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A Practical Use of Ligand Efficiency Indices Out of the Fragment-Based Approach: Ligand Efficiency-Guided Lead Identification of Soluble Epoxide Hydrolase Inhibitors

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Ligand efficiency is frequently used to evaluate fragment compounds in fragment-based drug discovery. We applied ligand efficiency indices in a conventional virtual screening-initiated lead generation study of soluble epoxide hydrolase inhibitors. From a considerable number of screening hits, we carefully selected a compound exhibiting relatively weak inhibitory activity but high ligand efficiency. This ligand efficiency-guided selection could reveal compounds possessing preferable lead-like characteristics in terms of molecular size and lipophilicity. The following hit-to-lead medicinal chemistry campaign successfully led to a more potent, ADMET-clean, lead-like compound preserving high ligand efficiency. Retrospective analyses, including consideration of the more recently proposed indices of ligand efficiency, shed light on the validity of our hit triage and hit-to-lead studies. The present work proposes a practical methodology for lead generation using the concept of ligand efficiency.

Introduction

The concept of ligand efficiency (LE),[a] defined as biological activity (affinity) per molecular size, was originally discussed by Andrews et al.[1] and later by Kuntz et al.,[2] and then its utilization in lead assessment was proposed by Hopkins et al.[3] and subsequently by Abad-Zapatero and Metz.[4] Since then, LE has become a useful yardstick to evaluate a ligand's ability to effectively bind to a target protein, especially in fragment-based drug discovery (FBDD).[5,6] Even with a relatively weak activity, it is expected that hits having high LE would, within a reasonable range of molecular size, offer greater potential for higher activity. Fundamentally, LE is a numerical representation of what medicinal chemists have conformed to, but as an unwritten and thus easily violable rule. We therefore believe that LE can be beneficial in conventional medicinal chemistry not only to seek a better starting point from screening hits but also to validate the track of medicinal chemistry efforts.[7] Herein, we present a lead identification study of soluble epoxide hydrolase inhibitors that was accurately guided by LE.

As a cytosolic enzyme, soluble epoxide hydrolase (sEH) is ubiquitously expressed in the body, particularly in the liver and kidney, and is responsible for conversion of epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatrienoic acids (DHETs) in the arachidonic acid cascade (Figure 1).[8] Inhibition of sEH results in elevation of plasma concentrations of EETs. EETs are known as lipid mediators that trigger a wide range of pharmacological responses, including vasodilation-induced hypotension, improvement in glucose tolerance, and anti-inflammatory reactions. Much effort has therefore been made to discover sEH inhibitors as potential new treatments for several therapeutic indications. Although a number of sEH inhibitors have already been reported, most of them are highly lipophilic compounds.[9] Extreme lipophilicity is well recognized as a risk factor of promiscuous association of a ligand with off-target molecules in the body, causing unexpected adverse effects.[10] We concluded that the key to success in the discovery of sEH inhibitors is to find a relatively small-sized, less lipophilic lead compound that would accommodate the increases

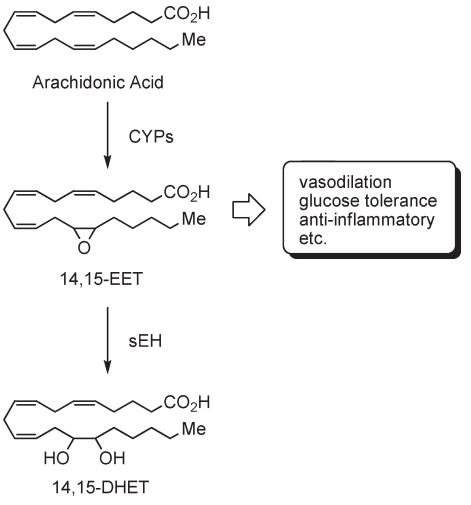

Figure 1. Role of sEH in the arachidonic acid cascade and the ensuing pharmacological effects.

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[a] Abbreviations: ADMET, absorption, distribution, metabolism, excretion, and toxicity; LE, ligand efficiency; FBDD, fragment-based drug discovery; sEH, soluble epoxide hydrolase; EET, epoxyeicosatrienoic acid; DHET, dihydroxyeicosatrienoic acid; LEI, ligand efficiency index; HAC, heavy atom count; BEI, binding efficiency index; H2L, hit-to-lead; SEI, surface binding efficiency index; PSA, polar surface area; VDW contact, van der Waals contact; CYP, cytochrome P450; LELP, ligand efficiency-dependent lipophilicity; FQ, fit quality; %LE, percentage ligand efficacy; SILE, size-independent ligand efficiency.

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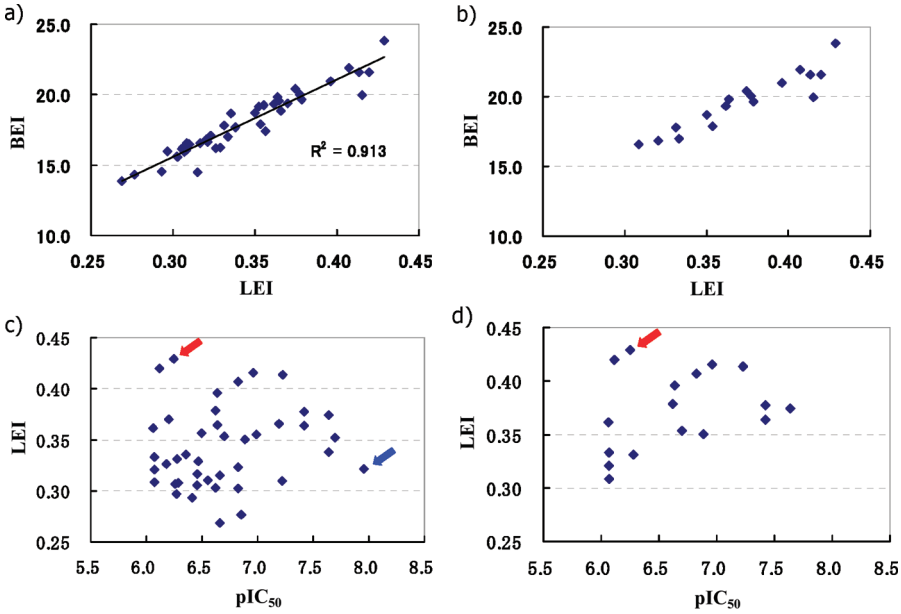
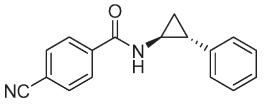


Figure 2. (a) Relationship between LEI and BEI of the 42 hit compounds. A clear linear relationship is observed. (b) After removal of relatively large (MW > 380) and lipophilic (calculated LogP > 3.5) compounds, 17 ligand-efficient (LEI > 0.31, BEI > 16.6) compounds remained as prominent hits. In particular, all highly ligand-efficient (LEI > 0.37, BEI > 19.5) compounds were retained. (c) Relationship between pIC_{50} and LEI of the 42 hit compounds. The selected hit **1** indicated by the red arrows is a highly ligand-efficient but weak sEH inhibitor. (d) After removal of the relatively large and lipophilic compounds, the most potent hit (blue arrow) was discarded.

Table 1. Structure and Profile of the Selected Hit **1**



1 (racemate)

M_w	IC_{50}	pIC_{50}	ACDlogP	AlogP	LEI	BEI	SEI
262	565 nM	6.25	2.38	2.76	0.43	23.8	11.8

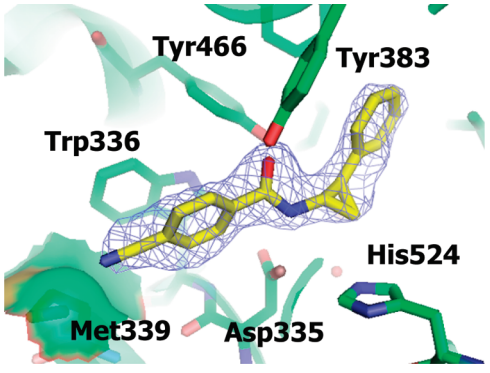


Figure 3. Crystal structure of the complex of the selected hit **1** and sEH hydrolase domain. Absolute configuration of **1** in the crystal structure could not be determined by electron density. Here it is represented by one enantiomer (PDB code 3ANS).

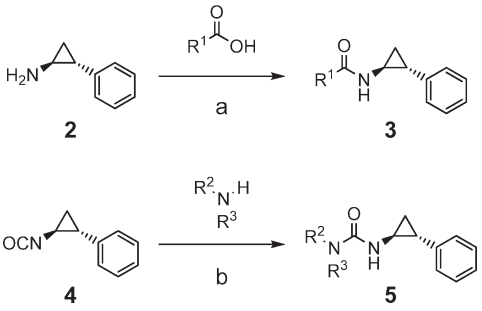
in molecular size and lipophilicity that often accompany activity enhancements during the ensuing lead optimization stage.[11]

Results and Discussion

Hit Identification. We initiated our study with a virtual screening of our internal compound collection using multiple crystal structures of the ligand-bound human sEH (PDB codes 1VJ5, 1ZD2, 1ZD3, 1ZD4, and 1ZD5) and then constructed a 735-member focused library composed of structurally diverse compounds. In vitro screening of this focused library for human sEH inhibitory activity identified 68 active compounds (IC_{50} < 1 μM). The high hit rate (9%) confirmed the relevance of our virtual screening campaign. Despite the diversity of the focused library, the identified compounds were generally amide or urea derivatives with only one exception (a β-hydroxyamine derivative, not shown). Moieties attached on either side of the amide or urea bond were structurally diverse but all highly lipophilic. These findings revealed the high specificity of the active site of sEH toward amides and ureas, presumably as transition state mimics,[8] and demonstrated the inherent promiscuity of the ligand-binding surface around the active site, reflecting the structural and physicochemical features of the endogenous lipid substrates (EETs). These results reinforced our concerns that exploration of sEH inhibitors is liable to stray into lipophilic chemical space.

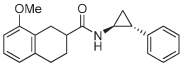
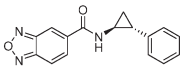
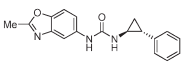
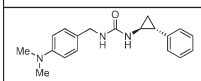
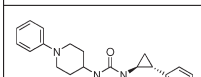
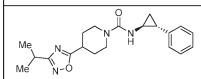
Hit Triage. Having a set of potent and structurally diverse hit compounds in hand, we turned our attention to the hit

Scheme 1[a]



[a] Reagents and conditions: (a) R^1CO_2H (1 equiv), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimidazole HCl (1 equiv), 1-hydroxybenzotriazole (1 equiv), triethylamine (2 equiv), *N*,*N*-dimethylformamide, room temp; (b) R^2R^3NH (1.5 equiv), toluene, room temp.

Table 2. Representatives of Potent and Ligand-Efficient Derivatives

Compound[a]	MW	ACDlogP AlogP	pIC_{50}[c] Human Rat Cell	LE[d] LEI BEI SEI	Insufficient physical and in vitro ADMET properties[e]
 **6**[b]	321	2.68 3.93	8.19 8.52 8.33	0.47 25.5 21.4	aqueous solubility metabolic stability (human, rat) CYP inhibition (2C19)
 **7**	279	2.50 2.45	7.21 6.62 NT[f]	0.47 25.8 10.6	aqueous solubility CYP inhibition (2C19)
 **8**	307	2.71 2.57	7.40 7.70 7.09	0.44 24.1 11.0	aqueous solubility metabolic stability (human, rat)
 **9**	309	2.38 2.94	7.52 NT[f] NT[f]	0.45 24.3 17.0	metabolic stability (rat) CYP inhibition (1A2, 2C19)
 **10**	335	3.31 2.99	8.64 8.77 8.10	0.47 25.8 19.5	aqueous solubility metabolic stability (rat)
 **11**	354	2.95 2.67	8.07 7.89 7.88	0.43 22.8 11.3	satisfactory as lead candidate

[a] All compounds are racemates. [b] Diastereomer mixture. [c] sEH inhibitory activities in human enzyme, rat enzyme, and human HepG2 cell-based assays. [d] Based on human sEH enzyme assay. [e] Insufficient properties: aqueous solubility $< 1.0\,\mu$g/mL at pH 7.4; metabolic stability (intrinsic clearance) > 0.1 mL/min/mg protein; CYP inhibition (IC_{50}) $< 10\,\mu$M. [f] Not tested.

triage phase. First, we visually inspected the structures of the hit compounds to discard non-lead-like structures and chemotypes known to be sEH inhibitors, leaving 42 hit compounds for further analysis. Among these compounds, we selected compounds possessing low molecular weight (MW), low lipophilicity, and high LE. Figure 2a shows the correlation between ligand efficiency index (LEI = ΔG/HAC)[3] and binding efficiency index (BEI = pIC_{50}/MW)[4] of the selected 42 hit compounds. Because free energy of binding (ΔG) was approximated from IC_{50} value ($\Delta G = -RT \cdot \ln IC_{50}$), ΔG is proportional to pIC_{50}. The principal difference between LEI and BEI is therefore the term representing molecular size, that is, heavy atom count (HAC) and MW. Since HAC and MW generally show good correlation, except when different numbers of heavier atoms (S, P, Cl, Br) are included in the molecules, a clear linear relationship between the two indices could be confirmed. Second, referring to the consensus of preferable molecular size and lipophilicity for leads,[11,12] we limited MW and the calculated LogP (from either ACDlogP or AlogP)[13] of the hit compounds to <380 Da and <3.5, respectively. Interestingly, this filtration exclusively removed less ligand-efficient compounds from the 42 hits and left a set of 17 ligand-efficient compounds (Figure 2b). This result encourages the use of LE-guided selection for pursuing small-sized and less lipophilic lead compounds (see Supporting Information). Among the 17 selected hits, we focused our interest on a cyclopropane derivative **1** that exhibited both the highest LEI and highest BEI.

In addition to the steps taken above, we also plotted LEI against sEH inhibitory activity (pIC_{50}) of the 42 hit compounds. As shown in Figure 2c, several of the most ligand-efficient compounds, including compound **1**, were not particularly potent sEH inhibitors; rather, such compounds were among the weakest inhibitors in this screening program. Often, weak hits are discarded in the hit triage phase and are not further investigated. However, having been inspired by the remarkable productivity of FBDD in lead generation, we were attracted to the high LE of **1**, as well as its simple molecular structure, and selected it for the ensuing hit-to-lead (H2L) study.

A series of analyses revealed that **1** possessed attractive features as a starting point for medicinal chemistry: low MW (262 Da), low lipophilicity (ACDlogP 2.38, AlogP 2.76), and high LE (Table 1). LEI and BEI of **1** were 0.43 and 23.8, respectively. The surface binding efficiency index (SEI = pIC_{50}/PSA),[4] which defines polar surface area (PSA) in terms of molecular size, was also in the preferable range (5−25), suggesting a prospect of good permeability. X-ray crystallography of the complex of **1** and the sEH hydrolase domain revealed the binding mode of **1** (Figure 3) in which the amide group of **1** created a hydrogen bonding network with the sEH catalytic triad composed of the side chains of Tyr383, Tyr466, and Asp335. This interaction mainly contributes to stabilizing the binary complex and is a highly conserved feature across the range of reported sEH inhibitors. In addition, a $\pi-\pi$ stacking

interaction between the benzene ring of the 4-cyanophenyl group and the indole ring of Trp336 and a van der Waals (VDW) contact between the cyano group and Met339 were observed. The phenyl group in the right-hand segment was loosely surrounded by lipophilic amino acid residues. Although it seemed possible to expand the inhibitor molecule to both sides, it was noticed that modification of the right-hand phenyl group would require a great deal of synthetic labor. We therefore prioritized synthetic tractability and thus chose to optimize the left-hand segment first.

Hit-to-Lead. Parallel synthesis was carried out to rapidly generate a series of derivatives of **1**. Due to the structural simplicity of **1**, the synthesis could employ concise reactions of commercially available coupling pairs: assorted carboxylic acids and *trans*-2-phenylcyclopropylamine (**2**) giving amide derivatives **3** or assorted amines and *trans*-2-phenylcyclopropyl isocyanate (**4**) giving urea derivatives **5** (Scheme 1). Carboxylic acids and amines within a limited MW and lipophilicity range were selected in order to keep the expected coupling products in the lead-like range (MW < 380, AlogP < 4). A set of 155 analogues was synthesized and evaluated for their in vitro activity. From this set, 38 potent sEH inhibitors (IC_{50} < 100 nM) were identified, and seven of them exhibited single-digit potency (IC_{50} < 10 nM). Significantly, all of the 38 potent inhibitors preserved good LE (LEI > 0.37, BEI > 20.0).

Potent and ligand-efficient representatives of the synthesized compounds are listed in Table 2. While LE was a primary concern in the selection of hits for further evaluation, for the purpose of the selection of a lead candidate (not the best screening hit) from ligand-efficient nominees, the weighting of LE was reduced relative to that of other properties, such as selectivity or ADMET profile. The derivatives **6**−**11** and others were tested in rat sEH and cell-based sEH assay (human HepG2). Among these compounds, the urea derivative **11** showed no species specificity (IC_{50} human, 8.5 nM; rat, 13 nM) and good membrane permeability (HepG2, 13 nM) and did not exhibit cytotoxicity up to 100 μM. In addition, in vitro ADMET screening of **11** showed improved aqueous solubility, CYP inhibition, and CYP induction compared to **1**. Other compounds were less appealing (for details, see Supporting Information).

Crystallography of the binary complex of **11** and sEH showed that the 4-(3-isopropyl-1,2,4-oxadiazol-5-yl)piperidine moiety was positioned in a large hydrophobic tunnel where the 4-cyanophenyl group of **1** was harbored (Figure 4a). The moiety created VDW contacts with one side of the pocket and left the other side open (Figure 4b). We hypothesized that the oxadiazole moiety of **11** forms a hydrogen-bonding interaction with Gln384 via water, which, while not visible here, was detected in different inhibitor-bound structures of sEH (see Supporting Information). Although one option to enhance the activity of **11** would be to fill the unoccupied (hydrophobic) space by increasing the volume of the molecule, such attempt would likely result in a major gain of lipophilicity. We therefore concluded that the left-hand segment in **11** had been optimized to meet the level of lead generation requirements and envisaged that further activity enhancement can be achieved by modification of the phenyl group on the right-hand side during the lead optimization stage. According to this view, we chose **11** as a lead for further structure optimization.

At this point in time, however, it was unveiled that a Merck group had submitted a patent application covering structurally close sEH inhibitors (more recently, they disclosed an array of sEH inhibitors with an identical scaffold).[14,15] We therefore decided to cancel further investigation of this chemotype and shifted our efforts to different chemotypes derived from a methodology analogous to that of the present study (vide infra).

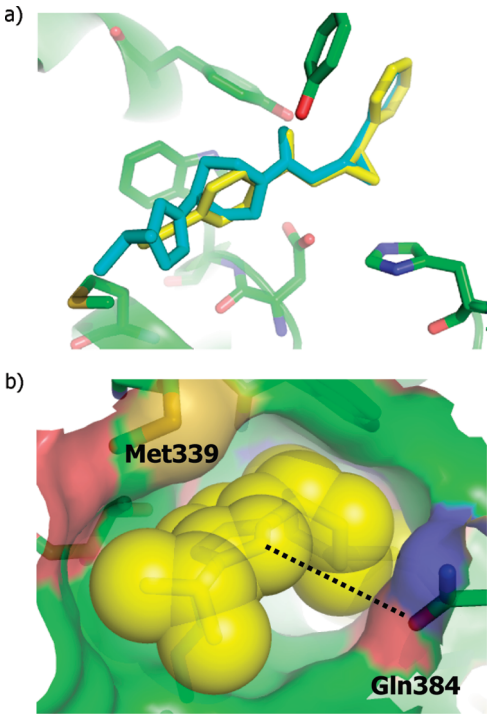


Figure 4. (a) Binding mode of **1** (yellow) in sEH superimposed with that of **11** (blue). Interaction between the CONH group of the inhibitors and the catalytic triad of sEH and binding mode of the phenylcyclopropane moiety were well conserved between the two inhibitors. (b) Binding of the 4-(3-isopropyl-1,2,4-oxadiazol-5-yl)-piperidine moiety of **11** (yellow space-filling model). In the hydrophobic tunnel of sEH, the moiety makes VDW contact with the left cleft. The carbamoyl group of Gln384 and nitrogen of the oxadiazole were ca. 5 Å apart (black dashed line). Presumably, a water molecule mediated hydrogen bonding between the carbamoyl group and the nitrogen atom. Other hydrophobic space around this moiety was left unfilled (PDB code 3ANT).

Retrospective LE Analysis. To better understand the use of the LE concept in medicinal chemistry, later in our campaign, we conducted a retrospective analysis of our hit triage and H2L studies (**1**→**11**), which included more recently reported indices (Table 3). Hajduk analyzed the transition of BEI in H2L studies at Abbott and reported decreasing BEI with increasing activity, even along ideal H2L paths.[16] Referring to his analysis, the decreasing rate of BEI from **1** to **11** was calculated to be 2.3%, which is almost the same as that reported by Hajduk (2.2%), demonstrating reasonable increase of molecular size in our H2L campaign. While BEI was slightly decreased, LEI was maintained throughout our H2L study. This fact could be attributed to the increased ratio of heteroatom count in HAC (15%→23%), enhancing the MW term in the BEI formula. As mentioned earlier, inhibitors of sEH are prone to be lipophilic, and we sought to minimize this propensity. Keseru and Makara recently proposed an LELP index that represents lipophilicity (LogP) per LE (LELP = calculated LogP/LEI); the higher the value of LELP the greater the lipophilicity of the compound.[17] Hit **1** exhibited LELP values of 5.56 (ACDlogP basis) and 6.43 (AlogP basis), which were in the range of preferable LELP values (0−7.5). Compound **11** also stayed in

Table 3. Changes in Parameters of the Selected Hit **1** and the Lead **11**

	pIC_{50}	MW	HAC	N+O[a]	LEI	BEI	SEI	LELP[b]	LELP[c]	FQ	%LE	SILE
1	6.25	262	20	3	0.43	23.8	11.8	5.56	6.43	0.92	69.2	2.54
11	8.07	354	26	6	0.43	22.8	11.3	6.92	6.26	1.13	82.4	3.04
Δ	+1.82	+92	+6	+3	0	−1.0	−0.5	+1.36	−0.17	+0.21	+13.2	+0.50

[a] Heteroatom count (nitrogen and oxygen). [b] ACDlogP basis. [c] AlogP basis.

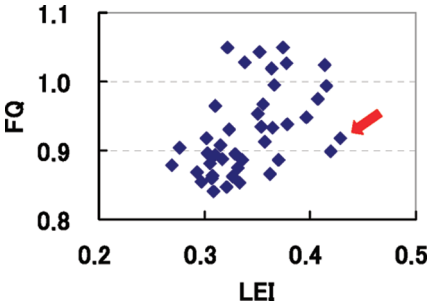


Figure 5. Relationship between LEI and FQ of the 42 hit compounds. No significant correlation was observed. The selected hit **1** is indicated by the arrow. Hit triage based on classic LE indices and that based on size-neutralized indices would have provided different outputs.

this range with 6.92 for ACDlogP basis and 6.26 for AlogP basis. Evidence supporting the reasonable trajectory of LE indices in our H2L study is that, via a one-step synthesis, **11** achieved greater inhibitory potency with higher ligand efficiency (LEI and BEI) than that of any of the potent screening hits (Figure 2c). These findings suggest that if the most potent hit compound were selected regardless of LE, it would be doubtful that its derivatives could have lead-like properties in terms of molecular size, lipophilicity, and LE. This consideration would be a useful prospective mechanism for medicinal chemists to prioritize compounds during both hit triage and H2L studies.

Recently, Reynolds et al. pointed out that LEI is affected by molecular size: maximum LE gets lower as molecules become larger.[18] To address this issue, they proposed the fit quality (FQ), which was later followed by the percentage ligand efficiency (%LE) proposed by Orita et al.[19] and the size-independent ligand efficiency (SILE) reported by Nissink.[20] FQ, %LE, and SILE of **1** were 0.92, 69.2, and 2.54, respectively. These values were not at the highest level among the selected 42 hit compounds but were improved during the H2L process leading to **11** (1.13, 82.4, and 3.04, respectively). It is fair to say that the LE of **1** was suboptimal compared with the theoretical maximum.[7] It might be possible that in the course of H2L studies, classic LE indices (LEI and BEI) tend to decrease, while size-neutralized indices (FQ, %LE, and SILE) tend to increase.[21] Since no significant correlation between the classic LE and the size-neutralized indices was observed in the present study, a selection from the 42 hit compounds, which was based on the latter indices, afforded different ranking lists (Figure 5, also see Supporting Information). Compounds ranked higher in the lists were more potent but larger inhibitors than the 17 compounds promoted by the classic indices. As a number of successful cases of FBDD have demonstrated, smaller compounds can be more advantageous in terms of synthetic tractability and steady improvements in activity. In fact, the evolution from **1** to **11** was rapidly achieved by concise synthesis using commercially available building blocks (Scheme 1). Exploration of the same 42-compound set based on size-neutralized indices is under way to learn its benefits. However, this is proving to be more labor-intensive because of increased structural complexity and multistep synthesis requirements. As long as the molecular size of hit compounds is limited according to the lead-likeness criteria, the influence of molecular size on LE indices would be minimal. Although this influence should be kept in mind, we believe that LEI and BEI are still useful and medicinal chemist-friendly concepts.

Conclusion

We presented here a practical application of LE in conventional lead identification. Selection based on MW and LogP criteria effectively led to highly ligand-efficient sEH inhibitors, demonstrating the virtue of LE-guided compound selection to achieve lead-likeness. We selected the hit compound **1** exhibiting relatively weak inhibitory activity but the highest LEI and BEI values, leaving behind more potent (double-digit nanomolar) compounds. Hit **1** was successfully derived into a potent, ADMET-clean, lead-like compound **11** by rapid H2L chemistry. Though **1** was fragment-sized (MW 262) and in fact the smallest among the 42 hits, its selection was not a coincidence but a logical consequence that arose from LE-guided hit triage. We believe that LE-guided hit triage is a useful way to identify and select hits that are preferable starting points for medicinal chemistry. In the present study, we have conducted neither high concentration nor biophysical screening of the fragment library (MW < 300); this approach could be called *fragment-inspired medicinal chemistry* in which the essence and advantages of FBDD are faithfully respected.

Experimental Section

General Remarks. All purchased reagents and solvents were used as received. Organic solutions were concentrated by rotary evaporation. ^{1}H NMR spectra were acquired on either a JEOL JNM-LA300 or a JNM-LA400 FT NMR system. Proton chemical shifts were reported in parts per million (ppm, δ scale) downshifted from tetramethylsilane (TMS) and were referenced to residual protium in the NMR solvent ($CDCl_3$, δ 7.26). LC-MS data were obtained with a combination of Perkin-Elmer Sciex API150EX mass spectrometer (40 eV) and Shimadzu LC 10ATVP. All purifications were performed using a Gilson HPLC System with a YMC CombiPrep ODS-A column (S-5 μm, 12 nm, 20 mm ×50 mm). Purity of all the key compounds (**1**, **6**−**11**) was confirmed to be >95% by HPLC analysis (at 220 and 254 nm UV detection).

Typical Preparation Procedure for Amide Compounds. To a solution of a carboxylic acid (0.2 mmol) in *N*,*N*-dimethylformamide (1.5 mL) was added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimidazole hydrochloride (38.3 mg, 0.2 mmol) followed by 1-hydroxybenzotriazole (27.0 mg, 0.2 mmol), triethylamine (84 μL, 0.6 mmol), and *trans*-2-phenylcyclopropylamine HCl (33.9 mg, 0.2 mmol). This reaction mixture was stirred at room temperature until completion (LC-MS monitoring). The resultant mixture was then partitioned between chloroform and saturated aqueous $NaHCO_3$. The separated organic phase was concentrated, and the residue was purified with the Gilson purification apparatus.

Typical Preparation Procedure for Urea Compounds. To a solution of an amine (0.3 mmol) in toluene (3 mL) was added *trans*-2-phenylcyclopropyl isocyanate (35 μL, 0.2 mmol). This reaction mixture was stirred at room temperature until completion

(LC-MS monitoring). The resultant mixture was concentrated, and the residue was transferred into methanol. Insoluble materials were then filtered off, and the filtrate was concentrated to give a crude product, which was purified with the Gilson purification apparatus.

4-Cyano-*N*-(*trans*-2-phenylcyclopropyl)benzamide (1). ^{1}H NMR (300 MHz) δ (ppm): 7.87−7.84 (m, 2H), 7.74−7.71 (m, 2H), 7.31−7.17 (m, 5H), 6.42 (br s, 1H), 3.10−3.04 (m, 1H), 2.21−2.15 (m, 1H), 1.40−1.34 (m, 1H), 1.31−1.24 (m, 1H). LC-MS: 263.3 (M + H).

8-Methoxy-*N*-(*trans*-2-phenylcyclopropyl)-1,2,3,4-tetrahydronaphthalen-2-carboxamide (6). ^{1}H NMR (400 MHz) δ (ppm): 7.30−7.24 (m, 2H), 7.22−7.15 (m, 3H), 7.10 (dd, 1H, J = 7.8, 8.1 Hz), 6.72(d, 1H, J = 7.8 Hz), 6.67 (d, 1H, J = 8.1 Hz), 5.82 (br s, 1H), 3.82 (s, 1.5H), 3.81 (s, 1.5H), 3.03 (dd, 1H, J = 5.4, 17.1 Hz), 2.95−2.89 (m, 1H), 2.89−2.75 (m, 2H), 2.75−2.64 (m, 1H), 2.49−2.38 (m, 1H), 2.15−2.03 (m, 2H), 1.90−1.78 (m, 1H), 1.27 (ddd, 1H, J = 6.1, 6.1, 7.6 Hz), 1.20−1.13 (m, 1H). LC-MS: 344.3 (M + Na), 322.3 (M + H).

***N*-(*trans*-2-Phenylcyclopropyl)benzo[*c*][1,2,5]oxadiazole-5-carboxamide (7).** ^{1}H NMR (300 MHz) δ (ppm): 8.17 (t, 1H, J = 1.1 Hz), 7.90 (dd, 1H, J = 1.1, 9.5 Hz), 7.83 (dd, 1H, J = 1.1, 9.3 Hz), 7.31−7.18 (m, 5H), 6.55 (br s, 1H), 3.13−3.07 (m, 1H), 2.25−2.18 (m, 1H), 1.43−1.28 (m, 2H). LC-MS: 280.5 (M + H).

***N*-(2-Methyl-1,3-benzoxazol-5-yl)-*N*′-(*trans*-2-phenylcyclopropyl)urea (8).** ^{1}H NMR (400 MHz) δ (ppm): 7.59 (d, 1H, J = 2.0 Hz), 7.37 (d, 1H, J = 8.8 Hz), 7.35−7.29 (m, 4H), 7.15−7.08 (m, 2H), 2.80−2.73 (m, 1H), 2.62 (s, 3H), 2.23−2.12 (m, 1H), 1.43−1.28 (m, 2H). LC-MS: 330.3 (M + Na), 308.4 (M + H).

***N*-(4-Dimethylaminobenzyl)-*N*′-(*trans*-2-phenylcyclopropyl)-urea (9).** ^{1}H NMR (400 MHz) δ (ppm): 7.31−7.09 (m, 5H), 6.95 (m, 2H), 6.70 (m, 2H), 4.99 (br s, 1H), 4.82 (br s, 1H), 4.40 (dd, 1H, J = 5.9, 14.1 Hz), 4.24 (dd, 1H, J = 4.9, 14.1 Hz), 2.94 (s, 6H), 2.58−2.51 (m, 1H), 2.05 (m, 1H), 1.31−1.18 (m, 2H). LC-MS: 332.4 (M + Na), 310.4 (M + H).

***N*-(*trans*-2-phenylcyclopropyl)-*N*′-(1-phenylpiperidin-4-yl)-urea (10).** ^{1}H NMR (400 MHz) δ (ppm): 7.31−7.18 (m, 5H), 7.05 (m, 2H), 6.92 (m, 2H), 6.85 (m, 1H), 4.76 (br s, 1H), 4.74 (br s, 1H), 3.90−3.78 (m, 1H), 3.59−3.45 (m, 2H), 2.94−2.81 (m, 2H), 2.56 (m, 1H), 2.13−1.98 (m, 3H), 1.62−1.42 (m, 2H), 1.34−1.23 (m, 2H). LC-MS: 358.2 (M + Na), 336.5 (M + H).

4-(3-Isopropyl-1,2,4-oxadiazol-5-yl)-*N*-(trans-2-phenylcyclopropyl)piperidine-1-carboxamide (11). ^{1}H NMR (400 MHz) δ (ppm): 7.29−7.23 (m, 3H), 7.21−7.14 (m, 2H), 4.85 (br s, 1H), 3.94 (m, 2H), 3.15−2.96 (m, 4H), 2.87−2.81 (m, 1H), 2.14−1.99 (m, 3H), 1.93−1.80 (m, 2H), 1.33 (d, 6H, J = 6.8 Hz), 1.22 (m, 1H), 1.14 (m, 1H). LC-MS: 377.5 (M + Na), 355.4 (M + H).

Virtual Screening. Crystal structures of human sEH in complex with inhibitors (PDB codes 1VJ5, 1ZD2, 1ZD3, 1ZD4, and 1ZD5) were used for virtual screening. Hydrogen atoms were added to each crystal structure, and the complex was subjected to a series of restrained partial minimizations using the OPLS-AA force field[22] with the Protein Preparation Wizard workflow in Maestro.[23] Virtual screening and docking were performed using Glide[24,25] in extra-precision mode.[26] The database of our internal compound collection was used as the source for virtual screening and preprocessed with LigPrep at pH 7.0.[27] Default settings were used for automated docking. The top 1000 compounds ranked by GlideScore XP were stored for visual analysis to check docking poses and interactions between ligands and receptor. A series of 735 structurally diverse compounds were then selected for bioassay.

Calculation and Analysis of LE. Compound physicochemical descriptors were calculated by ACD/PhysChem Batch Ver. 7.0 or Pipeline Pilot Ver. 7.5. Ligand efficiency (LEI, BEI, SEI, LELP, FQ, %LE, and SILE) was calculated and graphed in Microsoft Excel software.

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Supporting Information Available: Physicochemical and biological data of compounds, bioassay conditions, and peripheral discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

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