the basolateral to apical (BA) direction allows for calculation of the efflux ratio (ER = P_{app}BA/P_{app}AB), representing the involvement of efflux transporters (Zhang et al., 2003). Madin-Darby canine kidney cell systems (Irvine et al., 1999) and the parallel artificial membrane permeability assay (Kansy et al., 1998) are also used for assessing passive permeability. The three methods provide somewhat different permeabilities (Nozinic et al., 2010) and have different advantages and disadvantages (Avdeef, 2012; Balimane et al., 2006).

Influence of Physicochemical Properties on Oral Absorption

The processes of desolvation, diffusion, and resolvation required for passive permeability across a cell membrane are function of a few fundamental properties of a compound: size, polarity, lipophilicity, and conformational dynamics (Guimarães et al., 2012; Smith, 2010). In order for a compound to be orally bioavailable, these properties need to be balanced; for example, highly polar compounds fail to desolvate and enter the membrane, whereas highly lipophilic compounds may not dissolve in the GI tract or fail to partition out of the membrane (Wils et al., 1994; Yang et al., 2012). Absorption is thus a complex process, and in 1997, Lipinski et al. reported a simple rule of thumb that allowed in silico prediction of whether a compound falls into chemical space where solubility and permeability are likely to allow oral absorption. According to the Ro5, ≈90% of oral compounds pass three of four of the following rules: MW $\leq$ 500 Da, calculated LogP (cLogP) $\leq$ 5 and $\geq$ 0, hydrogen bond acceptors (HBAs) $\leq$ 10, and hydrogen bond donors (HBD) $\leq$ 5 (Lipinski et al., 1997, 2001). Subsequently, additional in silico properties such as polar surface area (PSA $\leq$ 140 Å^2), the number of rotatable bonds (NRotBs $\leq$ 10–20), and more complex 3D properties have been added, thereby expanding the correlations with absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters (Egan et al., 2000; Ghose et al., 2012; Gleeson, 2008; Guimarães et al., 2012; Johnson et al., 2009; Lajiness et al., 2004; Meanwell, 2011; Veber et al., 2002; Wager et al., 2010; Waring, 2009; Yang et al., 2012). The Ro5 and later additions are used ubiquitously in early stages of drug discovery to identify oral "drug-like" compounds in the pharmaceutical industry (Lipinski, 2004), to an extent where the Ro5 has come to be known as "commandments" (Abad-Zapatero, 2007). However, overinterpretation and misuse of the guidelines, for example, requiring all guidelines to be met at hard cutoffs (Lipinski, 2012) and the fact that a number of orally bioavailable drugs exist far beyond these boundaries, have led to commentaries questioning the application of the guidelines and their value to the industry (Abad-Zapatero, 2007; Bennani, 2011; Lipinski, 2012; Medina-Franco et al., 2014; Terrett, 2013; Walters, 2012; Zhang and Wilkinson, 2007). Recently, there has been a focus on decreasing the emphasis of hard-cutoff, rule-based classification of compounds, and continuous scales such as the quantitative estimate of drug-likeness (QED) (Bickerton et al., 2012) have been introduced.

Opportunities with bRo5 Compounds

Analyses of average ligand properties (such as MW and cLogP) by target class reveal that highly explored classes such as ligand gated ion channels (MW 359 Da, cLogP 3.0) and aminergic G-protein-coupled receptors (GPCRs) (MW 378 Da, cLogP 3.8) have ligands with lower MWs and/or more appropriate lipophilicity compared with classes such as peptidic GPCRs (MW 514 Da, cLogP 4.3), nuclear hormone receptors (MW 398 Da, cLogP 5.1), and serine proteases (MW 467 Da, cLogP 2.7) (Morphy, 2006;

Paolini et al., 2006; Vieth and Sutherland, 2006). Therefore, finding high-affinity orally available ligands for these difficult target classes and for protein-protein interactions (Surade and Blundell, 2012) may be enhanced if ligands with physicochemical properties at the borders or beyond Ro5 were explored to a larger extent. Recent approximations suggest that there are $\approx 10^{33}$ compounds <500 Da (heavy atom count [HAC] < 36) compared with $\approx 10^{78}$ compounds with a MW < 1,000 Da (HAC < 72) (Polishchuk et al., 2013). Hence, there is potential for higher degrees of novelty, diversity, and complexity in bRo5 compounds. Although it is also true that we have yet to comprehensively sample Ro5 space (Ruddigkeit et al., 2012; Virshup et al., 2013), there is room for further expansion if bRo5 compounds are considered. Strategies for lead generation such as natural-product-derived approaches (Li and Vederas, 2009; Newman, 2008), diversity-oriented synthesis (DOS) (Kesavan and Marcaurelle, 2013; O'Connor et al., 2012; Schreiber, 2000), and macrocycles (Driggers et al., 2008; Giordanetto and Kihlberg, 2014; Mallinson and Collins, 2012; Marsault and Peterson, 2011; Yu and Sun, 2013) all constitute steps toward capitalizing on opportunities in bRo5 space.

So far, bRo5 chemical space remains relatively unexplored, most likely due to the perceived non-oral properties, increased complexity of compounds in this space, and the synthetic chemistry challenges associated with its navigation. We therefore set out to analyze approved drugs and clinical candidates that belong to bRo5 space, with emphasis on finding the *current* limits of oral bioavailability and identification of what allows compounds close to the limits to be orally deliverable. In this context, it should also be pointed out that compounds that are delivered orally should also be expected to be cell permeable and thus useful for manipulation of intracellular targets. No agreed definition of bRo5 space exists, but in order to capture the majority of compounds, we used MW > 500 Da as the criteria to mine databases for bRo5 compounds and also imposed an upper limit (MW < 3,000 Da) to remove large biologics such as insulin, heparin, and large polypeptides from the data set. Many of the oral drugs and clinical candidates in the resulting data set clustered into what can be considered an extension of Ro5 space. We therefore conducted a deeper analysis of a significant subset of compounds that reside in chemical space close to the limits of oral bioavailability; for the purpose of this review, "bRo5" refers to this subset, although other publications may refer to bRo5 as compounds breaking one or more of Lipinski's guidelines.

Mapping bRo5 Space

The data set of marketed drugs was compiled using the GVK-BIO online structure-activity database as described in our recent review of macrocyclic drugs and clinical candidates (Giordanetto and Kihlberg, 2014; GOSTAR, 2013). Compounds in phases I, II, and III clinical trials and in the preregistration phase were retrieved from the Pharmaprojects database (Pharmaprojects, 2013) and Thomson Reuters Integrity (Thomsonreuters, 2013). In cases of missing structure, these were supplemented from Chemistry Connect (Muresan et al., 2011) or the primary literature. Compounds for which structures could not be found were excluded from the data set. Filtering out veterinary products, contrast agents, mixtures (with the major component of mixtures chosen if it accounted for >75% of the mixture), biologics (MW > 3,000 Da), and counterions for salts gave a list of compounds for further annotation. Each compound was then categorized with regards to the route of administration, as oral or parenteral. Agents acting locally in the GI tract that had low systemic bioavailability, e.g., antibiotics for GI tract infections, were classed as parenterals. In addition, each compound was classified by indication (chosen based on the disease areas with the most advanced clinical trials and the highest activity if a compound was progressed for several indications) and into one of four chemical classes (peptides and peptidomimetics, natural products and derivatives, de novo designed, or prodrugs). Classification into each chemical class was based on the origin and largest motif of the compounds. Where available, the year of approval, average dose for an adult (mg/day), permeability in the Caco-2 cell line in the AB direction (P_{app}AB, $\times 10^{-6}$ cm/s) and bioavailability (F%) in humans or preclinical species were extracted from the literature. To the largest extent possible, chemical structures and all other data were retrieved from the primary literature or from multiple quality databases (ClinicalTrials.gov, Micromedex 2.0, Food and Drug Administration [FDA], DailyMed). These efforts resulted in a data set of 485 annotated compounds. The percentage of drugs approved that had MW > 500 Da was calculated by comparison to data from the literature (Mullard, 2013). Statistics were calculated in Graphpad Prism v.5.00.

Prediction of physicochemical properties and relevant 3D conformations is not straightforward, especially in bRo5 space where higher molecular size and flexibility increase complexity and requires more accurate methods. However, by comparing multiple, widely used, and well-established methods, as described in the following, we sought to minimize any potential bias linked to the arbitrary choice of a single tool. Physicochemical properties were predicted using Instant J Chem 6.2.1 (ChemAxon, 2014), as it is freely available for academics and provides access to an array of standard computed properties for analysis. However, the predictions are not entirely accurate and in some cases are far from the experimentally determined values. For example, posaconazole has cLogP of 5.4, while the experimentally determined LogP is 2.4 (Saha and Kou, 2000), illustrating the need to interpret the predictions with caution. Different LogP predictors (such as MlogP, AlogP, XlogP) (Mannhold et al., 2009) were evaluated but did not significantly alter the overall conclusions and outcome of the present analysis. In order to allow comparisons and to facilitate use in prospective design, we have chosen to use cLogP rather than experimental LogP in the following discussions of compounds bRo5. QED was calculated according to the literature (Bickerton et al., 2012). 3D structures were generated in Omega 2.5.1.4 (OpenEye, 2014), Instant J Chem 6.2.1 (ChemAxon, 2014), and Corina (Sadowski et al., 1994). Normalized principal molecular moments of inertia (PMI) were calculated according to the literature (Sauer and Schwarz, 2003); however, the average normalized PMI of up to 100 structures found within 2 kcal/mol of the identified minima was used in our analysis. No distinct differences in the overall distributions of normalized PMI were found using the different software for conformation generation. Herein, PSA refers to the topological PSA (Ertl et al., 2000). As compounds increase in size and complexity, greater differences in 3D and topological PSA occur (Guimarães et al., 2012); however, the widespread use of topological PSA and difficulty in accurately predicting 3D conformations of larger bRo5 compounds led us to use topological PSA.

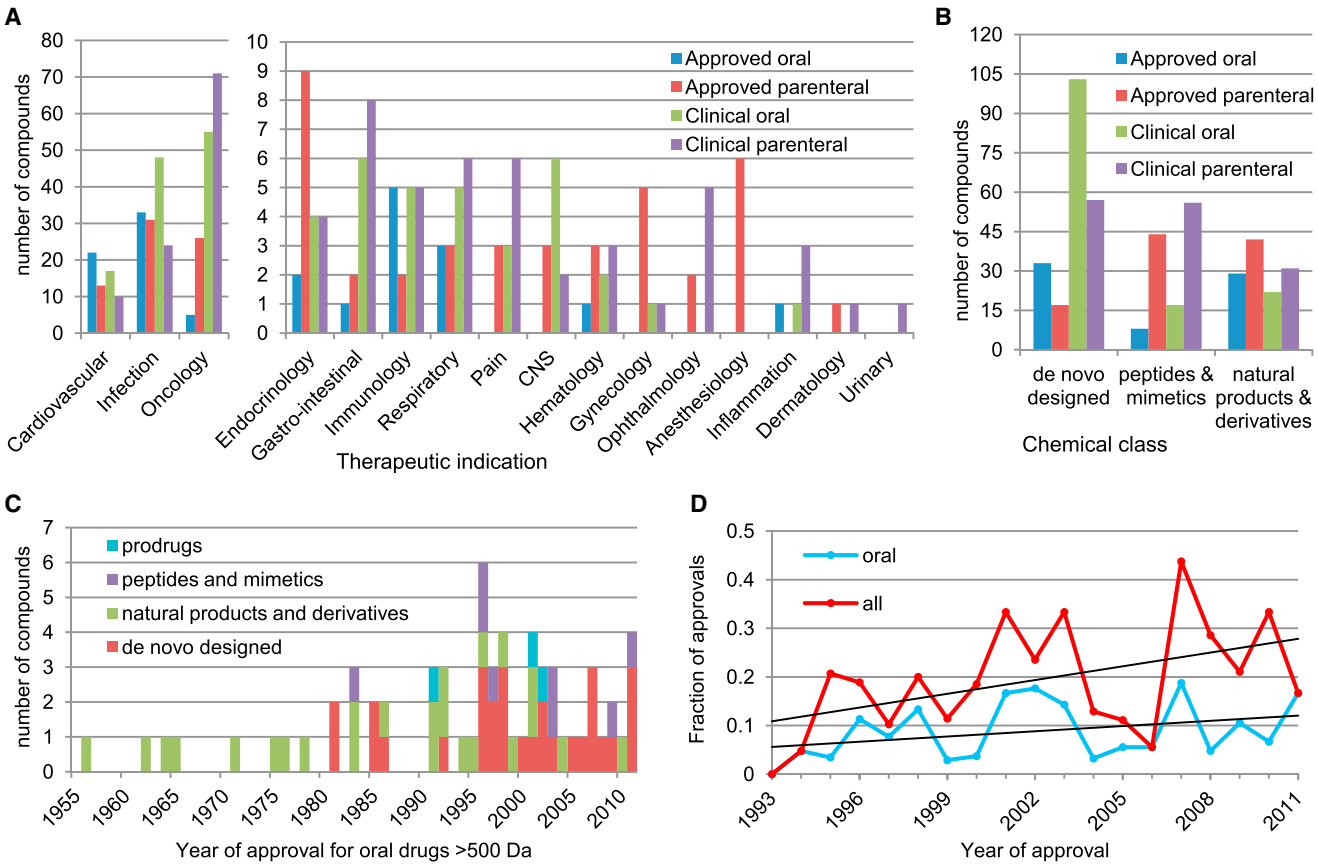


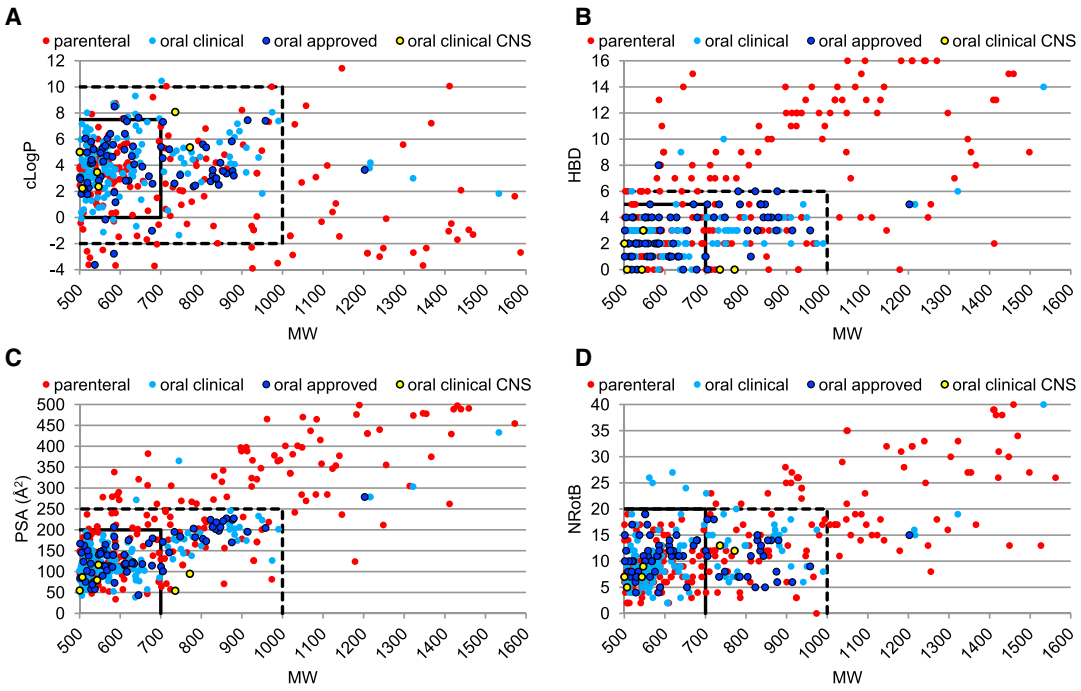
Figure 1. Distribution of Approved Drugs and Clinical Candidates Having MW > 500 Da
(A) Across therapeutic indications and by route of administration.
(B) Across chemical classes and by route of administration.
(C) Year of approval for oral drugs >500 Da from different chemical classes.
(D) Fraction of drugs approved by the FDA having MW >500 Da.

As the experimental data analyzed here were extracted from multiple literature sources, our analysis will be affected by discrepancies caused by differing protocols and experimental inconsistencies, and conclusions must therefore be judged with appropriate caution. In order to mitigate some of these concerns, average values or comparisons from the same source were used where possible. Caco-2 permeability ($P_{app}AB$) is known to vary between sources (Artursson et al., 2012), and our classification of poor/low and good/high was defined as $<1 \times 10^{-6}$ and $>10 \times 10^{-6}$ cm/s, respectively. These cutoff values define the region where oral bioavailability shifts from consistently low to consistently high values (Balimane et al., 2006; Li et al., 2007; Veber et al., 2002).

General Overview of All Drugs and Clinical Candidates with MW > 500

Database mining gave a set of 182 approved drugs and 303 compounds currently being evaluated in clinical trials that had a MW > 500 Da. Oncology, infection, and cardiovascular indications accounted for the majority of these compounds (n = 157, 32%; n = 136, 28%, and n = 62, 13%, respectively; Figure 1A). Each of the remaining indications does not exceed 4% of the data set. Forty percent of the approved drugs (n = 73) and 50% of clinical candidates (n = 153) are administered orally, but the split between orals and parenterals varies significantly between the therapeutic indications (Figure 1A). Among the three major indications, oncology relies mainly on parenteral administration of drugs and clinical candidates (62%), whereas infection and cardiovascular see a larger proportion of orally administered compounds (60% and 63%, respectively). Interestingly, clinical candidates in oncology are increasingly being evaluated for oral administration (44% versus 16% for approved oncology drugs). Only three CNS-approved drugs were found in the current data set, all of which are administered parenterally, but it is interesting to note that six of eight compounds in clinical trials are orals.

The drugs and clinical candidates analyzed here can be classified into three main chemical classes: peptides and peptidomimetics, natural products and derivatives, and de novo designed compounds. There is also a minor class of prodrugs (Figure 1B). De novo designed compounds constitute the majority of the compounds in the data set (n = 210, 43%), followed by equal numbers of natural products (n = 124, 26%) and peptides and mimetics (n = 125, 26%). The majority of de novo designed compounds are orally administered (64%), whereas, in line with expectations, peptides and natural products are mainly parenteral



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Class	Delivery	N	MW (Da)	cLogP	HBD	HBA	PSA (Å^2)	NRotB	Fsp^3
all	Oral	226	642(621-662)	4.1(3.8-4.3)	2.6(2.4-2.9)	7.7(7.3-8.1)	137(130-145)	10.4(9.8-11.1)	0.49(0.46-0.52)
	Parenteral	259	1059(983-1136)	-1.4(-2.3- -0.5)	10.5(9.2-11.8)	15.6(14.2-17.0)	357(320-394)	21.9(19.3-24.6)	0.52(0.50-0.54)
peptides & mimetics	Oral	25	815(700-929)	3.2(2.6-3.9)	4.5(3.5-5.5)	8.7(7.6-9.9)	199(169-229)	15.1(12.5-17.7)	0.56(0.50-0.62)
	Parenteral	100	1473(1336-1610)	-6.9(-8.2- -5.6)	19.3(17.1-21.5)	22.1(19.7-24.5)	582(518-645)	35.6(30.2-40.9)	0.56(0.54-0.58)
natural products	Oral	51	713(670-756)	3.7(3.0-4.3)	3.3(2.8-3.8)	10.2(9.2-11.2)	168(153-182)	9.2(8.0-10.5)	0.68(0.62-0.73)
	Parenteral	73	883(765-1002)	0.7(-0.6-2.0)	6.8(5.1-8.4)	14.3(11.8-16.9)	270(219-322)	13.6(10.1-17.2)	0.59(0.54-0.63)
de novo designed	Oral	136	590(574-606)	4.4(4.1-4.7)	2.1(1.8-2.3)	6.6(6.2-6.9)	113(107-120)	9.3(8.8-9.9)	0.40(0.37-0.43)
	Parenteral	75	733(662-803)	3.0(2.1-3.9)	3.7(2.8-4.6)	9.4(7.8-11.0)	176(146-206)	12.2(10.5-14.0)	0.39(0.34-0.43)
prodrugs	Oral	14	572(534-611)	3.8(2.2-5.4)	2.1(1.6-2.7)	7.6(6.6-8.6)	149(135-164)	17.1(13.6-20.6)	0.62(0.49-0.74)
	Parenteral	11	699(558-840)	4.6(1.6-7.6)	2.2(0.3-4.0)	7.2(5.2-9.2)	126(85-168)	19.6(14.9-24.2)	0.60(0.53-0.66)
CNS	Oral	6	601(474-727)	4.4(2.1-6.7)	0.8(-0.6-2.2)	5.0(3.9-6.2)	81(56-106)	8.8(5.6-12.1)	0.42(0.36-0.49)

Figure 2. Physicochemical Property Space of Drugs and Clinical Candidates Having a MW > 500 Da

Approved oral drugs are in dark blue. Oral clinical candidates are in light blue. Oral clinical candidates for CNS are in yellow, and parenteral drugs and clinical candidates in red. Extended Lipinski's Ro5 space (solid box, 62% of oral compounds) and limits of oral bRo5 space (dashed box, 93% of compounds) are indicated. The graph limits have been set so as to include all orals in the data set, resulting in an upper MW cut off at 1,600 Da and a number of parenterals fall outside of the limits.

(A) cLogP as a function of MW.

(B) Number of HBDs as a function of MW.

(C) PSA as a function of MW.

(D) NRotBs as a function of MW.

(E) Physicochemical property distribution of chemical classes with mean (95% CI of mean) of MW, cLogP, HBD, number of hydrogen bond donors (HBD) and acceptors (HBA), PSA, NRotB, and Fsp^3 carbons.

(80% and 59%, respectively; Figure 1B). These trends are consistent across drugs and clinical candidates and highlight that oral compounds can be designed in chemical space close to the borders or bRo5 with adequate chemical manipulation. As is discussed further below, at least part of the difference between the chemical classes may be explained by oral de novo designed compounds being closer to Ro5 chemical space than oral peptides and natural products (cf. Figure 2E). It should also be mentioned that de novo designed compounds dominate among oral drugs and clinical candidates in the cardiovascular and oncology indications (72% and 73% of orals in their therapeutic indication, respectively). Among peptides and mimetics, all peptidomimetics are oral, providing further evidence of the ability of medicinal chemists to design orally administered compounds with high MW. In contrast, all peptides are administered parenterally with the exception of a few cyclic peptides, consisting of cyclosporin A and derivatives thereof.

Analysis of the year of approval for oral drugs with MW > 500 Da reveals that approvals increased in the early 1990s, peaked between 1996 and 1998, and then stabilized at a somewhat lower level in the 2000s (Figure 1C). An increasing number of oral de novo designed drugs have been approved in the past 2 decades, whereas natural products featured more prominently prior to 1995. Moreover, when considering the fraction of FDA drug approvals that originate from MW > 500 Da compounds, the past 2 decades have seen a constant or slow increase in

introduction of orals, while the total share with MW > 500 Da has increased steadily (Figure 1D).

Extent of Oral Druggable Space for Drugs and Clinical Candidates with MW > 500 Da

Whereas Lipinski's rules delineates chemical space with compounds "more *likely* to be orally absorbed," we were interested in defining a more lenient "*possible* to be orally absorbed" space. An understanding of the approximate limits of this chemical space should help to guide the design of cell permeable and orally available ligands for less tractable targets, where working within the Ro5 is less likely to be successful. As a first step toward establishing this insight, the present data set of drugs and clinical candidates with MW > 500 Da were analyzed with respect to calculated physicochemical parameters (Figure 2). Of the 226 orally administered drugs and clinical candidates in this study, a large proportion (62%, n = 141) cluster into what can be considered as an extension of Ro5 space and a natural tail of the distribution of compounds based on Ro5 properties (Figure 2, solid boxes mark extended Ro5 space; MW $\leq$ 700, 0 $\leq$ cLogP $\leq$ 7.5, HBD $\leq$ 5, PSA $\leq$ 200 Å^2, NRotB $\leq$ 20). No clear and agreed definition of bRo5 chemical space exists, but for the purpose of this review, "bRo5 space" refers to chemical space outside the extended Ro5 space that clearly deviates from the Ro5 model. When applying the QED score (Bickerton et al., 2012), compounds within the extended Ro5 space had a mean QED score of 0.31 (95% confidence interval [CI] of mean 0.29–0.32, SD 0.11) while compounds bRo5 displayed a significantly lower mean of 0.16 (95% CI of mean 0.14–0.17, SD 0.07). The QED scores of these two data sets are significantly lower than the mean QED of 0.49 (SD 0.23) for compounds classified as "unattractive compounds" by medicinal chemist (Bickerton et al., 2012), which further confirms both data sets. The majority of orals in the data set (93%, n = 211/226; Figure 2, dashed boxes) possess physicochemical properties defined by MW $\leq$ 1000 Da, $-2 \leq$ cLogP $\leq$ 10, HBD $\leq$ 6, HBA $\leq$ 15, PSA $\leq$ 250 Å^2, and NRotB $\leq$ 20. Even though these cutoffs are somewhat arbitrary, we propose that this defines the *current* outer limits of physicochemical space where orally absorbed compounds may have a reasonable chance of being designed. Limits for size (MW) and lipophilicity (cLogP) as compared with Ro5 have increased by a factor of two (MW: 1,000 Da versus 500 and cLogP: 10 versus 5), while the number of HBDs has only increased by factor of 1.2 (HBD: 6 versus 5). The cutoff for PSA has increased by a factor of 1.8, from a value of 140 to 250 Å^2 (Palm et al., 1997); this could be expected based on the correlation of PSA to size. The increase in PSA is mainly due to an increased number of HBAs, and not HBDs. Because of the correlation of PSA with size, scaling PSA by calculating PSA/MW revealed no difference between compounds <700 Da (95% have PSA/MW 0.10–0.35) and those >700 Da (92% have PSA/MW 0.15–0.30). CNS penetration typically requires more stringent properties, for example, PSA $\leq$ 90 Å^2, MW $\leq$ 450 Da, HBD $\leq$ 3 (Ghose et al., 2012; Wager et al., 2010). Only six oral CNS compounds are found within the full data set; it is therefore not surprising that all of them are in clinical trials. Four of these compounds are close to Ro5 space being relatively small and nonpolar. The remaining two compounds despite being larger in size (735–771 Da) remain nonpolar (PSA 54–95 Å^2, 0 HBD) and lipophilic (cLogP 5.4–8.1). These results clearly indicate that there are substantial opportunities for design of non-CNS targeted orally available drugs both in extended and in bRo5 chemical space, as long as the number of HBDs is kept under control. CNS-targeted compounds, however, will be much more difficult to design in extended and bRo5 space.

Nevertheless, it is noteworthy to point out that none of the calculated molecular descriptors are able to clearly delineate oral and parenteral property space. For example, a large number of parenterals are found within the extended Ro5 space (Figures 2A–2D). However, when the data set is analyzed based on mean property values (95% CIs of mean), significant differences between orals and parenterals are found. Oral compounds are smaller, more lipophilic, have fewer polar atoms, and are less flexible (Figure 2E). These trends are generally maintained within classes; however, the ranges differ depending on class. Oral de novo designed compounds are significantly smaller and have lower HBD, HBA, and PSA than oral peptides and natural products. Furthermore, de novo designed compounds and natural products have a similar NRotB, which could be interpreted as a similar molecular flexibility in spite of the significant difference in MW. However, the flexibility of natural products is probably higher than the NRotB suggests, as macrocyclic bonds, frequently found in natural products, are not counted as rotatable in this context. Additionally, lipophilicity, as measured by cLogP, is the property that varies the least across oral chemical classes, thus confirming its relevance to oral absorption, as previously noted by several others (Egan et al., 2000; Gleeson, 2008; Veber et al., 2002). Fraction of sp^3 carbons (Fsp^3) increases progressively from de novo designed to peptides to natural products, and oral natural products have a higher Fsp^3 than parenteral natural products. Prodrugs, however, show less significant differences between oral and parenteral. Physicochemical-driven design can therefore be used for optimization of compounds in extended Ro5 and bRo5 space, preferably within the limits indicated by the dashed boxes in Figures 2A–2D.

As previously discussed, calculated physicochemical descriptors are useful but have limitations in their ability to predict oral bioavailability. However, passive permeability across a cell membrane is inversely proportional to the compounds hydrodynamic radius; i.e., it depends on the size and shape of the compound (Guimarães et al., 2012). The distribution of molecular shape, approximated by the normalized PMI (Sauer and Schwarz, 2003), was therefore investigated for the compounds in the current data set. The drugs and clinical candidates were found to predominantly populate rod- and disk-like shapes, notably due to de novo designed compounds. Peptides and natural products in the data set displayed a higher proportion of sphere-like shapes. However, no significant distinction in shape could be found between oral and parenteral compounds.

Apart from the physicochemical and shape characteristics, the administered dose could affect oral absorption of compounds that are substrates for efflux transporters as these become saturated at moderate to high doses (Padovan et al., 2012). Information about both human oral bioavailability and therapeutic doses in humans was found for 61 of the drugs and clinical candidates in the data set, but were not correlated (r_s 0.04). Interestingly, close to half of the studied oral compounds (42%, n = 74/175 compounds with oral dosage data) require only low doses (<50 mg/day) to be

orally efficacious. Furthermore, anti-infectives displayed the highest doses, some of which were ≥1 g/day, most likely due to the concentrations required to obtain satisfactory efficacy and the greater therapeutic window often found for anti-infectives. From the compiled Caco-2 data, 57% (n = 37) of oral compounds with MW > 500 Da had poor permeability ($P_{app}AB < 1 \times 10^{-6}$ cm/s), and only 9% of compounds had good permeability ($P_{app}AB > 10 \times 10^{-6}$ cm/s). Oral bioavailability was found for 76 compounds >500 Da and was low (F% ≤ 30) for 54%, similar to results from poor permeability; however, the two were not correlated (r_s 0.14). Thus, oral bioavailability was not found to be correlated to dose, nor to cell permeability, for the current data set.

Analysis of Oral Compounds in bRo5 Chemical Space

As discussed above, almost two thirds of the 226 orally administered drugs and clinical candidates in our data set cluster in what we considered as an extended Ro5 space (Figure 2, compounds within solid boxes). The remaining 85 oral compounds are found in what we refer to as bRo5 chemical space; i.e., they have at least one calculated physicochemical property within the following ranges: MW > 700 Da, cLogP < 0 or > 7.5, HBD > 5, HBA > 10, PSA > 200 Å^2, or NRotB > 20. This set of bRo5 compounds resides in chemical space close to the limits of oral bioavailability, and a comprehensive analysis of their properties was undertaken in order to provide knowledge that can be of use for design of orally available and cell-permeable compounds bRo5. Additional information on absorption, transporter-mediated efflux, and uptake, as well as use of formulations to enhance oral uptake for these compounds, was therefore compiled and analyzed.

Cardiovascular, infection, and oncology are still the major indications within this set of 85 oral bRo5 compounds, but the proportion of anti-infectives (58%, n = 49) has increased significantly as compared with the orals in the data set as a whole (36%, n = 81). When considering chemical class, a lower proportion of de novo designed compounds (19%, n = 16) and higher proportions of natural products (40%, n = 34) were observed in oral bRo5 space. This originates from oral de novo designed compounds being on average closer to Ro5 space than natural product and peptide classes (Figure 1). Macrocycles are more prominent among oral bRo5 (38%, n = 32) than in the full oral data set (15%, n = 34) and full >500 Da set (25%, n = 126), in line with previous observations (Giordanetto and Kihlberg, 2014). Additionally, native or modified sugars are also present in 21% (n = 18) of oral bRo5 compared with 8% (n = 19) of all oral compounds and 11% (n = 52) of all >500 Da compounds. A closer examination of the bRo5 data set revealed that the majority of compounds could be classified as belonging to a few specific chemical classes or by being directed to certain targets (cf. Figure 9). Hence, our subsequent analysis of the oral bRo5 will review compounds by class. The analysis will focus on the trends observed within and between classes, with higher emphasis being put on phase III and approved drugs. This differentiation is due to more data, in particular human bioavailability and established dose in the public domain for phase III and approved drugs and expected attrition of compounds in early development.

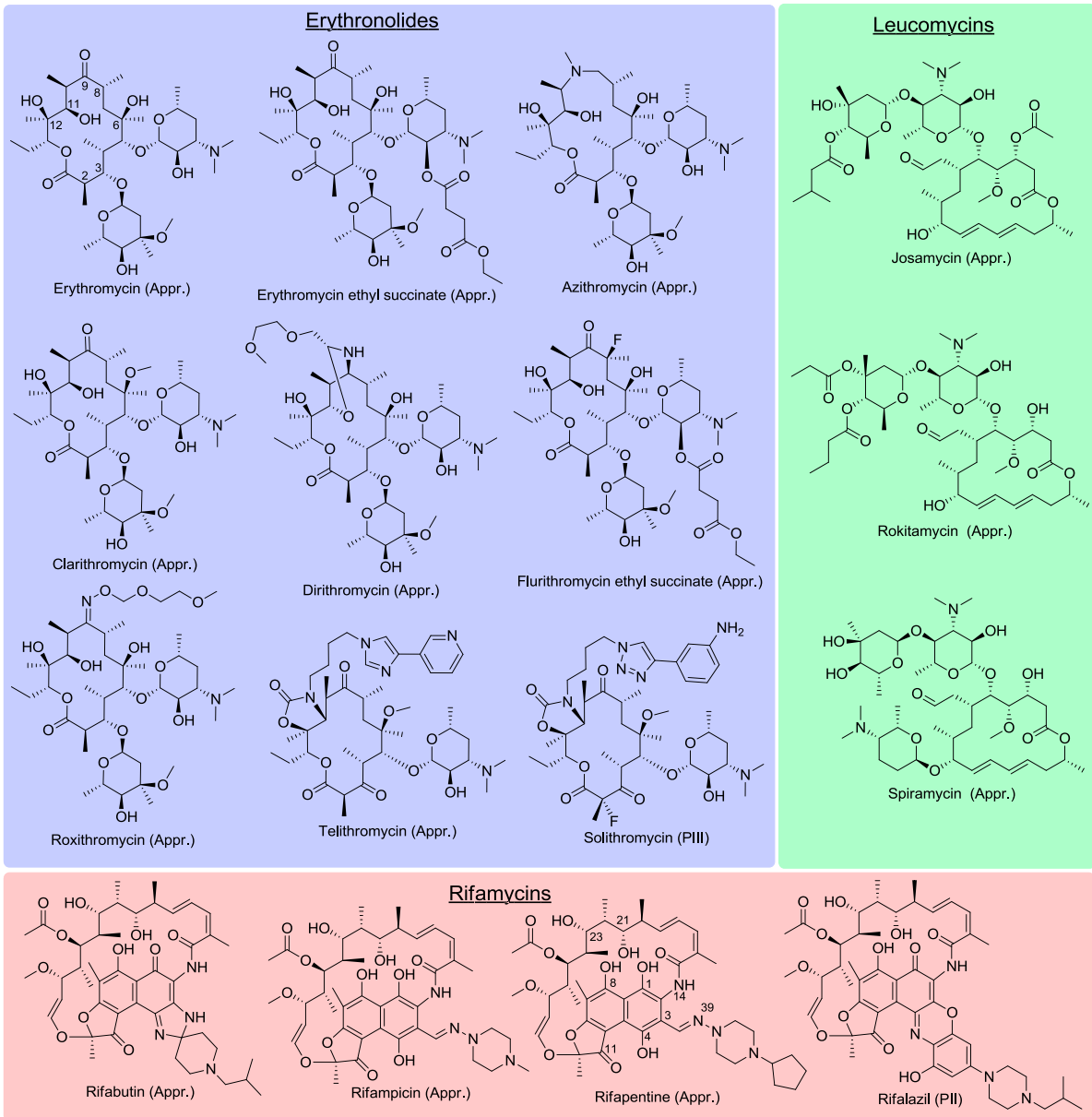
Erythronolides

The erythronolides are broad-spectrum antibacterial agents that inhibit bacterial polypeptide synthesis via binding to the 50S bacterial ribosome. They are 14- to 15-membered macrocyclic macrolide natural products and have MW > 700 Da and HBA > 10, often in combination with a PSA > 200 Å^2 (Figure 3). Erythromycin, telithromycin, azithromycin, clarithromycin, and roxithromycin constitute the most studied drugs in this class. Development of analogs has focused on increased acid stability by modifications at C6, C9, C11, and C12 and decreased metabolism by modifications of C8 and C2, which ultimately lead to improved pharmacokinetic properties, particularly less variable bioavailability (Omura et al., 2002; Zhanel et al., 2001). Removal of the C3 L-cladinose as in telithromycin and solithromycin improves stability and activity against bacterial efflux-mediated resistance (Zhanel et al., 2001).

Erythronolides generally have poor to moderate oral bioavailabilities (10%–57%), except for roxithromycin, which is 78% orally bioavailable (Giordanetto and Kihlberg, 2014; Nozinic et al., 2010). The Caco-2 cell permeability in the AB direction ($P_{app}AB$) is low or at best moderate, but as erythronolides are substrates of the efflux protein Pgp (Munić et al., 2010; Nozinic et al., 2010), they have a higher permeability in the BA direction ($P_{app}BA$ 6.4–16 × 10^{-6} cm/s). In line with these observations, erythronolides display a concentration dependency in their $P_{app}AB$, which is proposed to occur via saturation of efflux transporters such as Pgp at clinically relevant concentrations (Pachot et al., 2003; Padovan et al., 2012). This concentration dependency is particularly relevant as erythronolides are given at high doses, and saturation of intestinal efflux may therefore account for oral bioavailability higher than predicted from $P_{app}AB$ values. In addition, erythronolides have been linked to hepatic organic anion transporting polypeptide (OATP) 1A2, 1A4, 1B1, 1B2, 1B3 and organic anion transporter (OAT) 5 (Franke et al., 2008; Garver et al., 2008; Lam et al., 2006; Lan et al., 2009; Seithel et al., 2007). Finally, erythronolides have high volumes of distribution and an uneven tissue distribution. They accumulate in lung fluid, possibly due to Pgp efflux (Rodvold et al., 2011; Togami et al., 2012), and are also enriched in phagocytic cells, presumed due to accumulation in lysosomes (Ahmad et al., 2010; Bosnar et al., 2005; Wildfeuer et al., 1996), both of which lead to higher concentrations at their sites of action.

Leucomycins

The leucomycin macrolides are 16-membered macrocycles that are analogs to the erythronolides that have a high MW, PSA, and HBA (Figure 3). Just as the erythronolides, their antibacterial activity involves inhibiting the 50S bacterial ribosome. Less information is available regarding the PK of leucomycins than for the erythronolide antibiotics. However, in spite of the high MW and large PSA, josamycin has an excellent human bioavailability, whereas spiramycin is only moderate (Brook, 1998; Giordanetto and Kihlberg, 2014). Spiramycin is also known to have a large volume of distribution, similar to that of the erythronolides, and has higher accumulation in lung fluid than erythromycin (Brook, 1998). A number of transporters are associated with leucomycin PK, including Pgp, multidrug resistance-associated protein (MRP) 2, and OATP2 (Ito et al., 2007; Tian et al., 2007); however, high dosage of leucomycins likely results in saturation of intestinal efflux and contributes to oral bioavailability.



Compound	Phase	MW	HBD	HBA	cLogP	PSA	Dose	F (%)	P_{app}
Erythromycin	Approved	734	5	13	2.6	195	2500	25	0.09
Erythromycin ethyl succinate	Approved	862	4	14	3.4	227	500	55	
Azithromycin	Approved	785	5	13	2.4	182	500	37	0.22
Clarithromycin	Approved	748	4	13	3.2	184	1000	55	3.84
Dirithromycin	Approved	835	5	15	3.0	202	500	10	
Flurithromycin ethyl succinate	Approved	880	4	14	3.2	227	750		
Roxithromycin	Approved	837	5	16	3.0	218	300	78	0.52
Telithromycin	Approved	812	1	11	5.4	173	800	57	1.94
Solithromycin	III	845	2	12	5.8	199	400		
Josamycin	Approved	828	3	13	3.2	207	1000	95	
Rokitamycin	Approved	828	3	13	3.6	207	300		
Spiramycin	Approved	843	4	15	2.5	198	1500	35	
Rifabutin	Approved	847	5	13	4.2	206	300	53	9.5
Rifampicin	Approved	823	6	14	2.8	224	600	50	3.1
Rifapentine	Approved	877	6	14	3.6	224	750	70	10.7
Rifalazil	II	941	5	15	4.6	233		50(p)	

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Rifamycins

Rifamycins are macrocyclic ansamycin natural product antibacterials with high MW, HBA, HBD, and PSA (Figure 3). They bind to bacterial DNA-dependent RNA polymerase and prevent RNA synthesis via occlusion of the elongating RNA strand (Selva and Lancini, 2010). All four rifamycins are derivatives of the natural product rifamycin B/SV, which has poor cell-based activity and pharmacokinetic properties (Sensi, 1983). The ansa bridge, i.e., the aliphatic portion of rifamycins, particularly C21 and C23 as well as the napthoquinone C1 and C8 polar groups, was found to be important for antibacterial activity, whereas derivatization at C3 and C4 can be used to modulate PK to allow oral administration (Selva and Lancini, 2010).

Despite their in silico properties, the three approved rifamycins have good oral bioavailability (50%–70%), although the literature reports a large variability in bioavailability (Blaschke and Skinner, 1996; Burman et al., 2001; Ellard and Fourie, 1999; Loos et al., 1985; Rothstein et al., 2006; Selva and Lancini, 2010). In line with this, moderate to high permeabilities (P_{app}AB) have been reported for the three drugs (Gertz et al., 2010; Gonçalves et al., 2012; Ranaldi et al., 1992). Intramolecular hydrogen bonds (IMHBs) between the substituents on C1–C8 and C21–C23 have been reported (Agrawal et al., 2004; Bacchi et al., 1998; Brufani et al., 1964, 1967; Casey and Whitlock, 1975), with rifampicin and rifapentine having also been postulated to make C4–C11 substituent and N14–N39 hydrogen bonds (Pyta et al., 2012). Formation of these IMHB will reduce polarity and contribute to rifamycin's surprisingly good epithelial cell permeability and oral bioavailability considering their high MW and polarity. Rifamycins have been linked to Pgp efflux with Caco-2 ERs of 3.3–6 (Gertz et al., 2010), but as dosage is high (300–750 mg/day), efflux may be saturated and therefore have low impact on bioavailability. Inhibition of hepatic transporters OATP1B1 and OATP1B3 (Williamson et al., 2013) as well as induction of higher expression levels of Pgp, MRP1, MRP2, and CYP3A4 also occurs. These interactions may be the source of the numerous drug-drug interactions (Baciewicz et al., 2013), variable bioavailability, and decreases in bioavailability during chronic dosage that have been reported for rifamycins (Blaschke and Skinner, 1996; Ellard and Fourie, 1999; Loos et al., 1985). Higher concentrations of the rifamycins also occur in the lungs (Hosoe et al., 1996; Selva and Lancini, 2010) and macrophages (Mor et al., 1995), aiding their therapeutic potential for tuberculosis, a common indication for these antibacterial agents.

HCV NS3/4A Protease Inhibitors

The hepatitis C virus (HCV) NS3/4A protease is a chymotrypsin-like serine protease that is essential for the cleavage of the viral polyprotein during the HCV replication process. It has also been shown to mediate viral evasion of the host immune system by cleavage of key proteins in the innate immune system. The NS3/4A protease has a shallow and extended substrate-binding cleft that most likely explains why all ten inhibitors in clinical studies, as well as the recently launched simeprevir, have MW > 700 Da (Figure 4). NS3/4A inhibitors were designed starting from a weak hexapeptide lead (Lamarre et al., 2003), and extensive structure and property-based optimization has led to both linear and macrocyclic peptidomimetics. Macrocyclization has been achieved either between P1–P3 (cf. simeprevir) or P2–P4 (cf. vaniprevir). Interestingly, a comparative study of some linear and macrocyclic inhibitors revealed that macrocycles were generally more potent than linear compounds (Ali et al., 2013). In addition, P1–P3 macrocyclic inhibitors were found to be less susceptible to drug resistance compared with linear and P2–P4 macrocyclic inhibitors.

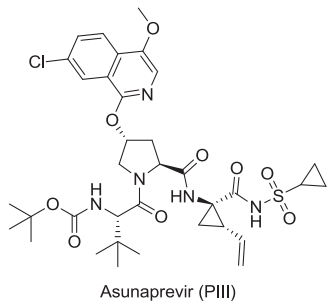
A moderate (41%) oral bioavailability in humans has been reported for narlaprevir (Gao et al., 2011). Similarly, most of the other NS3/4A protease inhibitors in phase II and III, as well as the recently launched simeprevir (Rosenquist et al., 2014), display either low or moderate bioavailabilities in preclinical species (Arasappan et al., 2010; Duan et al., 2012; Jiang et al., 2014; Liverton et al., 2010; Scola et al., 2014; Sheng et al., 2012; Summa et al., 2012). Caco-2 cell permeabilities (P_{app}AB) are also low to moderate (Duan et al., 2012; Jiang et al., 2014; McPhee et al., 2012; Rosenquist et al., 2014; Sheng et al., 2012). Significant efflux has been reported for asunaprevir (McPhee et al., 2012), danoprevir (Jiang et al., 2014), and vedroprevir (Sheng et al., 2012), the three inhibitors having low P_{app}AB values, and this was linked to Pgp for asunaprevir. As doses of HCV protease inhibitors are moderate to high in order to sustain low viral loads, it can be speculated that efflux transporters in the intestinal lumen will be saturated and that absorption will not be affected by efflux to a large extent (Bergström et al., 2009). Significant accumulation in the liver, with liver/plasma ratios typically ranging from 30-fold to several hundred fold have been reported for almost all of the inhibitors in preclinical species, which is important, because the liver is the organ affected by the HCV virus (Duan et al., 2012; Jiang et al., 2014; Liverton et al., 2010; Rosenquist et al., 2014; Scola et al., 2014; Summa et al., 2012; Yang et al., 2014). For several of the inhibitors, i.e., simeprevir, faldaprevir (Ramsden et al., 2014), danoprevir (Jiang et al., 2014), and MK 5172 (Summa et al., 2012), accumulation has been linked to uptake by OATPs such as 1B1 (Kalliokoski and Niemi, 2009). OATP-mediated uptake is likely to affect many NS3/4A inhibitors, particularly due to the carboxylic acid (faldaprevir) or bioisosteric cyclopropanesulfonamide in all but narlaprevir. Another consequence of this active uptake is that oral bioavailabilities are most likely low to moderate because of significant first-pass clearance to the liver.

HCV NS5A Inhibitors

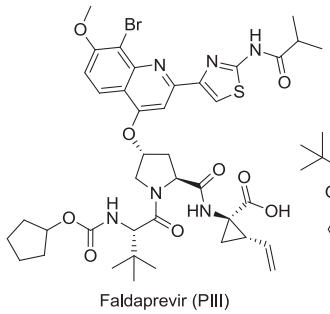
NS5A is essential for the HCV replication cycle, but its precise function is unknown (Manns and von Hahn, 2013). As a consequence, the mode of action of NS5A inhibitors is also unclear. However, as first demonstrated by daclatasvir, NS5A inhibitors provide a rapid decline in viral load and development of resistance to them has been mapped to NS5A (Gao et al., 2010). Leads for development of inhibitors of NS5A have been

Figure 3. Structures, Calculated Physicochemical Properties, and Data of Relevance for Oral Administration of Erythronolide, Leucomycin, and Rifamycin Antibiotics

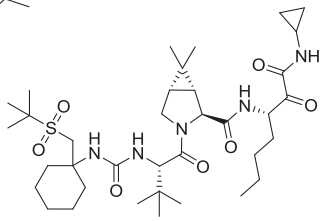
Dose, F, and P_{app} are average adult dosage in mg/day, oral bioavailability in human or preclinical species (p), and Caco-2 AB permeability in 10^{-6} cm/s where found.



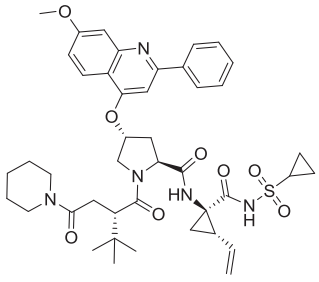
Asunaprevir (PIII)



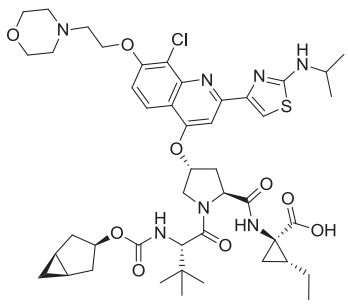
Faldaprevir (PIII)



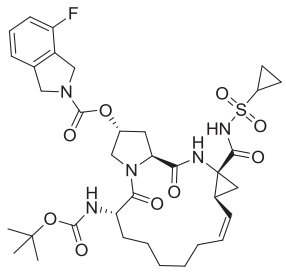
Narlaprevir (PII)



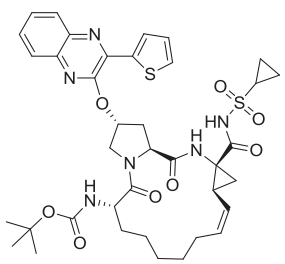
Sovaprevir (PII)



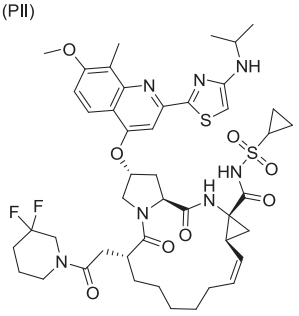
Vedroprevir (PII)



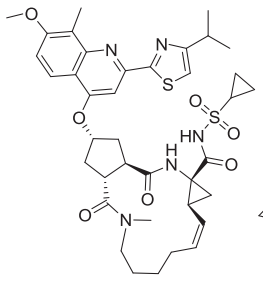
Danoprevir (PII)



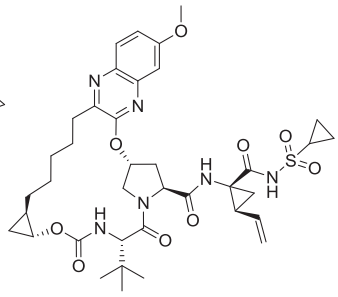
Veruprevir (PII)



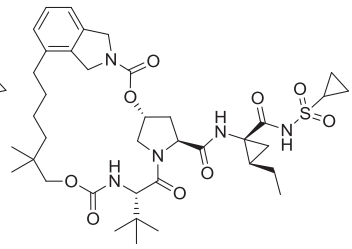
Neceprevir (PI)



Simeprevir (Appr.)



MK-5172 (PII)



Vaniprevir (PIII)

Compound	Phase	MW	HBD	HBA	cLogP	PSA	Dose	F (%)	P_{app}
Simeprevir	Approved	750	2	9	4.6	154	150	25-72 (p)	8.4
Asunaprevir	III	748	3	9	3.4	180	200	12-50 (p)	1.1-5.4
Faldaprevir	III	870	4	10	6.5	201	180	26-39 (p)	8.7
Vaniprevir	III	758	3	7	4.2	178	900	<15 (p)	
Danoprevir	II	732	3	7	2.6	178		15-78 (p)	<2
MK-5172	II	767	3	10	3.4	192		12.5 (p)	
Narlaprevir	II	708	4	7	3.5	171		41	
Sovaprevir	II	800	2	9	2.7	162			
Vedroprevir	II	911	4	12	3.5	197		49-143 (p)	0.8
Veruprevir	II	779	3	9	4.4	183			
Neceprevir	I	911	2	10	5.3	174			

(legend on next page)

discovered using high-throughput phenotypic screens, and optimization revealed that symmetry is important for potent antiviral activity. All five NS5A inhibitors in the oral bRo5 set have high MWs and PSA with their structures characterized by a rigid linear aromatic core that has derivatives of the dipeptide Val-Pro attached at both ends (Figure 5).

Daclatasvir was found to have good human oral bioavailability (66%–79%) (Jiang et al., 2012), while the other four NS5A inhibitors all displayed bioavailabilities that ranged from low to moderate in preclinical species (Coburn et al., 2013; DeGoey et al., 2014; Kazmierski et al., 2014; Link et al., 2014). In line with the observed bioavailability, daclatasvir has intermediate Caco-2 permeability (P_{app}AB) and a moderate ER of 5.8 (Bilello et al., 2011). A recent study indicates that an IMHB is formed between the imidazole NH and the carbamate carbonyl group in each of the dipeptidic parts of daclatasvir in nonpolar environments (Wakenhut et al., 2014). Identical or similar IMHB could also be formed for the other four NS5A inhibitors and may contribute to increasing their membrane permeability and oral bioavailability.

HIV-1 Protease Inhibitors and Related Pharmacoenhancers

Inhibitors of HIV type-1 (HIV-1) protease represent an important component of successful antiretroviral therapies for HIV infections. These inhibitors are normally coadministered with pharmacoenhancers (additional compound/s to improve the pharmaceutical properties of the parent drug) to maximize their therapeutic efficacy. Because of the structural requirements imposed by the HIV-1 protease active site, all HIV-1 protease inhibitors have MW > 500 Da. The two HIV protease inhibitors atazanavir and TMC-310911 and two pharmacoenhancers ritonavir and cobicistat stand out by having MW > 700 Da (Figure 5).

While no pharmacokinetic information has been disclosed for TMC-310911, atazanavir is reported to have a good oral bioavailability in the range of 60%–68% (Hsu et al., 1998; Kis et al., 2013; Le Tiec et al., 2005; Patel et al., 2004). Because of its limited and pH-dependent solubility, atazanavir absorption can be enhanced by coadministration with food, but absorption is significantly reduced by proton pump inhibitors (Kiser et al., 2006; Luber et al., 2007; Tomilo et al., 2006). Atazanavir's cellular permeability is greatly affected by transporters and metabolizing enzymes, and it has been reported to interact with Pgp, MRPs, breast cancer resistance protein (BCRP), OATPs, CYP3A, and UDP glucuronosyltransferase (UGT) 1A1 in vitro (Bousquet et al., 2008; Kis et al., 2013; Perloff et al., 2005; Zhang et al., 2005a). Because of its broad transporter profile and transporter activity saturation, atazanavir's permeability is highly concentration dependent (Kis et al., 2013). Atazanavir has been identified as a compound that can form IMHBs in nonpolar solvents; this property may also contribute to epithelial cell permeability and thereby to oral absorption (Alex et al., 2011).

Ritonavir and cobicistat are mechanism-based CYP3A inhibitors that may be administered to enhance the pharmacokinetic profile of drugs metabolized by these enzymes. While ritonavir also displays intrinsic HIV-1 protease inhibition, cobicistat is devoid of anti-HIV activity (Xu et al., 2010). The absorption profile of both drugs is modulated by several transporters, including Pgp, BCRP, and OATPs (Alsenz et al., 1998; Annaert et al., 2010; Lepist et al., 2012; Profit et al., 1999). Accordingly, saturation of transporters resulting in disproportional absorption has been observed (Parker and Houston, 2008). Based on the observed biological profiles at transporters and metabolizing enzymes, HIV protease inhibitors and associated pharmacoenhancers display a high tendency for additional drug-drug interactions, requiring careful coadministration monitoring (Chauvin et al., 2013; Deeks, 2014; Fukuda et al., 2013; von Hentig, 2008).

Ascomycins and Rapamycins

The three rapamycins (sirolimus, everolimus, and ridaforolimus) and the ascomycin tacrolimus are macrolides that have high MW, HBA, and PSA (Figure 6). They are immunosuppressants primarily used to reduce rejections after organ transplants. Tacrolimus (FK-506) binds to the immunophilin FK506 binding protein 12 (FKBP12), creating a new complex that binds to calcium, calmodulin, and calcineurin, thus inactivating the phosphatase activity of calcineurin and activation of T cells (Patel et al., 2012; Pirsch et al., 1997; Tada et al., 2005). The rapamycins, however, inhibit mammalian target of rapamycin (mTor) through formation of a complex with FKBP12, arresting T lymphocyte growth (Kirken and Wang, 2003; Sehgal, 2003). Rapamycins also have application in cancer therapy through inhibition of the PI3K/akt/mTor signaling pathway, which regulates cell proliferation, survival, and angiogenesis (Dancey, 2010; Gabardi and Baroletti, 2010). Everolimus and ridaforolimus are derivatives of sirolimus at position 40, developed to improve the PK, particularly solubility and oral bioavailability (Kirchner et al., 2004).

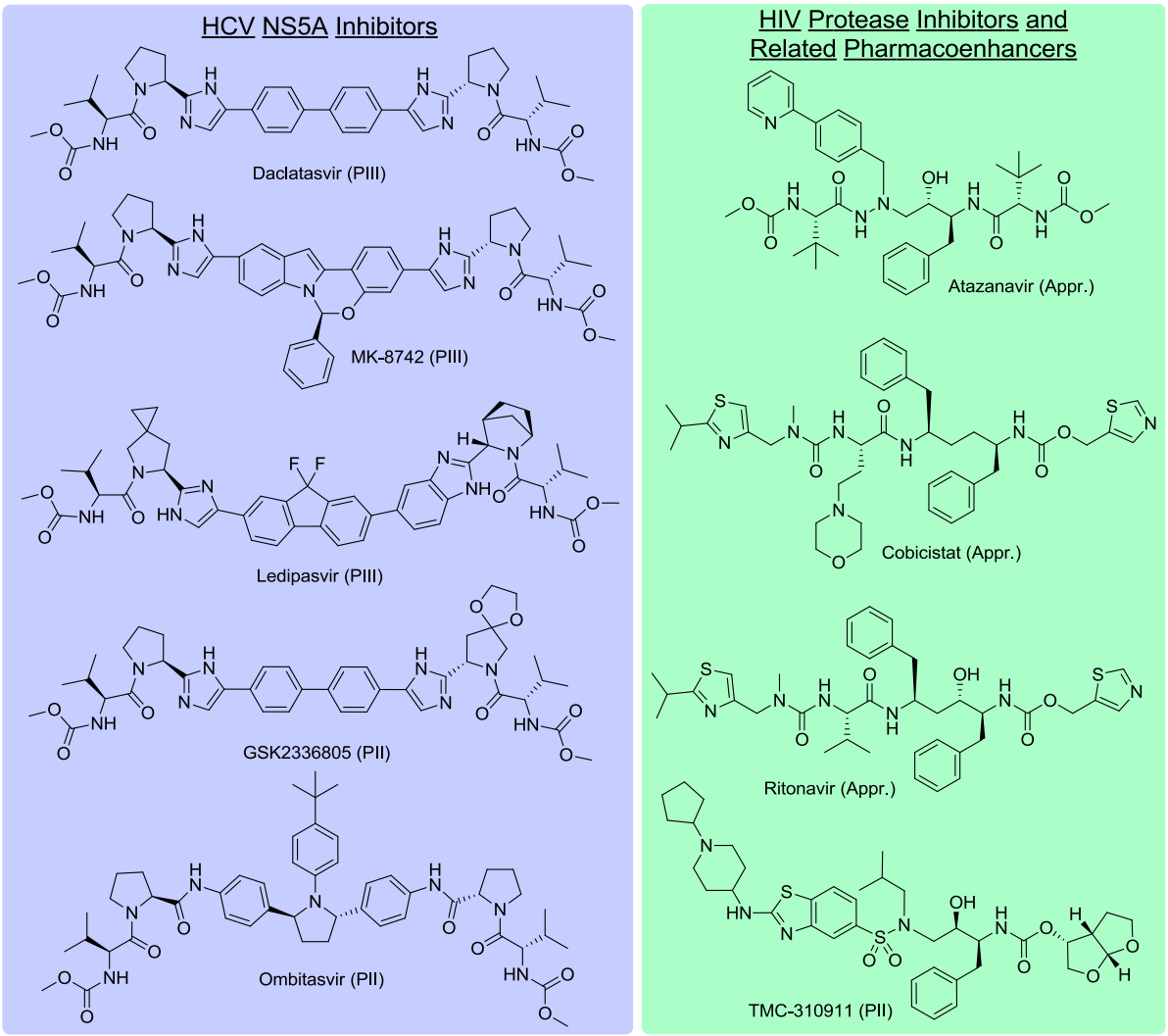
Tacrolimus has variable and low bioavailability partly due to its low solubility; therefore, several formulation approaches such as oil solutions, solid dispersions, complexation with cyclodextrins, and liposomes have been investigated (Patel et al., 2012), as well as prodrugs such as temsirolimus. Tacrolimus is also known to interact with Pgp, BCRP, and MRP-1 (Pawarode et al., 2007). Rapamycins have low bioavailability (14%–20%), and Caco-2 studies of sirolimus and everolimus indicate moderate P_{app}AB with saturation of efflux at higher concentrations (Lamoureux et al., 2012). Sirolimus and everolimus are substrates of Pgp and inhibitors of OATP1A2, OATP1B1, and OATP1B3 which are expressed in the intestine and liver (Picard et al., 2011). Rapamycins are administered at low doses; hence, efflux is unlikely to be saturated and will play a role in their absorption from the intestine. They are also enriched in the liver via Pgp, and there are speculations of additional involvement of transporters (Lamoureux et al., 2012).

Cyclosporins

Cyclosporin A is an 11-residue cyclic peptide immunosuppressant and the only approved cyclic peptide drug that is

Figure 4. Structures, Calculated Physicochemical Properties, and Data of Relevance for Oral Administration of HCV NS3/4A Protease Inhibitors

Dose, F, and P_{app} are average adult dosage in mg/day, oral bioavailability in human or preclinical species (p), and Caco-2 AB permeability in 10^{-6} cm/s where found.

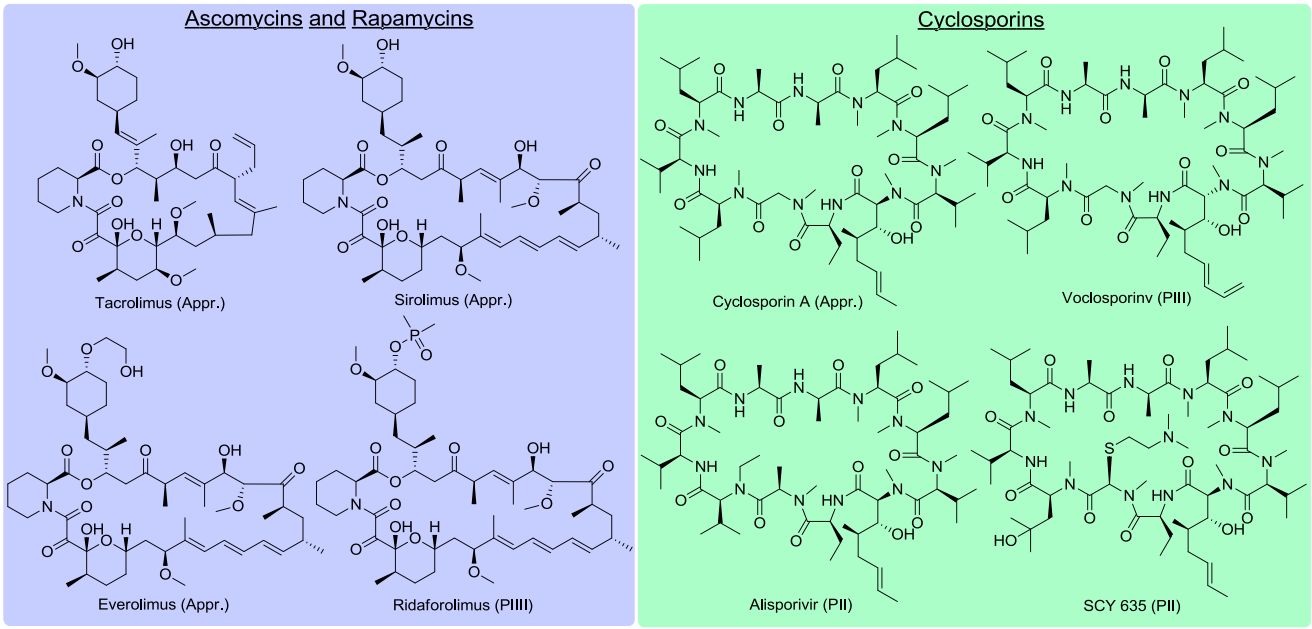


Compound	Phase	MW	HBD	HBA	cLogP	PSA	Dose	F (%)	P_{app}
Daclatasvir	III	739	4	6	5.1	175	60	66-79	7.2
Ledipasvir	III	889	4	6	7.2	175	90	32-53 (p)	
Ombitasvir	III	894	4	7	6.6	207		6.2-57 (p)	
GSK2336805	II	797	4	8	4.6	193		13-51 (p)	
MK-8742	II	882	4	7	6.1	189		9-35 (p)	
Atazanavir	Approved	705	5	7	4.5	171	400	60-68	1.2
Cobicistat	Approved	776	3	7	5.0	138	150	11-33 (p)	
Ritonavir	Approved	721	4	6	5.2	146	1200	70	0.52
TMC-310911	II	756	3	9	5.3	144	300		

Figure 5. Structures, Calculated Physicochemical Properties, and Data of Relevance for Oral Administration of Inhibitors of HCV NS5A Inhibitors and HIV Protease Inhibitors and Related Pharmacoenhancers

Dose, F, and P_{app} are average adult dosage in mg/day, oral bioavailability in human or preclinical species (p), and Caco-2 AB permeability in 10^{-6} cm/s where found.

administered orally. Cyclosporins bind to cyclophilin, the complex of which then inhibits calcineurin, which inhibits T cell activation. Elongation of the original (4R)-4-[(*E*)-2-butenyl]-4,*N*-dimethyl-L-threonine residue at position 1 of cyclosporin A by one carbon atom to give a terminal diene yields voclosporin, a significantly more potent immunosuppressive agent (Aspeslet et al., 2001; Giordanetto and Kihlberg, 2014) (Figure 6). In contrast to cyclosporin A and voclosporin, SCY 635 and alisporivir are not immunosuppressive, but are potential treatments for hepatitis C due to their inhibitory effect on cyclophilin (Flisiak et al., 2012; Hopkins et al., 2010; Watashi, 2010). All four cyclosporins have a very high MW, HBA, and PSA and have only minor variations at amino acids 1, 3, and 4.



Compound	Phase	MW	HBD	HBA	cLogP	PSA	Dose	F (%)	P_{app}
Tacrolimus	Approved	804	3	11	5.6	178	21	19	2.5
Sirolimus	Approved	914	3	12	7.5	195	2	14	2.8
Everolimus	Approved	958	3	13	7.4	204	2.75	20	1.6
Ridaforolimus	III	990	2	12	7.4	202	40	16	
Cyclosporin A	Approved	1203	5	12	3.6	279	950	5-60	1.6
Voclosporin	III	1215	5	12	3.8	279	90		
Alisporivir	II	1217	5	12	4.2	279			
SCY 635	II	1322	6	14	3.0	303		11-23 (p)	1.9

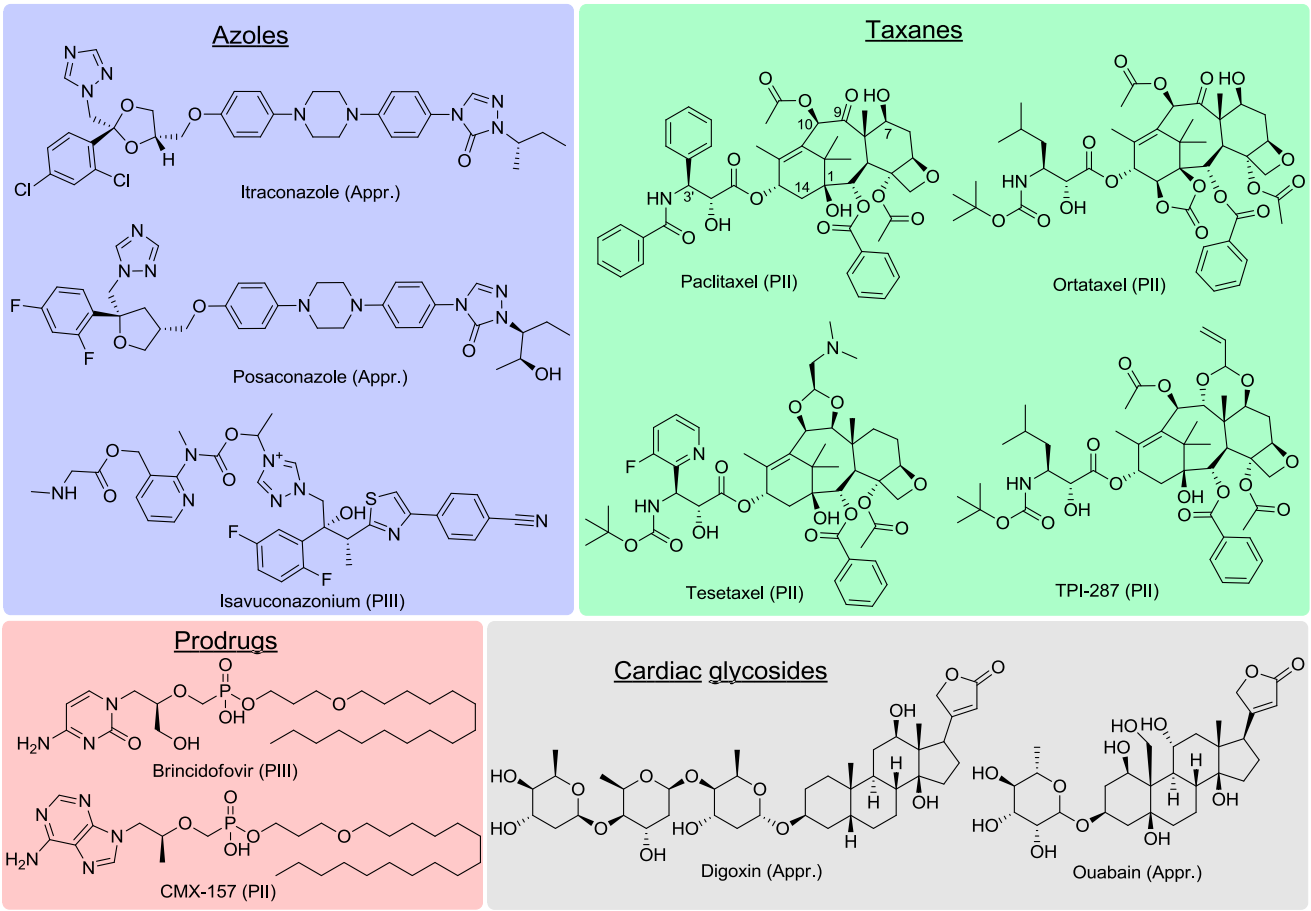
Figure 6. Structures, Calculated Physicochemical Properties, and Data of Relevance for Oral Administration of Ascomycins, Rapamycins, and Cyclosporins

Dose, F, and P_{app} are average adult dosage in mg/day, oral bioavailability in human or preclinical species (p), and Caco-2 AB permeability in 10^{-6} cm/s where found.

Cyclosporin A is a well-known substrate of Pgp; however, it also interacts with a number of additional efflux transporters such as MRP-1 and BCRP and consequently has a highly variable oral bioavailability (Fahr, 1993; Pawarode et al., 2007; Ptachcinski et al., 1985). The ERs for the analogs can be substantially lower, as demonstrated for SCY 635 (ER = 16.5) compared with cyclosporin A (ER = 279), indicating that SCY 635 is transported less efficiently (Hopkins et al., 2010). When considering its MW and polarity, cyclosporin A has an unexpectedly high permeability and bioavailability. Extensive nuclear magnetic resonance (NMR), small molecule X-ray, and computational studies indicate that these properties originate from conformational flexibility that allows formation of IMHBs that shield polarity during passage across nonpolar environments such as cell membranes (Alex et al., 2011; Augustijns et al., 1993; El Tayar et al., 1993; Khojasteh et al., 2011; Lown et al., 1997; Ptachcinski et al., 1985). The specific pattern of N-methylation of nonintramolecular hydrogen bonded amides has also been linked to reducing polarity in the intramolecular hydrogen bonded conformation. As voclosporin, SCY 635, and alisporivir are close analogs of cyclosporin A, it can be assumed that their cell permeability and oral bioavailability are also facilitated by the same type of IMHB (Giordanetto and Kihlberg, 2014). Various formulation strategies ranging from soft gelatine capsules, oral solutions (Sandimmune), a microemulsion (Neoral), topical emulsions, and inhaled forms have been employed to obtain more consistent plasma levels of cyclosporin A (Fatouros et al., 2007; Iacono and Bartley, 2002).

Azoles

Azole antifungals inhibit lanosterol 14-α-demethylase, thereby inhibiting synthesis of ergosterol, an essential component of the fungal membrane (Maertens, 2004). The three azoles in the bRo5 data set all have MW > 700 Da, but isavuconazonium is an oral prodrug of ravuconazole with improved solubility and as such is not discussed further (Thompson and Wiederhold, 2010) (Figure 7). A number of lower MW azole antifungals (MW < 500 Da), such as fluconazole and voriconazole, that have excellent bioavailability (>90%) exist on the market but have a reduced spectrum of activity (Li et al., 2010). Development of azole antifungals has centered on obtaining a broad spectrum of activity, reduced toxicity, and drug-drug interaction caused by binding to human CYP enzymes. For example, posaconazole has an improved spectrum of activity and reduced binding to human CYP enzymes compared with itraconazole, making it less



Compound	Phase	MW	HBD	HBA	cLogP	PSA	Dose	F (%)	P_{app}
Itraconazole	Approved	706	0	9	7.3	101	400	72	
Posaconazole	Approved	701	1	9	5.4	112	800	52 (p)	34
Isavuconazonium	III	717	2	9	0.5	159	328	95	
Paclitaxel (oral)	II	854	4	10	3.5	221	300	6	0.97
Ortataxel	II	872	3	11	4.1	246		25	2.80
Tesetaxel	II	882	3	11	4.1	203		63 (p)	
TPI-287	II	870	3	10	4.7	212		47 (p)	
Digoxin	Approved	781	6	13	2.4	203	0.95	70	0.86
Ouabain	Approved	585	8	11	-2.8	207	24	1	0.11
Brincidofovir	III	562	3	8	3.9	147	29	88 (p)	
CMX-157	II	570	2	8	2.7	137			

Figure 7. Structures, Calculated Physicochemical Properties, and Data of Relevance for Oral Administration of Azole Antifungals, Taxanes, Prodrugs and Cardiac Glycosides

Dose, F, and P_{app} are average adult dosage in mg/day, oral bioavailability in human or preclinical species (p), and Caco-2 AB permeability in 10^{-6} cm/s where found. The bioavailability is given for the cyclodextrin formulation of Ortataxel and Tesetaxel.

prone to toxicity and drug-drug interactions (Lipp, 2010; Torres et al., 2005).

The oral bioavailability of itraconazole capsules is variable with a mean of 55%, potentially due to poor solubility (Peeters et al., 2002) and dependence on dosage with food/gastric pH. Hence, an oral suspension with cyclodextrins was developed, which reduced variability and improved the mean bioavailability to 72% (Prentice and Glasmacher, 2005). Posaconazole has a high permeability; however, it also suffers from poor solubility (Saha and Kou, 2000) and benefits from cyclodextrin formulation-improving oral bioavailability from 14% to 52% in monkeys (Courtney et al., 2003; Nomeir et al., 2000). Both itraconazole and posaconazole are substrates of Pgp (Keogh and Kunta, 2006; Li et al., 2010; Merck, 2006, NOXAFIL-posaconazole suspension label), but as doses of both compounds are high, it can be postulated that efflux does not have a major impact on oral absorption. Finally, itraconazole has a nonlinear dose dependency, postulated to be caused by saturation of liver metabolism, which contributes to its good oral bioavailability at the high dose given (Prentice and Glasmacher, 2005).

Taxanes

Paclitaxel is probably most well known for its semisynthetic method of production and the novel mechanism of anticancer activity, i.e., β tubulin binding and microtubule stabilization that prevents G2-M cell progression (Cragg, 1998) (Figure 7). It is administered intravenously, but suffers from poor solubility and requires long infusion times with Cremophor EL. Cremophore EL is an ≈3 kDa polyethylene glycol derivative used as a formulation aid for a number of drugs and is thought to cause toxicity and hypersensitivity reactions as well as nonlinear kinetics of paclitaxel (Gelderblom et al., 2001). Consequently, substantial effort has been placed on the development of analogs that are orally bioavailable and that also overcome drug resistance, mainly Pgp-mediated efflux and tubulin mutations (Ferlini et al., 2008). Three such taxanes with high MW (852–882 Da) and high PSA (203–246 $Å^2$) are in phase II trials. Changes to the paclitaxel structure in positions C7, C10, C14, and C3′ have been found to improve potency and/or modulate the Pgp-mediated efflux of taxanes (Jing et al., 2014). It is noteworthy that all three analogs have a somewhat increased cLogP (4.1–4.7 versus 3.5) as compared with paclitaxel and a reduced number of HBD (3 versus 4).

The epithelial cell permeability ($P_{app}AB$) of paclitaxel is low; however, to a large extent, this is due to Pgp-mediated efflux as demonstrated by an ER of ∼34 (Jing et al., 2014). The role of efflux is illustrated by the increase in oral bioavailability of paclitaxel from a low 6% to 47% when dosed with cyclosporin A, a Pgp inhibitor (Meerum Terwogt et al., 1999). Phase I/II clinical trials of paclitaxel codelivered with the Pgp inhibitor HM30181 are underway and may provide a viable oral delivery of paclitaxel. Ortataxel, in contrast, has ≈3-fold greater $P_{app}AB$ and a reduced ER ≈7 (Jing et al., 2014; Vredenburg et al., 2001), with a corresponding improvement in human oral bioavailability to 25%. TPI-287 has shown potential of blood-brain barrier penetration (Fitzgerald et al., 2012) and mouse oral bioavailability (McChesney et al., 2008); however, clinical trials currently focus on i.v. administration.

Cardiac Glycosides

Digoxin and ouabain are natural products and possibly endogenous hormones (Nicholls et al., 2009) acting as inotropic agents. They inhibit the membrane bound α-subunits of the Na^+/K^+-ATPase, leading to an increased Ca^{2+} level in heart muscle cells, which ultimately results in stronger contractions of the heart (Schoner and Scheiner-Bobis, 2007). Digoxin is included in the bRo5 data set because of high MW, HBD, and PSA, whereas the cLogP remains acceptable despite the trisaccharide moiety. Oubain is smaller but highly polar (PSA = 207 $Å^2$, HBD = 8) with a low cLogP (−2.8) due to its polyhydroxylated nature.

The oral bioavailability of ouabain is very low (1%) and varies between individuals and therefore oral treatment with ouabain is rare (Belz et al., 1984; Nordqvist et al., 2004). Digoxin has a higher and less variable bioavailability (Cohen et al., 1993; Ochs et al., 1981) and is still prescribed, although the identification of angiotensin-converting enzyme inhibitors, β-adrenergic blockers and angiotensin-receptor blockers has significantly reduced its clinical use. Digoxin is one of the best analyzed substrates of Pgp, as it is frequently used as reference compounds in transporter studies (Cavet et al., 1996; Xu et al., 2003). Digoxin is also described as a substrate of MDR1 and MDR2, but with contradictory information on effects on OATP transporters (Khojasteh et al., 2011; Taub et al., 2011). It is interesting to note that, in spite of Pgp-mediated efflux, low-dose and a low epithelial cell permeability the oral bioavailability of digoxin is still high.

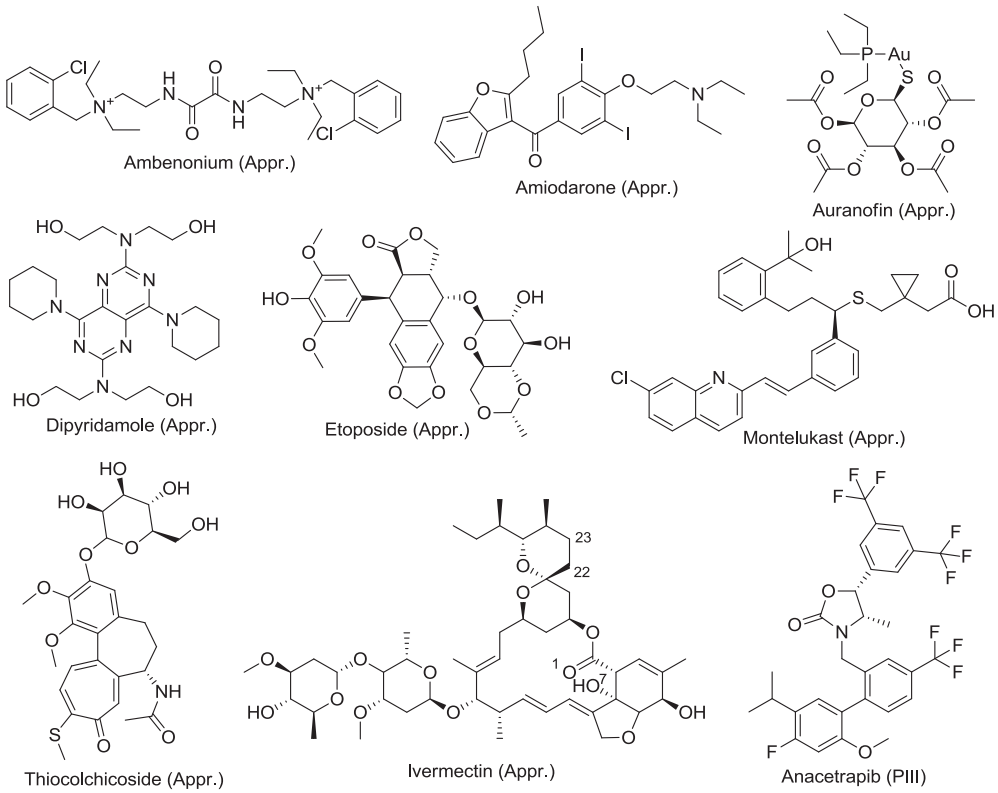
Prodrugs

Six prodrugs are included in the oral bRo5 set, the majority due to a high NRotBs. Four of these conform to well-established ideas for improving solubility or providing extended release. However, the antiviral prodrugs brincidofovir (hexadecyloxypropyl-cidofovir, CMX-001) (Marty et al., 2013) and CMX-157 (Lanier et al., 2010) (Figure 7) are lysophosphatidylcholine mimetics that are proposed to be absorbed through endogenous fatty acid uptake pathways to the blood or lymph (Painter and Hostetler, 2004).

The oral bioavailability of brincidofovir is 88% in mice (Ciesla et al., 2003) compared with the parent drug, cidofovir, which is <5% in humans (Wachsman et al., 1996). Once absorbed into the bloodstream, brincidofovir and CMX-157 remain as the prodrug, thereby avoiding uptake in the kidneys by the organic anion transport protein hOAT1, which has been linked to the nephrotoxicity of cidofovir (Ciesla et al., 2003; Hostetler, 2010). Cellular uptake then occurs via endocytosis or with the aid of flippases in the membrane, followed by cleavage and phosphorylation to give the active, antiviral drug in cells (Hostetler, 2010). While not typical of the compounds in the oral bRo5 data set, these two prodrugs illustrate an interesting development in prodrug technology where rational design for incorporation into uptake pathways provides better oral absorption and decreased toxicity in spite of increasing the MW > 500 Da.

Selected Compounds that Have No Analogs in the Oral bRo5 Data Set

In addition to the classes of compounds discussed above, there are 26 compounds that have no close analogs in the oral bRo5 data set. Analysis of these focuses on the eight approved drugs and the only compound in phase III, with illustrative points rather than detailed discussion of all (Figure 8). As less information is available for the compounds in phases I and II (phase I: 6, phase II: 11), these will not be covered here. The nine late-stage compounds have varying indications, and in some cases, their exact mechanism of action is poorly understood (e.g., Auranofin [Walz et al., 1983; Eisler, 2003]). Their physicochemical properties also differ with no common property leading to their inclusion in bRo5 space (Figure 8). Bioavailabilities and Caco-2 permeability are low to moderate, i.e., similar to the other major classes of bRo5 drugs (ambenonium chloride [Havard and Fonseca, 1990], amiodarone [Shukla et al., 1994], auranofin [Blocka, 1983; Walz et al., 1983], dipyridamole [Bjornsson and Mahony, 1983; Terhaag et al., 1986; Elsby et al., 2008; Zhang et al., 2005b], etoposide [Hande, 1998; Guo et al., 2002], ivermectin [Fox, 2006; Griffin et al., 2005], montelukast [Mougey et al., 2009],thiocolchicoside [Sandouk et al., 1994; Trellu et al., 2004], anacetrapib [Kumar et al., 2010; Tan et al., 2010]). Three also display highly variable bioavailability (amidoarone, dipyridamole, and etoposide). Some compounds have been linked to efflux transporters such as Pgp, BCRP, MRP1, and OATP



Compound	Phase	MW	HBD	HBA	cLogP	PSA	Dose	F (%)	P_{app}
Ambenonium	Approved	538	2	2	-3.6	58	45	12	
Amiodarone	Approved	645	0	3	7.6	44	700	20-80	
Auranofin	Approved	678	0	5	-1.0	114	6	25 (p)	
Dipyridamole	Approved	505	4	12	1.8	145	400	11-75	15
Etoposide	Approved	589	3	12	1.2	161	100	40-75	~0.75
Ivermectin	Approved	875	3	13	5.8	170	9	55	0.81
Montelukast	Approved	586	2	4	8.5	73	10	64	~1.5
Thiocolchicoside	Approved	563	5	10	-0.2	164	8	25	
Anacetrapib	III	638	0	2	9.3	39	100	<20	

Figure 8. Structures, Calculated Physicochemical Properties, and Data of Relevance for Oral Administration of Other Approved and Phase III Compounds in the Oral bRo5 Set

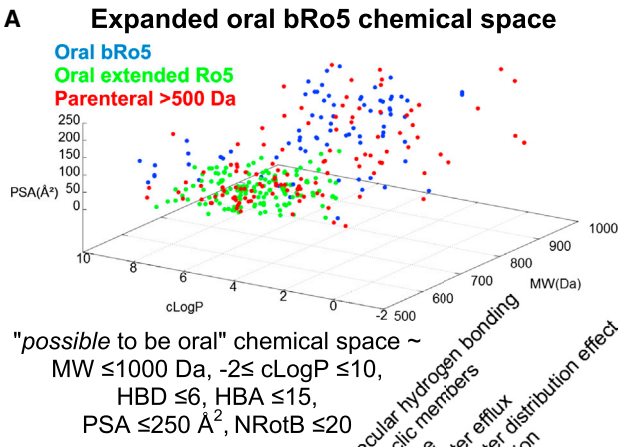
Dose, F, and P_{app} are average adult dosage in mg/day, oral bioavailability in human or preclinical species (p), and Caco-2 AB permeability in 10^{-6} cm/s where found.

(amiodarone [Segawa et al., 2013; Cermanova et al., 2010], etoposide [Stephens et al., 2002], and montelukast [Roy et al., 2009]). Of all the bRo5 compounds investigated, only montelukast has data suggesting that it is actively taken up in the intestine (Mougey et al., 2009; Mougey et al., 2011); however, conflicting data also exist (Chu et al., 2012; Kim et al., 2013). A number also display poor solubility and can benefit from alternate formulations or dosage with food (amiodarone [Meng et al., 2001], dipyridamol [Kostewicz et al., 2002], ivermectin [Edwards et al., 1988], montelukast [Okumu et al., 2008], anacetrapib [DiNunzio et al., 2012; Geers et al., 2011; Kumar et al., 2010]). From comparison to the avermectin B_{1a} crystal structure (Springer et al., 1981), intramolecular hydrogen bonding has been suggested between O1 and O7 of ivermectin, but NMR does not support this (Neszmélyi et al., 1989). For thiocolchicoside, the glycoside itself is not detectable in blood; rather, the aglycone is found (which is Ro5 compliant) and thus may be the absorptive species (Sandouk et al., 1994; Trellu et al., 2004).

The Landscape of bRo5 Space

It has been argued that strict implementation of the Ro5 may have resulted in lost opportunities in drug discovery (Abad-Zapatero, 2007; Walters, 2012; Zhang and Wilkinson, 2007). As an extension of our previous review of macrocycles (Giordanetto and Kihlberg, 2014), we therefore performed an exhaustive review of all known drugs and clinical candidates having a MW > 500 Da. As discussed in greater detail in this section, our analysis of how oral bioavailability is related to the physicochemical properties and in vitro cell permeability of the compounds in the data set led to several key findings. First, it

A



B

Major oral bRo5 Class							Origin
Erythronolides		✓	✓	✓	✓		natural product
Leucomycins		✓	✓	✓			natural product
Rifamycins	✓	✓	✓	✓	✓		natural product
HCV NS3/4A protease inhibitors		✓	✓	✓	✓		peptide
HCV NS5A inhibitors	✓			✓			HTS
HIV-1 protease inhibitors	✓		✓	✓			peptide
Ascomycins and Rapamycins		✓		✓		✓	natural product
Cyclosporins	✓	✓	✓	✓		✓	natural product
Azoles				✓	✓		✓	*de novo*
Taxanes				✓	✓		✓	natural product
Cardiac glycosides					✓			natural product
Lipid mimetic prodrugs						✓		*de novo*

Figure 9. Overview of Chemical Space for Drugs and Clinical Candidates with MW > 500 Da and Trends Found for Orals bRo5

(A) Oral and parenteral categories of drugs and clinical candidates having a MW > 500 Da and extent of "possible to be oral" chemical space.

(B) Major classes of oral drugs and clinical candidates bRo5, common trends that affect oral bioavailability, and origin of leads for the different classes.

was established that the chemical space where orally bioavailable compounds may be designed extends far bRo5. In addition, hits and leads for compounds currently found in this bRo5 space were found to predominantly originate from natural products and peptides, with several interesting approaches being explored preclinically. Opportunities to improve compound properties and thereby facilitate oral administration in design of compounds bRo5 were identified, e.g., intramolecular hydrogen bonding and tailor-made formulations. Finally, risks in this chemical space, such as efflux at the blood-brain barrier of compounds intended for CNS applications, are summarized.

Extent of Chemical Space Where Oral Drugs Can Be Found

Our analysis reveals that the proportion of approved drugs that have a MW > 500 Da has increased steadily during the last 2 decades. This increase has mainly been driven by approval of increasing numbers of de novo designed compounds, whereas natural products previously dominated approvals in this chemical space. Even though ADMET may be a hurdle when molecular size and complexity increases, analysis of the current data set suggests that the limits for oral bioavailability extend to approximately MW $\leq$ 1000 Da, $-2 \leq$ cLogP $\leq$ 10, HBD $\leq$ 6, HBA $\leq$ 15, PSA $\leq$ 250 Å^2, and NRotB $\leq$ 20 (Figure 9A). This represents a significant expansion of the traditional "*likely* to be oral" Ro5 chemical space to a substantially larger "*possible* to be oral" space. It should be pointed out that whereas most calculated physicochemical properties can be increased significantly as compared with the Ro5, this is not the case for the number of HBD. Consequently, HBA rather than HBD are largely responsible for the increased PSA of high-MW orally available compounds. Interestingly, there are still a few oral compounds that have properties outside the "possible to be oral" space, such as cyclosporin A and derivatives. Without doubt, additional compounds with properties far outside Ro5 space will therefore be discovered in the future and contribute to our understanding of the extent of oral druggable space.

The Origin of Drugs and Clinical Candidates bRo5

It is important to define the sources of hits and leads for drugs and clinical candidates close to the limits of oral bioavailability (Figure 9B). Traditionally, natural products have been a rich source of drugs in oral bRo5 space, with many being discovered over 2 decades ago. As the parent natural product often had nonoptimal ADME properties, modification was required to provide satisfactory oral bioavailability (cf. erythronolides, rifamycins, rapamycins, and taxanes). More recently, peptides have appeared as starting points for discovery of oral bRo5 compounds even though few have properties suitable for oral delivery, with cyclosporin A being a notable exception. However, optimization based on a combination of structure based design and use of peptidomimetics tactics to improve PK has led to the successful oral delivery of bRo5 compounds at the border of the peptidomimetic and de novo designed classes. HCV NS3/4A and HIV protease inhibitors are prominent examples of oral compounds in bRo5 space developed from peptides. For more peptidic compounds, formation of IMHBs and N-methylation of amide bonds has been demonstrated to be important for oral bioavailability (Rezai et al., 2006a; White et al., 2011). High-throughput screening has also been applied to identify leads in bRo5 space, with HCV NS5A inhibitors originating from phenotypic screens. The azole antifungals exemplify how a continued development starting from the 1944 discovery that benzimidazole had antifungal activity may lead into bRo5 space.

The recent advances in development of antivirals reveal that we are able to design oral compounds in bRo5 space from starting points other than natural products. Hence, the low proportion of de novo designed compounds bRo5 is likely a result of perceived "bad ADMET bRo5", which has led to less effort in this chemical space. Our understanding of ADMET has progressed substantially over the last 15 years; hence, the lack of de novo designed compounds bRo5 indicates a significant area of potential future growth. Success will partly rely on our ability to discover hits and leads that are substantially different from those originating from traditional small molecule screening collections. To facilitate this, several interesting approaches are already being explored, including use of small tethered oligopeptides (Terrett, 2010), larger cyclic peptides (Josephson et al., 2014; Obrecht et al., 2012), diversity-oriented synthesis (O'Connor et al., 2012; Schreiber, 2000), and a resurged interest in

natural product derived approaches (Li and Vederas, 2009; Newman, 2008). Alternately, traditional small molecule screening collections may be used, provided that a significant change in the mindset and goals of the optimization is allowed.

Intramolecular Hydrogen Bonding

Formation of IMHBs is a structural feature that has been reported to contribute to improvement of cell permeability and oral bioavailability for several chemical classes in bRo5 space (Figure 9B). Incorporation of IMHB during design therefore represents an important opportunity to improve compounds properties bRo5. In the current data set, natural products such as cyclosporins, rifamycins, and ivermectin as well as de novo designed peptidomimetic HIV protease inhibitors and HCV NS5A inhibitors have been reported to form IMHB. Cyclosporins and rifamycins stand out due to their high calculated polarity (5–6 HBD, 12–15 HBA, and PSA >200 $Å^2$) yet maintain moderate to good oral bioavailabilities of 30%–70%. For cyclosporin A, extensive investigations demonstrate formation of several IMHB in nonpolar solvents, effectively shielding polar groups, and improving cell permeability (Alex et al., 2011; El Tayar et al., 1993). Additionally and importantly, solubility is maintained as IMHB dissociate in aqueous environments exposing polar groups. Rifamycins have been less extensively investigated; however, studies indicate formation of two to four IMHB (Agrawal et al., 2004; Bacchi et al., 1998; Brufani et al., 1964, 1967; Casey and Whitlock, 1975). HIV protease and HCV NS5A inhibitors are less polar (3–5 HBD, 6–9 HBA, PSA 145–205 $Å^2$), but formation of IMHB may also improve cell permeability and absorption for these drugs. It should also be noted that all 17 bRo5 compounds that may form IMHB have high MW (705–1,322 Da), but 11 maintain cLogP < 5, possibly indicating that larger compounds require more polar groups to maintain cLogP and subsequently form IMHB to reduce the number of exposed polar atoms when passing through a cell membrane.

A number of studies describe design or investigations of IMHB in compounds for improved permeability, including linear and cyclic peptides (Beck et al., 2012; Bockus et al., 2013; Rafi et al., 2012; Rand et al., 2012; Rezai et al., 2006a; Rezai et al., 2006b; White et al., 2011), as well as drug discovery leads (Ashwood et al., 2001; Ettorre et al., 2011; McDonagh and Lightner, 2007; Over et al., 2014; Sasaki et al., 2003; Wu et al., 2001). This further demonstrates the opportunity of using IMHB to tune and improve compound properties during design. It also highlights the need for methods that can predict and identify IMHB (Goetz et al., 2014; Jansma et al., 2007; Shalaeva et al., 2013), particularly for compounds requiring CNS penetration or having a high HBD count. A systematic review of crystal structural data revealed a number of preferred IMHB geometries (Kuhn et al., 2010), which were dominated by flat 5- and 6-membered rings, that may be utilized in compound design. In addition to geometry, the use of different acceptors and donors should also be considered to adjust the strength of IMHB in order to provide compounds possessing appropriate properties (Desai et al., 2012). In such efforts, medicinal chemists can also draw on the understanding gained from *inter*molecular hydrogen bonding (Bissantz et al., 2010; Laurence et al., 2009).

Macrocyclization

Macrocyclization is another structural feature that has been highlighted to convey drug-like properties, including oral bioavailability, in bRo5 space (Brandt et al., 2010; Driggers et al., 2008; Giordanetto and Kihlberg, 2014; Mallinson and Collins, 2012; Marsault and Peterson, 2011). This finding is further supported by the observation that macrocycles are enriched in oral bRo5 space (Figure 9B), with 38% (n = 32 of 85) found bRo5 as compared to 15% (n = 34 of 226) in the entire oral data set. Moreover, intramolecular hydrogen bonding in macrocycles such as cyclosporins is generally believed to improve absorption from the intestine. Improved diffusion coefficients across membranes have been demonstrated for macrocyclic model compounds compared to acyclic matched pairs (Bogdan et al., 2011; Rezai et al., 2006b). Generally, this is thought to be due to changes in conformation and shape; however, this remains to be proven experimentally. Additional advantages of macrocyclization have been postulated in drug discovery, including improved affinity for the target due to a reduction in the entropy of binding (Driggers et al., 2008; Mallinson and Collins, 2012). However, studies indicate that this relationship is complex and does not necessarily occur by default but requires careful design and screening of macrocycle linker types (Delorbe et al., 2010). Improved selectivity and reduced metabolism have also been reported for a number of specific examples (Driggers et al., 2008; Mallinson and Collins, 2012).

Transporter-Mediated Efflux and Saturation

The majority of the classes in bRo5 space have been linked to the Pgp efflux transporter, as either substrates or inhibitors. Interactions with additional transporters such as MRPs, BCRP, and OATPs also feature in several classes (Figure 9B). These transporters may reduce the oral bioavailability of low-dose drugs through efflux toward the GI tract, uptake into the liver, or excretion in the kidneys. As efflux transporters are saturable, the effect on efflux from the GI tract is commonly overcome by the high local concentration obtained in the intestine on administration of moderate to high doses. For instance, saturation of intestinal efflux has been suggested for erythronolides, HCV NS3/4A protease, and HIV protease inhibitors (Bergström et al., 2009; Padovan et al., 2012; Parker and Houston, 2008) and may also occur for a number of other bRo5 drugs. It is little surprise that 22 of 26 oral bRo5 drugs with high dosage are anti-infectives, as high dosage is often administered to prevent development of resistance. In addition, anti-infectives target proteins not found in humans, leading to large therapeutic windows and hence opportunities to administer high doses without toxicities.

As noted above, there are few oral CNS-penetrant compounds in the data set. This is consistent with the conclusion that Pgp and other efflux transporters are much more important for CNS-targeted drugs as drug plasma concentrations rarely reach saturation levels at the blood-brain barrier (Desai et al., 2013; Hitchcock, 2012). Tumor, testis, placenta, and retina also express high levels of efflux transporters, and drugs targeting these organs/tissues may also have a greater dependence on efflux compared with drug targeting other organs/tissues. Efflux may also be taken advantage of to reduce CNS side effects, as seen with some classes of drugs (Chen et al., 2003; Hitchcock, 2012).

While Pgp efflux has a limited impact on intestinal absorption, expanding the role of bRo5 drugs to CNS targets will require significant effort. Pgp has been extensively studied, and a number of in silico parameters are correlated to Pgp efflux, including increasing MW, HBA, HBD, PSA, and the most basic pKa (Desai et al., 2013; Hitchcock, 2012; Demel et al., 2008). Consequently, strategies that influence theses parameters can be used to overcome Pgp efflux at the blood-brain barrier and also in the intestine (Desai et al., 2013; Hitchcock, 2012). Another approach illustrated in the current data set involves administration of pharmacoenhancers, such as ritonavir and cobicistat. These act by inhibition of CYP3A4 metabolism and Pgp-mediated efflux and are administered to enhance the bioavailability of other anti-HIV drugs. Coformulations of Pgp inhibitors with paclitaxel for improved bioavailability are also in development. However, in this context, it should be noted that saturation and inhibition of transporters and metabolism can have disadvantages, leading to potential drug-drug interactions complicating the therapy of patients on multiple medications. Our knowledge of the importance of transporters in ADMET is constantly growing (DeGorter et al., 2012; Giacomini et al., 2010; Sugano et al., 2010; Varma et al., 2010), and computational models for investigating the role and saturation of transporters have recently been disclosed (Fenu et al., unpublished data), both of which should also facilitate drug discovery bRo5.

Active Uptake and Distribution Effects

Active uptake from the intestine has been suggested only for montelukast among oral bRo5 drugs and clinical candidates (Mougey et al., 2009). This reveals that active uptake is not a common route to improved bioavailability for oral bRo5 compounds and indicates that it may be difficult to capitalize on in future drug discovery, a conclusion that is consistent with several other reviews (DeGorter et al., 2012; Giacomini et al., 2010; Sugano et al., 2010; Varma et al., 2010). Variable bioavailability caused by genetic and environmental variation in transporters is a noted disadvantage of reliance on active uptake. Interestingly, the two prodrugs Brincidofovir and CMX-157 that mimic the active absorption of lysophosphatidylcholine (Painter and Hostetler, 2004) could represent a more generally applicable method for introducing active uptake of drugs.

The current bRo5 data set reveals that improved distribution to target organs through interaction with transporters is of greater importance than active drug uptake from the intestine. Erythronolides, leucomycins, and rifamycins accumulate in lung fluid, likely due to Pgp-mediated efflux for erythronolides and leucomycins (Brook, 1998; Rodvold et al., 2011; Togami et al., 2012). Erythronolides and rifamycins have also been noted to accumulate in phagocytic cells, thought to be due to accumulation of the cationic species in lysosomes (Ahmad et al., 2010; Bosnar et al., 2005; Mor et al., 1995; Wildfeuer et al., 1996). HCV NS3/4A inhibitors are enriched in the liver, which has been linked to uptake via OATP1B1 and a sodium-dependent uptake mechanism (Kalliokoski and Niemi, 2009). Transporters are found throughout the body, but given the prevalence of efflux transporters in the liver and kidneys, it is reasonable to speculate that compounds with sites of action in these organs may benefit from improved uptake by transporters such as OATP and Pgp.

Formulation Improvements

Improved formulations have been used for some of the drugs and clinical candidates in the oral bRo5 data set to overcome poor solubility resulting in low and variable bioavailability (Figure 9B). Thus, formulation of cyclosporin A and tacrolimus has been investigated extensively, resulting in major reductions in the variability of human plasma levels (Fatouros et al., 2007; Iacono and Bartley, 2002; Patel et al., 2012). Reformulation of the azole anti-infective itraconazole with cyclodextrin raised the oral bioavailability and reduced variability in humans; this was also achieved for administration of posaconazole to monkeys. For anacetrapib, the use of an optimized amorphous dispersion gave improved bioavailability (DiNunzio et al., 2012; Geers et al., 2011). A number of improved pharmacokinetic formulations are currently on the market for bRo5 drugs such as cyclosporin A (Neoral) and ritonavir (Norvir) employing lipid-based formulations (O'Driscoll and Griffin, 2008). In addition to use of pharmaceutical formulations, administration with or without food has long been recognized to effect bioavailability; often administration with food improves the solubility of a drug in the intestine, thereby enhancing bioavailability. However, food may also lead to competition with and/or upregulation of efflux transporters and metabolizing enzymes, which alters bioavailability. Similarly, the use of cyclodextrin formulations can cause undesired side effects (Stella and He, 2008). It can, however, be expected that the use of tailor-made formulations to improve low and/or variable bioavailability will become increasingly important for future development of drugs in bRo5 space.

Risks with Compounds bRo5

Working in bRo5 space will most likely result in increased risks, some of which have been mentioned above. Here we therefore endeavor to summarize and discuss risks often touted in the literature as being correlated to bRo5 properties. First, low *solubility* is correlated to increasing MW and cLogP, especially for uncharged compounds (Lipinski, 2000; Meanwell, 2011). While a number of compounds in the current oral bRo5 data set have poor solubility, intramolecular hydrogen bonding and formulation may compensate for this issue. In addition, natural products as well as drugs and clinical candidates have been found to have improved solubility if the proportion of saturated carbons (Fsp^3) and the number of chiral centers is increased, i.e., they are made less flat (Lovering et al., 2009). Solubility may therefore be one reason why de novo designed compounds, which are commonly flatter than natural products and peptidomimetics, are predominantly found within the extended oral Ro5 space and less so in the oral bRo5 data set (cf. Figure 2E). However, with increasing complexity, synthetic tractability must also be considered, and systematic exploration of complex structures represents a challenge for medicinal chemistry in a drug discovery context. Poor *permeability* across intestinal epithelial cells has been correlated to increasing MW and polarity and to decreasing lipophilicity and reaches an optimum for cLogP between 0 and 5 (Guimarães et al., 2012; Johnson et al., 2009; Meanwell, 2011; Waring, 2009; Yang et al., 2012). The majority of bRo5 compounds in this analysis did display low to moderate permeability in the Caco-2 cell assay, but for the 28 drugs where human bioavailability and Caco-2 cell permeability were available, no correlation was found

between permeability and oral bioavailability. Most likely this finding is due to saturation of efflux transporters, as concentration-dependent Caco-2 cell permeability has been described for a number of these bRo5 drugs. The lack of correlation between permeability and oral bioavailability, in combination with the noted variability in the Caco-2 cell assay between laboratories (Artursson et al., 2012), indicates that prediction of oral bioavailability based on epithelial cell permeability data for bRo5 compounds is currently poorly understood. Additionally, the multitude of membrane transporter interactions reported for bRo5 compounds might constitute a potential drug-drug interaction risk during the development of compounds for multidrug therapies. Issues related to *metabolism* have also been commonly associated with bRo5 compounds. A number of relationships between enzymes belonging to the CYP family and in silico compound properties have been published in the literature; the primary concern for bRo5 space is inhibition of CYP3A4, which is correlated to increasing MW and cLogP as well as decreasing Fsp^3 (Gleeson, 2008; Johnson et al., 2009; Lovering, 2013; Meanwell, 2011). This may lead to issues with clearance as well as drug-drug interactions. *Toxicity* is also cited as a reason not to work in bRo5 space, predominantly because of increasing cLogP being correlated to toxicity and promiscuity (Hopkins et al., 2006; Leeson and Springthorpe, 2007; Meanwell, 2011; Price et al., 2009). Studies, however, show that as MW increases promiscuity decreases (Hopkins et al., 2006), in line with the idea that higher MW compounds are more complex and hence less likely to bind to multiple targets. Inhibition of the hERG ion channel is also correlated with increasing cLogP and with a larger value for the fraction of the molecular framework (number of atoms in the molecular framework divided by the total number of atoms in the molecule) (Yang et al., 2012). Hence, small lipophilic molecules are more likely to have off-target effects resulting in toxicity than larger complex ones. In summary, increased lipophilicity appears to be associated with greater risks than high MW in bRo5 chemical space. However, as molecular complexity usually increases for large compounds, higher lipophilicity may be more acceptable in bRo5 space than within Ro5 space; the finding that oral druggable space bRo5 may extend up to a cLogP of 10 provides some support for this speculation (cf. Figure 2A).

Conclusions and Future Oulook

Failure of clinical candidates because of lack of efficacy in phase II trials constitutes one of the current major problems in drug discovery and has been proposed to originate from poor target selection (Bunnage, 2011). In part, this might be due to the fact that effective drugs already exist for a number of diseases, necessitating expansion into new and higher risk therapeutic areas (Scannell et al., 2012). Lack of success in efforts to develop ligands for target classes, such as peptidic GPCRs, nuclear hormone receptors and proteases but also for protein-protein interactions may also contribute to these difficulties. We suggest that a too strict reliance on the Ro5 has led to reduced exploration of bRo5 space and subsequently to poor success in efforts to find ligands for such difficult targets. The observation that ligands for difficult targets have higher MW and greater lipophilicity than highly explored classes (Morphy, 2006; Paolini et al., 2006; Vieth and Sutherland, 2006) lends support to this hypothesis. As discussed herein, the existence of a significant number of orally bioavailable drugs and clinical candidates in this space further points to the opportunities bRo5. Moreover, the low-QED scores found for compounds bRo5 in our data set indicates that a change in mindset will be required for drug discovery in this chemical space.

Discovery of oral drugs bRo5 remains largely uncharted territory and can be expected to be associated with increased risks. Problems with solubility, cell permeability, metabolism, and toxicity have all been correlated to increases in physicochemical properties. However, both low solubility and issues with toxicity have been observed to decrease with higher Fsp^3, i.e., increasing molecular complexity, indicating that these problems may be less severe than otherwise expected. Synthetic chemistry and purification challenges associated with iterative and exhaustive modifications of complex molecules must also be considered, especially for natural product leads. Recent and future advances in synthetic methods may reduce this concern (Marsault and Peterson, 2011). Intestinal efflux can also be an issue, which can be overcome by appropriate dosage and formulations. However, as increased dosage will have little impact on CNS-targeted drugs, further strategies to overcome efflux are required (Hitchcock, 2012). Some drugs, however, benefit from efflux transporters, enabling increased distribution to specific organs such as the liver, kidneys, and lungs.

The current analysis of drugs and clinical candidates contributes insight on how bRo5 space could be explored in the future. Hit finding strategies based on natural products, DOS, structure-based methods, macrocycles, and peptides are of high interest in order to provide starting points of sufficient diversity and complexity for oral bRo5 drug discovery. Moreover, our ability to design oral drugs bRo5 may not be limited to medicinal chemistry starting from bRo5 leads. Simply adjusting guidelines and metrics such as QED scores and LE, could allow projects originating from high-throughput screening and fragment-based lead discovery to progress into this space. Optimization, using structure-based design when possible, and based on our current understanding of ADMET bRo5, can then provide oral clinical candidates, even though this may be more challenging than in Ro5 space. Our analysis indicates that the number of HBD may increase only slightly on moving into bRo5 space, while a larger lipophilicity window appears to be available for optimization, and significant expansions in MW, PSA, and HBA are allowed. Use of intramolecular hydrogen bonding and macrocyclization to tune compound properties, or use of alternate formulations and moderate to high dosage to improve oral bioavailability are tactics that appear to be of particular value for drug discovery in this space.

AUTHOR CONTRIBUTIONS

All authors collated data and prepared the manuscript. J.K. and F.G. initially formulated the concept of the review.

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