feature

A Sorcerer's apprentice and The Rule of Five: from rule-of-thumb to commandment and beyond

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Scientists working in medicinal chemistry, computational chemistry, chemo-informatics or other areas of drug discovery might think that Lipinski's 'Rule of Five' [1] is a concept strictly related to their profession. It is now so familiar that it has been abbreviated to a well-recognized acronym, 'Ro5' [1,2]. It is a concept connected to weeding out small molecules with undesirable properties on the long path toward identifying the most promising candidates for clinical studies. Chris Lipinski and coworkers proposed this simple concept as a rule-of-thumb to predict whether or not a molecule was likely to have absorption problems because of poor solubility and/or permeability. The Ro5 is typically implemented by flagging compounds that violate two or more of the following physico-chemical thresholds: molecular weight (>500), ClogP (>5), number hydrogen bond acceptors (number of N and O atoms) (>10), number of hydrogen bond donors (sum of OH and NH groups) (>5). Other criteria were added later related to the number of rotatable bonds in the ligand (≤10) and the polar surface area (PSA < 140 Å^2) [3]. Variations of the Ro5 for smaller fragments soon followed (Rule of Three, Ro3) [4].

The simplicity of these criteria to remove outlier molecules using software, made them very easy to implement. Thus, the Ro5 moved rapidly in the hierarchy of medicinal chemistry concepts from being a set of 'alerting' criteria in the minds of the medicinal chemists to a commandment engraved in the high altars of 'do's' and 'don'ts' of drug seekers. I am not a medical doctor nor am I a savvy drug-discoverer; I am just an apprentice. However, I suggest that ten years after the publication of the Ro5, it might be time for a collective reflection.

Currently, the Ro5 is used almost indiscriminately. I think that we should be very cautious about relying too heavily on these criteria, for two reasons. First, it is worth pointing out that there are examples of successful drugs (i.e. Lipitor™, Atorvastatin™) that are notable violators of the Ro5 and we and others should never underestimate the impact of the highly improbable event in our theories and preconceived notions [5]. Second, it is well recognized in the drug discovery field that in spite of these magic rules, and the introduction of ingenious methods to discover new drugs [6], the number of new chemical entities reaching the market has remained constant or continued on a downward trend [7]. One may ask: Where is the power of those magic rules? Are they helping us to focus on the right molecules? Or are they preventing us from discovering new opportunities? Do they represent something deep and profound about drug discovery? Or are they preventing us from a deeper understanding of the drug discovery variables?

Ever since the insights of Pasteur, Koch and Ehrlich (among many others) into the biology and chemistry of drug discovery, we have marveled at the power of those 'magic bullets' to cure disease and even save patients from certain death. We have been mesmerized by the power of a few basic concepts of how living organisms attack a patient or by the few successes that followed after extensive testing of drugs *in vitro* and *in vivo*. It has barely been a century since we first tried to unveil the secrets of drugs discovery in a concerted manner. With the establishment of medicinal chemistry as a discipline and the initial drafts of the human and mouse genomes at the turn of the 21st century, it has been suggested that drug discovery is contemplating its golden age. I have a much more humble view of the current state of affairs in the field. The humility of considering the little we know and the landscape that is still for us to discover.

I see the historical successes of our illustrious predecessors more like the discoveries of early sky watchers. They discovered the early stars and planets and through careful observations were able to trace their passages through the sky. Like them, we have discovered certain patterns in the firmament of drug discovery as they relate to various chemical entities with therapeutic properties, and characterized the molecules in the biological universe to which they relate. However, I would not go any further than that. In trying to understand the universe of drug discovery, I am not even ready to affirm whether we know with certainty if the system is geocentric (ligand at the center, as it would be suggested by medicinal chemists) or heliocentric (target in the center as proposed by biologist, macromolecular crystallographers or geneticists). Moreover, although we have a sense of what the forces that bring the two together are, robust calculations that can accurately predict how one relates to the other still elude us. We know there is a key parameter (i.e. K_i, their relative affinity) that

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2007.10.022

connects this crucial pair but we cannot calculate it accurately. Consequently, the number of experimental observations (*in vitro* and *in vivo*) relating the two dominant poles of the drug-discovery universe is extensive and continues to grow in the existing databases (public and proprietary) at an exponential rate. All these measurements remind me of the careful observations made by Tycho Brahe (circa 1600) that were crucial for Kepler's insights.

We have used with some success the relative affinity (i.e. variables related to K_i) to guide us in our drug-discovery efforts; especially combined with parameters related to the toxicity, absorption, metabolism, accumulation and secretion of small molecular entities in animal models and humans. We have also synthesized, screened and tested a relatively large number of chemical entities, looking for the needle in a haystack. However, searching for a needle in a haystack does not indicate a reliable knowledge of where the needle might be or even the size and number of haystacks that need to be screened. I would argue that we have not figured out yet the 'rules of planetary motion' within the drug discovery universe. We do not even know for certain who has the strongest pull over the other: the ligand or the target. We might have a sense of what the trajectories are and have used a substantial number of variables to describe them *in vitro* and *in vivo* with success but only for certain systems. In my view, these observations, models and predictions are akin to the early descriptions of the movement of the planets in terms of epicycles. They may have some predictive value; however, they do not unveil the underlying logic of the system that for astronomy was revealed by the laws of planetary motion proposed by Johannes Kepler (1571–1630) using Brahe's observations.

Re-reading some of the essays of one of the masters of scientific writing, the late Steven J. Gould (1941–2002), I found to my delight an essay entitled 'The Rule of Five' within his collection entitled 'The Flamingo's Smile' [8]. Gould's essay does not relate to Lipinski's Ro5 but to two taxonomic systems that were very popular in the decades before Darwin published the *Origin of Species* in 1859. Gould's observations resonated in my mind and I cannot resist quoting a few excerpts. The opening of the essay sets the ground:

"THE HUMAN MIND DELIGHTS in finding pattern – so much so that we often mistake coincidence or forced analogy for profound meaning. No other habit of thought lies so deeply within the soul of a small creature trying to make sense of a complex world not constructed for it"

And another thought follows, relevant to our task at hand of understanding the complex interactions between small molecules and their therapeutic properties:

"No other error of reason stands so doggedly in the way of any forthright attempt to understand some of the world's most essential aspects – the tortuous paths of history, the unpredictability of complex systems and the lack of casual connection among events superficially similar [. . .] We delight in catalogs of disparate items united by the same number, and often feel in our gut that some unity must underlie it all"

He goes on to review how our ancestors pondered the mystique of seven (number of planets, the deadly sins, etc.), and the connections in sets of five (related to the number of our fingers or toes): the smooth stones that David selected to slay Goliath and others.

Rules, commandments or absolute certainties are dangerous. Even if they can be found to be approximately true at the beginning, they are eventually superseded by a deeper understanding of the problem. They might provide an initial guidance of our decisions or actions but not a solid compass for long and tortuous journeys, because if we follow them strictly they might obstruct our way to discovery. Navigating through reality is much more subtle and requires a delicate balance between rules and insight.

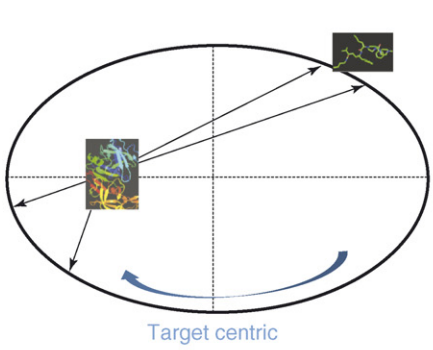
In the same way that there are no absolutely safe drugs, there are no absolute commandments on how to find them yet. All of our chemical wizardry (elegant medicinal chemistry, combinatorial, parallel synthesis and others) is far more advanced than our understanding of the biological machinery that we target. After the first draft of the human genome and the genomes of many of our model organisms, the impact of the multiple 'omics' is still rather limited. There is, of course, a time-delay between the conception and development of any new technology and its direct impact on the drug discovery process. High-throughput methods have had some impact. However, it might be once again that 'brute force' methods are not the most suitable way to make inroads into the subtleties of living organisms. The old (somebody might say 'out of fashion') tools and methods of careful observation, painstaking and dedicated laboratory work and serendipity are to be maintained fresh and alive.

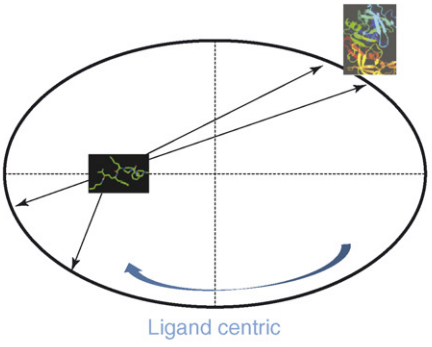
The conclusion of the brief intellectual journey of Gould's essay is also worth quoting here:

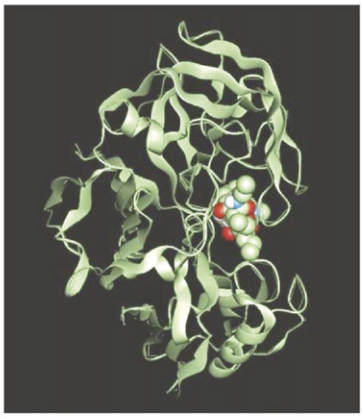
"Darwin destroyed the rule of five forever because he removed its rationale by reconstructing nature. His agent of destruction was not evolution itself [. . .] Darwin's exterminating angel was, simply, history. Evolution does not unroll according to simple laws specifying necessary results. It follows the vagaries of history. Its pathways are twisted and churned by changing environment [. . .]"

In my view, our efforts to effectively discover and develop drugs are also subject to the vagaries of evolution, thereby adding to the complexity of the search. Firstly, our 'magic bullets' are directed toward targets that are being crafted constantly at the atomic level by the forces of evolution, following Darwin's insight at a scale that he would never had imagined. Secondly, our ideas, concepts, methods and tools to find those 'magic bullets' are also evolving constantly – and so they should. Evolution, as we know, does not produce perfectly engineered pieces. The molecules resulting from our searches and designs are typically chimeras with multiple heads and different active groups. They are the product of human ingenuity in different laboratories, artificially selected for their efficacy, lack of toxicity and bioavailability among others. They have developed from screens, resulted from analogy or serendipitous observations and followed by careful analytical and clinical laboratory experiments. There is an element of history and contingency in the drug discovery process. Perhaps these reflections might help to enlarge our conceptual framework to find new drugs in a more predictable and effective way.

I would argue that we have to look for more subtle variables and concepts than simple 'quinarian' or 'ternarian' rules in order to guide our efforts to discover better drugs more effectively. We need to combine and reduce the multitude of variables spreading through the myriad of columns of our spreadsheets, much like Kepler did in trying to uncover the regularities of the solar system. We have to look deeper into the underlying principles that govern the interactions between drugs and targets. We have been doing this complex job relatively for a short time.







$\Delta G = -RT\ln K_i$

Drug Discovery Today

FIGURE 1

It was not easy for Kepler to find the laws of planetary motion with the technology of his time and when he found them, they came disguised in a very clever quantitative language: 'the squares of the periods of the planets are proportional to the cubes of their mean distances from the Sun' (Figure 1). These were not rules-of-thumb having to do with integer numbers. They were subtle, well hidden, patterns in Nature that led the way to the Newtonian revolution. They reflected the insight of finding the right combinations of variables to illuminate the problem and solve it. Can we unveil and describe numerically the equivalent of these laws at the center of our drug discovery universe? Whether this universe is ligand-centric, target-centric or a combination of both might be revealed within those hidden patterns. The search must go on and none of us should accept without questioning any stone-engraved commandments that might appear to simplify the search, when in reality they might be obscuring our path or clouding our vision toward the ultimate goal of making drug discovery more effective.

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