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The Rule of Five Revisited: Applying Log *D* in Place of Log *P* in Drug-Likeness Filters

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Abstract: The much publicized "Rule of 5" has been widely adopted among the pharmaceutical industry. It is used as a first step filter to perform virtual screening of compound libraries, in an effort to quickly eliminate lead candidates that have poor physicochemical properties for oral bioavailabilty. One of the key parameters used therein is log *P*, which is a useful descriptor, but one that fails to take into account variation in the lipophilicity of a drug with respect to the ionic states present at key biological pH values. Given that the majority of commercial pharmaceuticals contain an ionizable moiety, we propose that log *D* is a better descriptor for lipophilicity in the context of the Rule of 5. It gives more physiologically relevant results, thereby reducing the number of potential false-negatives incorrectly eliminated in screening. Using a series of commercial compound libraries, this study showed that the adapted Rule of 5 using log *D* instead of log *P* provides notable improvement in pass rate for compounds that have the desired lipophilicity at a relevant physiological pH.

Keywords: Rule of 5; log *P*; log *D*; partition coefficient; distribution coefficient; lipophilicity; drug-likeness; physicochemical property prediction; property profiling; library screening; oral bioavailability; permeability

Introduction

Since its publication in 1997,[1] the empirically derived Rule of 5 (Ro5) has become widely accepted by the pharmaceutical industry as a quick profiling tool for drug-likeness for orally administered therapeutics that are passively transported to the site of action. While this simple set of rules does not provide an exhaustive study of all the descriptors that define overall compound drug-likeness, the Ro5 specifically offers a fair estimate of drug-likeness with regard to good *in vivo* permeability. The term "drug-likeness" specifically refers to drug-like permeability properties, for the purpose of this investigation. The key factors used to describe drug-likeness in the Ro5 are molecular weight <500; partition coefficient (log *P*) <5; <5 hydrogen bond donor atoms; and a sum of nitrogen and oxygen atoms <10. Molecules that violate these rules, hence showing poor pharmacokinetic properties for oral administration, are filtered out early in discovery to allow efforts to be focused upon more promising candidates. In subsequent years, several groups have researched and published profiling techniques to further define drug-likeness[2−6] and lead-likeness[7,8] in order to increase the efficiency of drug discovery. At the heart of all these profiling

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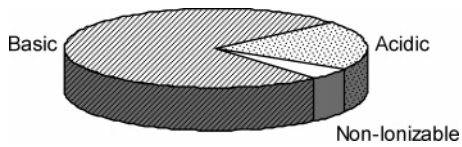

Figure 1. Overall Composition of marketed drugs.

conventions lie critical physicochemical properties, like lipophilicity, that helps to predict *in vivo* behavior of potential drugs.

Lipophilicity is widely accepted as having a significant impact on the absorption, distribution, metabolism, and excretion (ADME properties) of compounds.[2] Compounds that are too lipophilic are not only more likely to be rapidly metabolized and bioaccumulate, they also have low aqueous solubility and often poor absorption properties. Early determination of lipophilicity (either through experimental measurement or prediction) highlights potential liabilities, and aids better decision-making both in hit-to-lead identification and lead optimization. It has become standard practice to measure and discuss lipophilicity in terms of log *P* (the partition coefficient), a descriptor of the differential partitioning of a neutral compound between two immiscible solvents, most commonly octan-1-ol and water.

Since ionization of a molecule results in decreased lipophilicity with respect to the neutral state, it is necessary to take the ionic state of the compound into account when describing the lipophilicity of potential drugs. While log *P* is a useful reference point for the comparison of overall trends, its inability to account for the ionization of compounds under physiological conditions means it should be used cautiously in drug discovery. As represented in Figure 1, it is approximated that 95% of all drugs are ionizable (~75% are bases and 20% acidic).[9] The changing pH environment in the body means compounds will often be found as a mixture of ionic species with the exact distribution being dependent on the pH of immediate physiological conditions. While biochemical mechanisms maintain the pH of blood at 7.4, the pH along the GI tract varies from the stomach (fasted pH 1−2, fed pH 3−7) to the colon (pH 5−8). Oral drug absorption occurs in the small intestine at ~pH 5.5. Instead of using lipophilicity parameters related to only the neutral species, we need to use parameters that account for both neutral and ionic states. Log *D*, a pH dependent version of log *P*, is a descriptor of the octanol−water distribution coefficient for partitioning of ionizable species in biphasic media. It reflects the true behavior of ionizable compounds in solution at a given pH value or range. Log *D* accounts for mixtures of species that may exist at any given pH and is, therefore, the descriptor that should be used to truly understand the lipophilic nature of molecules in physiological systems.

At the time of publication of the Ro5, log *P* had to be used as the descriptor for lipophilicity since there was no convenient and/or reliable log *D* prediction capability available. Over the last 10 years the technology behind physicochemical predictors has improved greatly, as has the choice of vendors offering software for this specific research. There are now *in silico* models that predict not only log *P* but also pK_a and log *D* with high degrees of accuracy. Since we understand that log *D* is a more relevant term than log *P* to describe lipophilicity for drug-likeness profiling, we are proposing that predicted log *D* should be used in place of log *P* when filtering compounds for Ro5, and other drug-likeness filters.

Table 1. Summary of Libraries Screened

library	no. of compds	no. of compds used for log *D* Ro5 study[a]
PDR[b]	352	351
DSSTOX[c]	1217	1204
AMES	2400	2350
ChemDiv (60% Tanimoto)[c,d]	4010	3918
Specs (60% Tanimoto)[c,e]	6548	6313
Enamine (90% Tanimoto)[c,f]	63016	61764
Frinton (90% Tanimoto)[c,g]	806	797
ComGenex (90% Tanimoto)[c,h]	10180	10156
IBS (80% Tanimoto)[c,i]	25207	24399

[a] Compounds for which both log *P* and log *D* were predicted by ACD/PhysChem Batch. [b] Reference 13. [c] Libraries obtained from the ZINC[11] database. Tanimoto percentage indicates the level of chemical similarity allowed within a given library and identifies the specific library used. [d] Reference 14. [e] Reference 15. [f] Reference 16. [g] Reference 17. [h] Reference 18. [i] Reference 19.

Hypotheses. Since log *P* is the descriptor of lipophilicity for neutral compounds, it represents the most lipophilic state of a molecule. Ionization of any compound makes it more water soluble and, by default, less lipophilic. If we predict log *D* at a physiologically relevant pH we would expect to (1) observe an increase in the number of compounds that pass lipophilicity screening (log $D \leq 5$) compared to log *P* and (2) see a subsequent increase in the number of compounds identified as drug-like in a given library/dataset when assessing compounds for overall drug-likeness (other Ro5 violations not withstanding)

This study is an investigation of whether computationally predicted lipophilicity descriptors, log *P* and log *D*, can reproduce the known physical phenomena stated above, and is an examination of how the widely used Ro5 can be modified to improve current *in silico* profiling techniques for compound screening.

Computational Methods

An initial study to test the hypotheses was carried out using two libraries of known pharmaceutical compounds: the Physicians Desktop Reference (PDR), a compilation of all prescribable drugs available to physicians; and a subset of the DSSTOX library identified as drugs by the U.S. Food and Drug Administration (FDA). This was followed by further testing to substantiate the application of log *D* Ro5

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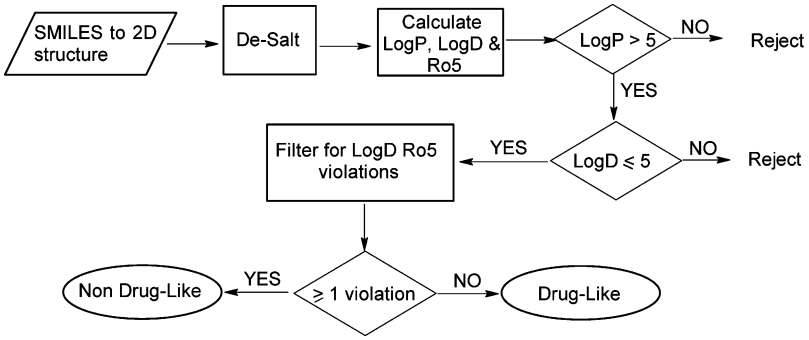
Scheme 1. Workflow of Computational Methodology



as a selective drug-likeness filter using the AMES library,[10] a mixed set of compounds previously tested for mutagenicity via *in vitro* assay. The study was then extended to screening of six chemically diverse libraries available from ZINC.[11] A summary of the libraries used, their size, and other relevant information is given in Table 1. ACD/PhysChem Batch (version 10.0)[12] was used for calculation of log *P* and log *D* values, and for flagging a number of Rule of 5 violations. Statistical workup of results was undertaken using Microsoft Excel.

Libraries were imported as SMILES strings and directly converted to 2D structures using ACD/PhysChem Batch, along with application of a standard desalt procedure to automatically remove additional components (such as counterions, hydrates, and solvates) from compounds upon import. (Settings used in the software to achieve this were as follows: convert covalent salts to ionic; remove all smaller components; minimize and remove charges; and convert pentavalent nitrogen oxides). The Batch software was used to calculate log *P*, log *D* (without ion-pair partitioning) at pH 5.5 (relevant for intestinal absorption of orally administered therapeutics), and Ro5 properties for the library. The dataset was filtered to remove all compounds with log $P <$ 5, since these already comply with Ro5 requirements of lipophilicity. All compounds with log $P > 5$ and log $D \leq 5$ at pH 5.5 (log $D_{5.5}$) were selected for further study, resulting in the removal of neutral compounds where log $P =$ log D. The conventional Ro5 filter results (using log *P* as the descriptor for lipophilicity) were used to identify the number of violations for this subset. Ro5 violations using log $D_{5.5}$ as the lipophilicity descriptor (log $D_{5.5}$ Ro5) were computed manually. While in the standard application of the Ro5 more than two violations classifies a compound as non-drug-like, for these studies failure of a compound for Ro5 was defined as one or more violations. Lipinski's Ro5 has been modified and tweaked over the years to better fit different areas of research. In neuro therapeutic research, for example, log *P* of ~2 is optimal, in other areas MW >500 may be tolerated. Our choice of one or more violations constituting Ro5 failure highlights the maximum impact of using log *D* as the descriptor of lipophilicity and the minimum effect of lipophilicity on library screening pass rates. This stringent application of the Ro5 focuses on the influence of liphophilicity descriptors on drug-likeness. The overall workflow for the methodology is shown in Scheme 1.

Results

Testing the Hypotheses. Results obtained from screening of the PDR (351 drugs) and DSSTOX[20] (1204 drugs) libraries supported the hypotheses set out at the outset of this work (see Table 2). We observed an overall increase of 5.3% of compounds exhibiting satisfactory lipophilicity upon application of log $D_{5.5}$ as the descriptor compared with log *P*. Further, an average of 4.6% more drugs passed the stringent drug-likeness guidelines of Ro5 violations.

For these known drug libraries, approximately 10% of the dataset failed drug-likeness because their log *P* was greater than 5. Of the subset of compounds that failed with log $P >$ 5, ~55% passed when using the log $D_{5.5}$ filter. For the larger DSSTOX dataset, a 4.5% increase in the number of compounds that passed the overall Ro5 test was observed when using log $D_{5.5}$ instead of log *P*, which translates to 54 false-negatives with respect to known drugs. It is also worth noting that an average difference of ~3 log units, between log $D_{5.5}$ and log *P*, was observed for compounds that failed drug-likeness for high lipophilicity. By comparison, you would have to increase the log *P* filter cutoff to 6.25 or 6.16, respectively, to observe a similar increase in the number of known drugs to be identified as drug-like.

The objective of the next study was to investigate the effect of a change in predicted lipophilicity (using the descriptor log $D_{5.5}$) on Ro5 profiling on a mixed library of compounds. The Ames mutagenicity library (2350 compounds) is pri-

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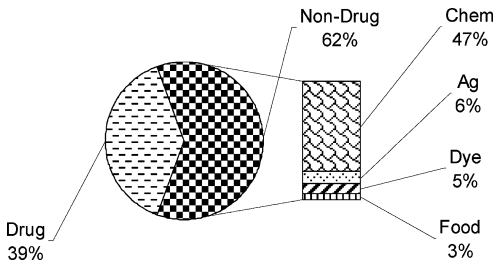

Figure 2. Composition of the AMES library (2350 compounds).

Table 2. Results of Drug Library Screens: PDR and DSSTOX

library	compds passing lipophilicity log $D_{5.5} \leq 5$	additional drug-like compds identified from log $D_{5.5}$ Ro5 filter
PDR	5.41%	4.84%
DSSTOX	5.32%	4.49%

marily based on compounds published in the Handbook of Carcinogenicity, Potency and Genotoxicity Databases[21] (with compounds listed in the Merck index[22] classified as drugs). The different classes of data contained in this library are depicted in Figure 2. Results of the screening using log *P* and log $D_{5.5}$, respectively, are shown in Table 3.

For the complete dataset, 3.32% more compounds were classified as drug-like using the log $D_{5.5}$ lipophilicity filter, compared to log *P*. Of the compounds that passed lipophilicity requirements, 63% (49 out of 78) were drugs, while the AMES library consists of ∼40% drugs. This represents an approximate 1.6-fold enrichment of drugs passing lipophilicity requirements. While an increase in the number of non-drugs passing the lipophilicity log $D_{5.5}$ filter were observed, the improvement factor for drugs was markedly better. This same trend was observed on examination of results from the log $D_{5.5}$ Ro5 screen. An additional 2.81% of compounds identified as drug-like in the AMES library came from a greater enrichment of the drug subset. By raising the cutoff of log *P* to 6.25, an additional 137 compounds are classified as drug-like. Of these, 87 are non-drugs and 50 are drugs. The enrichment observed by increasing the acceptable log *P* value is therefore 0.92, compared to 1.6 when log *D* is used as the descriptor of lipophilicity. Randomly picking 137 compounds from the dataset we would expect on average to select ∼55 drug-like compounds since the dataset is 40% drugs. Simply changing the log *P* parameter, therefore, is not an effective measure to selectively increase the number of drug-like compounds identified from a dataset. Using log $D_{5.5}$ as the descriptor for lipophilicity increased our ability to preferentially identify drugs from a chemically diverse library for both the log $D_{5.5}$ filter and log $D_{5.5}$ Ro5.

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Table 3. Results of the AMES Mixed Library Screen

AMES subsets	compds passing lipophilicity log $D_{5.5} \leq 5$	additional drug-like compds identified from log $D_{5.5}$ Ro5 filter
full library	3.32%	2.81%
drugs	5.37%	4.93%
non-drugs	2.02%	1.46%

Application of the Log $D_{5.5}$ Ro5 Filter to Commercially Available Libraries. To corroborate the trends observed so far, the effect of the log $D_{5.5}$ Ro5 filter on a collection of six commercially available high-throughput screening (HTS) libraries was investigated. The ZINC library offers access to a list of currently purchasable compounds for structure-screening, so that companies can perform structure-based virtual screening prior to purchase. This library collection is thus directly relevant to the current materials being screened by the drug industry and is expected to have been designed to adhere to drug-likeness (or lead-likeness) rules for commercial viability. Table 4 provides a summary of the subsets that were examined along with calculation statistics for lipophilicity predictions. The objective of this investigation was to provide an estimate of the real "cost" of false-negatives which may occur when log *D* is not used as the lipophilicity descriptor in library profiling.

To obtain a good estimate of general trends, libraries with a generous size and spread were chosen. These varied from the smallest (Frinton) containing 797 compounds to Enamine containing 61 764 compounds. The exact composition of these libraries (percentage of drug-like versus non drug-like) is unknown.

The effect of switching log *D* for log *P* varies with the dataset selected, but generally ranges from 0.6% to a 2.3% increase in compounds passing the conventional Ro5 filter using log *P* as the lipophilicity descriptor. Lipophilicity screening using the log $D_{5.5}$ descriptor resulted in a 10−20% reduction in compounds failing drug-likeness. The average difference between log *P* and log *D* was 2 log units, a 100-fold disparity. The fact that the results above are near-identical (from the lipophilicity log $D_{5.5}$ column to log $D_{5.5}$ Ro5) suggests that the difference in Ro5 screening is primarily based on lipophilicity. The number of unique compounds from this collection of datasets was 103,505, and the total number of additional compounds that pass log $D_{5.5}$ Ro5 screening is 904. While these numbers may initially seem insignificant, when taken in the context of library

Table 4. Summary of Screening Results for Six Commercial Libraries

library	% compounds passing lipophilicity log $D_{5.5} \leq 5$	additional drug-like compounds identified from log $D_{5.5}$ Ro5 filter
ChemDiv	0.74%	0.71%
Specs	1.46%	1.35%
Enamine	0.64%	0.64%
Frinton	0.63%	0.63%
ComGenex	2.25%	2.25%
IBS	0.78%	0.78%

screening the 0.6% to 2.3% increase in drug-like permeability represents 600−2300 additional leads for every 100 000 compounds screened. Even a small chance of excluding a potential block-bluster drug as a false-negative hit from virtual screening due to unrealistic values is unacceptable.

Summary

Results from screening libraries containing known drugs showed that utilizing pH dependent log *D* as a descriptor for lipophilicity in place of log *P* significantly increased the number of compounds correctly identified as drug-like using the log $D_{5.5}$ Ro5 drug-likeness filter. Additional screening of a mixed dataset showed a greater increase in the number of known drugs being predicted as drug-like compared to known non-drugs, indicating this to be a selective filter for drug-like compounds. Screening a collection of uncharacterized HTS libraries further confirmed a significant effect of log *D* on lipophilicity and drug-likeness filters.

From this work we conclude that log *D*, at a relevant pH, has significant effects on virtual screening because it offers a more realistic descriptor of lipophilicity under the relevant pH environments in the body. Log *D* should be used preferentially over log *P* as the descriptor for lipophilicity, especially when looking at compounds that are likely to ionize in physiological media. While we have only shown the application of this descriptor in a Ro5 filter, the effects apply to any drug-likeness filter that currently uses log *P* as its lipophilicity parameter.

Supporting Information Available: Raw summarized data of lipophilicity and Ro5 violations for all libraries used in the study (Table A-1) and detailed analysis comparing lipophilicity and Ro5 violations for log *P* and log $D_{5.5}$ (Table A-2). This material is available free of charge via the Internet at http://pubs.acs.org.

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