There are several different ways drugs can be administered.

- **intravenous administration (iv)**
  - Drugs are directly and fully available in the bloodstream.

- **oral administration (po)**
  - Drugs have to cross some barriers to reach the systemic circulation, so that drugs can be significantly reduced before getting on the bloodstream.
  - Several properties must be optimized, such as permeability (across the intestinal wall) and metabolic stability.

Bioavailability $F$ represents the efficiency of po against iv.

![Bioavailability](image)

\[
F_{abs} = 100 \cdot \frac{AUC_{po} \cdot D_{iv}}{AUC_{iv} \cdot D_{po}}
\]

**Lipinski’s rule of five (Ro5)**
- Proposed by Dr. Christopher A. Lipinski, a medicinal chemist at Pfizer, in 1997.
- Based on the observation that most orally administered drugs are relatively small and moderately lipophilic.
- Outlined that 90% of oral drugs (at that time) obeyed three out of four of the following guidelines: $MW < 500$ Da, $cLogP \leq 5$, $HBD \leq 5$, and $HBA \leq 10 \rightarrow "Ro5"$
- Compounds residing within Ro5 space are more likely to be orally absorbed.
- Quickly became a staple of medicinal chemistry.

**During the last two decades, design of orally-absorbed drugs has mainly focused on the Ro5 space.**

**Small molecules in Ro5 space**
Great potential for oral availability.

The human proteome is estimated to have $100,000 \sim 1,000,000$ binary protein-protein interactions (PPIs), and half of all drug targets cannot be modulated with compounds in the Ro5 space.

**Biologics such as antibodies**
Known as an useful modality for such difficult targets.
Lack the cell permeability required to reach intracellular targets and oral bioavailability.

The shortcomings of small molecules and biologics has recently led to an interest to explore the chemical space between these two.

- **beyond the rule of 5 (bRo5) space**
  - **bRo5**
    - $MW > 500$ Da and at least one of:
      - $MW 700-3000$ Da, $cLogP < 0$ or $> 7.5$,
      - $HBD > 5$, $HBA > 10$, $PSA > 200$ Å²,
      - $NRotB > 20$

- **extended the rule of 5 (eRo5)**
  - $MW 500-700$ Da, $cLogP 0-7.5$,
  - $HBD \leq 5$, $HBA \leq 10$, $PSA \leq 200$ Å²,
  - $NRotB \leq 20$

- **Ro5**
  - $MW < 500$ Da, $cLogP 0-5$,
  - $HBD \leq 5$, $HBA \leq 10$
More than 35 oral drugs have been approved in bRo5 space since 1990. 27 drugs of them belong to the following seven major classes.

- **erythronolides** (antibiotics)
- **rifamycins** (antibiotics)
- **azoles** (fungi)
- **HIV-1 protease inhibitors** (HIV)
- **HCV NS3/4A protease** (HCV)
- **NS5A inhibitors** (HCV)
- **ascomycins or rapamycins** (prevent rejection of organ transplants)

**~1990:**
- approved infrequently
- typically from natural products or derivatives thereof

**2010 ~:**
- became more frequent
- de novo designed took over

More than 35 oral drugs have been approved in bRo5 space since 1990. 27 drugs of them belong to the following seven major classes.

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- **rifamycins** (antibiotics)
- **azoles** (fungi)
- **HIV-1 protease inhibitors** (HIV)
- **HCV NS3/4A protease** (HCV)
- **NS5A inhibitors** (HCV)
- **ascomycins or rapamycins** (prevent rejection of organ transplants)
striking differences in the distribution of binding site shapes Ro5 drugs: display a preference for internal and pocket binding sites eRo5 drugs and clinical candidates: more evenly distributed to groove, tunnel, pocket, and internal sites bRo5 drugs and clinical candidates: mainly the groove sites can bind to even flat binding sites
plot of buried ligand surface area (SA) against total ligand SA

**Ro5 compliant drugs:**
close to 1.0 line, which means Ro5 drugs use their SAs efficiently to bind their targets

**bRo5 drugs and clinical candidates:**
- larger buried SAs than Ro5 drugs can be seen
- proportion of buried ligand SA (against total SAs) is lower compared to Ro5 drugs
  - bRo5 molecules exhibit lower ligand efficiencies (LE) when bind to flat- or groove-shaped sites than Ro5 compliant drugs which bind to pockets or internal sites.

shapes of the ligands (in their target bound conformations)

**Ro5 compliant drugs:**
predominantly rodlike

**bRo5 drugs and clinical candidates:**
more widely distributed and more spherelike
  - Different chemical space can be reached by the use of bRo5 molecules

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**Macrocycles (MC) have a trend to be less rodlike and more spherelike than non-macrocycles (non-MC).**

Only MC molecules in bRo5 space bind to flat sites.
**Beyond the Rule of 5**

**Kyohei Hayashi**

**Baran Group Meeting 8/21/2021**

**Oral bioavailability of bRo5 molecules.**

Main two barriers in oral administration:
1. Hepatic metabolism
2. Permeability against intestinal wall

Permeability is a well-known problem for bRo5 space molecules. Permeability is an essential property to achieve intracellular targets as well.

<table>
<thead>
<tr>
<th>MW (Da)</th>
<th>Oral available molecules can be seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW &lt; 1000 Da:</td>
<td>A sharp drop-off in proportion of oral compounds</td>
</tr>
<tr>
<td>MW &gt; 1000 Da:</td>
<td></td>
</tr>
</tbody>
</table>

**Solutions for the permeability problem**

- **Shielding free HBD by lipophilic side chains.**

Permeability, oral bioavailability in rat: hexa-peptide > penta-peptide

Structures determined by NMR and X-ray indicated the followings;
- The pentapeptide: The Leu projected outward from the plane of the macrocycle, exposing the backbone amides to the solvent.
- The hexapeptide: The Leu shielded the HBDs from the surrounding environment.

Based on the current data set about bRo5 molecules, the limits for oral bioavailability can be extended as following.

<table>
<thead>
<tr>
<th>MW (Da)</th>
<th>likely to be oral (from Ro5 molecules)</th>
<th>possible to be oral (from bRo5 molecules)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>≤ 500</td>
<td>≤ 1000</td>
</tr>
<tr>
<td>cLogP</td>
<td>≤ 5</td>
<td>-2 ≤ cLogP ≤ 10</td>
</tr>
<tr>
<td>HBA</td>
<td>≤ 10</td>
<td>≤ 15</td>
</tr>
<tr>
<td>HBD</td>
<td>≤ 5</td>
<td>≤ 6</td>
</tr>
</tbody>
</table>

- **Note that no big difference in number of HBD**

**Well-known cells/membrane for permeability tests**
- **Caco-2 cell:** expresses P-gp (a well-known efflux transporter)
- **MDCK and RRCK cell:** low expression levels of drug transporters compared to Caco-2 cell
- **PAMPA:** parallel artificial membrane permeability assay (PAMPA) is also often used

**MW 566 Da**
- PSA 146 Å
- RRCK $P_{app}$: $1.7 \times 10^{-6}$ cm/s
- F (rat): 4%

**MW 679 Da**
- PSA 175 Å
- RRCK $P_{app}$: $12 \times 10^{-6}$ cm/s
- F (rat): 17%

---

Neopentyl group reduced EPSA (lipophilicity in hydrophobic environment) and improved permeability.

<table>
<thead>
<tr>
<th>R</th>
<th>CXCR7 Kᵢ (nM)</th>
<th>ClogP</th>
<th>EPSA</th>
<th>MDCK P_app [10⁻⁶ cm/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HN₀</td>
<td>23</td>
<td>8.7</td>
<td>104</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>9.3</td>
<td>89</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>7.1</td>
<td>89</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>9.2</td>
<td>79</td>
<td>4.8</td>
</tr>
</tbody>
</table>

**✓ IMHB formation to mask free HBD**

Py-nitrogen atom formed an intramolecular hydrogen bond (IMHB) to mask the free polar NH.

Temperature coefficients of amide proton NMR (ΔδNH/ΔT) is known as useful way to see existence of IMHB.

large chemical shift change means “not involved in IMHB”

N-methylation of such residues often give significant improvements in permeability and bioavailability

A data set of 475 drugs and clinical candidates with MW > 500 Da MC are likely to have advantage for oral bioavailability compared to non-MCs.

macrocycle structure tends to form IMHB and facilitates bioavailability?

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Chameleonic property

CsA can undergo a conformational change from "open form" to "closed form" according to the environments (suggested by NMR and X-ray).

- in hydrophobic environment: closed form
  - four IMHB are formed
  - expected to have high affinity to membrane interior
- in aqueous media: open form
  - no IMHB is observed
  - binds cyclophilin with this open form

Min (blue) or Max (green) 3D PSA: calculated from structures determined by X-ray crystallography.

Chameleonic property of bRo5 drugs:

oral bRo5 drugs

- Atazanavir (705 Da)
- Ritonavir (721 Da)
- Erythromycin A (734 Da)
- Clarithromycin (748 Da)
- Azithromycin (749 Da)
- Tacrolimus (804 Da)
- Telithromycin (812 Da)
- Rifampicin (823 Da)
- Roxithromycin (837 Da)
- Rifabutin (847 Da)
- Paclitaxel (854 Da)
- Ivermectin (875 Da)
- Rapamycin (914 Da)
- Cyclosporine A (1203 Da)

This conformational flexibility would be facilitated by high numbers of rotatable bonds of CsA (NRotB = 15). It is called “chameleonic property”.

Chameleonic property is thought to be an interesting feature of bRo5 molecules.

Beyond the Rule of 5

Kyohei Hayashi

PROTAC molecules in bRo5
- attracted many attentions in recent years
- expected to dominate the clinical trial population over the next years
- PROTACs consist of a warhead, a linker, and a ligand that recruits an E3 ubiquitin ligase
  - PROTACs are prone to reside in the bRo5 space

Relatively small two oral PROTACs (ARV-110 and ARV-471) recently reached Phase II

Cell permeable larger PROTAC molecule (a VHL-based anticancer PROTAC)

It has a large number of rotatable bonds (NRotB = 27) so that a high flexibility can be expected.

NMR analysis
- Environment dependent conformations

In polar solvents
- extended conformers are present
- In CDCl₃*
  - folded conformations are predominantly found.

Extended conformers
- larger and polar
- Folded conformers
- smaller and less polar

* CDCl₃ was used to mimic the interior of a cell membrane; dielectric constant of chloroform (ε = 4.8) is close to lipid bilayer (ε = 3.0)

Radius of gyration (Rgyr) versus solvent accessible 3D polar surface area (SA 3D PSA) for all conformations. The area of each circle is proportional to the population of each conformation.

CDCl₃: green, DMSO-d6: yellow, DMSO-d6-D₂O: cyan

ACS Med. Chem. Lett. 2021, ASAP
Molecules in bRo5 space have complex structure (SMCM: synthetic molecular complexity metric proposed by Oprea)

e.g.) a complex oral bRo5 drug, Simprevir

- an inhibitor of the hepatitis C virus (HCV) NS3/4A protease
- HCV NS3/4A protease catalyzes most of the cleavages required to convert the newly synthesized viral polypeptide into the different viral proteins
- the MW is around 750 Da
- demonstrates bioavailability
- binds to a flat and featureless site at the surface of the viral protease
- The synthesis is relatively complicated due to the presence of four stereocenters and 14 membered macrocyclic ring

Conducted SAR exploration

- Quinolines
- Isouguinolines
- Quinazolines
- Carbamates
- Pyrimidines
- Other aromatic rings
- Substituent effects
- Ring size
- Heteroatom incorporation

Overlay of simprevir (yellow) and vaniprevir (cyan) bound to the NS3/4A protease.

Compared with Ro5 space, bRo5 space has multiple problems to overcome, such as permeability, bioavailability and synthesis. However, in terms of expanding targetability, it is fair to say that drug discovery in bRo5 space would become more important and more attractive.