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Preface

Understanding the Challenge of Beyond-Rule-of-5 Compounds

The genesis of this theme issue and the meeting around which it is based, was a conversation on the way to the opening reception at the AAPS annual meeting in San Diego 2014. In it we started to discuss the challenges of beyond-rule-of-5 (bRo5) compounds and to what extent we could expect oral administration to provide a viable means to support therapeutic concentrations of such molecules. The central point of the discussion was the argument that the reason for compounds ending up in the bRo5 space was biological and that target biology would dictate the molecular properties of the ligand. Thus, for targets with highly lipophilic binding pockets or targets directed to broad protein-protein interactions where large, flat binding pockets are common, it was highly unlikely that ligands could be identified that were Ro5 compliant. Against this backdrop, it seemed likely that bRo5 compounds would persist, and if anything become more prevalent, and therefore that as delivery scientists we needed to recalibrate our views as to how to enable their oral delivery. For example, what zones in the bRo5 chemical space are actually orally tractable and how can we identify them? To what extent do we understand the molecular characteristics of bRo5 compounds that may be orally bioavailable? How do we make use of this information to identify, early in development, compounds in this chemical space that are sufficiently drug-like to be translated into well-functioning oral medicines? What is the state-of-the-art in delivery technologies to help support oral absorption for these challenging molecules? As we walked, we realized we had many more questions than answers and so the discussions began that led to the 49th Journées Galéniques de St. Rémy de Provence meeting in June 2015 and the collection of reviews that comprise this theme issue - the latter based largely (but not exclusively) around the St. Rémy meeting.

The hypothesis that the properties of the target ultimately dictate, at least in part, the properties of the molecule is explored here by Bergström and colleagues in the first article in this theme issue [1]. In this analysis, 1620 molecules were sampled that were recently patented to give an indication of the properties of relatively contemporary hits and leads (rather than relying on marketed compounds alone). Their molecular properties were calculated using in silico tools and correlated with the target biology. The data suggested, at least in the case of lipophilicity, that consistent with the suggested hypothesis, patented compounds against some targets are almost universally lipophilic. Of the targets examined, approximately 20% had ligands with mean log P values above 5 and approximately 50% had ligands with mean log P values of 4 and above. Realising this inherent propensity for drugs at certain targets to move into bRo5 space, Bergström et al. subsequently explored recent progress in the use of in silico tools to identify functional properties such as solubility and permeability. This type of in silico biopharmaceutical profiling can be used to provide an early signal of the need for enabling formulation strategies for discovery compounds; in this case in particular for identifying the potential to increase exposure via the use of lipid-based formulations. The computer-based biopharmaceutical profiling methods employed were suggested to be highly complementary to other recently developed in silico approaches (multivariate data analysis models and molecular dynamics (MD) simulations) that seek to guide the formulator towards the most appropriate formulation pathway for a new drug molecule. The review stresses the importance of understanding the properties of the target biology early in the discovery process and provides guidance on how in silico tools may be used as decision gates to facilitate the development of oral dosage forms for targets with ligands in the bRo5 space.

Trends in molecular properties and the drivers of a move for drug candidates towards the edge of Ro5 space (and beyond) are discussed in more detail by Paul Leeson [2]. He describes the concept of molecular inflation and presents data showing moderate increases in lipophilicity and significant increases in molecular size over time [2]. Whilst the definition of Ro5 compliant compounds (and therefore bRo5 molecules) is based on limits in molecular weight, lipophilicity and hydrogen bond donors and acceptors, Leeson stresses the importance of additional physicochemical properties such as ionization behaviour, molecular flexibility (number of rotatable bonds) and aromaticity as well as topological indices such as the fractional molecular framework (non-hydrogen (heavy) atoms in the molecular core divided by the total number of heavy atoms) and composite measures such as the property forecast index (a combination of log P and aromaticity) to probe drug-likeness. Updated analyses of the discovery pipelines from large pharmaceutical companies almost 20 years after the introduction of the Ro5 further indicate that, on average, lead optimization practices are leading to the pursuit of larger, more lipophilic and less complex (e.g. less numbers of chiral centres) molecules when compared to marketed drugs. Leeson also reminds us that moves to increasingly lipophilic compounds have been correlated with negative development properties beyond those captured by Ro5, for example toxicity. As such he cautions that before launching into the 'exception', or bRo5 space, efforts to optimise leads within mainstream property space should have been exhausted and that the deliberate use of parameters such as ligand efficiency and lipophilic ligand efficiency, should enable more effective identification of improved leads - even when addressing challenging targets.

Paul Leeson´s analysis is complimented by the commentary provided by Chris Lipinski, the original architect of the Ro5 [3]. Lipinski continues the discussion of molecular properties and the trends towards identification of molecules that sit close to or beyond Ro5 space. While Ro5 is commonly used to identify issues with solubility and permeability, Lipinski reiterates the relationship between Ro5 parameters and target biology, and points out that three out of four Ro5 properties are fundamental to the structure of the binding site. Thus, the molecular

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size cut-off of 500 correlates well with the binding pocket volume for typical protein targets and the descriptors of hydrogen bonding capacity (acceptors and donors) relate to the energy penalty resulting from unsatisfied hydrogen bonding patterns in the binding pocket. Although some targets clearly have ligands that are more lipophilic than others [1], Lipinski claims that log P cannot be related to the biophysics of the interaction between ligand and protein in the same way as molecular weight and hydrogen bonding capacity. The reason for this lies in the way lipophilicity is determined. The artificial system comprising water saturated n-octanol (and n-octanol saturated water) has little or no direct relevance to the lipophilic interactions in the binding pocket. Lipinski subsequently identifies that the majority of successful examples of bRo5 compounds can be found in the natural product chemical space. He cites cyclosporin A as an example of a macrocyclic compound with unique, chameleon-like molecular properties that are rarely found, even among natural products. Cyclosporin A has the potential to form a network of intramolecular interactions that facilitate its permeation through membranes in a manner that is unexpected for a large peptide-like molecule. Furthermore, when aqueous solvation is required, cyclosporine A makes use of the same hydrogen bonds to interact with water. In this way, organisation of hydrogen bonding interactions can facilitate both solubility and permeability in a manner that has been proven difficult to replicate with synthetic peptides. (The role of intramolecular hydrogen bonds in membrane permeability is discussed further by Matsson et al. [4] and Krämer et al. [5] later in this issue).

Lipinski concludes that although functional bRo5 compounds are most likely to be found among natural products, the exploration of protein-protein interactions using structure-directed, fragment-based screening may also identify bRo5 compounds that can be delivered orally. Interfaces of protein-protein interactions are usually flat and relatively large (1,000-2,000 $Å^2$) when compared to the deep clefts and cavities (300-500 $Å^2$) that more commonly accommodate Ro5-compliant compounds. The discovery of compounds that modulate such targets and that are deliverable through the oral route is complex, but possible, and Lipinski provides the example of Navitoclax®. He reiterates, however, that such successes are dependent on exceptional skills in the discovery team; in particular strong communication between medicinal chemists and the in vivo biology team.

Our understanding of the membrane permeability properties of bRo5 compounds is explored in detail by Matsson et al. who discuss the relationship between a range of molecular properties and cell permeability [4]. They remind us that only ~ 50% of all drug targets have been suggested to be tractable by Ro5-compliant compounds and hence, a large fraction of targets are expected to have ligands from the 'difficult' or bRo5 chemical space. They describe the 'extended Ro5' (eRo5) chemical space, a concept introduced as a means to identify molecules that sit outside, but close to, the strict definitions of Ro5 chemical space. In comparision to eRo5 compounds, bRo5 molecules differ more significantly from Ro5 and therefore pose even greater challenge in terms of delivery. The Ro5, eRo5 and bRo5 provide information as to the likely limits (based on current technologies) of chemical space where cell permeable and orally bioavailable drug compounds are likely to be found. From these analyses it becomes evident that orally bioavailable drugs can be found in chemical spaces significantly exceeding the cut-off values of Ro5. Thus, the current outer limit of chemical space, within which cell permeable and orally bioavailable compounds may still be found, appears to be molecular weights as high as 1,000 Da, polar surface areas to 250 $Å^2$, hydrogen bond acceptors up to 15 and log P values ranging from -2 to 10 (albeit commonly centred around 4). Importantly hydrogen bond donors appear to demand stricter control and few orally bioavailable compounds have been identified that have >6 hydrogen bond donors. Although systematic analyses of permeability for large, structurally diverse bRo5 compounds are rare, a recent analysis of 200 macrocycles (non-peptide) reveals fragments that are responsible for driving or restricting permeability. Phenyl and pyridyl groups, isoxazoles and tertiary amines typically increase cell permeability, whereas carbonyls and secondary amines are unfavourable. Based on these findings, Matsson et al. provide a detailed update of permeability enhancing design strategies including strategies to promote conformational shielding of polar groups by lipophilic substituents, covalent modification of amide bonds, formation of intramolecular hydrogen bonds (similar to those discussed for cyclosporin A) and macrocyclization. They note that increases in permeability often occur at the expense of aqueous solubility, and that balancing permeability and solubility remains a challenge. A number of successful examples of molecular approaches to permeability enhancement are provided and underscore the importance of flexibility, stereochemistry and conformation. Their analysis also suggests that bRo5 compounds are more likely to be substrates for efflux proteins of the ABC family (e.g. MDR1, MRP2, BCRP) than influx transporters of the SLC family. More data is needed to draw definitive conclusions around the potential effect of drug transporters, however transporter-specific size restrictions suggest that not all transporters will have the capacity to transport compounds in the (far) bRo5 chemical space. Passive membrane permeability is therefore likely to remain crucial for many bRo5 compounds.

In the subsequent review by Krämer et al. [5] the extent to which passive lipoidal diffusion occurs for polar bRo5 compounds is further explored. In agreement with Lipinski [3] and Matsson et al. [4], Krämer et al. identify the most restrictive of the Ro5 properties to be hydrogen bond donors. They therefore set out to analyse to what extent compounds with 6-10 hydrogen bond donors can be absorbed (and thus provide oral bioavailability) at the same time restricting the data set to compounds with reasonable potency (defined as bioassay activity at concentrations <10 μM). These criteria identified ~100 bRo5 compounds from the entire PubChem database, of which only 10 were found to be significantly absorbed after oral administration. The molecular structures resulting in good absorption were the tetracyclines, macrocycles (e.g. rifampicin), folates and digoxin. Using tetracycline and rifampicin as exemplar bRo5 compounds the authors used a liposomal permeation assay to show that bRo5 compounds can show high passive lipoidal diffusion. Indeed, permeability values in the same range as metoprolol, a commonly used biopharmaceutics classification system (BCS) class I (high permeability and high solubility) model compound, was obtained for rifampicin in this system. The authors conclude that the molecular features required to promote permeability for bRo5 compounds are the ability to neutralize hydrogen bond donors and acceptors in the apolar membrane environment (through e.g. intramolecular hydrogen bonding); the potential to adopt an elongated shape so that the molecule can penetrate the sterically hindered lipid bilayer; and the presence of a high fraction of neutral species. Molecular targeting of influx transporters was also suggested to have the potential to increase absorption but at the risk of an increased incidence of drug-drug interactions, non-linear kinetics and variability related to genetic polymorphism.

Using standard drug-likeness filters, such as the Ro5, bRo5 compounds are more lipophilic, larger and/or have greater hydrogen bonding propensity than is ideal for oral delivery. The permeability limitation in the bRo5 chemical space is most pronounced for large and hydrophilic compounds (or at least compounds with significant hydrogen bonding capacity), whereas solubility issues are typically related to high lipophilicity. The BCS is commonly used to capture the collective impact of solubility and permeability on drug absorption. In the BCS the likelihood of solubility problems emerging is based on the potential for a drug dose to be soluble in a nominal volume of water (typically 250 mL) that might be taken on dosing. It is increasingly apparent however, that this may be an overestimation and the European Medicines Agency (EMA), for example, state that in the fasted state, solubility/dissolution studies should be conducted in ≥150 mL. In this theme issue, Weitschies and coworkers examine water volumes in the human GI tract in some detail [6]. They present recent human in vivo studies of GI volumes,