



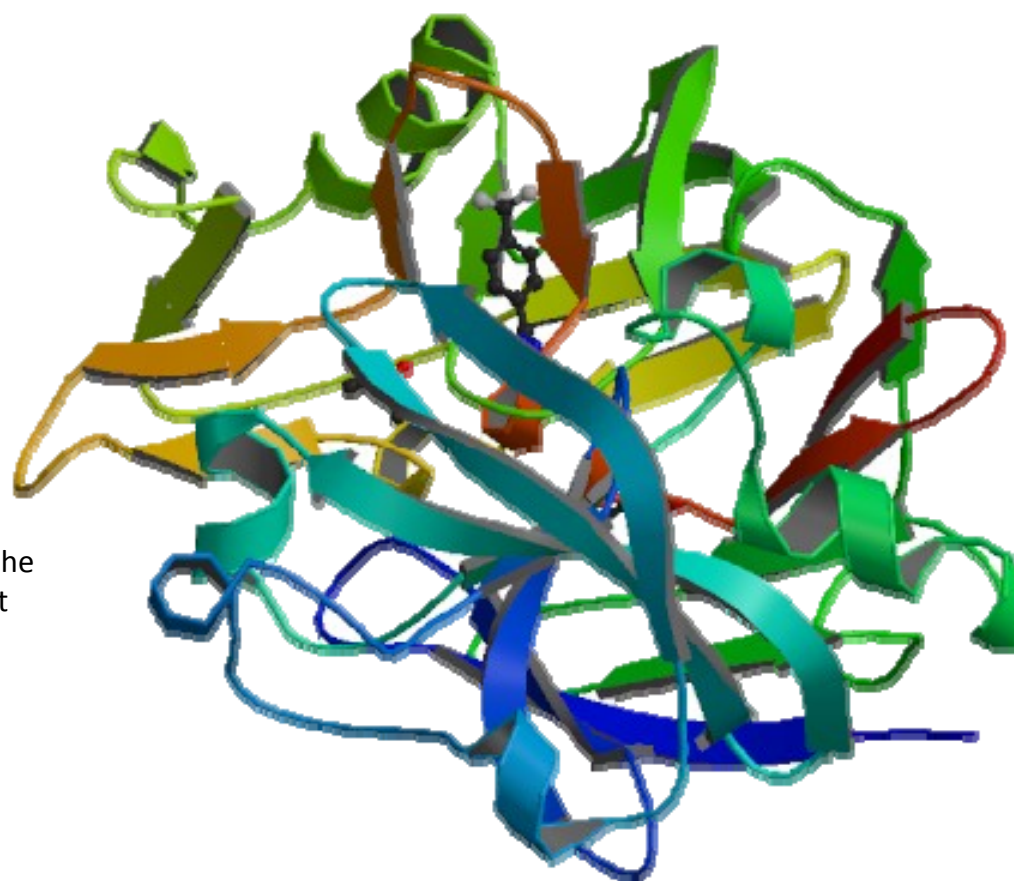
**KEEP
CALM
AND
FOLLOW
THE RULES**

J Med Chem. 2011 Nov 24;54(22):7784-96. doi: 10.1021/jm200642w. Epub 2011 Oct 25.

A small nonrule of 3 compatible fragment library provides high hit rate of endothiapepsin crystal structures with various fragment chemotypes.

Köster H¹, Craan T, Brass S, Herhaus C, Zentgraf M, Neumann L, Heine A, Klebe G.

Biological Assembly Image for 3PCW
Endothiapepsin in complex with a fragment
Protein chains are colored from the N-terminal to the
C-terminal using a rainbow (spectral) color gradient



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- library of 364 fragments - average properties within Ro3 guidelines (with the exception of “Lipinski acceptors,” which would include the nitrogen of a tertiary amide) + some outliers
- performed a fluorescence-based competition screen against the model protein endothiapepsin, resulting in 55 fragments that inhibited at least 40% at 0.5 or 1 mM concentration
- then crystallography trials - 11 structures
- lots of nice analysis of how these fragments bind to the protein
- It also notes that:

Only 4 of the 11 fragments are consistent with the rule of 3. Restriction to this rule would have limited the fragment hits to a strongly reduced variety of chemotypes.



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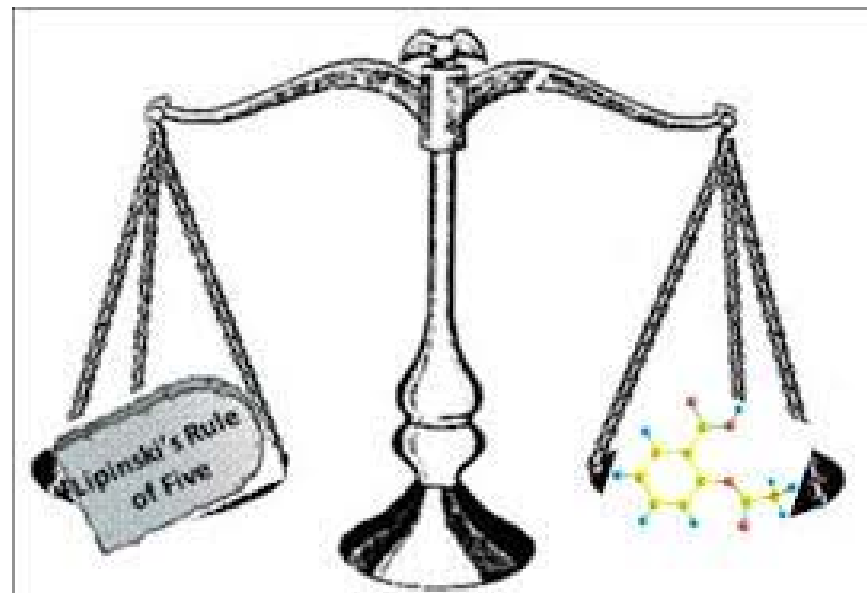
Drug-likeness

- the values of simple physicochemical and structural compound properties that successful drugs have in common
 - hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility
 - bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others



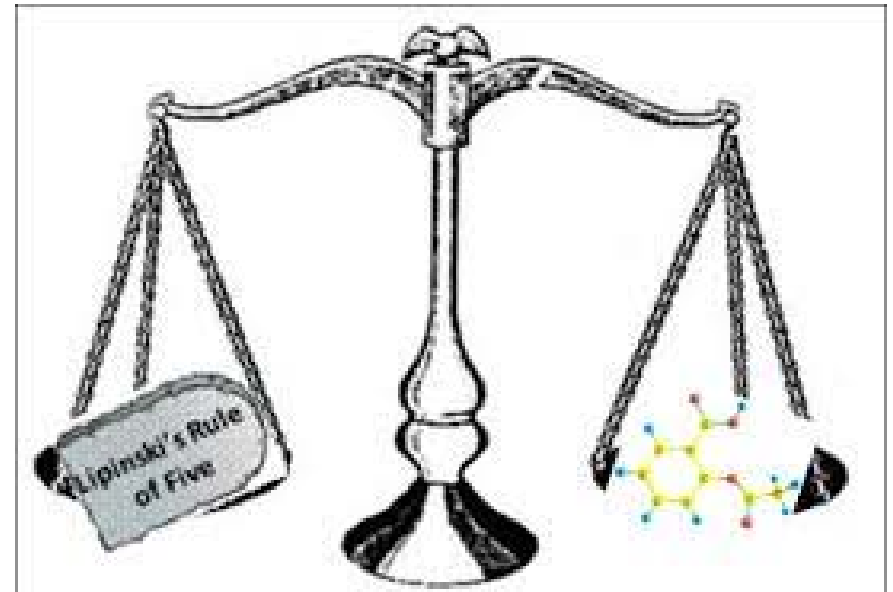
Lipinski's rule of five

- or Pfizer's rule of five or the Rule of five (RO5)
- to evaluate druglikeness or determine if a chemical compound has properties that would make it a likely orally active drug in humans
- derived because of the realization that HTS was identifying large numbers of hit compounds, many of which did not possess 'drug-like' properties
- by Christopher Lipinski in 1997, most orally administered drugs are relatively small and moderately lipophilic



Lipinski's rule of five

- Ro5 identifies molecular properties important for a drug's pharmacokinetics in the human body: absorption, distribution, metabolism, and excretion ("ADME")
- however, the rule does not predict if a compound is pharmacologically active
 - important when a pharmacologically active lead structure is optimized to increase the activity and selectivity



Lipinski's rule

```
graph TD; A[Lipinski's rule] --- B[No more than 5 hydrogen bond donors (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds)]; A --- C[A molecular mass less than 500 daltons]; A --- D[Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)]; A --- E[An octanol-water partition coefficient log P not greater than 5];
```

No more than 5 hydrogen bond donors (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds)

A molecular mass less than 500 daltons

Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)

An octanol-water partition coefficient log P not greater than 5

*an orally active drug has no more than one violation of the following criteria

As with many other rules of thumb, there are many exceptions to Lipinski's Rule

Other rules to define drug-like properties

- Veber, *et al.* (*Journal of Medicinal Chemistry*, 2002): majority of compounds with good oral bioavailability in rats had less than 10 rotatable bonds (ROTB) and polar surface area (PSA) less than 140 Å².
- Hughes, *et al.* (*Bioorganic and Medicinal Chemistry*, 2008): compounds with logP less than 3 and PSA greater than 75 Å² were six times less likely to exhibit adverse events in *in-vivo* tolerance studies
- Ritchie, *et al.* (*Drug Discovery Today*, 2009): number of aromatic rings greater than 3 significantly increases the risk of compound attrition
- Lovering, *et al.* (*Journal of Medicinal Chemistry*, 2009) : "flatness" of compounds, as defined by the fraction of carbons that are sp³ hybridized, guarantees a success in clinical development
- Omran, *et al.* (*Eur. Biophys J.*, 2014): application to drug design and targeting in cancer



- very simple to apply
- properties to which they relate are easily calculated
- easy to see when a compound meets the criteria in each case
- guidance regarding potential risk factors and indicate strategies for improvement



- tendency to apply them as filters or hard cut-offs
- simple compound properties have only a weak correlation with the objectives to which they relate, such as oral bioavailability or toxicity
- the risk of rejecting good compounds or considering non-oral drugs also meet the Ro5

Lead-likeness

- Ro5 provides criteria for drug-like compounds, not appropriate for identifying lead molecules for drug discovery program
- Teague et al. compared lead-drug pairs as well as the results of multiple high-throughput screens that were run at AstraZeneca
- After examining sources of lead molecules - advantage of starting a drug discovery program with compounds that are smaller (molecular mass 100 -- 300) and less lipophilic (CLogP 1 -- 3).
- a number of authors: comparing drugs to the corresponding leads and found that drugs tend to be larger and more lipophilic than the lead molecule
- Perola came to somewhat different conclusions and found that the lipophilicity of drugs was very similar to that of the initial lead

Fragment-based design and the rule of 3

- in F-bd, small lead molecules are identified by using biophysical methods (typically NMR or X-ray crystallography) or high concentration screening
- these fragment leads are then elaborated to produce higher affinity compounds
- to derive definitions of what constituted a fragment and examine the relationships between the potency of a fragment and the potency of a subsequently optimized compound
- in 2003, Congreve et al. analysed the results of fragment screens at Astex and proposed a modification to the Ro5 known as the 'Rule of 3'

A rule of three

```
graph TD; A["A rule of three"] --- B["octanol-water partition coefficient log P not greater than 3"]; A --- C["molecular mass less than 300 daltons"]; A --- D["not more than 3 hydrogen bond donors"]; A --- E["not more than 3 hydrogen bond acceptors"]; A --- F["not more than 3 rotatable bonds"];
```

octanol-water
partition
coefficient $\log P$
not greater than 3

molecular mass
less than 300
daltons

not more than 3
hydrogen bond
donors

not more than 3
hydrogen bond
acceptors

not more than 3
rotatable bonds



- efficient tool in the search for new drugs
- FBDD an alternative approach to traditional lead identification via high-throughput screening (HTS)
 - better chance for the final lead compound to have common drug-likeness parameters
 - relatively small (few thousands) collection of fragment compounds of which pair wise combinations cover larger chemical space compared with HTS
- authors do not say how they define hydrogen bond acceptors
- derivation of Ro3
- issues with cut-offs
- Ro3 is too restrictive

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Only 4 of the 11 fragments are consistent with the rule of 3. Restriction to this rule would have limited the fragment hits to a strongly reduced variety of chemotypes.

- This may be an overstatement!!!!
 - all fragments have molecular weights less than 300
 - only one has ClogP > 3
 - these aspects of the Ro3 are the most important (library did contain larger, more lipophilic members)
 - all 11 fragments have 3 or fewer hydrogen bond donors and TPSA < 60 Å²
 - only two of the fragments have more than 3 rotatable bonds
 - majority of the fragments fail to pass Ro3 is in the number of “Lipinski acceptors,” where 6 of the 11 have > 3.
 - if you count hydrogen bond acceptors more judiciously (ie, compound 291 would have 3 acceptors rather than 4, since the aniline nitrogen would not be counted), only 1 of the 11 fragments has more than 3 acceptors.

Summary

- Ro5 was one of the most influential recent medicinal chemistry publications
- multiple groups have refined and expanded methods for predicting 'drug-likeness' are already having a major impact on the design and selection of compounds in several pharmaceutical companies
- Ro5 has motivated many other studies of drugs and related molecules
 - development of screening libraries and virtual screening strategies in pharmaceutical research.

Future

- pharmaceutical productivity continues to lag, and the industry is exploring new models to improve its output
- to move beyond simple models based on lipophilicity and gain a deeper understanding of molecular interactions and why some compounds violate common rules, but are still bioavailable
- new targets and other drug discovery challenges may require the development of new 'rules'



Thank you!

