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REVIEW

Ligand efficiency metrics in drug discovery: the pros and cons from a practical perspective

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ABSTRACT

Introduction: Ligand efficiency metrics are almost universally accepted as a valuable indicator of compound quality and an aid to reduce attrition.

Areas covered: In this review, the authors describe ligand efficiency metrics giving a balanced overview on their merits and points of weakness in order to enable the readers to gain an informed opinion. Relevant theoretical breakthroughs and drug-like properties are also illustrated. Several recent exemplary case studies are discussed in order to illustrate the main fields of application of ligand efficiency metrics.

Expert opinion: As a medicinal chemist guide, ligand efficiency metrics perform in a context- and chemotype-dependent manner; thus, they should not be used as a magic box. Since the 'big bang' of efficiency metrics occurred more or less ten years ago and the average time to develop a new drug is over the same period, the next few years will give a clearer outlook on the increased rate of success, if any, gained by means of these new intriguing tools.

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1. Introduction

Developing a new medicine takes more than 10 years, with costs exceeding US$2500 million, and less than 12% of human tested compounds being approved for marketing [1]. Nevertheless, the above figures are solely related to the investments of corporate companies and thus the real-life social cost of a new drug is even higher [2]. Even rationally conceived compounds have few chances of being clinically relevant with a success rate halved in the last 10 years [3]. As a result, the costs of new launched drugs are more than doubled in the last decade to cover what wasted for failures [1].

AQ2

The observed failures are basically due to increased complexity of clinical trials (mainly for regulatory purposes) [1], adoption of counterproductive strategies [4], difficulty in identifying adverse reactions or limited efficacy in early steps of development [2], intrinsic high risk of failure in some research areas (e.g. chronic and degenerative diseases) [5], or focus on poorly validated new targets that are less druggable than expected [6]. Nowadays, it is widely acknowledged the need for a higher quality of investigational compounds achievable by improving the selection of candidate drugs in early stages of development to reduce attrition at later stages [7]. At a practical level, this means choosing hits whose biological profile (e.g. potency and selectivity) can be easily optimized by facile chemical modifications without escaping from the 'drug-like' space [8].

The quest for high-quality investigational compounds is an iterative process, generally described as design–make–test–analyze cycle [9]. A key role is played by quantitative structure–activity relationships [10] and molecular modeling [11,12] studies as well as by chemical intuition and expert feeling [13,14]. Besides limitations imposed by environmentally sustainable practices [15], this process may be biased by prejudices when assessing the merit of hit or lead compounds, and their optimization through rational chemical modifications [16,17].

However, over the last two decades the medicinal chemist's panoply has been strengthened with new weapons enabling to better state the quality of starting hit compounds and control their physicochemical properties during development: ligand efficiency metrics. These composite parameters relate compound potency and relevant structural and/or physicochemical features. Herein we propose an informed synopsis of the most commonly used ligand efficiency measures and related drug-like properties.

This review has been inspired by the seminal works of several scientists who are quoted as 'the founding fathers' in the next section, where the timeline of ligand efficiency metrics will be outlined. For the sake of clarity, the discussion will be supported by some recent and successful case studies. We will focus on fragment-based drug discovery (FBDD) and on few other examples where the attrition is high. The possibility of using efficiency metrics to improve molecular docking is also reviewed. Then, we will address some notes of criticism. Finally, the Expert opinion section draws some personal statements about ligand efficiency metrics as effective guideposts in drug discovery and development.

2. Historical notes: the founding fathers

Ligand efficiency metrics may be defined as the result of our attempts to capture in simple numerical frameworks a

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Article highlights

- Ligand efficiency metrics have been proposed as a valuable aid to face the dramatic reduction of the rate of success observed in drug discovery campaigns in the last few decades.
- Ligand efficiency metrics are almost universally accepted as a valuable indicator of compound quality whose benefits are mostly in the early stages of drug discovery projects.
- Ligand efficiency metrics have been successfully applied in fragment-based drug discovery (FBDD), hit to lead optimization, deconstruction exercises and may be useful to improve molecular docking.
- Regardless of questionable formal aspects, the work of the 'founding fathers' and their epigones has evolved the classical way of thinking about SAR and drug design.
- Rooms for other use is still there but with a certain level of misuse risk.

This box summarizes key points contained in the article.

series of empirical rules stemmed from successful drug design programs in the course of the last 20 years [18]. To underline the plethora of ligand efficiency metrics now available, Shultz [19] referred to their rise as a 'Big Bang' of properties spurred by the work of Lipinski and co-workers who coined the famous rule of five (Ro5) [20]. Despite Kenny's opinion [21], however, the literature on ligand efficiency metrics cannot be considered as a mere 'Ro5 envy'. Indeed, the roots of ligand efficiency metrics protrude far behind the seminal work of Lipinski's group. A schematic timeline illustrating the progress of the main theoretical breakthroughs and drug-like properties related to efficiency metrics is reported in Table 1.

In the seventies, Page and Jencks wondered about the reasons behind the exceptionally high rate of enzyme-catalyzed reactions in comparison with uncatalyzed reactions. They concluded that translational and rotational motions represent the driving force for enzymatic reaction rate enhancement [62]: the catalytic properties of enzymes come from their ability to act as 'entropy traps' [22], that is, to employ highly oriented substrate-binding interactions [23] to overcome the unfavorable energetic barrier typical of chemical reactions. To evaluate the intrinsic binding energy of the substrate, the 'anchor principle' was introduced: the true binding energy of a group of atoms (or a molecule, A) may be obtained as the difference between the $\Delta G_{binding}$ of the molecule presenting A as a substituent (A-B) and the $DG_{binding}$ of the corresponding unsubstituted compound (B, the anchor molecule). Indeed, the observed difference in binding energy reflects all of those factors associated with the interaction of A, with the exception of the entropy loss associated with the initial binding of the anchor (B). Thus, Page and Jencks [63] first suggested that by linking two fragments the affinity of the joined molecule would be greater than the sum of the affinity of the separated moieties.

The word 'anchor' echoes in the work of Rejto and Verkhiver [25] who postulated that the primary event in the interaction between a small molecule and its target protein binding site is granted by a 'recognition nucleus', that is, a core fragment that serves as a 'molecular anchor'. As a corollary, we may assume that most of the unfavorable binding entropy loss is paid by the molecular anchor, thus reverberating what previously discussed by Page and Jencks.

The relationship between the works of these founding fathers was later revisited by Murray and Verdonk [64] who afforded an accurate estimation of the rigid body entropy barrier, that is, the loss of translational and rotational entropy that accompanies the binding of a small molecule to its binding site (DG_{rigid}). This amount of energy (4.2 kcal/mol) had been previously overestimated by Page and Jencks [63] and represents the cost a fragment have to pay (entropic barrier) to bind its target pocket. These considerations recall the ones previously afforded by Rejto and Verkhiver [25] when illustrating the concept of molecular anchor.

Murray and Verdonk referred also to the intrinsic binding affinity associated with a fragment, a concept present *in nuce* in the papers of Page and Jencks and further explored by Andrews who extended the anchor principle to the study of drug–receptor interactions [24].

Acknowledging the Page and Jencks' seminal work, Andrews examined a series of 200 biologically relevant small molecules and attributed an average contribution (intrinsic binding energy, E_x) to a series of common functional groups or substituents (X). Different E_x values were also given to carbon atoms, depending on their hybridization (tetrahedral or trigonal), and nitrogen atoms, depending on their ionization state (neutral or positive). Andrews proposed E_x's as reference values to roughly estimate the average binding affinities for any putative ligand by simply adding the contribution of each constituting part [65].

Andrews' binding energy may be assumed as the maximal theoretical affinity of a ligand, that is, the maximum free energy of interaction that a ligand is expected to achieve when interacting with its target binding site, provided that all of its constituting parts contribute optimally. This concept inspired the study of Kuntz and colleagues who examined the free energy of binding of more than 100 small, high-affinity ligands [26] and showed that the maximum DG change per non-hydrogen atom (DG/HA) in organic compounds is −1.5 kcal/mol. However, the relationship between ΔG and HA was not linear and the gain in potency per added HA drops for molecules formed by more than 15 HA.

In the meantime, Lipinski's group developed Ro5 framework as a guide to obtain orally bioavailable drug candidates (Table 2) [20]. Oprea and co-workers proposed that the lead-like space should be populated by compounds with less molecular complexity (less MW, less number of rings and rotatable bonds) and lower hydrophobicity with respect to drug-like space to allow optimization [66]. This milestone work generated the rule of three (Ro3) which was the scaled down version of Ro5, suited for lead-like compound discovery in high-throughput screening (HTS) campaigns [28], which was in turn followed by several new versions (cf. Table 2).

Benefiting of the efforts of all previous works, Hopkins [30] conceived in 2004 the first ligand efficiency metric (ligand efficiency, LE) thus inaugurating the new era of ligand efficiency metrics. The original definition of LE recalled the Kuntz et al. [26] binding energy per non-hydrogen atom. Afterwards, this view was enlarged to comprise experimentally derived measures of potency. In Table 3 details for LE and succeeding

Table 1. Schematic timeline illustrating the main theoretical breakthroughs and drug-like properties (left-hand side), and efficiency metrics (right-hand side).

Theoretical milestones and drug-like properties	Year	Efficiency metrics
Entropy trap [22]	1962	
Anchor principle [23]	1977	
Intrinsic binding energies of functional groups [24]	1984	
Molecular anchor [25]	1996	
Rule of five (Ro5) [20]	1997	
Free energy of binding per atom [26]	1999	
Veber's rules [27]	2002	
Rule of three (Ro3) [28]	2003	
Aliphatic indicator [29]		
Aromatic indicator [29]		
	2004	Ligand efficiency (LE) [30]
		Serum-free IC_{50} ($IC_{50,free}$) [31]
	2005	Binding efficiency index (BEI) [18]
		Percentage efficiency index (PEI) [18]
		Surface (binding) efficiency index (SEI)[18]
	2007	Ligand efficiency index (LEI) [32]
		Lipophilic ligand efficiency (LLE) [33]
		Group efficiency (GE) [34]
		Enthalpic efficiency (EE) [35]
Rule of four (Ro4) [36]	2008	Fit quality (FQ) [38]
3/75 rule [37]		
Complexity (Fsp^3 + chirality centers) [39]	2009	Lipophilicity-corrected ligand efficiency (LELP) [43]
Number of aromatic rings (NAR) [40]		Size-independent ligand efficiency (SILE) [44]
Golden ratio [41]		Percentage ligand efficiency (%LE) [41]
Pfizer metabolism index (PMI) [42]		
Metabolism-lipophilicity efficiency (MLE) [42]		
Central nervous system multiparameter optimization (CNS MPO) [45]	2010	Drug efficiency index (DEI) [46]
		Size-independent enthalpy efficiency (SIHE) [47]
		Surface (binding) efficiency index per polar atom (NSEI)[a] [48]
Property forecast index (PFI) [49]	2011	Enthalpy efficiency (LE H) [51]
Aromatic carbon atom minus sp^3 hybridized carbon atom counts (Ar-sp^3) [50]		Entropy efficiency (LE S) [51]
Absorption, distribution, metabolism,		Astex ligand lipophilicity efficiency (LLE_{AT}) [52]
excretion, and toxicity score (ADMET score) [8]		Kinetic efficiency (KE) [53]
Quantitative estimate of druglikeness (QED) [54]	2012	
Relative drug likelihood (RDL) [55]	2013	
Lipophilic metabolism efficiency (LipMetE) [56]		
Patient rule induction method (PRIM) [57]	2014	Lipophilic enthalpy efficiency (LLE H) [58]
		Lipophilic entropy efficiency (LLE S) [58]
Fluorine-corrected molecular weight (MW_{FC}) [59]	2016	ADMET efficiency index (AEI) [60]
		Ligand specific efficiency (LSE) [61]

[a]Temptative definition.

metrics are reported. The reader may find an exhaustive discussion in a recent Hopkins' review [58].

An obvious criticism may arise against LE because it does not discriminate between different HAs [19] thus introducing a bias against isologs bearing lighter atoms. Thus an alternative metric was proposed, that is the binding efficiency index (BEI), where the number of HA was replaced by MW [18]. BEI may not compensate for differences in atom-dependent contributions to potency [76]. As an example, two oxygen atoms give the same contribution to MW as one sulfur atom but the former would contribute differently to potency (on average, 2.2 kcal/mol for two O's, 1.1 kcal/mol for one S) [65]. Furthermore, all atoms in a molecule are not necessarily involved in binding interactions, although increasing the MW. This observation could explain the deviation from linearity observed when relating ΔG and MW.

A more sensitive metrics, group efficiency (GE) [34], echoes the anchor principle and differs from LE because the variation in energy of binding (ΔΔG) refers only to the atoms (ΔHA) that are added when moving from a given compound to its more complex analog.

LE did not take into account another crucial parameter – van der Waals polar surface area (PSA). Surface (binding) efficiency index (SEI) was introduced and its use in combination with BEI [18] was suggested. Further on, a new version of SEI (NSEI) relating the activity to the sum of polar atoms (N and O) and acknowledging Ro5 was introduced [48].

The detrimental effect of high lipophilic content was encoded in the lipophilic ligand efficiency (LLE = pIC_{50} – cLog *P*) [33] metric, which states that the higher the LLE the lower the probability that binding is a mere result of the ligand tendency to leave the aqueous medium. LLE is size-independent and was proven as the most robust of all metrics [19]. Thus, all previously reported drug-like properties found their respective suitable metric.

Finally, to overcome the size dependency of LE (i.e. the same ΔHA corresponds to a different gain in potency depending on MW) that makes smaller compound intrinsically more efficient, size-independent metrics were proposed, including fit quality (FQ) [38] and size-independent ligand efficiency (SILE) [44].

The years from 2008 to 2011 were characterized by the concern about the controversial role of high sp^2 carbon atom count. Flatness was envisaged as a major cause of attrition while compounds with higher degree of saturation are more likely to enter clinical use, taking advantage from increased solubility [39]. Chirality was found to be more prevalent in later stages of development than in earlier ones. Building on these observations, several back-of-the-envelope-calculations

Table 2. Drug-like properties and guides.

Definition and abbreviation	Formulation[a]	Description, main features, and applications
Rule of five (Ro5) [20]	HBDs ≤ 5; HBAs ≤ 10; MW ≤ 500 Da; cLog P ≤ 5	Framework for the development of orally bioavailable drug candidates; based on the analysis of calculated properties for a large set of approved drugs
Veber's rules [27]	RBs ≤ 10; PSA ≤ 140 Å^2 or (HBDs + HBAs) ≤ 12; RBs ≤ 10	Derived from a data set of 1,100 compounds with oral bioavailability in rat; a maximum of seven RBs seem to be optimal for oral bioavailability; these rules should guarantee rat oral bioavailability > 20–40%
Rule of three (Ro3) [28]	HBDs ≤ 3; HBAs ≤ 3; MW ≤ 300 Da; cLog P ≤ 3; RBs ≤ 3	Framework scaled-down with respect to Ro5, useful for the identification of hit compounds suitable for optimization (lead-like); based on an analysis of a dataset of 96 lead-drug pairs [66]; an analysis of a diverse set of fragment hits then suggested PSA ≤ 60 might also be a useful criterion for fragment selection [28]
Aliphatic indicator [29]	Number of sp^3 hybridized carbon atoms/total carbon atom count	Doomed to be more popular as Fsp^3 after the Lovering's [39] (see below); descriptor of relative aliphatic degree of a molecule, one of the 40 parameters used by Yan and Gasteiger to predict water solubility
Aromatic indicator [29] or aromatic proportion (AP) [67]	Number of aromatic carbon atoms/HAC	Descriptor of relative aromatic degree of a molecule, one of the 40 parameters used by Yan and Gasteiger to predict water solubility; more recently used in combination with MW to predict aqueous solubility [67]
Rule of four (Ro4) [36]	cLog P ≤ 4; MW ≤ 400	Rule of thumb to obtain improved ADMET profile; based on the analysis of a proprietary database
3/75 rule [37]	TPSA > 75 Å^2; cLog P ≤ 3	Based on *in vivo* toleration studies on 245 preclinical proprietary compounds, addresses promiscuity (i.e. off-target pharmacology)
Complexity (Fsp^3 + chirality centers) [39]	Fsp^3 (= aliphatic indicator) > ~0.2	Solubility increases and melting point lowers with increasing Fsp^3; this developability indicator generally rises during development; chirality is more prevalent in later stages of development than in preceding ones
Number of aromatic rings (NAR) [40]	NAR < 3	An increased number of aromatic rings have a detrimental impact on compound developability; based on 280 compounds in the pipeline of a pharmaceutical company
Golden ratio (ϕ) [41]	$HAC_{lead}/HAC_{fragment}$	~1.6; inspired by the finding that generally the ratio between the size of a lead-like compound and that of the corresponding scaffold approximates ϕ ('phi' ~ 1.6, the golden ratio); based on the analysis of 30 FBDD successful examples from the literature
Pfizer metabolism index (PMI) [42]	% HLM Cl_{int} decrease − % HLM Cl_{int} increase	indicates structural changes that introduce metabolic liability (PMI < 0) or reduce metabolism rate (PMI > 0); a 2-twofold variation in Cl_{int} was chosen as a normalizing factor, thus PMI = |100| would be for a group that gives a 2-twofold variation in HLM value; based on a matched molecular pair analysis over 150,000 HLM Cl_{int} values stored within a proprietary database; the difference observed for each couple of compound was expressed as a percentage (% decrease, % no change, % increase); a modified Topliss tree including PMI values was proposed
Metabolism-lipophilicity efficiency (MLE) [42]	PMI + 25cLog P	The 25-fold scaling factor was used to attribute the same weight to both parameters; obtained as a derivation of PMI (see above); plotted against ΔcLog P, gives hints on the dependence of the effect of the same substituent depending on its position in a ring
Central nervous system multiparameter optimization (CNS MPO) [45]	Desirable range: cLog P ≤ 3, cLog D ≤ 2, MW ≤ 360, TPSA > 40 Å^2 and ≤ 90 Å^2, HBD ≤ 0.5, and pK_a ≤ 8 undesirable range: cLog P > 5, cLog D > 4, MW > 500, TPSA ≤ 20 Å^2 and > 120 Å^2, HBD > 3.5, and pK_a > 10	Parameter for CNS penetration obtained by linear combination of six parameters, each weighted 1 if in the desirable range, 0 when in the undesirable range, and scaled when falling between the targeted values; CNS MPO desirability score ≥ 4, using a scale of 0–6; based on the analysis of 119 marketed CNS drugs, 108 proprietary CNS candidates, and 11,303 diversity set of proprietary compounds
Property forecast index (PFI) [49]	Log $D_{pH7.4}$ (or Log P) + #Ar (i.e. NAR)	<7 (or <5 on the cLog P scale); based on chromatographic hydrophobicity measurements in a data set of 100,000 proprietary compounds; performed better than lipophilicity values alone as an indicator of promiscuity liability
Difference between aromatic and sp^3 carbon atom counts (Ar-sp^3) [50]	Number of aromatic carbon atoms minus number of sp^3 hybridized carbon atoms	Alternative to both AP and aliphatic indicator which are inversely related fractional terms; an Ar-sp^3 based analysis of over 2000 orally bioavailable drugs and over 10,000 patented compounds revealed that the latter present a higher number of aromatic rings (i.e. higher flatness, lower solubility, and higher lipophilicity) than oral drugs [50]
Absorption, distribution, metabolism, excretion, and toxicity score (ADMET score) [8]	$\frac{\lvert 2.5-ALOGP\rvert}{2.0}+\frac{\lvert 330-MW\rvert}{120}$ if MW < 330, MW = 0 is used for calculation	<2 in 86% oral drugs; indicates the deviation of the properties of a compound from the mean values found in oral drugs; the higher the score, the higher the probability of attrition due to ADMET liability; the use of lipophilicity and size parameters in combination should perform better as prioritizing filters than each parameter taken individually; based on the analysis of thousands of drug discovery and marketed oral drug compounds from ChEMBL database
Quantitative estimate of druglikeness (QED) [54]	f(MW, ALOGP, HBDs, HBAs, PSA, RBs, NAR, and ALERTS) $= \exp\left(\frac{\sum_{i=1}^{8} w_i \ln d_i}{\sum_{i=1}^{8} w_i}\right)$ w = weight applied to each function d = individual desirability function	Index of esthetic beauty of drug molecules obtained by evaluating 771 orally administered FDA drugs; QED values range from 0 to 1 with 1 corresponding to the most drug-like and 0 to the least drug-like; obtained as a combination of eight parameters weighted by their relative significance; weights were those that maximized information content; each parameter was rendered as a desirability function (d) obtained from the property distribution data modeled as asymmetric double sigmoidal; proposed to overcome some shortcomings of Ro5

(*Continued*)

Table 2. (Continued).

Definition and abbreviation	Formulation[a]	Description, main features, and applications
Relative drug likelihood (RDL) [55]	$f(\text{MW, ALOGP, HBDs, HBAs, PSA, RBs, NAR, and ALERTS}) = \exp\left(\frac{\sum_{i=1}^{8} \ln d_i}{8}\right)$ d = individual desirability function	>1; desirability score obtained employing Bayesian methods over the same eight properties used in QED; for each parameter, a desirability function (d) was obtained considering the likelihood that a certain value for that parameter is found in drugs and quoting the so-obtained function on a similarly obtained function expressing the probability that a certain value for the same parameter is found in non-drugs; performed better than QED in identifying 771 orally administered small-molecule drugs from a negative set of >650,000 compounds (ChEMBL database)
Lipophilic metabolism efficiency (LipMetE) [56]	$\log D - \log (Cl_{int,u})$ where $Cl_{int,u} = Cl_{int,app} / f_{u,mic}$	Complex metric that describes the efficiency of the metabolic stability of a compound relative to its lipophilicity; can indicate the contribution of lipophilicity to metabolic stability vs. other factors, such as intrinsic chemical stability; based on a matched molecular pair analysis of 19 in-house cyclic ethers
Patient rule induction method (PRIM) [57]	Box covering algorithm	Method to identify rules from multidimensional data for selection of compounds with an improved chance of success as oral dosed drugs; based on set of boxes in property space, iteratively identified in order to select the maximum number of desirable compounds within; each box corresponds to a rule; performed similar to RDL in differentiating a set of 247 orally administered drugs from 1000 randomly selected compounds (ChEMBL database)
Fluorine-corrected molecular weight (MW_{FC}) [59]	MW – #F	While increasing MW, the addition of fluorine atoms does not introduce multidrug resistance efflux ratio liability, does not impair passive permeation, and does not reduce HLM stability; thus MW_{FC} more strongly correlates with above ADMET aspects than MW; based on data obtained from 149,420 compounds containing zero to five fluorine atoms

[a]HBD: hydrogen bond donor, i.e. that is, oxygen or nitrogen atoms with at least one hydrogen atom; HBA: hydrogen bond acceptor, i.e.that is, oxygen and nitrogen atoms; RB: rotatable bond; PSA: polar surface area; HAC: heavy (i.e., non-hydrogen) atom count; TPSA: topological polar surface area (some authors refer 'T' to 'total'); HLM: human liver microsome; ALOGP: lipophilicity estimated by atomic based prediction of octanol-water partition coefficient; ALERTS: structural alerts, i.e.that is, chemical features considered undesirable as a result of chemical reactivity or perceived toxicity issues; Log D: distribution of all species present at a given pH, generally evaluated at pH 7.4; $Cl_{int,u}$: unbound intrinsic clearance; $Cl_{int,app}$: bound intrinsic clearance; $f_{u,mic}$: unbound fraction (i.e., nonspecific binding) in HLMs.

Table 3. Efficiency metrics: abbreviations, analytical definition, suggested ranges, fields of application.

Metric	Abbreviation (s)	Definition(s)[a]	Proposed optimal values, main features, and applications
Ligand efficiency [30]	LE	$\frac{-\Delta G^{\circ}_{binding}}{HAC} = \frac{-RT\ln K_i/C^{\circ} \text{ or } -RT\ln K_d/C^{\circ}}{HAC}$ $\approx 1.37\frac{pK_i \text{ or } pK_d \text{ or } pIC_{50}}{HAC}$	>~0.3 kcal per mole per heavy atom (based on an ideal reference compound displaying K_d < 10 nM and having a HAC of 38, i.e. MW ≈ 500 Da) [30]; binding energy per atom (excluding hydrogens); widely accepted as a tool to compare fragments and prioritize the most size efficient ones for development; LE should be kept constant during optimization; given its size dependency, the use of LE to make comparison across wide size ranges is discouraged
Serum-free IC_{50}	$IC_{50,free}$	Free fraction × $IC_{50,total}$	Based on experimentally observed IC_{50} values ($IC_{50,total}$) and free fractions for an appropriate concentration range; potency corrected for nonspecific binding to proteins under the assumption that only molecules that are not bound to serum proteins may be efficacious; several lines of evidence demonstrate that the last statement is not true [68], thus this metric is doomed to obsolescence
Binding efficiency index [18]	BEI	$\frac{pK_i \text{ or } pK_d \text{ or } pIC_{50}}{MW}$	27.0 (based on an ideal reference compound displaying pK_d, pK_i, or pIC_{50} of 9.0 and having MW = 0.333 kDa, with the latter limit corresponding to the mean MW of a 25 HAC compound) [18]; over 15 HAC Kuntz et al. [26] found no significant increase in the free energy of binding, thus the limit of 25 considered a suitable limit to ideally truncate MW increase; alternative to LE in the early stages of development
Percentage efficiency index [18]	PEI	$\frac{\%\text{inhibition at a given [compound]}}{MW}$	1.5 (based on an ideal reference compound displaying 50% inhibition, 0.5 on a 0–1 scale, at a given screening concentration, and having MW = 0.333 kDa) [18]; alternative to LE in high throughput screening (HTS) studies where activity is generally evaluated at a certain concentration of each compound
Surface (binding) efficiency index [18]	SEI	$\frac{pK_i \text{ or } pK_d \text{ or } pIC_{50}}{PSA/100Å}$	18 (based on an ideal reference compound displaying pK_d, pK_i, or pIC_{50} of 9.0 and having PSA = 50 Å) [18]; 100 Å was chosen as a normalizing factor, in agreement with the sharp change in oral bioavailability observed for compounds with PSAs approximating this value [69]; suggested use in combination with BEI during lead optimization [18] to map ligands in Cartesian planes
Ligand efficiency index [32]	LEI	$\frac{pK_i \text{ or } pK_d \text{ or } pIC_{50}}{HAC}$	>~0.2 (based on an ideal reference compound displaying K_d < 10 nM and having a HAC of 38, i.e. MW ≈ 500 Da) [70]; having the number of HAs as its only unit, is considered as a 'unitless' metric [71], and obviously affords slightly lower figures than LE [70]; not to be confused with Abad-Zapatero's ligand efficiency indices which are pair of complementary ligand efficiency metrics relating to size and polarity, and assumed as coordinates of a Cartesian plane [72]
Lipophilic ligand efficiency [33] (lipophilic efficiency)	LLE (LipE)	pK_i (or pK_d or pIC_{50}) –cLog *P* (or Log *D*)	5–7 (based on an oral drug database containing 2,118 agents approved worldwide up to 2007, with cLogP ~2.5 and potency in the range ~1–10 nM); potency of a compound with respect to its lipophilicity; LipE was proposed as an alternative notation to remark the difference between this metric (size-independent and based on lipophilicity) and other stigmatized metrics based on HAC (e.g. LE) [19]
Group efficiency [34]	GE	$\frac{-\Delta\Delta G}{\Delta HAC}$	Defines the efficiency of an added moiety; binding energy per atom (excluding hydrogens) of the added group; each group is assumed as contributing independently (i.e. additively); useful in the optimization process
Enthalpic efficiency [35]	EE	$\frac{\Delta H}{MW}$	Provides a quantification of the ligand's net bond forming capability: the higher the absolute value, the higher the developability
Fit quality [38]	FQ	LE/LE_Scale where $LE\_Scale = \frac{7.5328}{HAC} + \frac{25.7079}{HAC^2} + \frac{361.4722}{HAC^3}$	Scaled so that for the most efficient ligands FQ approximates 1; allows comparison between differently sized compounds, thus outperforming LE in the analysis of HTS campaign results; based on the analysis of more than 8,000 small molecules with reported potency measures and encompassing a 10–50 HAC range; underestimates the value of compounds with HAC < 15
Lipophilicity-corrected ligand efficiency [43] (ligand efficiency dependent lipophilicity)	LELP	$\frac{\text{Log P}}{LE}$	–10 < LELP < 10 for acceptable leads (based on a reference compound displaying LE = 0.3 and lipophilicity in the range − 3 < Log *P* < 3); desirable range: 0 < LELP < 7.5 (based on an ideal reference compound displaying LE = 0.4 and Log *P* < 3); contemperates LE with lipophilicity depicting the price of ligand efficiency paid in Log *P*; LELP is negative when Log *P* is negative; the higher the absolute value of LELP, the less drug-like the lead compound; when LELP > 0, the closer LELP is to zero, the better; derived from the analysis of hundreds of hit-lead pairs from the literature
Size-independent ligand efficiency [44]	SILE	$\frac{pK_i \text{ or } pK_d \text{ or } pIC_{50}}{HAC^{0.3}}$	≥2.5; allows comparison between differently sized compounds, thus outperforming LE in the analysis of HTS campaign results; based on the analysis of Reynolds' LE/HAC curve [38]; proposed as a simpler alternative to FQ; should increase during optimization regardless of initial ligand efficiency of the starting point
Percentage ligand efficiency [41]	%LE	(LE/max LE) × 100 where $\max LE = 1.614^{\log2(10/HAC)}$	~1.6; inspired by the finding that generally, when HAC is halved, LE increases by ~1.6-fold (a number that approximates ϕ, the golden ratio); based on Kuntz's data [26], was proposed to overcome the main limitation of FQ

(*Continued*)

Table 3. (Continued).

Metric	Abbreviation (s)	Definition(s)[a]	Proposed optimal values, main features, and applications
Drug efficiency index [46]	DEI	$DEI = pX_{50} + Log D_{eff}$ where $pX_{50} = pIC_{50}$ or pK_d or pK_i $D_{eff} = \frac{[drug]_{biophase}}{Dose}$	>$pX50_{50}$ ($D_{eff} > 1$ for the majority of orally bioavailable drugs); sum of pX_{50} (i.e., a measure of potency) and base 10 logarithm of dose scaled free plasma concentration; presented as affinity corrected by the *in vivo* pharmacokinetic potential; D_{eff} has been defined as the free concentration at the site of action (biophase) relative to dose; the concentration is expressed in mg/ml; dose is given in mg per g body weight; thus, D_{eff} is unitless if 1 ml of biophase is assumed to weigh 1 g; originally introduced as a measure of both drug efficacy and unbound concentration, it has been then extended to the early development stages where *in vitro* measurements are used [73]; it has been claimed that LLE correlates well with DEI [32] but this statement sounds as a tautology since both metrics incorporate a potency measurement; [71] the criticism arisen against $IC_{50,free}$ (see above), casts doubts on the usefulness of these metrics as a tool to optimize efficacy
Size-independent enthalpy efficiency [47]	SIHE	$\frac{pK_H}{40} HAC^{0.3}$ where $pK_H = -\Delta H/(2.303 \times RT)$	→1; size unbiased metric (see SILE); alternative to QED; based on the analysis of data for 1232 complexes referring to 648 ligands; the normalizing factor 40 was chosen since potency shows an increasing trend up to about 40 HAs where it reaches a plateau; thus this metric, in contrast to SILE definition, is normalized to 1; the different power of HAC with respect to SILE is worth noting; studying this metric, it was demonstrated that enthalpic and entropic effects are reciprocally related to size (the former decrease, the latter increase with increasing MW); proposed for use in hit and lead selection, and in monitoring optimization processes; because of a series of factors conditioning ligand binding thermodynamics at both macroscopic and microscopic levels, some authors disagree about the routine use of raw thermodynamic data and related metrics which should be considered less robust than LLE for optimization runs [74]
Surface (binding) efficiency index per polar atom [48][b]	NSEI	$\frac{pK_i}{NPol}$ where NPol = #N + #O	1.5 (based on an ideal reference compound displaying $pK_i = 9.0$ and having NPol = 6); introduced as a more intuitive version of SEI (see above) to take into account polarity; suggested use in combination with NBEI, nBEI, and mBEI (see below) to map ligands in Cartesian planes; used to map 1283 target–ligand complexes (PDBBind database) in the so-called 'fan-plots' (given their fan-like or harp-like appearance)
Binding efficiency index per heavy atom [48][b]	NBEI	$\frac{pK_i}{HAC}$	0.36 (based on an ideal reference compound displaying $pK_i = 9.0$ and made of 25 HA); formally identical to LEI (see above); a modified definition – nBEI = $p(K_i/HAC)$ – allowed better separation of the compounds in the nBEI–NSEI plane; the reference value of 10.25 has been suggested for the latter definition; similarly, a new version of BEI was introduced: mBEI = $p(K_i/MW)$; the reference value of 11.5 has been suggested for the latter definition
Enthalpy efficiency [51]	LE H	$\frac{\Delta H}{HAC}$	Similar to EE (see above)
Entropy efficiency [51]	LE S	$\frac{-T\Delta S}{HAC}$	The study of the trends of variation of both this metric and enthalpy efficiency (LE H = EE, see above) revealed that size dependency is related to enthalpy more than entropy, with larger molecules presenting relatively large buried surface areas that do not contribute to binding
Astex ligand lipophilicity efficiency [52]	LLE_{AT}	$0.11 + \frac{-\Delta G^*}{HAC} \approx 0.11 + 1.4\frac{LLE}{HAC}$	≥0.3 (based on an ideal reference compound displaying $K_d = 10$ nM, HAC of 36, and cLog *P* of 3, the latter consistent with the profile of known oral drugs for which average cLog *P* is 3); LLE adjusted for HAC, i.e., hybridization product of LE and LLE; ΔG* denotes the free energy change of the specific binding of a ligand to the target protein (i.e., $\Delta G - \Delta\Delta G_{lipo}$, with the latter corresponding to the free energy accompanying the transfer from aqueous solution to a hydrophobic environment); the constant term was added so to obtain the same ideal value above indicated for LE; if significantly lower than LE, it indicates that lipophilicity is the driving force of binding; applicable to fragments, hits, and leads, shares applications and features with GE; size-independent; expressed in units of kcal mol^{-1} per non-hydrogen atom (thus differing from LLE which is a unitless metric)
Kinetic efficiency [53]	KE	$\frac{\tau}{HAC} = \frac{t_{1/2}}{0.693 \times HA}$	Suggested values are context-dependent with slow dissociating ligands (high KE) being superior; residence time (τ) per heavy atom count ($t_{1/2}$ = half life for dissociation); limited utility in the early stages of development where residence times are normally too low (too low affinity) to allow discrimination; where toxicity concern arises (e.g. antipsychotics), fast dissociating ligands (lower residence times) are preferable; it has been suggested that in matched molecular pair analysis the kinetic component is built into LLE analysis [75]
Lipophilic enthalpy efficiency [58]	LLE H	pK_H – cLog *P*	Is to LLE as LE H (EE) is to LE; plotted versus cLog *P* in a Cartesian plane to demonstrate that size dependency is related to enthalpy
Lipophilic entropy efficiency [58]	LLE S	pK_S – cLog *P*	Is to LLE as LE S is to LE; plotted versus cLog *P* in a Cartesian plane to demonstrate that size dependency is not related to entropy

(*Continued*)

Table 3. (Continued).

Metric	Abbreviation(s)	Definition(s)[a]	Proposed optimal values, main features, and applications
ADMET efficiency index [60]	AEI	$\frac{p\mathit{K}_i \text{ or } p\mathit{K}_d \text{ or } pIC_{50} - \lvert \text{Log P} \rvert}{\text{PSA}} \times 100$	>7 based on 57 recent LLE optimized compounds; encompasses LLE and the Biopharmaceutical Drug Disposition Classification System (BDDCS) space; focused on PSA (cf. SEI) because of its ability to score transporter liability and unveil 'ugly' compounds; it is worth noting the use of the modulus of Log *P* which makes an important difference with respect to LLE (it takes into account the negative consequences of negative cLog *P* values on absorption); the higher the value the lower the maximum daily dose; applied to HIV protease inhibitors, allowed differentiation of drugs on an ADMET basis; a decision tree was proposed as a useful framework to facilitate the use of the metric to anticipate attrition due to ADMET liability
Ligand specific efficiency [61]	LSE	$\frac{p\mathit{K}_d \text{ or } pIC_{50}}{\text{CHI(IAM)}}$	≥5.4 [based on the analysis of 16 clinically relevant position emission tomography (PET) tracers]; affinity normalized to nonspecific binding, useful for the optimization of PET tracer candidates; CHI(IAM) = chromatographic hydrophobicity index on immobilized artificial membranes, experimental measure of nonspecific binding

[a]HAC: heavy (i.e. non-hydrogen) atom count; R (ideal gas constant): 1.987×10^{-3} kcal/K/mol; T: temperature in Kelvin (K), generally 300 K; K_i: inhibition constant; K_d: dissociation constant; C°: standard concentration, generally 1 M; IC_{50}: half-maximal inhibitory concentration; PSA: polar surface area.

were proposed, including the aromatic proportion (AP [67], that is, Yan and Gasteiger's aromatic indicator [29]), the fraction of sp^3 hybridized carbon atoms (Fsp^3 [39], that is, Yan and Gasteiger's aliphatic indicator [29]), and the difference between aromatic and sp^3 carbon atom counts (Ar-sp^3) [50].

With the diffusion of the isothermal titration calorimetry (ITC) technique, the enthalpies and entropies of binding have become increasingly available thus fueling the birth of new metrics in which the above thermodynamic parameters are used in lieu of affinity measures (Tables 1 and 3) [51]. These new metrics provided insights in the phenomena at the basis of the trends observed between ligand efficiency and molecular size and reinforced the conventional drug designers' wisdom (a strong inverse correlation exists between ΔH and ΔS of binding).

In the last lustrum, a series of more complex measures have been proposed with the aim to control physicochemical properties involved in absorption, distribution, metabolism, and toxicity (ADMET). The discussion of these multidimensional, ADMET oriented metrics falls out of the scope this review. The interested reader may catch just a glimpse by browsing Table 2 whereas full details are in Meanwell's [70,77] and Hetényi and colleagues' [78] reviews.

In the mean time, several new efficiency metrics have been proposed, thus crowding an already overpopulated topic (Tables 1 and 3). To deal with this overwhelming amount of information, several graphical tools have been proposed [79], including bar graph profiles [80,81], flower plots [82], scatter plots [83], efficiency maps [48], oral bioavailability estimation maps [84], a golden triangle [85], egg plots [86], traffic lights [87], pie charts [40,79,83], box plots [40], Chernoff faces [79,88], time series plots [79,89], network-like similarity graphs [90], radar (or spider or cobweb) plots [66,91], and a chemical global positioning system (ChemGPS) [92]. However, sometimes the remedy may be worse than the disease and the availability of numerous graphical tools may further confuse a mid-level practitioner. The risks related to the plethora of available metrics and visualization tools has been pointed out [19,21] and will be briefly treated in Chapter 4.

3. Ligand efficiency metrics: what do they have to say?

Originally proposed as guidance in selecting and optimizing lead compounds, ligand efficiency metrics are nowadays mostly applied in FBDD and hit to lead optimization. The main reason resides both in the ever increasing credit attributed to FBDD as a reliable approach and in the relatively high ligand efficiency of fragments in comparison with larger compounds [93]. Thus, starting with good fragments (high LE) and controlling lipophilicity during development by means of suited lipophilic efficiency metrics (e.g. LLE) attrition should be lowered. Several recent successful FBDD campaigns [94–96] use efficiency measures and refer to the anchor-fragment concept [23,25]. The following two sections will illustrate further recent examples. Then, an example will be given that illustrates the fruitful use of ligand efficiency metrics in the deconstruction approach, that is, moving from high-sized molecules to their constituting fragments. Finally, some less explored applications of efficiency metrics will be illustrated in Sections 3.4 and 3.5.

3.1. Ligand efficiency metrics and FBDD

The use of ligand efficiency metrics is not alternative to other well-validated approaches commonly used in drug design and discovery campaigns. The following example illustrates the synergistic application of LE/GE and X-ray crystal structure analyses in a FBDD program.

Nearly two million people die each year from tuberculosis but a robust pipeline of potential therapies to combat this disease is still missing. Looking for *Mycobacterium tuberculosis* pantothenate synthetase (PS) inhibitors as new drugs against tuberculosis, GE analysis was used in a FBDD approach [97].

Starting from fragments **1** and **2** (Figure 1), both fragment growing and linking approaches rapidly led to the hit compounds **3** and **4**, which were dissected in four main building blocks whose corresponding binding contributions were then calculated from K_d values obtained by ~~isothermal~~ ITC. Most of the binding energy results from the initial indole fragment (GE = 0.75) and charged acetate side chain (GE = 0.43 and 0.35 for **3** and **4**, respectively), with the acylsulfonamide, methylpyridine, and benzofuran groups being inefficient binding components (GE = 0.16–0.17).

Although the aim was to replace the less efficient parts of the molecule to improve the inhibition potency, the acylsulfonamide linker was still retained as an effective functional group for properly targeting the two main pockets of the binding site [98]. Probably, this is why amide **5** was not optimized though its better profile compared to **4** (lower K_d and higher LE values). A series of indole acylsulfonamide compounds were then prepared thus obtaining five submicromolar inhibitors, with those bearing an electronrich *p*-tolyl (**6**) and a lipophilic *p*-trifluoromethyl phenyl (**7**) being the most interesting ones. Conversely, the replacement of the methyl pyridine and benzofuran groups of **3** and **4**, respectively, with more hydrophilic moieties led to a drop in potency and LE values.

The X-ray cocrystal structures of the most potent compounds with PS confirmed the binding at the active site, with a conserved binding mode for the indole sulfonamide fragment core. Furthermore, the left side of the molecules bound the lipophilic P1 pocket of the enzyme thus clashing with the nearby Met40 wall. Hence, further optimization focused on introducing a methylene spacer between the aromatic and sulfonyl groups of the most potent compound of the series (**7**) in order to allow the aromatic group to slide below Met40 residue and probe more deeply into the P1 pocket. Compound **8** was thus obtained and its X-ray cocrystal structure confirmed the complementarity of the molecule to the binding site, with favorable hydrophobic interactions of CF_3 group with Val139, Val142, and Val143. Compound **8** inhibited PS with an IC_{50} value of 250 nM, significantly improved compared to **7** (IC_{50} = 5.7 μM). Furthermore, a cell-based assay against *M. tuberculosis* showed on-target inhibitory activity leading to cell death.

Unfortunately, the authors did not report the ITC-derived K_d value for the final compound **8** and calculated the

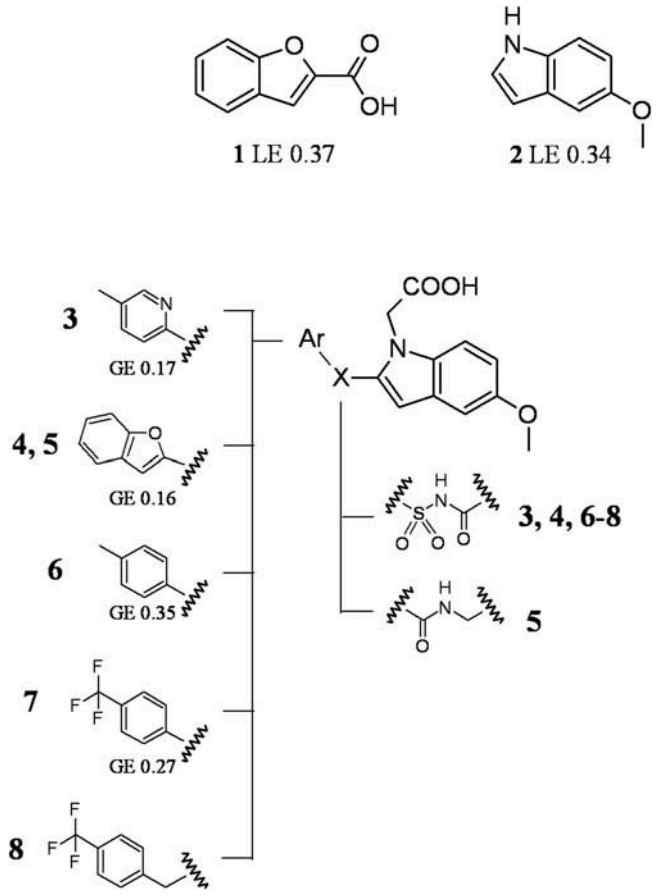

Figure 1. Structures and relevant metrics for *Mycobacterium tuberculosis* pantothenate synthetase inhibitors 1–8.

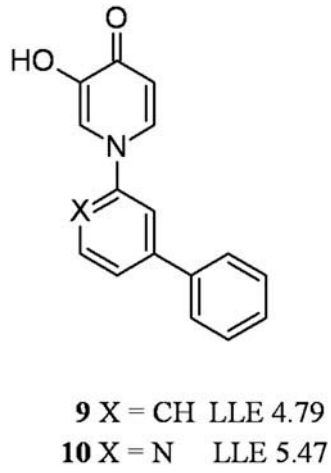

Figure 2. Structures and LLE values for COMT inhibitors 9,10.

corresponding LE score (0.28) on the basis of the IC_{50} value. Thus, we cannot argument on the curious finding of a relatively low potency (5.7 μM) coupled to a relatively high affinity (K_d = 0.2 μM) for compound **7**.

3.2. Ligand efficiency metrics and hit to lead optimization

It has been shown that LLE is a reliable metric regardless of size and chemical class under study. Indeed, it has resisted invalidation attempts [19]. LLE has been shown to correlate with binding enthalpy, offering a potential explanation for its validity as a useful efficiency metric [77].

On the other hand, it has been revealed that the three dimensionality of compounds, expressed as the mean Fsp^3 count, increased as a compound progressed through development [39]. Possibly, the higher is the Fsp^3 value the lower are promiscuity and metabolic liability [99]. The following two examples will illustrate the above statements.

Catechol-*O*-methyltransferase (COMT) inhibitors have been recently proposed for the treatment of cognitive symptoms associated with schizophrenia, a chronic and debilitating brain disorder with 1% prevalence [100]. Increase of cortical dopamine levels, due to central modulation of COMT activity, shows positive effects in both rodent models and humans [101].

By controlling lipophilicity through LLE and cLog *P*, Merck researchers developed *N*-heterocyclic pyridinones as brain-penetrant COMT inhibitors possibly endowed with suitable drug-like properties for *in vivo* studies. With respect to the reference compound **9** (Figure 2), preliminary *in vitro* potency proved sensitive to biphenyl replacements, with the 4-phenyl-pyridin-2-yl assemblage (**10**) leading to a 0.68 unit increase in LLE while maintaining the potency. When the phenyl ring was replaced with 5- or 6-membered heteroaryl moieties, either polar and less active or more lipophilic with lower LLE analogs were obtained. Therefore, pyridinone **10** emerged as the lead compound of the series showing the better *in vitro* potency together with excellent LLE. The *in vivo* effect of **10** was confirmed by measuring the levels of two dopamine metabolites, biomarkers for central COMT inhibition, in rat cerebrospinal fluid.

Unfortunately, despite a small improvement in human and rat intrinsic clearance with respect to **9**, the lead compound **10** exhibited poor metabolic stability with an observed plasma clearance as high as 94 ml/min/kg and a half-life as low as 0.4 h. Pyridinone glucuronidation, located distal to the site of structural changes in these analogs, should be the major route of metabolism. Thus, this compound can be considered as a useful starting point that needs further modifications at its metabolically labile sites rather than a clinical candidate. Incidentally, it should be noted that the replacement of a phenyl with a pyridyl ring (i.e. passing from **9** to **10**) was beneficial, as generally observed in numerous biologically relevant chemotypes [102].

Recently, metabotropic glutamate receptors (mGluRs) have also emerged as potential targets for the treatment of schizophrenia. In particular, mGluR2 agonists showed clinically significant antipsychotic properties, thus providing a new option for the treatment of schizophrenia [103]. The optimization of positive allosteric modulators of mGluR2 represents an interesting application of what can be accomplished when lipophilicity is controlled [104].

Starting from aryl azabenzimidazolone **11**, a series of *N*- and *C*-(nonaryl)-linked analogs were investigated as new chemotypes endowed with favorable ADME properties (Figure 3).

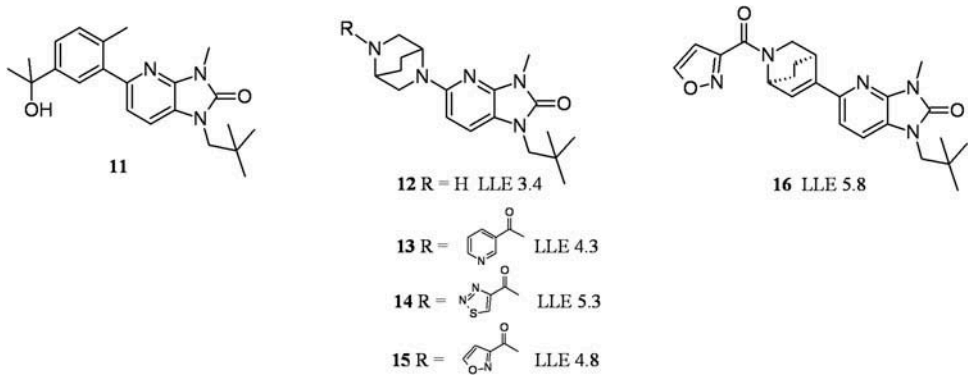

Figure 3. Structures and LLE values for mGluR2 positive allosteric modulators 11–16.

Incorporation of a [2.2.2]bicyclic piperazine scaffold led to piperazine **12** which, although three-fold less potent than the corresponding piperidine derivative, exhibited reduced cLog *P* and 1.4 unit increase in LLE, thus resulting a superior lead. Decoration of this scaffold by acylation led to the nicotinoyl amide **13** which emerged as a new lead compound because of a higher LLE. It showed high solubility at neutral pH (183 μM), attractive rat oral bioavailability and clearance.

Over 100 heteroaryl amides of **12** were then synthesized, with the two most intriguing being the optically active thiadiazole **14** and isoxazole **15**. They exhibited a fourfold improvement in potency, together with higher LLE, compared to pyridyl amide **13**. Furthermore, both compounds featured excellent cell permeability and none of them was a P-gp substrate (rat or human).

Although they were almost indistinguishable in terms of *in vitro* profiles, different pharmacokinetic profiles indicated **15** as suitable for once-daily dosing. Thus, its efficacy in a rat behavioral model of antipsychotic activity was assessed [105,106] and full efficacy at low-micromolar plasma exposure was observed. Although the *in vitro–in vivo* correlation was excellent, further progression of isoxazole **15** was discontinued because of significant metabolic liability resulting from a potent time-dependent inhibition of CYP 3A4, with high potential for drug–drug interaction. The aniline nitrogen atom of **15** was the most likely cause of the CYP inhibition, presumably by forming a reactive nitroso metabolite.

The bioisosteric replacement of the CH_2N group with a potency-enhancing endocyclic olefin led to the optimized analog **16** whose (1*S*,4*R*)-eutomer showed excellent drug-like properties and the highest LLE within the azabenzimidazolone structural class (LLE = 5.8). Furthermore, robust *in vivo* efficacy, high subtype selectivity, as well as oral bioavailability due to mitigated metabolic liability were observed, thus making **16** the most interesting compound in the series. It should be noted that Fsp^3 raised during the study and two chirality centers were added in good agreement with the general trend observed by Lovering [39].

3.3. Ligand efficiency metrics and deconstruction approach

The usefulness of ligand efficiency metrics at the early stages of drug development is illustrated by the following example where they helped determining the moieties most contributing to potency in relatively complex starting hit compounds.

Apoptosis signal-regulating kinase 1 (ASK1) inhibitors may be useful for the treatment of heart failure. ASK1 is a mitogen-activated protein kinase family member responsible for heart failure and acute ischemia/reperfusion injury [107]. Thus, selective ASK1 inhibitors represent potential agents for heart failure and have been investigated at Takeda [108].

At first, key interactions between two known ASK1 inhibitors (**17, 18**, Figure 4) and the ATP binding site were identified. In order to determine the relative contributions of the different binding elements to potency, a deconstruction approach was applied to the hit compound **18** by employing LLE, LE, and GE calculations. The removal of the cyclopropyl imidazole together with the methyl group and fluorine atom to obtain an unsubstituted benzamide as well as the truncation of the *N*-isopropyl triazole ring resulted in a drop of both potency and LLE, while LE remained unchanged. Furthermore, calculation of GE showed that the *N*-isopropyl triazole at the right side of the molecule as well as fluorine and methyl substituents on the benzoyl moiety mostly contribute to the ASK1 inhibition. On the other hand, a study based on the overlay of a model of **18** and the cocrystal structure of **17** in hASK1 had already highlighted that the hydrogen bond between 5-membered nitrogen heterocycles and the side-chain of Lys709 is one of the key interactions and the *ortho*-fluorine atom might favor the coplanarity between the benzamide group and the rest of the core. Besides, the *t*-butyl group and cyclopropyl imidazole moieties of **17** and **18**, respectively, allow the molecules to lean toward the solvent exposed regions and are sandwiched between Arg705 and Lys769 residues. Therefore, further studies looked for more efficient ASK1 inhibitors able to interact with both Lys709 and Lys769 and/or Arg705 while maintaining a planar conformation. Several compounds were prepared and the isoindolinone **19** displayed the highest pIC_{50} and LLE values. The crystal structure of **19** bound to ASK1 showed a binding mode similar to **18**.

Higher LLE and pIC_{50} values were obtained when a terminal hydroxyl group was introduced in the isopropyl substituent on the triazole ring (**20**). This chiral compound also showed stereoselectivity, with the R enantiomer being the eutomer.

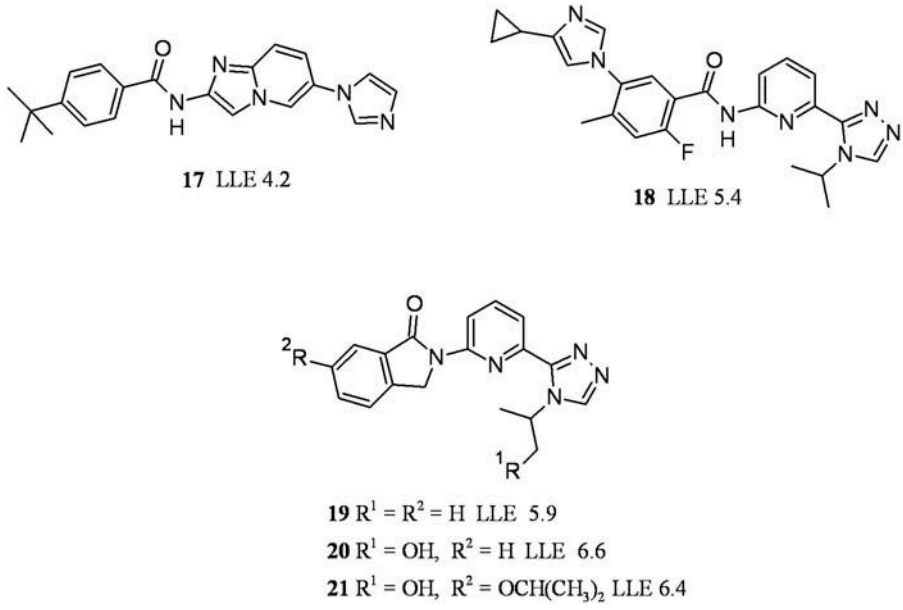

Figure 4. Structures and LLE values for ASK1 inhibitors 17–21.

In order to target the solvent exposed region of ASK1, the 6-position of the isoindolinone ring was modified with aliphatic groups bearing hydrogen bond acceptors. These compounds were tested in an ASK1 enzyme activity assay as well as in a cell-based assay and (*R*)-**21** showed the highest cell potency (pEC_{50} = 8.2) together with good LLE. Displaying good overall *in vitro* ADME properties, (*R*)-**21** was also tested in the Langendorff perfused *ex vivo* heart model and displayed reduction of infarct size thus claiming a place as a novel, potent, and orally bioavailable ASK1 inhibitor with favorable physicochemical properties.

3.4. Ligand efficiency metrics: an aid for rescuing the overlooked

Ligand efficiency metrics may be useful to gain new insights during drug research campaign and retrospectively evaluate previous positions. The following example will illustrate how GE, LE, and LLE value analyses may draw attention on a congener that might have been underestimated.

Voltage-gated sodium channel (VGSC) blockers, traditionally known as local anesthetic, antiarrhythmic, and anticonvulsant agents, are also used for the symptomatic treatment of both chronic pain and skeletal muscle syndromes [81].

In the last decade, an academic group has developed a series of both α- and β-proline 2,6-xylydides (**22** and **23**, respectively) [109] as cyclic analogs of tocainide (Figure 5). When compared to the lead compound, both series were endowed with 10-fold higher VGSC blocking potency toward the open and/or inactivate states of VGSCs, a property useful to selectivily block VGCS affected by pathological activation, as are found in myotonic muscles.

Further improvements were obtained via benzylation of the pyrrolidine nitrogen atom (**24**) [110], simplification of the structure (**25**), and replacement of the benzyl with an α-naphthylmethyl group (**26**) [111]. The latter was the most potent compound of the series and has recently displayed

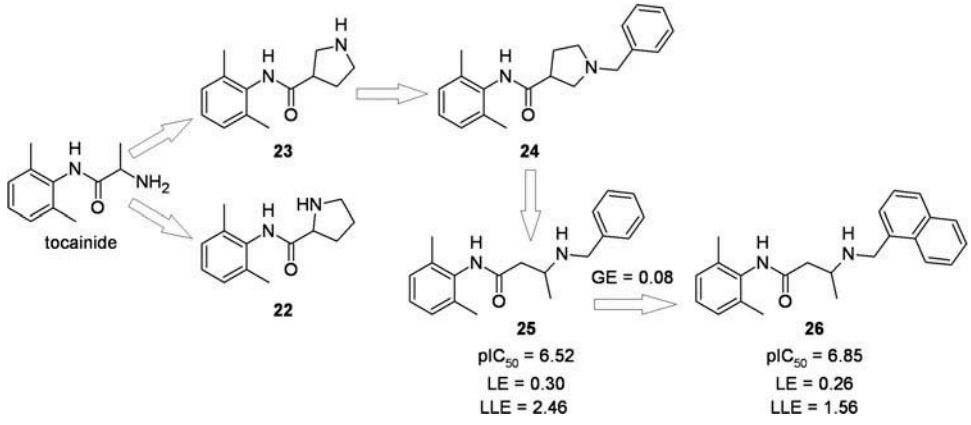

Figure 5. Structures, biological data, and relevant metrics for tocainide and its analogs 22–26.

interesting activity in an animal myotonia model [112]. However, when considering ligand efficiency metrics (e.g. LE, GE, or LLE; cf. Figure 5) compound **25** seems as promising as **26** and further development of **25** might be suggested.

3.5. Improving molecular docking through ligand efficiency metrics

LE and its size-adjusted modifications have been applied for identifying new hits in molecular docking campaigns (MDC). MDC is a valuable cost-effective option largely pursued by both academia and industry in lead discovery and optimization programs [113,114].

Unlike standard use of ligand efficiency metrics, MDC does not need any experimental data since metrics are based on computed binding energies (i.e. docking scores). For the purposes of drug discovery, a typical MDC procedure is carried out on large databases, typically comprising dozens of thousands of drug-like molecules exploring a chemical space as large as possible. The ultimate aim is that of prioritizing, for a given target, new potential initial hits for experimental testing [115–117].

Despite their high chemical diversity, compounds are usually ranked on the basis of the sole docking scores and thus irrespective of their size and predicted ligand efficiency metrics [118,119]. Noteworthy, increasing the molecular size often implies an increment of docking score, a value substantially rewarding hydrophobic complementarity as driving force in determining protein-ligand affinity. Thus, an uninformed use of MDC could encourage the selection of compounds intrinsically unsuitable for hit-calling (e.g. too large-sized), hence causing an unjustifiable waste of time and money since early stages of drug discovery process. To smooth such a bias toward large-sized compounds, Garcia-Sosa et al. [120] suggested employing some ligand efficiency metrics. By comparing experimental values to predicted binding free energies, it was demonstrated that normalizing docking scores with respect to molecular weight, number of heavy atoms, and Wiener index strongly improves docking fitness functions. This approach proved effective also in recently published MDC [121–123].

Several molecular modeling suites allow compound prioritization based on docking scores normalized considering different metrics. A valuable example is given by Autodock and Autodock Vina, open source software suites widely employed to perform MDC. Together with the docking score, the user can extract from the output file a value of 'ligand efficiency' calculated by dividing the score obtained in the docking experiment by the total number of non-hydrogen atoms of the ligand.

Furthermore, AudockVina is provided with a graphical user interface to facilitate the selection of compounds showing δLE > 1 or δLE ≥ m + 3s where δLE is computed as follows:

$$\delta_{LE} = LE_{ligand}/LE_{standard}$$

Note that $LE_{standard}$ is the ligand efficiency computed for the standard compound (e.g. cognate ligand of the considered X-ray solved crystal), m is the average value of δ_{LE} for all the compounds, and s the associated standard deviation.

Several metrics are instead computed by grid-based ligand docking with energetics (GLIDE), software available from Schrodinger suite. Specifically, the user can rank compounds based on:

- Docking score/(number of heavy atoms), termed 'ligand efficiency' in the output file;
- Docking score/(number of heavy atoms)$^{2/3}$, termed 'ligand efficiency sa' in the output file;
- Docking score/[1 + ln(number of heavy atoms)], termed 'ligand efficiency ln' in the output file.

The second efficiency metric is acknowledged to approximate the effect of surface area. It is worth to point out that both the herein considered software suites do not allow the computation of metrics accounting for physicochemical properties (e.g. molecular weight and partition coefficient), despite their growing importance has been highlighted by several papers.

4. Ligand efficiency metrics: notes of criticism

'The human mind delights in finding pattern', recalled Abad-Zapatero [124] quoting Stephen Jay Gould. Humans have a natural inclination to readily perceive visual patterns [79]. In particular, the chemist's ability to envisage structural similarities (gestalt pattern recognition) [125] is probably his peculiar skill [126,127]. On the other hand, this pattern recognition ability has been stigmatized as one of the cause of the 'correlation inflation' (i.e. exaggerating trends in data analysis) observed in the context of molecular optimization [21].

In fact, ligand efficiency metrics have been used also for deriving supposedly predictive models. Basically, the pIC_{50} response value is replaced by metrics such as LE under the assumption that LE normalizes biological activity with respect to size-dependent physicochemical properties. In this respect, a gain in predictivity has been claimed when using LE instead of pIC_{50} as QSAR activity [128–132]. Actually, the improvement of predictions of LE over pIC_{50} is substantially due to mere statistical artifacts. Efficiency metrics are in fact the results of user-desired transformations of pIC_{50}, initially conceived to make data interpretation easier. As a consequence, using LE over pIC_{50} has the effect of inflating correlation and noise. On the other hand, such transformations make models intrinsically inaccurate since scaling activity involves the propagation, to a higher extent, also of the associated uncertainty.

Soon after the seminal paper by Lipinski et al. [20], experts sparked a passionate debate to weigh the so-called Rule of 5 (Ro5) and suggested new metrics based on retrospective data analysis. The success of Ro5 has been a goad for researchers suffering of the so-called 'Ro5 envy' [21]. Many metric definitions have been proposed with the intention to inaugurate a new era of property-focused medicinal chemistry. These research efforts allowed the scientific community, including the users of ligand efficiency metrics, to critically approach metrics, including Ro5, but many questions remain still unanswered: (1) are there metrics correlating with successful hit-to-

lead decision-making? (2) Are the mathematical definitions of ligand efficiency metrics interpretable from a chemical point of view? (3) Which metric should be used in a typical medicinal chemistry program? In two recent papers [19,75], Shultz has attempted to properly answer all these questions on the basis of existing literature. Importantly, the author noticed that the widely employed LE is not correlated with successful optimization, whereas a good trend can be found if, among others, LLE is considered. In addition, LLE is a highly interpretable metric since it seems to correlate with the enthalpy of binding and therefore should be preferred for ligand optimization. However, LLE includes cLog *P* values which are generally affected by at least half an order of magnitude of error [133]. On the other hand, several *in vitro* activity measurements are not more accurate than cLog *P*. Thus, LLE values may vary for ±2 units depending on the software chosen for cLog *P* calculation and experimental errors.

Although Schultz's contribution brings in the foreground a specific metric by means of convincing arguments, it should be noted that one must be careful before drawing conclusions from correlation resulting from retrospective data analysis. The risk of get lost in chance correlations instead of deriving causative relationships is indeed behind the corner, as properly pointed out by Kenny and Montanari [21]. Often drug discovery comes to misleading conclusions on the basis of illusory trends based on inappropriate data treatment. An example is given by the common practice of partitioning data into subsets prior to analysis.

We are still far from answering the mentioned questions; however, the increasing criticism toward the misuse of metrics has contributed to curbing the general and wrong tendency of using metrics more as a magic box than as a rationally driven route.

5. Conclusions

Ligand efficiency metrics are typical elements of property-based design whose primary use should be for measures of compound quality. The role of physicochemical properties such as lipophilicity and molecular size is of utmost relevance for tuning lead optimization and ADMET profile. On the other hand, moving from hit or fragment sized compounds is very much effective for sampling, being intrinsically higher the chance of smaller molecules to exert complementarity to a given target [134,135]. Ligand efficiency metrics are almost universally accepted as a valuable indicator of compound quality whose benefits are mostly in the early stage of drug discovery projects. Rooms for other use are still there but with a certain level of misuse risk.

After a duly acknowledgement of the seminal work of the founding fathers, we have tried to give a balanced overview on efficiency metrics merits and points of weakness to enable the readers to gain an informed opinion. In the next section, we will present our own convincement.

6. Expert opinion

It has been underlined that ligand metrics are generally questionable from the mathematical and statistical point of view [136]. However, more than the numbers obtained from calculations, the work of the founding fathers and their epigones has evolved the classical way of thinking about SAR and drug design. Is the game worth the candle? It much depends on the real-life applications of metrics, which should be discussed case-by-case.

The paradigm shift divulgated by Hopkins' group [30] undoubtedly supports the expanding role of FBDD and hit to lead development where efficiency metrics are effective irrespective of specific measurements. In early stages of drug discovery pipeline, identifying light chemical structures provided with a significant target activity represents an unprecedented opportunity for medicinal chemists. Indeed, it opens the door to rationally inspired molecular decorations, which imply not only the increase of molecular bulkiness of dozens of Da but hopefully also a jump of activity from the μM to the nM range. In most cases, LE decreases in the optimization step, thus making difficult the conversion of a fragment into a drug-like compound. As a result, prioritizing fragment hits based on LE scores allows also smaller low affinity compounds to be attractive for further optimization [137] even considering that ADMET parameters could deteriorate with either increasing MW or Log *P* [36]. Ideally, an efficient fragment growth should reflect constant LE values so that the increase of binding affinity should linearly follow the increase of MW [138].

'To generalize is to be an idiot' was William Blake's contribution to conventional wisdom. This well-known aphorism sounds a warning also to medicinal chemists engaging with SAR. A methyl may be 'magic' if it allows unexpectedly high gain in potency when substituting a hydrogen in a reference compound [30]. However, 'magic methyls' are rarely found in the literature [139]. More often, methyls perform as lipophilic ballast conferring only modest improvement in potency [139] or are detrimental [140,141]. The above quote is suited to warn about the context- and chemotype-dependent performance of ligand efficiency measures [77]. As an example, LE works well when comparing molecules in the same MW range, but it should not be used to choose the best candidate over a wider MW range of compounds; in this case, size-independent metrics such as SILE would be preferred.

A common criticism against rules and metrics is that they would restrain the possibility of choice thus limiting a priori chances of finding out a new drug beyond the conventional drug-like space [142]. This would happen if guideposts are intended as inviolable commandments [124]. Several recent examples of successful drugs emerged from regions beyond the borders of what is traditionally considered to be drug-like [77]. Hardly, they may be presented as exemplary 'attractive' molecules but some of them contribute to the recently developed oral therapy of chronic hepatitis C – a medical triumph [143].

Physicochemical recommendations should not be used as hard cutoffs when looking for protein-protein interaction inhibitors (PPI). In fact, it has been proposed that the average MW required for this interesting class is higher than molecular size found in other target classes since PPI would block large and diffuse binding sites [144].

Similar considerations should be kept in mind when pursuing polypharmacology, where agents are deliberately designed to be promiscuous. In this case, compounds are not optimized for just one single target [145,146] and thus they can not show high efficiency [147].

It would be ungenerous to discriminate natural compounds through rigid property filters since nature produces and stocks in aqueous environment molecules that are extraordinary efficient, diverse, and elegant [148], while being outside Ro5 space. Imposing a physicochemical scotoma over these classes of compounds would thus reduce the rate of success.

Furthermore, the hypothesis has been formulated that the active transport across membrane has been underestimated so far [142]. Thus, the deleterious role of exceeding molecular size could have been exaggerated. This implies that both synthetic and natural compounds violating the normal property range including oral bioavailable drugs could be endowed with good permeability.

On the other hand, size-based efficiency metrics such as LE and GE should be used in deconstruction studies, thus applying also to natural products. Then, LLE would be used to control the effects of additions to the so-individuated highly efficient cores.

Finally, we should escape from the temptation to find predictive models giving the apparently right answer without a rigorous quantitative validation [149]. Distorting user perception, QSAR models based on ligand efficiency metrics can be misleading and elusive [71,150].

The 'big bang' [19] of efficiency metrics occurred more or less 10 years ago. Since the average time to develop a new drug is over the same period, the next few years will say a clearer word on the increased rate of success, if any, gained by means of these new intriguing tools, provided they are used *cum grano salis*.

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