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(Article begins on next page)

Intramolecular Hydrogen Bonding - An opportunity for improved design in medicinal chemistry

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Running head: Intramolecular hydrogen bonding in drug discovery

Abstract

Recent literature shows that intramolecular hydrogen bond (IMHB) formation can positively impact upon the triad of permeability, solubility and potency of drugs and candidates. IMHB modulation can be applied to compounds in any chemical space as a means for discovering drug candidates with both acceptable potency and ADME-Tox profiles. Integrating IMHB formation in design of drugs is therefore an exciting and timely challenge for modern medicinal chemistry. In this review we first provide some background about IMHBs from the medicinal chemist's point of view and highlight some IMHB-associated misconceptions. Second, we propose a classification of IMHBs for drug discovery purposes, review the most common in silico tactics to include IMHBs in lead optimization and list some experimental physicochemical descriptors which quantify the propensity of compounds to form IMHBs. By focusing on the compounds size and the number of IMHBs that can potentially be formed, we also outline the major difficulties encountered when designing compounds based on inclusion of IMHBs. Finally, we discuss recent case studies illustrating the application of IMHB to optimize cell permeability and physicochemical properties of small molecules, cyclic peptides and macrocycles.

Introduction

An analysis of the literature (Figure 1) performed with Scopus (<https://www.scopus.com/>) shows that the number of papers which include discussions of intramolecular hydrogen bonding (IMHB) has increased significantly in recent years. This analysis thus supports the growing interest that researchers have in IMHB. Notably, two well-known small molecule drugs, amlodipine (a long-acting 1,4-dihydropyridine calcium channel blocker) and sildenafil (a specific inhibitor of phosphodiesterase type 5), form IMHB^{1,2} but the crucial role played by IMHB in governing their ADME-Tox profile was only investigated recently.^{3,4} But why are IMHBs so important for medicinal chemists right now? The emerging interest for IMHBs can be summarized as follows; IMHB formation can mask polar groups and thus impact upon the triad of permeability, solubility and potency of drugs and candidates. IMHB modulation is therefore a promising strategy that can be applied to any chemical space (small molecules and beyond rule of 5 (bRo5)) as a means of discovering drug candidates with both acceptable potency and ADME-Tox profiles.^{5,6 7, 8,9 10}

Figure 1. Number of publications that match the query "Intramolecular Hydrogen Bond" in Scopus up to 2017. Source Scopus: <https://www.scopus.com/> July 2018.

Currently, peptides are probably the most appreciated bRo5 derivatives by pharmaceutical researchers. They are easy accessible via solid phase synthesis, highly selective, relatively safe and well tolerated.¹¹ Unfortunately, only a few of them show a bioavailability (F) \geq 10%.¹² Their transport pathways (both active and passive) across biological membranes have not yet been fully clarified. However it was shown that the physicochemical properties (e.g. lipophilicity and charge) and flexibility of cyclic hexapeptides direct their membrane permeation mechanisms.^{13,14,15} IMHBs which modulates conformation and thus molecular lipophilicity is therefore an accepted key factor impacting on the permeability of cyclic hexapeptides,^{5,16} which can provide medicinal chemists with significant help by acting as an additional tool that facilitates their design efforts.

However, several questions remain; which projects are ideal for incorporation of IMHBs? How can it be decided whether an IMHB is a desirable feature or not? Which IMHB motifs are most suited for optimization of a given lead? This review will attempt to stimulate discussion and give answers to these, and other, questions by critically evaluating the current state of the field and by providing personal, and sometimes provocative, points of view.

We are aware that even though most medicinal chemists are very knowledgeable about IMHB, three main misconceptions are often found in the current literature. Firstly, IMHBs and intramolecular interactions are not synonymous (see below). It follows that IMHBs are only one of several possible intramolecular interactions that involves polar groups.¹⁷ A second fallacy about IMHB concerns the assumption that all that is needed to form an IMHB is one hydrogen bond acceptor (HBA) and one donor (HBD) group in the molecule in poorly-defined “good positions”. This neglects the directionality of IMHBs which is also detailed below. Finally, some still believe that a compound either forms IMHBs or does not. Lack of experience and time are often responsible for these misconceptions which can be overcome by recalling the definition of hydrogen bonding (HB) and IMHB from a medicinal chemistry point of view.

IMHB definition

A hydrogen bond is a non-covalent bond between a donor pair D-H (in which the hydrogen atom H is bound to a more electronegative atom D) and a neighboring electronegative acceptor A (Figure 2A).¹⁸ An HB is defined by three quantities showed in Figure 2A; the (covalent) bond length between atoms D and H (named d_{DH}), the (non-covalent) bond length between atoms H and A (named d_{HA}) and the angle Φ between the two bonds. A HB is formed when the three quantities are comprised in well-defined numerical ranges (Fig. 2B).¹⁹ The proton-carrying partner is often an NH or OH group in many drug discovery projects. The opposite group is an electronegative atom with a partial negative charge such as a carbonyl group or a tertiary amine.

Figure 2. HB and IMHB: A) Schematic representation of the three quantities that define an HB; B) allowed ranges for IMHB formation as reported by Kuhn and coworkers²⁰ and C) the equilibrium governing the formation of an IMHB.

If both the HBA and the HBD belong to the same molecule, an IMHB can be formed, allowing the formation of a pseudo-ring (Figure 2C). In practice, an IMHB is not different than any other hydrogen bond, but the simultaneous presence of HBD and HBA groups within the same molecule imposes additional constraints on HB formation. If the structure is flexible, it must allow an energetically favourable positioning of the HBA and HBD so that the bond lengths and the angle fall within the allowed ranges for formation of a HB (Figure 2B). Otherwise the IMHB formation is not possible or not favorable. Also for rigid structures, the HBA and HBD must be correctly oriented if an IMHB is to be formed. It is worth remembering that the formation of any HB is governed by an equilibrium (Fig. 2C) described by a thermodynamic constant, here named K_{IMHB} , which quantifies the relative amount of closed (IMHB) and open (no IMHB) forms ($K_{\text{IMHB}} = [\text{closed form}]/[\text{open form}]$).

HBD and HBA properties of chemical moieties are crucial to modulate the formation of IMHBs. Several scales have been defined to characterize the different HBD and HBA capacities of chemical groups,²¹ with Abraham's scales being the most widely used in drug discovery.²² In short, Abraham provided a general solute HBD scale and an HBD descriptor for each solute named α_2^{H} . For example, the α_2^{H} value for phenol (0.60²³) is about twice that of methanol (0.37²³). This reflects the better propensity of an aromatic hydroxyl group to act as an HB donor compared to an aliphatic OH. An analogous scale was also introduced for HBA solutes and an HBA descriptor named β_2^{H} was defined as well. If aromatic and aliphatic amines are considered as examples, aniline shows a β_2^{H} value of 0.38²⁴ whereas an aliphatic amine has 0.70.²⁴ In this case, Abraham's descriptors reflect the higher propensity of aliphatic amines to act as HBA with respect to the aromatic ones. However, it should be noted that any scale refers to the formation of intermolecular HBs and their application to IMHBs is not straight forward.

Factors influencing IMHB formation in biomedica

As in any chemical equilibrium, a number of factors can shift the equilibrium position (Fig. 2C) either to the right, forming more of the closed form (larger K_{IMHB}), or to the left giving the open form (smaller K_{IMHB}). Solutes' ionization, although sometimes underestimated in medicinal chemistry,²⁵ is a factor that can significantly impact upon molecular properties,²⁶ and thus also K_{IMHB} , as many molecules contain acidic and basic groups. For instance, an HBD group may be a base (e.g. a secondary amine), which may be neutral or protonated at physiological pH depending on its exact nature (Fig. 3). Such protonation may reinforce IMHB formation. On the other hand, an HBD group may also be an acidic moiety (e.g. a carboxylic acid), the deprotonation of which at physiological pH prevents the formation of any IMHB.

Figure 3. A schematic representation of the impact of ionization on the propensity of compounds to form IMHBs. A) The molecule has no ionization centers, B) the HBD group is a base which is protonated at physiological pH.

A second crucial factor that influences K_{IMHB} is the polarity of the environment. The human body displays a variety of environments which can be roughly, but efficiently, characterized by their polarity. There are aqueous environments ($\epsilon = 80$), apolar environments (e.g. the membrane/protein interior, $\epsilon=2^{27,28}$) and a number of intermediate situations. Since the open form is more hydrophilic, polar media will shift the equilibrium towards the open form (Fig. 4A), whereas the reverse is true for apolar media which favor IMHB formation (Fig. 4C).

Figure 4. The impact of the environment on K_{IMHB} . A) a polar environment favors the formation of the open form (no IMHB), B) the environment is not considered and C) an apolar environment favors the formation of IMHBs.

The effect of HBA and/or HBD properties of the solvent molecules on IMHBs are related to that the solvent may form intermolecular HBs with the solute and thus compete with the solutes IMHB formation. This was experimentally proven by Kamlet et al.²⁹ using 2-nitrophenol (2-NP) as an example, which can form an IMHB between the nitro group (HBA) and the phenolic moiety (HBD). Kamlet et al. showed that the IMHB exists in solvents without HBA properties (e.g. tetrachloromethane), whereas 2-NP cannot form the IMHB in a solvent with HBA properties like DMSO (Fig. 5).

Figure 5. Open-closed form equilibrium of 2-nitrophenol in DMSO. DMSO can form an HB with the open form of the solute.

Finally, the isotropy or anisotropy of the environment may also impact on the formation of IMHB. The environment is isotropic in a solution, meaning that it is reasonable to assume that IMHB formation is only weakly affected by the reorientation of solvent molecules. A different situation may be found in anisotropic environments (e.g. membranes, solid state).

Classifying IMHBs for drug discovery purposes

The complexity of HB interactions calls for methods to differentiate and rank the different types of HBs. Traditionally HBs are classified using energetic criteria. HBs cover a wide range of energy from around 2 to nearly 167 kJ/mol. For instance, Desiraju proposed a classification resulting from a consensus of different proposals: very strong (bond energy ranging from 63 to 167 kJ/mol); strong (bond energy ranging from 17 to 63 kJ/mol); and weak (bond energy less than 17 kJ/mol).¹⁹ Since any energy cutoff could be used, the classification of strong and weak HBs is arbitrary and questionable.^{19,30} Moreover, this classification is poorly suited for drug discovery purposes since it focuses on the strength of the IMHB bond and does not provide any information on the ease of IMHBs formation. In other words, it does not consider the entropic contribution to K_{IMHB} .³⁰

Kihlberg and coworkers⁹ distinguished dynamic from static IMHBs based on if their formation depends on the environment or not. In more general terms, a molecule for which the closed form dominates in any

environment is forming a static IMHB. Conversely, a molecule for which the closed form is preferred in apolar environments and the open form in aqueous media forms a dynamic IMHB and can be considered a molecular chameleon. As discussed in more details in the next sections, this classification is well suited for medicinal chemistry.

Although recently taken up in the literature, the concept of molecular chameleon is not new. In 1991 Carrupt et al. suggested the possibility of morphine glucuronides to exist as molecular chameleons and that this behavior could account for their comparatively good brain penetration and the slow urinary excretion of the two metabolites.³¹ The paper by Alex et al.,⁷ published in 2011, is truly key to understanding the potential of dynamic IMHBs in drug discovery. The authors discussed two experimental conformers of the cyclic peptide cyclosporin A (CsA) and used the concept of a molecular chameleon to rationalize the unexpected bioavailability of this bRo5 immunosuppressant.⁷ It was hypothesized that the reason for the unexpectedly high permeability of CsA is a conformational change from an “open” conformation in water (where the backbone amides mostly form intermolecular HBs with the solvent) to a “closed” conformation in the membrane interior (where IMHBs are formed). According to this hypothesis, the closed IMHB conformations should be populated to some extent already in the polar environment in order to facilitate membrane insertion and diffusion by reduction in the energetic cost associated with the desolvation of the amide N-Hs.⁵ Rafi and coworkers did not specifically mention dynamic IMHBs but suggested that in some cases suitably positioned HBA functionalities can improve the membrane permeability of peptidic small molecules without removing otherwise important HBD functions.³²

The implementation of the concept of static and dynamic IMHB in drug discovery programs is also supported by the results obtained by Rossi Sebastiano et al.³³ who investigated the influence of dynamically exposed polarity on cell permeability and aqueous solubility for a structurally diverse set of drugs and clinical candidates far beyond the Ro5. In this study, the authors firstly collected the available crystal structures for the investigated dataset and showed that all compounds populated multiple distinct conformations. Less than half of the 24 investigated structures contained 1–4 static IMHBs (i.e. present in all crystal structures). Eight compounds formed dynamic IMHBs (i.e. they were present in some but not all

X-ray structures). Then the authors calculated the polar surface area (PSA) of all structures and verified its variation with conformers. The average solvent-accessible 3D PSA hidden by dynamic IMHBs was 27 Å², but this number varied considerably from 9 to 77 Å² between different compounds. Overall this study, supports the impact on passive permeability of dynamic IMHBs in bRo5 drugs and is in line with Whitty and coworkers³⁴ who postulated that some degree of chameleonic behavior is required for large drugs to be orally available. In conclusion, such molecules change their conformation to expose polar groups in aqueous solution, but bury them in apolar environments for instance when traversing lipid membranes.¹¹

Incorporating IMHB in drug design

During the optimization phase of drug discovery, focus lies on increasing both potency and the ADME-Tox profile of drug candidates and IMHB strategies may be crucial to achieve these goals. For instance, a pseudo-ring due to a static IMHB could be used to replace a heterocycle and improve potency.^{35,36} In fact the introduction of an IMHB that leads to a pseudo-nine atom ring in a peptidic structure was recently used by Yang and coworkers to obtain a best-in-class APC-Asef interaction inhibitor.³⁷ Alternatively, to increase permeability and possibly oral absorption, the medicinal chemist is expected to reduce molecular polarity, i.e. the number of HBD and HBA groups. This can be done by alkylation of polar groups, but usually results in reduced solubility. An alternative to the permanent removal of a polar HBD through alkylation is the introduction of a complementary HBA functionality. Through the formation of an IMHB in apolar environments, the HBD and HBA groups are effectively shielded and thus polarity is lowered. Conversely, in water, the HBD and HBA groups can fully expose their polarity and thus not reduce solubility. Generally speaking, a static IMHB is often used to improve potency, whereas a dynamic IMHB could improve permeability and thus the ADME-Tox profile of drug candidates (see Case studies). These two alternatives were also mentioned by Kuhn together with their benefit profiles.⁶

In practice, designing an effective static or dynamic IMHB for optimization purposes is not a trivial task and mainly depends on the size of the compound and the number of IMHBs that it can potentially form. Simplistically, most drug discovery programs can be seen as managing four types of compounds: a) small molecules (MW<500, often Ro5 compliant), b) cyclic peptides (which are macrocyclic if they contain four or

more amino acids in the ring), c) non-peptidic macrocycles (here we define macrocycles as those molecular structures that contain one or more rings of at least 12 atoms) and d) non-macrocylic compounds. Compounds in the three latter classes often reside in bRo5 space.

In general, small molecules can usually form only one or two IMHBs and candidate optimization via IMHB strategies is often easier to obtain than for more complex compounds. For instance, substituted benzamides which are privileged scaffolds in drug discovery can also be exploited due to their potential to form IMHBs. Moreover, pseudo-ring formation is generally straightforwardly detectable by standard NMR experiments performed to assess the chemical structures of new compounds.

Cyclic peptides usually have complex IMHB networks due to the presence of many HBD and HBA groups both on the backbone and on the side chains.^{38,15,39} Moreover, the cyclic peptide IMHB network is easily perturbed by the external environment.⁷ The complexity and peculiarity of IMHB patterns in cyclic hexapeptides were recently illustrated by Ermondi et al.⁴⁰ who studied three cyclic hexapeptides differing by a single amino acid. Using experimental and computational tools the authors showed that in water, the IMHB network involves the side chains of *Asn* and *Asp*, but not that of *Lys* which is therefore free to interact with the surroundings through its charged amino group. Conversely, in chloroform and in a bilayer environment, the *Lys* side chain is folded and not exposed as in water. Overall, this and other studies support that any peptide has a complex and specific IMHB network, that is highly sensitive to the environment. This behavior has two main consequences for cyclic peptides. Firstly, it is very difficult to generalize the properties of cyclic peptides, even within a series. And secondly, using specific IMHBs to modulate the overall IMHB impact on potency and permeability is a most risky strategy.⁴¹ N-methylation¹⁶ and more recently Lipophilic Prodrug Charge Masking (LPCM)¹⁵ and hydrophobic shielding⁴² are reported in the literature as alternatives to IMHB formation to increase lipophilicity and thus permeability of polar peptides. N-Methylation is a common transformation used by medicinal chemists to mask an HBD that causes poor ADME properties. However, N-methylation and IMHB formation are strongly linked. In fact N-methylating amides involved in the formation of IMHBs generally induce an increase in solubility and a decrease in lipophilicity and thus it is not favorable when attempting to improve permeability.^{43,44,45,46}

Non-peptidic macrocycles are complex structures which are attracting major interest in drug discovery efforts aimed at discovering orally available drugs for challenging protein targets that cannot be modulated by Ro5-compliant small molecules.^{47,48,49} The macrocyclic core exhibits a certain level of rigidity,⁹ which often may exceed that of cyclic peptides. For this reason, and because non-peptidic macrocycles usually contain fewer HBDs and HBAs than cyclic peptides, IMHBs are formed to a lesser extent for the former. Formation of IMHBs within the macrocycle core can thus be expected to be rare, but notable exceptions are found, for instance in a derivative of bryostatin 2⁵⁰ (Fig. 6A). However substituents and coupling groups that connect to the macrocyclic ring can be expected to participate more frequently in IMHB formation.³³ The ansamycin antibiotic rifampicin, which is absorbed in the intestine,⁴⁸ provides an example of formation of IMHBs outside the macrocyclic core, as revealed by X-ray crystallography⁵¹ (Fig. 6B). Several members of the erythronolide class of antibiotics, e.g. erythromycin, azithromycin and roxithromycin, also display IMHBs that involve their side chains.⁵²

Figure 6. Crystal structures of A) bryostatin 2 7-(p-bromobenzoate) (CSD code: SIVWAV) which forms IMHBs within the macrocycle core; B) rifampicin (CSD code: MAPHIW) which forms IMHBs outside the macrocycle core and C) faldaprevir (CSD code: MEBYEZ), a non macrocyclic bRo5 compound. IMHBs are in light blue.

IMHB formation in non-macrocyclic bRo5 drugs has been described for the HCV NS3/4A protease inhibitors faldaprevir and asunaprevir.³³ For faldaprevir, one X-ray structure (CSD code: MEBYEZ) reveals the formation of IMHBs shielded by the adjacent aliphatic substituents (Fig. 6C). Notably, in this closed conformation a significant part of the solvent-accessible 3D polar surface area (PSA) is buried, as shown by its reduced value (194 Å²)³³ compared to that (267 Å²)³³ calculated for an open conformation adopted by faldaprevir when bound to the hepatitis C virus NS3-NS4A protease (PDB code: 3P8N). Designing cell permeable non-peptidic macrocycles, as well as non-macrocyclic bRo5 compounds, using IMHB based strategies is expected to be a complex task. However, as compared to cyclic peptides both classes show

fewer IMHBs, both of static and dynamic nature, and can thus be expected to share some common feature at least within series.

Which tactics to use when including IMHB in drug design?

In this part we describe the most common tactics to implement IMHBs in drug discovery programs and a few physicochemical tools that can be used experimentally to evaluate the efficiency of the design procedure early in projects.

Kuhn's topologies

Crystal structures are generally considered as good guides to conformational preferences in solution.^{53,54,55}

In this respect the paper by Kuhn et al.⁶ is a milestone when considering to introduce IMHBs in medicinal chemistry. In this paper, HBD and HBA functionalities, as well as the IMHBs in which they participate, were identified from crystal structures from the Cambridge Structural Database (CSD) and the Protein Databank (PDB) using IMHB distance and angle criteria (Figure 2B). This information was used to compile a list of the topologies of five- to eight-membered ring systems, considered relevant to drug discovery, and their corresponding propensities to form IMHBs.⁶ The propensity was expressed as “the ratio between the number of entries that fulfill the geometric criteria for an IMHB and the total number of entries in the database containing this substructure”. Figure 7 shows three typical Kuhn's topologies endowed with different propensities.

Figure 7. Examples of Kuhn's topologies with their different propensity to form an IMHB.⁶ The first column shows the number of atoms involved in the pseudo-ring motif. In the Topology column the linker between the HBD and HBA groups is characterized. In particular sp^2 – hybridized linker atoms are indicated whereas the circle denotes a cyclic bond. Examples of crystal structures from the CSD or PDB that show the motif reported in Topology are then provided. Finally, the corresponding propensity values (defined in the text) are in the last column⁶

Although conformations adopted in the solid and solution states are not always identical, it is reasonable to assume that topologies with low Kuhn's propensities can form dynamic IMHBs whereas topologies with high Kuhn's propensity can form static IMHBs. Therefore, to design an IMHB with the desired profile (either static or dynamic), the medicinal chemist could submit the 2D representation of the candidates to an application which identifies Kuhn's topologies and ranks structures for their propensity to form IMHBs. If a molecular chameleon is required, candidates showing topologies with low/average propensity to form IMHBs should be selected. The reverse is true if a static IMHB is sought for.

Unfortunately, this strategy has some drawbacks in spite of its tempting simplicity. Firstly, Kuhn's topologies are based on a limited set of five- to eight-membered ring systems and requires an update with more motifs. For instance, motifs including sulfur⁵⁶ and fluorine⁵⁷ atoms still need be evaluated for their propensity to form IMHBs. Secondly, the impact of solvent polarity, together with the isotropic/anisotropic properties of the environment (see previous section) could affect the propensity of forming an IMHB. Finally, designing dynamic IMHBs in cyclic peptides, using Kuhn's topologies, is not recommended. The generic cyclic hexapeptide shown in Fig. 8 can be considered as an illustrative example. IMHBs between the amidic NHs (HBDS) and the carbonyl C=O groups (HBAs) present in the backbone could originate from a number of different chemical motifs. Some of them are present in Kuhn's list, others not. For instance, it is possible to find five- (in blue in Fig. 8) and seven-membered motifs (in green in Fig. 8) with Kuhn's propensities of 8% and 0.8%, respectively. An eight-membered pseudo-ring could also be formed (in red in Fig. 8), but the sequence of atoms in the linker does not correspond perfectly with any of Kuhn's motifs. Additional IMHBs may also be formed among the side chains and between the side chains and the backbone.

Figure 8. Cyclic peptides can form IMHB between the amidic NHs (HBDS) present in the backbone and the carbonyl C=O groups (HBAs). For a generic cyclic hexapeptide topologies present in the Kuhn's list are indicated in in blue and green. An eight-membered pseudo-ring not reported in Kuhn's list is shown in red.

Quantum/Molecular mechanics-based tools

Another strategy to design molecular chameleons (or compounds with static IMHBs if needed) is based on the use of computational tools. In practice, after designing an IMHB in a compound undergoing optimization the difference between the energy of the closed and open conformations need to be calculated to evaluate its dynamic or static nature.⁵⁸ A careful evaluation of the most stable conformations of the two forms is required to reach this aim. This evaluation includes two steps: a conformational sampling procedure to identify an ensemble of conformers containing the biologically relevant ones and an appropriate method, i.e. a force field, for ranking of their energy. A plethora of conformational sampling methods have been reported in the literature⁵⁹ as well as a large number of Quantum and Molecular Mechanics/Dynamics methods to evaluate energies in different environments (their review is beyond the scope of this paper).^{60,60} As discussed above, the influence of the environment cannot be neglected in sampling and ranking of energies. In many Quantum Mechanics calculations, molecular optimization is obtained applying a continuum solvent model⁶⁰ but this approximation underestimates the effect of the interaction of the solutes with solvents bearing HBA or HBD moieties. The use of explicit solvent molecules⁶⁰ could improve the reliability of the simulation. Overall, a standard procedure has not yet been defined that can be applied in drug discovery to effectively identify the dynamic/static nature of an IMHB in any given situation.

A few computational methods that originally were not setup to handle the IMHB problem, but that could be used to describe IMHB patterns in different environments, have been implemented in macrocyclic and cyclic peptides research. Rezaei et al.⁵ adopted an extensive conformational sampling method in low (mimicking a membrane environment) and high (mimicking a water environment) dielectric environments to select the most probable conformations of a series of cyclic peptides. Then, the energy of the most stable conformers common to the two environments was used to obtain a descriptor correlated with cell permeability. The method also provided a qualitative but, in terms of drug discovery, useful analysis of the IMHB patterns in the two environments. Riniker and coworkers applied a molecular dynamics (MD) approach⁶¹ to obtain information about the permeability of Cyclosporin A. In this case, MD simulations of

Cyclosporin A in water and in chloroform were performed and used to characterize the metastable conformational states by means of the Markov state model theory. The conformational landscapes in the two solvents show significant overlap but also net differences. Two metastable sets of conformations in water were close in structure, as defined by the RMSD (the root-mean-square deviation of backbone-atom-positions) to a couple of metastable sets in chloroform and shared their IMHB patterns. These two metastable sets were defined as “congruent” sets and their existence was related to the permeability of Cyclosporin A. “Non-congruent” sets in the two solvents showed different IMHB patterns and could be used to study the influence of the environment on the IMHB profile of the investigated molecule. Notably, both Rezai and Riniker focused on finding the conformations present both in water and in an apolar solvent mimicking a membrane environment. This conformation, or this set of conformations, was then used to predict the permeability of the cyclic peptides.

Physicochemical descriptors to validate design including IMHBs

As discussed above, both Kuhn’s topologies and computational methods can fail in the identification of the static/dynamic nature of the IMHBs of the candidates and thus experimental methods are required to validate the design. NMR techniques, from the simpler amide chemical shift temperature coefficients to the more sophisticated description of the solution ensemble of flexible structures, are applied mostly in late stages of drug discovery. Because of their speed and the small amount of substance required, physicochemical descriptors are preferred in the early phases of drug discovery. In fact during lead optimization, medicinal chemists evaluate the absorption, distribution, metabolism and excretion (ADME) properties of a candidate by monitoring the variation in its physicochemical property profile following chemical modifications.⁶²

Nowadays a number of IMHB descriptors have been implemented in property-based drug design²⁶ as recently reviewed.⁶³ In summary, these include a) $\Delta \log P_{\text{oct-tol}}$, i.e. the difference between $\log P_{\text{oct}}$ and $\log P_{\text{tol}}$ (i.e. the logarithm of the partition coefficient P in the toluene/water system)⁶⁴, b) EPSA, an experimental descriptor of molecular polarity that can be obtained from supercritical fluid chromatography (SFC) retention (normal phase conditions)^{65,66} and c) $\log k'_{80}$ PLRP-S, a molecular descriptor obtained using a

reverse-phase (RP) chromatographic system with a polystyrene/divinylbenzene polymeric column as a stationary phase and an acetonitrile:buffer mixture (80:20) as a mobile phase.⁶⁷

$\Delta \log P_{\text{oct-tol}}$ is a clean descriptor of exposed HBD properties⁶⁸. The presence of IMHBs produces low $\Delta \log P_{\text{oct-tol}}$ values. When a compound has a single HBD group (and at least one HBA), the compound has high propensity to form IMHBs if $\Delta \log P_{\text{oct-tol}}$ is close to 0. In the presence of more HBD groups, the experimental $\Delta \log P_{\text{oct-tol}}$ is not often equal or near to 0 and it is not trivial to establish a net threshold that discriminates when IMHBs are present or not. In these situations, a pairwise approach could help.

EPSA measures the exposed polarity of compounds^{65,69}. Molecules that can form an IMHB can hide their polarity and therefore show lower retention than similar compounds that cannot. Through a pairwise approach, EPSA allows one to distinguish compounds with propensity to form IMHBs (lower EPSA value) from those with no propensity to form IMHBs (higher EPSA values). However, distinguishing the residues involved in IMHBs from the others is not an easy task. In a paper by Sciabola et al.⁴⁶ EPSA was used as a filter (100 was used as the cutoff) to not miss potentially permeable peptides.⁶⁶ To distinguish the residues involved in IMHBs from the others it is possible to evaluate the difference in EPSA (ΔEPSA) between the parent and the differently methylated derivatives.⁴⁶ If ΔEPSA is negative a decrease in exposed polarity is found (i.e. N-methylation has been performed on a residue not involved in IMHB) and thus the permeability of the derivative is expected to be higher than that of the parent. The reverse is true if ΔEPSA is positive.⁴⁶

$\log k'_{80}$ PLRP-S measures the lipophilicity of neutral compounds in an apolar environment.⁷⁰ Like EPSA, $\log k'_{80}$ PLRP-S should be used in a pairwise way. Indeed, for a pair of close analogues, we can assume that both the control and the sample have similar size and hydrophobic properties and their difference in $\log k'_{80}$ PLRP-S is mostly due to the difference in the exposed HBD properties. Therefore, $\log k'_{80}$ PLRP-S for samples with propensity to form IMHBs (less polar) are higher retained than controls with no propensity to form IMHBs (more polar).

The three methods listed above create environments with low polarity and thus can identify both dynamic and static IMHBs. Recently, Grumetto and coworkers⁷¹ introduced $\Delta \log K^w_{\text{IAM}}$, an Immobilized Artificial Membranes (IAM) chromatography derived descriptor. As shown by Ermondi et al. $\Delta \log K^w_{\text{IAM}}$ is an effective

descriptor of polarity⁷² and thus in principle it can also be used to characterize the propensity of compounds to form IMHBs.

Case studies

As shown in Fig.1, the number of published papers discussing IMHBs as a medicinal chemistry strategy has increased considerably in recent years. Most studies describe the potency and/or physicochemical properties of new IMHB-motif-bearing leads, whereas some offer retrospective analysis of the impact of IMHBs on the permeability and physicochemical properties of selected compounds. Finally, a few articles discuss how IMHB formation can be integrated in drug design. Below we review studies in which molecules (bioactive or not bioactive in themselves) were designed to influence the relationship between IMHB and cell permeability, and sometimes also with solubility and lipophilicity; all three of which are major ADME determinants. We focus on those studies that we think provide the best insight on the practical application of IMHB in drug discovery. In particular, we discuss studies strongly supported by experimental data. For the sake of clarity, it is preferable to distinguish applications concerning small Ro5 compliant molecules from bRo5 compounds, and to distinguish the latter into cyclic peptides and macrocycles. Since cyclic hexapeptides are the most studied, we decided to focus on them as examples of cyclic peptides in bRo5 space. Compound numbering is the same as used in the original papers.

Small molecules

The incorporation of an IMHB in a small Ro5 compliant compound during optimization has been described in a few papers, most of which report about the impact of IMHB on potency.^{36,73,74} Notably, in these investigations the use of Kuhn's topologies in design provided good results.

However, as stated above, our main focus lies on studies that provide insight about the application of IMHB to optimize permeability and related physicochemical properties. In 2011 Ettore and coworkers described the use of IMHBs to increase solubility and membrane permeability in a series of compounds with hNK2 antagonist properties.⁷⁵ The authors transformed the original active but poorly permeable compound **1** (Fig. 9) into derivatives which could form IMHBs and thus mask the polarity of one of the amide bonds. The presence of an IMHB was verified by NMR spectroscopy in polar (water and DMSO) environments.

Compound **7** showed a higher permeability and solubility compared to **1** (the control), which could be due to the formation of an IMHB. However, since **1** and **7** are significantly different from a structural point of view, it is difficult to come to a clear conclusion about to what extent IMHB formation influences their difference in permeability (e.g. lipophilicity could also play a role). Conversely, matched pairs **4** and **5**, as well as **6** and **7**, allow investigations of the impact of IMHB on permeability. In both pairs a nitrogen atom has been introduced in the aromatic moiety and allows formation of an IMHB with the N-terminal amide bond. For both matched pairs the introduction of the nitrogen atom led to a reduction in potency at the hNK2 receptor by about 0.8 log units, a slight increase in solubility and a significant increase in permeability both in the Parallel Artificial Membrane Permeability Assay (PAMPA) and the Caco-2 system. The absence of NMR data in apolar media and experimentally determined physicochemical descriptors prevents us from performing an analysis of the static/dynamic nature of the IMHB, but nevertheless this study provides support for the integration of IMHB in drug design.

*Figure 9. Compounds described by Ettorre and coworkers:⁷⁵ **1** is the original active, but poorly permeable lead, the matched pairs **4** and **5**, and **6** and **7**, show how the introduction of a nitrogen atom allows the formation of an IMHB, which in turn is responsible for the increase in permeability.*

Labby et al. reported on the design and synthesis of new neuronal nitric oxide synthase (nNOS) inhibitors in 2012.⁷⁶ The aim of the study was to improve the Blood Brain Barrier (BBB) penetration of the lead (**3**), while maintaining potency, which was achieved by diffusing the overall charge of **3** (Fig. 10A) via the incorporation of an IMHB. This goal was reached in **4b** through the formation of a six-membered IMHB reinforced by protonation of the adjacent secondary amine (Fig 10B).

*Figure 10. A) Representative compounds taken from the paper by Labby and coworkers⁷⁶. Potency and permeability (RPI) are also reported (see text for more details); B) 3D structure of **4b** in D₂O as determined by NMR spectroscopy; C) 3D structure of **4b** in the bioactive conformation (pdb code: 3TYM).*

Fig. 10 A shows potency (expressed as K_i , lower values indicate higher activity) and permeability across the BBB (expressed by the relative permeability index (RPI), lower values indicate higher permeability). The study showed that the propensity of compounds to form IMHBs enhances BBB permeability, but **4b** is also somewhat less potent than **3**. The crystal structure of **4b** (Fig. 10C) showed that the conformation that is bound to nNOS does not form any IMHB. Conversely, NMR experiments in water revealed that **4b** has a high propensity to form an IMHB (Fig. 10B). In our opinion, this study suggests that a chameleon was needed to combine potency with BBB permeability since the “open” conformer maximizes potency whereas the “closed” conformer provides high permeability. However, the NMR experiments revealed that **4b** contains a strong IMHB, detected in a polar solvent and at relatively high temperatures. To enter the active site of nNOS, intermolecular binding interactions must overcome IMHB and this should be easier for a compound with a lower propensity to form IMHBs.

Tardia et al. described the design, synthesis and biological evaluation of a series of P-gp inhibitors in 2014.⁷⁷ Since high lipophilicity is required to obtain the conformation needed to maximize drug-receptor interactions, the authors included an IMHB to increase lipophilicity which, in turn, was expected to lead to an increase in potency (Fig. 11). Therefore, this study mostly focuses on the beneficial effects of IMHB on ligand-receptor binding, but lipophilicity and solubility data are also reported.

*Figure 11. Chemical structures of the two compounds present in the paper by Tardia et al.⁷⁷ and discussed in the text: **4a** is the reference whereas **4b** can form a static IMHB (indicated by the cyan circle). Potency, solubility and experimental and calculated lipophilicity data are also reported.*

NMR spectroscopy and computational studies revealed that **4b** can form an IMHB and is significantly more potent than **4a** that cannot form any IMHB. The increased potency agrees well with the higher lipophilicity determined for **4b** as compared to **4a**. However, the calculated lipophilicities of the two compounds are identical, which highlights the difficulties in the prediction of lipophilicity for compounds forming IMHBs.

Interestingly, the solubility of the two compounds is comparable, which could reflect that **4b** acts as a molecular chameleon that adjusts its conformation to the surrounding environment.

bRo5 compounds: cyclic peptides

A number of studies have investigated how IMHB formation affects permeability in cyclic peptide model systems (mostly not bioactive in themselves), as well as in natural product inspired analogues. Rezai, et al., studied the permeability of a set of stereoisomers of the cyclo-[Leu4-Pro-Tyr] hexapeptide model system⁷⁸ across a parallel artificial membrane (PAMPA). The authors found that permeability varied by two orders of magnitude, with peptide **1** being the most permeable and **9** one of the least (Fig. 12). Peptides **1** and **9** are stereoisomers and of course have identical calculated 2D properties, but their PAMPA permeability differs 100-fold, or possibly more. This was attributed to differences in the number and strengths of IMHB formed by the two compounds. NMR studies in CDCl₃ revealed that **1** and **9** formed four and three IMHBs, respectively. Moreover, the authors verified that the IMHBs formed by **1** were stronger than those in **9**.

Figure 12. Chemical structures of the stereoisomers 1 and 9 discussed by Rezai et al. ⁷⁸. Peptide 1 is 100 times more permeable than 9 since it forms more and stronger IMHBs.

Wang et al. synthesised 62 peptides, measured their permeability in the PAMPA and Caco-2 cell system and performed chromatographic and NMR experiments.⁷⁹ Then the authors correlated permeability with the logarithm of the capacity factor and the NMR amide temperature coefficients, which was considered a convenient overall measure of the hydrogen bonding potential of a peptide. The final model describes the interplay between peptide permeability, lipophilicity and hydrogen bonding potential. In this study, the authors uncovered at least two noteworthy findings; firstly, they experimentally verified that measuring hydrogen bonding potentials is a necessity since computed values (e.g. the number of HBA and HBD) are not reliable for modeling of permeability. Secondly, they showed that hydrogen bonding potential alone cannot model peptide permeability, but that also lipophilicity contributes to cyclic peptide permeability as might be expected.

Thansandote et al. published a study that explored strategies to reduce lipophilicity while maintaining permeability and good solubility of cyclic peptides in 2015.⁸⁰ MD simulations were used to design 6- and 7 membered cyclopeptidic models. The authors then prepared *ad hoc* series of compounds to explore different strategies to optimize properties. Besides backbone rigidification and N-methylation, they verified the importance of the backbone hydrogen bonding network to promote passive permeability. Moreover, chemical structures in which side chains are expected to participate in the hydrogen bonding network to improve solubility while maintaining permeability were designed. Cyclic peptides **9a–b** containing 2-pyridylalanine and **10a–b** containing L- or D-threonine were then prepared (Fig. 13). Experimental determinations of permeability (non-cellular assay), solubility and lipophilicity were performed with in-house tools. To enable a comparative analysis **8** and **1b** were used as controls. Data revealed that introducing and masking side chain polarity whilst successfully improving other physicochemical properties, such as solubility and lipophilicity, had a detrimental effect on permeability at least for the investigated series of compounds. The authors hypothesized that results are probably due to the fact that the expected IMHBs were not formed (i.e. the design failed) but no experimental data was provided to support this conclusion. Overall the authors suggested that increasing passive permeability often occurs at the expense of solubility and lipophilicity, but the available data do not allow to any straightforward correlations between these parameters to be drawn.

*Figure 13. Chemical structures of compounds reported in the paper by Thansandote and coworkers⁸⁰. **8** and **1b** were used as controls for **9a**, and for **10a** and **10b**, respectively. **9b** is an additional control for **9a**. According to the design procedures, **9a**, **10a** and **10b** are expected to form IMHBs involving the polar side chains. Permeability data revealed failures in the prediction since **9a**, **10a** and **10b** were less permeable than controls.*

Bockus et al. synthesised 14 cyclic hexapeptide diastereoisomers containing γ -aminoacids in 2015.⁴⁴ For these compounds the authors measured permeability (PAMPA and MDCK-LE cell system), solubility,

lipophilicity and some other in vitro properties. Then they used NMR spectroscopy to derive solution conformations in CDCl₃. The authors observed that the amides of the less permeable derivatives were significantly solvent-exposed whereas the reverse is true for the more permeable peptides. Interestingly, they also showed that NH had a larger impact (more exposed) than OH (more involved in IMHBs) on permeability. Overall, Bockus and coworkers verified that despite their overall structural similarity, the structures in an apolar solvent exhibited a variety of conformations and IMHB patterns.

Finally, Ahlback and coworkers⁸¹ focused their attention on the permeability of naturally occurring cyclic peptides. In this paper the authors reported the passive membrane permeabilities of 39 derivatives and interpret the results using a computational permeability prediction algorithm based on either available experimental (crystal or NMR) structures or calculated 3D conformations. Moreover, they found that the permeabilities of these compounds, measured in a PAMPA system, spanned a wide range and demonstrated the influence of conformation on membrane permeability. In particular, from the set of compounds for which an experimental structure was available, all but one of the 23 compounds classified as permeable exhibited at least one or more IMHBs. Furthermore, three out of the five most permeable compounds adopted solution conformations in which every amide is involved in an IMHB.

bRo5 compounds: macrocycles

A few studies report about the impact of IMHB on the permeability of macrocycles. In 2015, Over et al. published an investigation into the physicochemical properties and permeability across Caco-2 cell monolayers of a series of eight stereoisomeric lactam inhibitors of *T. cruzi* (Fig. 14).⁸² The aim of the paper was to understand the impact of stereospecific IMHBs on cell permeability and solubility of this set of inhibitors.⁸³

Figure 14. A) Chemical structures, permeability and solubility for the eight stereoisomeric lactam inhibitors of T. Cruzi, described by Over, et al.⁸² IMHBs that may form are indicated in pink. C8, C9 and C25 (in light blue) are the stereogenic centers of the compounds (the configuration is reported in the table); B and C)

conformations of 1 and 8, respectively, as deduced from analysis of energy minimized conformations and NMR data.

This study is a clear example of how a static IMHB can impact the ADME profile of a series of compounds, which is most illustrative even though the compounds are not macrocycles (the largest ring is eight-membered). In this retrospective paper, pK_a , $\log D_{oct}$, solubility and cell permeability data show that the compounds can be clustered into two groups according to the stereochemical relationship between C8 and C9 (Fig. 14A). Stereoisomers **1-4**, which have a trans-relationship of the C8, C9 substituents, have lower pK_a and solubility as well as higher $\log D_{oct}$ and permeability than the set of C8, C9 cis-isomers **5-8**.

Theoretical calculations for **1** and **8** (which represent compounds **1-4** and **5-8**, respectively) revealed that two IMHBs could be formed for both compounds. The first is between the amidic NH and the tertiary amino group (Fig. 14A). The second is between the amidic oxygen of the lactam and the side-chain OH group. The latter IMHB is not discussed in the original paper and is thus not considered further here. NMR spectroscopy in combination with energy minimization of conformations showed that compound **1** forms an IMHB between the amidic NH and the amino group in both $CDCl_3$ and $DMSO-d_6$. Even though not investigated experimentally, it was assumed that this was also the case for the other three C8, C9 trans-isomers (**2-4**). This static IMHB is thus responsible for their high lipophilicity, permeability and low solubility. The remaining four C8, C9 cis-stereoisomers (**5, 6, 7** and **8**) do not form this IMHB, neither in $DMSO-d_6$ nor in $CDCl_3$, and thus exhibit lower permeability and higher solubility. Interestingly, the high propensity to form an IMHB, shown by **1, 2, 3** and **4**, is also revealed by pK_a measurements. In fact, **1-4** are less basic than **5-8** probably because the free electron pair is involved in the formation of the IMHB and thus less available for protonation. This study reveals some issues with the use of Kuhn's topologies in drug design as the same topology can show different IMHB propensities, in this case because of stereoisomerism.

One of very few examples where it is well documented by experimental data that chameleons provide improved permeability and maintained (high) solubility, as compared to rigid analogues, was reported by

Over, et al. in 2016.¹⁴ The paper showed that the syn isomer **7** displayed greater conformational flexibility than anti isomer **12** (Fig. 15), leading to formation of a more dynamic intramolecular hydrogen bond network in an environment-dependent manner for **7**. The oscillation between a single ‘membranophilic’ conformation having two IMHBs in CDCl₃ and a set of hydrophilic conformations with single IMHBs in a polar environment (DMSO-d₆) explains why **7** displays both high cell permeability and high solubility. In contrast, the more polar stereoisomer **12** populates conformations with single IMHBs in both environments and consequently has lower permeability.

*Figure 15. Chemical structures of the compounds discussed by Over, et al. in 2016.¹⁴ Minimum energy conformations of **7** (the most permeable stereoisomer, top row) and **12** (the less permeable stereoisomers, bottom row) in CDCl₃ (left) and DMSO-d₆ (right). Intramolecular hydrogen bonds are indicated by dotted lines.*

In a most recent paper, we explored the variation in IMHBs when conformational sampling was performed with different softwares and in different media for a series of macrocyclic drugs. The results from two of the three softwares highlight that compounds able to form IMHBs are expected to do so to a greater degree in an apolar than a polar environment. Correlating these in silico results with cell permeability was, however, beyond the scope of the paper.⁵²

Outlook

Our ability to rationally use IMHBs in design could significantly affect the success rate of drug discovery projects. The directionality of hydrogen bonds and the strength of HBA/HBD groups are concepts to be kept in mind, just as the dependence of any IMHB network on the polarity of the surrounding medium. In principle IMHB design strategies can be applied to any compound belonging to any chemical space. However, different levels of complexity and success rates are expected to be found; the number of IMHBs that can be formed by the investigated compound is a critical factor.

The classification into static and dynamic IMHBs is essential to implement IMHB in design in drug projects (a molecule for which the closed form dominates in any environment is forming a static IMHB, a molecule for which the closed form is preferred in apolar environments and the open form in aqueous media is forming a dynamic IMHB). It is often found that the formation of a static IMHB is sought to improve potency, whereas use of dynamic IMHBs are convenient when permeability optimization is needed without compromising solubility. As drugs in bRo5 space are often affected by permeability and solubility issues incorporation of dynamic IMHBs is of particular interest in this chemical space. If successful, this could fully release the potential of cyclic peptides and macrocycles in drug discovery.

The application of Kuhn's topologies is a potentially valuable tool to implement IMHB in design in drug projects, at least for Ro5 compliant small molecule drugs. However, it is not recommended for large and flexible compounds in bRo5 space. Traditional conformational sampling procedures combined with force fields for calculating and ranking energies suffer from drawbacks related to the treatment of the solvent. Thus, no standard tool exists to predict the formation of IMHBs, at least not for compounds as complex as many drugs. Therefore, physicochemical descriptors like $\Delta \log P_{\text{oct-tol}}$, EPSA and $\log k'_{60}$ PLRP-S have recently been proposed to quickly assess the formation of IMHBs using a modest amount of compound.

Implementing IMHB strategies to design new permeable cyclic peptides is a very complex task since the large number of HBA and HBD groups enable the simultaneous formation of many IMHBs. In practice, it is the overall effect from co-operative interactions within the IMHB network which governs molecular properties,⁵ whereas the impact of a single IMHB may be less relevant. Several research groups have showed that also in the presence of an overall structural similarity, cyclic peptides can show a variety of intramolecular hydrogen bonding patterns. As a result, many findings are specific within series, or even for individual peptides, and thus not transferrable to other situations. Nevertheless, the synthetic accessibility of cyclic peptides emphasizes the need for continued efforts to implement IMHB in the design of this class of compounds.

Macrocycles are of great interest in drug discovery programs aimed at targeting protein binding sites that cannot be modulated by small, Ro5 compliant molecules. Although macrocycles are expected to be less

flexible than cyclic peptides, the complexity of their chemical structure prevents a safe application of Kuhn's topologies, just as for cyclic peptides. However, because of their lower flexibility and their lower number of HBA and HBD groups, it can be hoped that prediction of their IMHB network is somewhat easier than for cyclic peptides. Computational techniques may therefore be the privileged instruments to implement IMHBs considerations in the design of macrocycles. To fulfil this expectation protocols that account better for the properties of the surrounding environment need to be developed.

Overall, this review shows that IMHB considerations deserve to be included in medicinal chemistry projects, although difficulties can be expected to be encountered, especially for compounds at the borders of or beyond the Ro5. Stronger collaboration between academia and industry would significantly improve the integration of IMHB strategies into drug projects and should increase the impact on project success.

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Author biosketches

Giulia Caron studied at the University of Torino (Italy) where she received a B.Sc. in Pharmaceutical Chemistry and Technology in 1992, and a B.Sc. in Pharmacy in 1994. She then moved to the University of Lausanne where she was awarded a Ph.D. in Pharmaceutical Sciences in 1997 under the supervision of prof. B. Testa. After a postdoctoral period at the University of Lausanne, from 1999 to 2014 she was Assistant Professor at the Molecular Biotechnology and Health Sciences Department at the University of Torino where now she holds the position of Associate Professor. She teaches medicinal chemistry, pharmaceutical analysis and chemometrics also with blended learning technologies. Her primary scientific activity was lipophilicity, then she moved to the design, experimental determination and computational prediction of physicochemical properties related to ADME properties and to permeability measurements. The integration of Intramolecular Hydrogen Bonding (IMHB) considerations in drug design and the development and application of tools to provide a mechanistic interpretation of QSAR/QSPR models are two of her main fields of interest. To fit new drug discovery exigences she is now focusing on defining a set of experimental and in silico tools for molecular properties evaluation in the bRo5 chemical space. She is coauthor of about 85 papers, 4 book chapters and 2 software products.

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Figure legends

Figure 1. Number of publications that match the query “Intramolecular Hydrogen Bond” in Scopus up to 2017. Source Scopus: <https://www.scopus.com/> July 2018.

Figure 2. HB and IMHB: A) Schematic representation of the three quantities that define an HB; B) allowed ranges for IMHB formation as reported by Kuhn and coworkers²⁰ and C) the equilibrium governing the formation of an IMHB.

Figure 3. A schematic representation of the impact of ionization on the propensity of compounds to form IMHBs. A) The molecule has no ionization centers, B) the HBD group is a base which is protonated at physiological pH.

Figure 4. The impact of the environment on K_{IMHB} . A) a polar environment favors the formation of the open form (no IMHB), B) the environment is not considered and C) an apolar environment favors the formation of IMHBs.

Figure 5. Open-closed form equilibrium of 2-nitrophenol in DMSO. DMSO can form an HB with the open form of the solute.

Figure 6. Crystal structures of A) bryostatin 2 7-(p-bromobenzoate) (CSD code: SIVWAV) which forms IMHBs within the macrocycle core; B) rifampicin (CSD code: MAPHIW) which forms IMHBs outside the macrocycle core and C) faldaprevir (CSD code: MEBYEZ), a non macrocyclic bRo5 compound. IMHBs are in light blue.

Figure 7. Examples of Kuhn’s topologies with their different propensity to form an IMHB.⁶ The first column shows the number of atoms involved in the pseudo-ring motif. In the Topology column the linker between the HBD and HBA groups is characterized. In particular sp^2 – hybridized linker atoms are indicated whereas the circle denotes a cyclic bond. Examples of crystal structures from the CSD or PDB that show the motif reported in Topology are then provided. Finally, the corresponding propensity values (defined in the text) are in the last column ⁶

Figure 8. Cyclic peptides can form IMHB between the amidic NHs (HBDs) present in the backbone and the carbonyl C=O groups (HBAs). For a generic cyclic hexapeptide topologies present in the Kuhn’s list are indicated in in blue and green. An eight-membered pseudo-ring not reported in Kuhn’s list is shown in red.

Figure 9. Compounds described by Ettore and coworkers:⁷⁵ **1** is the original active, but poorly permeable lead, the matched pairs **4** and **5**, and **6** and **7**, show how the introduction of a nitrogen atom allows the formation of an IMHB, which in turn is responsible for the increase in permeability.

Figure 10. A) Representative compounds taken from the paper by Labby and coworkers⁷⁶. Potency and permeability (RPI) are also reported (see text for more details); B) 3D structure of **4b** in D₂O as determined by NMR spectroscopy; C) 3D structure of **4b** in the bioactive conformation (pdb code: 3TYM).

Figure 11. Chemical structures of the two compounds present in the paper by Tardia et al.⁷⁷ and discussed in the text: **4a** is the reference whereas **4b** can form a static IMHB (indicated by the cyan circle). Potency, solubility and experimental and calculated lipophilicity data are also reported.

Figure 12. Chemical structures of the stereoisomers **1** and **9** discussed by Rezai et al.⁷⁸. Peptide **1** is 100 times more permeable than **9** since it forms more and stronger IMHBs.

Figure 13. Chemical structures of compounds reported in the paper by Thansandote and coworkers⁸⁰. **8** and **1b** were used as controls for **9a**, and for **10a** and **10b**, respectively. **9b** is an additional control for **9a**. According to the design procedures, **9a**, **10a** and **10b** are expected to form IMHBs involving the polar side chains. Permeability data revealed failures in the prediction since **9a**, **10a** and **10b** were less permeable than controls.

Figure 14. A) Chemical structures, permeability and solubility for the eight stereoisomeric lactam inhibitors of *T. Cruzi*, described by Over, et al.⁸² IMHBs that may form are indicated in pink. C8, C9 and C25 (in light blue) are the stereogenic centers of the compounds (the configuration is reported in the table); B and C) conformations of **1** and **8**, respectively, as deduced from analysis of energy minimized conformations and NMR data.

Figure 15. Chemical structures of the compounds discussed by Over, et al. in 2016.¹⁴ Minimum energy conformations of **7** (the most permeable stereoisomer, top row) and **12** (the less permeable stereoisomers, bottom row) in CDCl₃ (left) and DMSO-d₆ (right). Intramolecular hydrogen bonds are indicated by dotted lines.