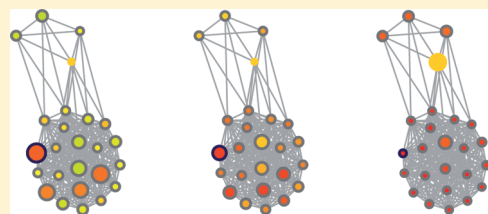


Assessing the Confidence Level of Public Domain Compound Activity Data and the Impact of Alternative Potency Measurements on SAR Analysis

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ABSTRACT: Publicly available compound activity data have been analyzed to distinguish between compounds for which single or multiple potency measurements were available and gain insight into data confidence levels. Different potency measurements with defined end points and alternative ways to represent multiple potency values for active compounds have been evaluated in the context of SAR analysis. Approximately 78% of all compounds with multiple potency measurements were found to represent high-confidence data, which corresponded to ~10% of all activity data. The use of different types of potency measurements and alternative representations of multiple potency values changed the SAR information content of compound data sets and resulted in different activity cliff distributions. Thus, the types of activity measurements that were available and how they were used substantially impacted SAR analysis. Compounds with multiple K_i measurements provided the most reliable basis for SAR exploration.



INTRODUCTION

Although bioassay results should generally be expected to be reliable, the potential variability of assay data and assay-dependent limitations are widely appreciated.^{1–3} Assay variations and potential sources of systematic measurement errors are also of major concern in high-throughput screening.^{4–6} Consequently, in order to address assay fluctuations, potentially inconsistent measurements, and limited reproducibility, a variety of statistical methods and data analysis concepts have been introduced.^{5,6} However, problems associated with data integrity and reproducibility are not only of concern in evaluating bioassays and high-throughput screening results. Moreover, these factors ultimately also influence the quality of compound activity data deposited in major compound repositories,^{7,8} which are a prime resource for data mining and SAR studies. The quality of large bodies of compound activity data extracted from literature sources is subject to experimental variability, which also affects subsequent analyses of bioactive compounds.

Data heterogeneity also is a major issue in SAR analysis, at different levels. For example, rather different assay systems might be used to characterize a class of active compounds in various laboratories. Furthermore, different types of activity readouts are often utilized such as equilibrium constants, compound concentrations to achieve half-maximal inhibition, or percent inhibition, which are not directly comparable and, in the latter two instances, dependent on assay conditions. Moreover, for many compounds, only single measurements are available. Hence, it is generally difficult to estimate the degree of compound data heterogeneity, which often is a limiting factor for subsequent studies.

In light of this situation, we have carried out a detailed analysis of public domain compound activity data to differentiate between

Table 1. Data Set Description^a

category	description
HCD	<ul style="list-style-type: none">compounds with multiple potency measurements (same biological activity)variation in potency values only within 1 order of magnitude
HET	<ul style="list-style-type: none">compounds with multiple potency measurements (same biological activity)variation in potency values of at least 1 order of magnitude
SGM	<ul style="list-style-type: none">compounds with a single potency measurement (same biological activity)
MPM	<ul style="list-style-type: none">all compounds with multiple potency measurements (same biological activity)HCD and HET data subsets
TOT	<ul style="list-style-type: none">all compoundsunion of SGM and MPM data sets

^a All compound categories discussed in the text are defined.

compounds with single and multiple potency values and evaluate the confidence levels of multiple activity measurements.

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Table 2. Data Set Statistics^a

category		compound pairs (ECFP4 Tc \geq 0.55)	potency records	AC compounds	ACs
K_i	HCD	37,260	28,211	11,769	225
	HET	3,492	10,955	2571	190
	SGM	652,870	92,736	92,736	222
	MPM	47,248	39,166	14,340	225
	TOT	846,481	131,902	107,076	225
IC_{50}	HCD	78,282	46,270	20,545	445
	HET	21,568	22,124	6693	372
	SGM	1,008,430	139,186	139,186	441
	MPM	129,017	68,394	27,238	445
	TOT	1,366,116	207,580	166,424	445

^aFor the definition of compound categories, see Table 1. "AC" stands for activity class. The activity classes consist of compounds belonging to one or more categories. For each category, the total number of compounds in all activity classes and the total number of their potency records (annotations) are reported. Results are separately shown for K_i and IC_{50} measurements.

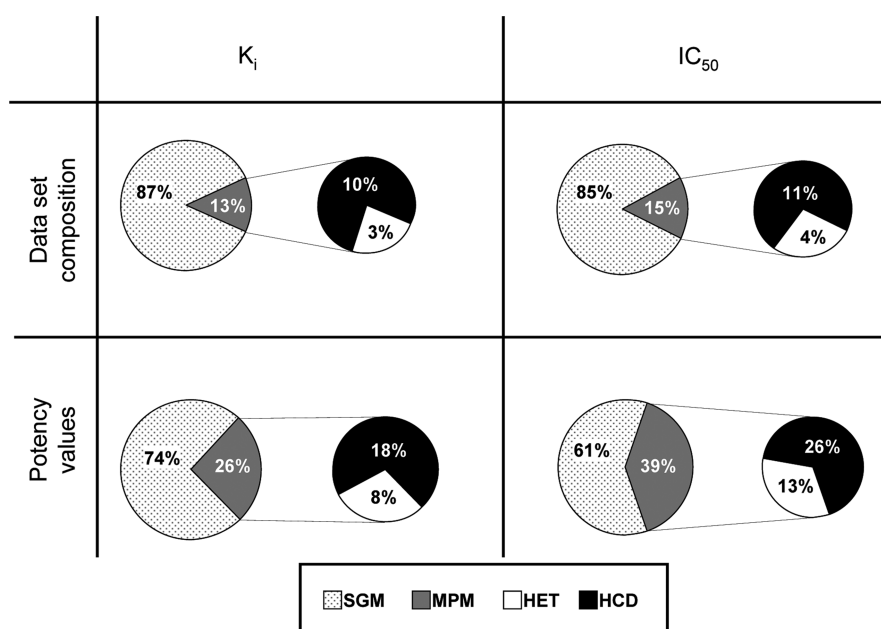


Figure 1. Compound set composition and potency value distribution. Reported is the composition of data sets extracted from BindingDB and the distribution of potency measurements over these data sets.

Furthermore, we have investigated how data variability might affect SAR features of compound data sets.

MATERIAL AND METHODS

We have analyzed the March 2011 version of BindingDB⁷ that also contains a major part of the ChEMBL⁸ compound collection for targets available in BindingDB. These databases predominantly collect compounds from the medicinal chemistry literature. For our analysis, only compounds with defined potency measurements were considered, i.e., K_i or IC_{50} values. By contrast, "percent inhibition" or other not well-defined readouts were not taken into account. Because K_i and IC_{50} values are not directly comparable, compound sets with exclusive K_i or IC_{50} measurements were assembled. Database compounds were divided into sets for which single (SGM) and multiple potency measurements (MPM) were available. MPM sets were further divided into compounds with high confidence activity data (HCD),

i.e., compounds with multiple measurements that did not differ by more than 1 order of magnitude, and compounds with heterogeneous measurements (HET), for which reported potency values varied by more than 1 order of magnitude. Table 1 summarizes these different compound data set categories. For compounds with multiple potency measurements, the minimum (MIN), maximum (MAX), and geometric mean (MEAN) potency values were separately considered in our analysis, as further discussed below.

Following categorization of all active compounds, activity classes (ACs) were selected for further analysis that contained at least 10 HCD compounds. Applying this selection criterion, a total of 225 ACs with K_i values and 445 ACs with IC_{50} values were obtained. Each AC represented a single biological activity. For these ACs, all HCD, HET, and SGM compounds were collected. Their combination represented the total number of compounds (TOT) per activity class.

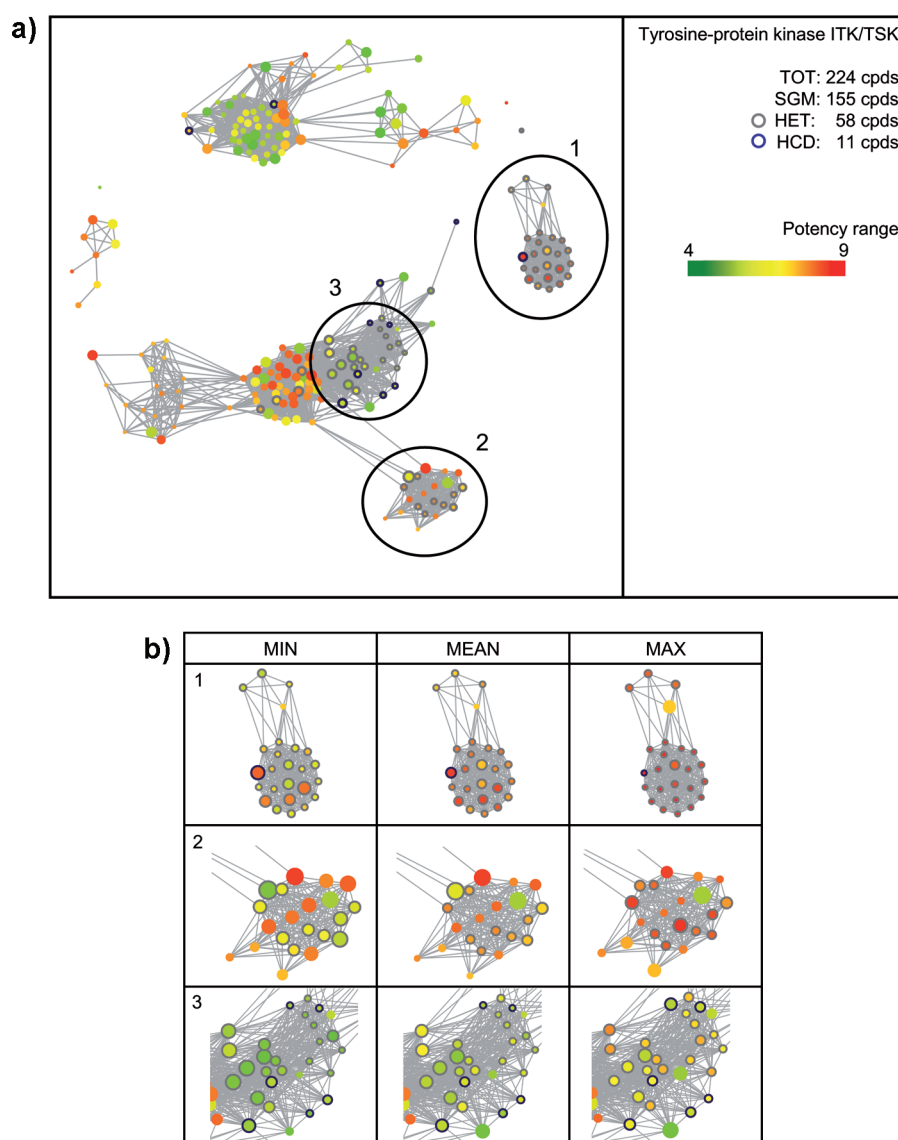


Figure 2. (a) Network-like similarity graphs based on alternative potency values. An NSG representation of a set of tyrosine kinase ITK/TSK inhibitors using MEAN potency (pIC_{50}) values is shown. A node represents a compound, and edges are drawn between compound pairs having an ECFP4 Tc value above 0.55. Nodes are color-coded according to potency values and scaled in size according to compound SAR discontinuity scores. Encircled compounds represent compounds with multiple measurements (dark-blue circles, HCD compounds; gray circles, HET compounds). (b) Three clusters with a large number of MPM compounds are shown in alternative NSG representations based on MIN, MEAN, or MAX potency values.

Pair-wise compound similarities were calculated using the “extended connectivity fingerprint with bond diameter 4” (ECFP4),⁹ as implemented in Pipeline Pilot.¹⁰ Through pair-wise compound comparison, activity cliffs^{11,12} were identified, i.e., structurally similar compounds with large potency differences. As a similarity threshold for activity cliff formation, an ECFP4 Tanimoto coefficient (Tc)¹³ value of 0.55 was applied, which generally indicates the presence of structurally very similar compounds. Furthermore, a pair of similar compounds was only considered to form an activity cliff if one compound had a pK_i or pIC_{50} value equal to or lower than five and the other a value was at least greater than seven, following a previously introduced classification scheme.¹⁴ Thus, the highly potent compound of a cliff-forming pair was required to have a potency of at least 100 nM, and there had to be an at least a 100-fold difference in potency between cliff forming compounds. Activity cliff statistics

were calculated on the basis of MIN, MEAN, and MAX potency values. In addition, network-like similarity graphs (NSGs)¹⁵ were generated for compound data sets using alternative potency values. These graph representations are designed to reveal global and local SAR features including activity cliffs, as further discussed below.

All calculations were carried out with in-house generated Perl and Java programs and Pipeline Pilot scripts.

RESULTS

Activity Data Survey. We initially separated potency measurements with defined end points into K_i and IC_{50} sets following the data set classification scheme described in the Methods section. A total of 147 727 K_i and 242 748 IC_{50} potency records were available for 81 013 and 161 704 compounds, respectively. A total of 70 377 of the compounds had single K_i values, and

Table 3. Activity Cliff Statistics^a

MIN		K_i MEAN		MAX	
ActCliffs	percentage	ActCliffs	percentage	ActCliffs	percentage
HCD					
104	0.28	103	0.28	103	0.28
HET					
52	1.49	10	0.29	4	0.11
SGM					
4055	0.62	4055	0.62	4055	0.62
MPM					
326	0.69	165	0.35	150	0.32
TOT					
6054	0.72	5328	0.63	5541	0.65
MIN		IC_{50} MEAN		MAX	
ActCliffs	percentage	ActCliffs	percentage	ActCliffs	percentage
HCD					
556	0.71	513	0.66	480	0.61
HET					
119	0.55	83	0.38	28	0.13
SGM					
9685	0.96	9685	0.96	9685	0.96
MPM					
1232	0.95	741	0.57	787	0.61
TOT					
14,894	1.09	13,304	0.97	14,442	1.06

^a The number of activity cliffs (ActCliffs) for all compound categories and potency value representations (MIN, MEAN, MAX) are reported. In addition, the percentage of all qualifying compound pairs (meeting the similarity threshold for activity cliff formation) that form activity cliffs is given. As in Table 2, results are separately reported for K_i (top) and IC_{50} (bottom) measurements.

137 010 had single IC_{50} values. A total of 10 636 compounds had multiple K_i values, and 24 694 had multiple IC_{50} values. Of compounds with multiple measurements, HCD compounds were on average annotated with 2.4 K_i or 3.9 IC_{50} values and HET compounds with 3.9 K_i or 3.2 IC_{50} values. The maximal potency difference, i.e., the difference between MIN and MAX, varied for HET compounds from 1 to 12 (K_i) and 1 to 10 (IC_{50}) orders of magnitude. For HCD compounds, the maximal difference between potency values was by definition below 1 order of magnitude. Table 2 summarizes the data and compound set distribution, and Figure 1 reports the proportion of compounds with single and multiple potency measurements and the relative distribution of potency values.

Activity Classes with Multiple Potency Measurements. The selected 225 K_i - and 445 IC_{50} -based activity classes with at least 10 HCD compounds represented approximately 20% of all BindingDB classes with the same type of potency measurements

(1017 K_i - and 1906 IC_{50} -based classes). K_i -based activity classes contained between 12 and 2921 compounds. They were found to consist of 2.3–100% HCD and 0–77.2% HET compounds. IC_{50} -based activity classes contained between 11 and 2220 compounds including 2.0–100% HCD and 0–42.9% HET compounds. Thus, the proportion of HET compounds was overall lower for K_i than for IC_{50} measurements.

High-Confidence Data. We next determined for all MPM compounds the percentage of compounds for which all multiple potency measurements were within an order of magnitude. These compounds and their annotations were considered high-confidence data. For our activity classes, 82% of all compounds with multiple K_i and 75% of all compounds with multiple IC_{50} measurements met the high-confidence criterion. These compounds corresponded to 11% (K_i) and 12% (IC_{50}) of all selected (MPM and SGM) compounds. Thus, the proportion of high-confidence data was overall higher for K_i than for IC_{50} measurements.

Data Variability and SAR Information Content. Compound similarity and potency relationships in data sets were graphically analyzed in NSGs for sets containing SGM, MPM, HCD, and HET compounds using different potency assignments, i.e., MIN, MEAN, or MAX values. For these alternative potency values, significant variations of SAR features were observed. This is illustrated in Figure 2a showing the NSG representation of an exemplary activity class, a set of tyrosine kinase inhibitors, using MEAN potency values.

In NSGs, nodes represent compounds, and edges represent pairwise similarity relationships. Nodes are color-coded according to compound potency values using a continuous spectrum from green (lowest potency) over yellow to red (highest potency). Furthermore, nodes are scaled in size according to the contribution of individual compounds to local SAR discontinuity, calculated using a previously reported per compound discontinuity score.¹⁵ Local SAR discontinuity is introduced when structurally similar compounds (nearest neighbors) have significantly different potency. In NSGs, pairwise combinations of large red and green nodes indicate the most prominent activity cliffs contained in a data set.

In Figure 2a, MPM compounds are encircled, with dark blue circles indicating HCD and gray circles HET compounds. Three compound clusters are outlined (1–3) that contain many MPM compounds and represent different local SAR environments. In Figure 2b, clusters are compared for alternative MIN, MEAN, or MAX potency values. It can be seen that the SAR character of these clusters and the distribution of activity cliffs changed for alternative potency values. For example, clusters 1 and 2 were characterized by a much higher degree of local SAR discontinuity when MIN values were used instead of MEAN or MAX. However, the situation was essentially reversed in the case of cluster 3 where MAX values introduced more SAR discontinuity than MIN or MEAN. Hence, alternative potency annotations notably affected the SAR information content of compound data sets and its interpretation. An important implication of these observations is that the results of qualitative or quantitative SAR analyses will inevitably vary depending on the use of alternative potency measurements.

Activity Cliff Statistics. Activity cliffs represent the extreme form of SAR discontinuity. As such, they are associated with high SAR information content and generally regarded as the most prominent features of activity landscapes of compound data sets.¹² Therefore, we also analyzed activity cliff distributions to assess to what extent alternative potency values altered SAR

features. Hence, for K_i - and IC_{50} -based data sets, the activity cliff distribution was determined for different potency measurements and compound subsets. The results for all sets are summarized in Table 3.

Applying MIN, MEAN, or MAX potency values to data sets containing HCD and HET compounds revealed that the largest number of activity cliffs was generally formed when applying MIN values. The HET compound subsets displayed a clear decrease in activity cliff numbers from MIN over MEAN to MAX, with 52 to 10 to 4 cliffs for K_i and 119 to 83 to 28 for IC_{50} values. By contrast, the distribution of activity cliffs in HCD sets was essentially constant for K_i measurements and MIN, MEAN, and MAX values. However, for IC_{50} measurements, there also was a statistically significant decline in cliff numbers from MIN over MEAN to MAX. The percentage of all qualifying compound pairs forming activity cliffs was 0.67% for K_i and 1.04% for IC_{50} values. Thus, for IC_{50} , more activity cliffs were formed than for K_i measurements. This implies that the analysis of activity

cliffs using IC_{50} values might be more prone to false-positive cliff detections than when using K_i values.

Conserved and Nonconserved Activity Cliffs. The use of alternative potency values led to significant changes in the distribution of activity cliffs. For K_i data, a total of 4987 conserved and 1962 nonconserved activity cliffs were detected and, for IC_{50} data, 12 159 conserved and 6163 nonconserved cliffs. These variations are illustrated in Figure 3. In Figure 3a, an NSG representation using MEAN potency values is shown for a set of dihydrofolate reductase inhibitors. This exemplary data set consists of 622 compounds including 178 HCD, 152 HET, and 292 SGM molecules. In this set, a total of 68 activity cliffs were detected in MIN, MEAN, and MAX value-based representations, only 28 of which were conserved. These 28 conserved activity cliffs were mainly formed by SGM and HCD compounds, but only one conserved cliff was formed by an HCD-HET compound pair. Figure 3b shows the subset of 82 compounds that were involved in the formation of the 68 conserved activity cliffs (upper panel).

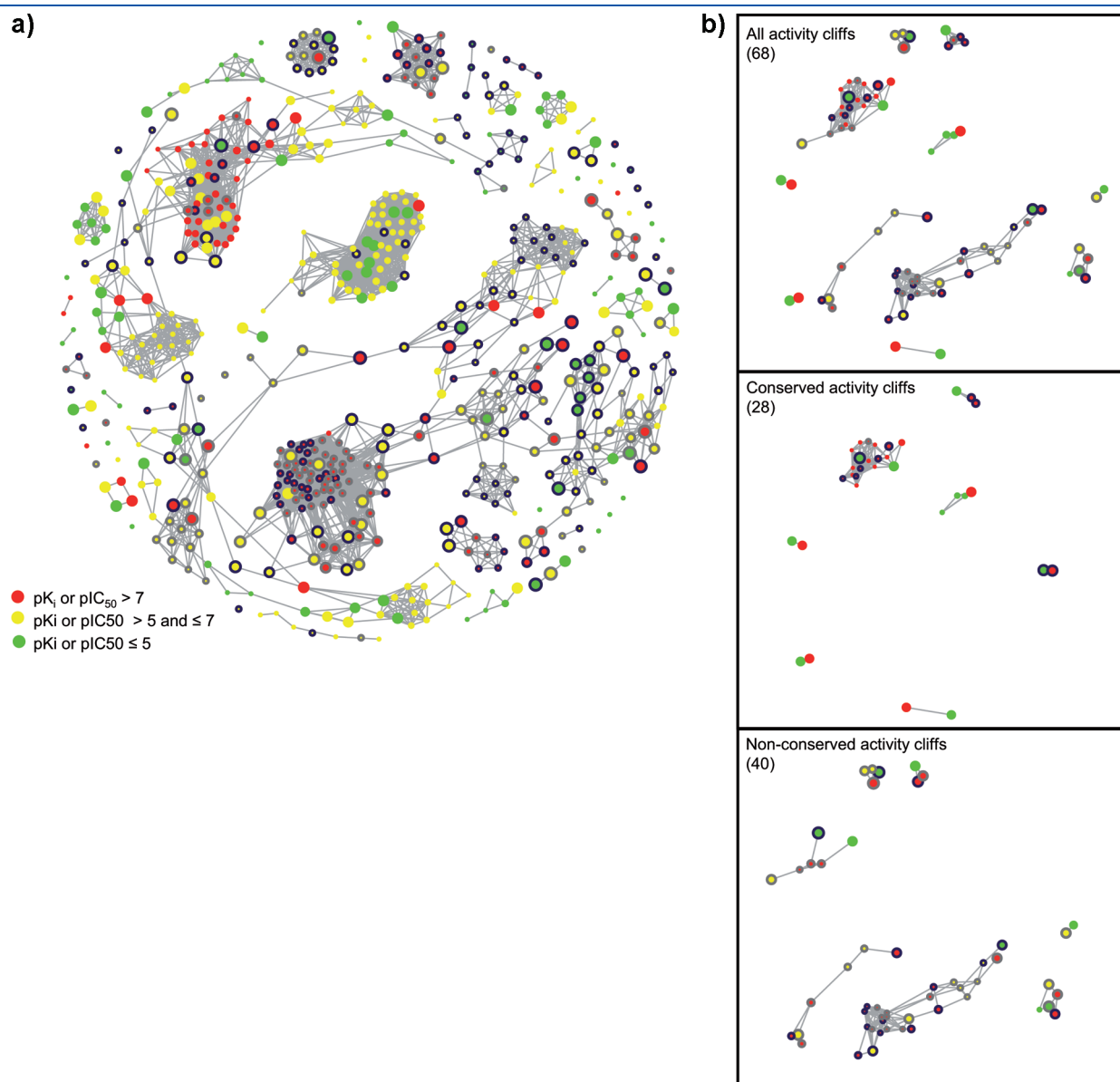


Figure 3. Continued

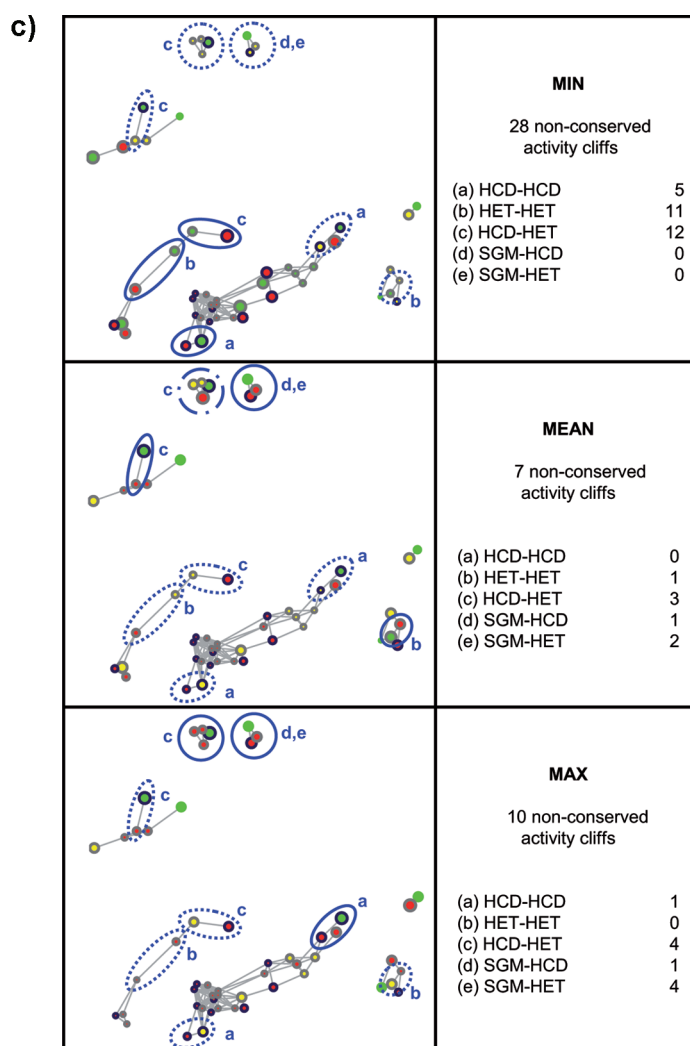


Figure 3. Distribution of conserved and nonconserved activity cliffs. (a) An NSG representation of a set of dihydrofolate reductase inhibitors is shown using MEAN potency values. In order to focus the representation on activity cliff formation, nodes are color-coded in this case according to different potency ranges: green, $pIC_{50} \leq 5$; yellow, $pIC_{50} > 5$ and ≤ 7 ; red, $pIC_{50} > 7$. Otherwise, the node representation is according to Figure 2. (b) All activity cliffs formed between dihydrofolate reductase inhibitors are extracted from the NSG with MEAN potency values (top). Then, NSGs were recalculated for MIN and MAX values. Activity cliffs conserved for all potency values are represented in the middle panel, and all nonconserved cliffs detected only for one or two of three potency values are shown at the bottom. (c) Nonconserved activity cliffs are separately shown in NSGs for MIN, MEAN, and MAX potency values and organized according to compound types. Nonconserved cliffs can be formed by two HCD compounds (labeled “a”), two HET (“b”), a combination of HCD and HET (“c”), SGM and HCD (“d”), or SGM and HET (“e”) compounds. Exemplary nonconserved activity cliffs are encircled using a solid line if they are formed in the case of MIN, MEAN, or MAX values (i.e., in each individual case) or with a dashed line if they are absent when using the respective potency values.

In the middle panel, conserved activity cliffs and in the bottom panel, nonconserved cliffs are displayed. In Figure 3c, nonconserved activity cliffs are separately analyzed for MIN, MEAN, and MAX values. We found that HCD compound pairs formed overall fewer activity cliffs than other compound types. However, most of the activity cliffs formed by HCD were conserved when multiple potency values were assigned to active compounds in different ways. Thus, activity cliff distributions were stable when high confidence activity data were used. These cliff distributions provide the most accurate basis for SAR studies.

DISCUSSION

The use of reliable data is essential for SAR analysis. Therefore, we have searched for publicly available high-confidence compound

activity data. Multiple potency measurements were considered to be of high confidence if their values were within 1 order of magnitude, taking anticipated limited assay variations into account. On the basis of our analysis, more than 80% and 70% of all preselected BindingDB/ChEMBL compounds with multiple K_i and IC_{50} measurements, respectively, fell into the high-confidence data category. Thus, 20–30% of compounds with multiple measurements are not accurately characterized, which represents a caveat for SAR analysis and a considerable source of potential errors. Extrapolating from these findings, one might anticipate a similar percentage of compounds with single measurements to have questionable potency assignments. K_i values generally yielded more high-confidence data than IC_{50} measurements. This should be expected because K_i values represent equilibrium

constants, whereas IC_{50} values do not. IC_{50} is in principle dependent on assay conditions, which limits the consistency of IC_{50} measurements. Furthermore, for compounds with multiple potency measurements, potency information might be used or combined in different ways, for example, in the form of minimum, mean, or maximum values. We assigned alternative potency readouts to data set compounds and subjected them to SAR monitoring.

We also determined activity cliff distributions as a proxy of SAR information content. Activity cliffs have high SAR information content and are a focal point of large-scale SAR analysis of compound data sets.^{12,14} The formation of activity cliffs is dependent on the steric and chemical requirements of specific ligand–target interactions that are met by one compound but not a structurally similar one. It has previously been shown that activity cliffs can often, but not always, be rationalized on the basis of ligand–target complex X-ray structures.¹⁶ In addition, activity cliffs have recently been systematically deduced from structural data.¹⁷ In our analysis, the use of alternative potency values was found to cause significant changes in local SAR characteristics and activity cliff distributions. IC_{50} values generally produced more activity cliffs than K_i values. However, for alternative potency assignments, the distribution of activity cliffs was essentially constant on the basis of K_i values, whereas significant changes were observed for IC_{50} values. Hence, compounds with multiple K_i values overall represent a preferred data source for SAR analysis. However, these compounds currently represent only ~10% of molecules with publicly activity data.

CONCLUSIONS

In summary, the variability of activity measurements critically influences the integrity of compound activity data, and the use of high-confidence data is an important quality criterion for SAR analysis. In addition, SAR characteristics of compound sets and activity cliff distributions are clearly affected by the types of potency measurements that are used and the way multiple measurements are represented. Thus, SAR information extracted from compound data sets must be considered taking these parameters into account. Upon publication, the compound data sets assembled for our study are freely available via <http://www.lifescienceinformatics.uni-bonn.de/downloads>.

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REFERENCES

- (1) Ostland, M. Characterization and Implications of Bioassay Variation. *Dev. Biol.* **2002**, *107*, 3–10.
- (2) Ren, S.; Frymier, P. D. Reducing Bioassay Variability by Identifying Sources of Variation and Controlling Key Parameters in Assay Protocol. *Chemosphere* **2004**, *57*, 81–90.
- (3) Wilson, J. F.; Barnett, K. Variation with Time in Components of Variance for Measurements of Therapeutic Drugs. *Clin. Chem.* **2005**, *51*, 2385–2387.

- (4) Parker, C. N.; Bajorath, J. Towards Unified Compound Screening Strategies: A Critical Evaluation of Error Sources in Experimental and Virtual High-Throughput Screening. *QSAR Comb. Sci.* **2006**, *25*, 1153–1161.

- (5) Brideau, C.; Gunter, B.; Pikounis, B.; Liaw, A. Improved Statistical Methods for Hit Selection in High-Throughput Screening. *J. Biomol. Screen.* **2003**, *8*, 634–647.

- (6) Malo, N.; Hanley, J. A.; Cerquozzi, S.; Pelletier, J.; Nadon, R. Statistical Practice in High-Throughput Screening Data Analysis. *Nat. Biotechnol.* **2006**, *24*, 167–175.

- (7) Liu, T.; Lin, Y.; Wen, X.; Jorissen, R. N.; Gilson, M. K. BindingDB: a Web-Accessible Database of Experimentally Determined Protein-Ligand Binding Affinities. *Nucleic Acids Res.* **2007**, *35*, D198–D201.

- (8) Warr, W. ChEMBL. An interview with John Overington, team leader, chemogenomics at the European Bioinformatics Institute Outstation of the European Molecular Biology Laboratory (EMBL-EBI). *J. Comput.-Aided Mol. Des.* **2009**, *23*, 195–198.

- (9) Rogers, D.; Hahn, M. Extended-Connectivity Fingerprints. *J. Chem. Inf. Model.* **2010**, *50*, 742–754.

- (10) Scitegic Pipeline Pilot; Accelrys Inc.: San Diego, CA. <http://accelrys.com/products/scitegic/index.html> (accessed July 1, 2011).

- (11) Maggiora, G. M. On Outliers and Activity Cliffs – Why QSAR often Disappoints. *J. Chem. Inf. Model.* **2006**, *46*, 1535–1535.

- (12) Wassermann, A. M.; Wawer, M.; Bajorath, J. Activity Landscape Representations for Structure-Activity Relationship Analysis. *J. Med. Chem.* **2010**, *53*, 8209–8223.

- (13) Willett, P. Searching Techniques for Databases of Two- and Three-Dimensional Structures. *J. Med. Chem.* **2005**, *48*, 4183–4199.

- (14) Wassermann, A. M.; Dimova, D.; Bajorath, J. Comprehensive Analysis of Single- and Multi-Target Activity Cliffs Formed by Currently Available Bioactive Compounds. *Chem. Biol. Drug Des.* **2011**, *78*, 224–228.

- (15) Wawer, M.; Peltason, L.; Weskamp, N.; Teckentrup, A.; Bajorath, J. Structure-Activity Relationship Anatomy by Network-like Similarity Graphs and Local Structure-Activity Relationship Indices. *J. Med. Chem.* **2008**, *51*, 6075–6084.

- (16) Sisay, M. T.; Peltason, L.; Bajorath, J. Structural Interpretation of Activity Cliffs Revealed by Systematic Analysis of Structure-Activity Relationships in Analog Series. *J. Chem. Inf. Model.* **2009**, *49*, 2179–2189.

- (17) Seebeck, B.; Wagoner, M.; Rarey, M. From Activity Cliffs to Target-specific Models and Pharmacophore Hypotheses. *ChemMedChem* **2011**, *6*, 1630–1639.