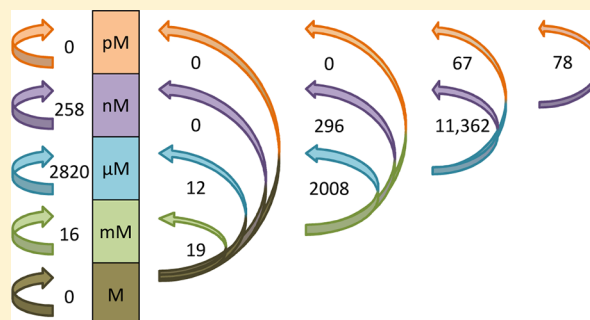


Frequency of Occurrence and Potency Range Distribution of Activity Cliffs in Bioactive Compounds

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ABSTRACT: We have systematically identified activity cliffs in bioactive compounds for which high-confidence potency data were available. Different molecular representations were utilized and similarity and potency difference criteria for activity cliffs were clearly defined. Cliff formation was studied across the global potency range observed for qualifying bioactive compounds. Depending on the specific representation of activity cliffs, between ~22% and 34% of all active compounds meeting the data selection criteria were involved in the formation of at least one activity cliff spanning a potency difference of at least two orders of magnitude. However, these cliffs involved only between ~4.7% and ~5.7% of all compound pairs meeting the similarity criteria. Hence, in light of these findings, the formation of well-defined activity cliffs is a relatively rare event. Moreover, the potency range distribution of activity cliffs was analyzed in detail, revealing that most activity cliffs were formed between compounds with micro- and nanomolar potency, consistent with the global distribution of potency data. On the basis of our analysis, we propose a general definition of activity cliffs for data mining.



INTRODUCTION

Activity cliffs are formed by similar compounds having large differences in potency^{1,2} and are increasingly studied in chemoinformatics and medicinal chemistry.^{2,3} For computational research, activity cliffs are of interest because their presence in compound series might limit the applicability of predictive structure–activity relationship (SAR) models.^{1,4} In addition, they can be used to assess the extent to which computational models cover SAR information.⁵ In medicinal chemistry, activity cliffs are mostly studied as sources of SAR information and to identify SAR determinants.^{2,6} Compound pairs forming cliffs are an obvious focal point of chemical optimization efforts given that they are characterized by small structural changes leading to large potency variations. Another topic in activity cliff research, which is of comparable relevance for chemoinformatics and medicinal chemistry, is modeling of activity landscapes^{6,7} for SAR visualization. Activity landscapes are generally defined as graphical representations that integrate compound similarity and potency relationships in a systematic manner⁶ and often used for large-scale SAR analysis.^{6–8} Activity cliffs are the most prominent features of activity landscapes and typically represent a starting point for landscape exploration.⁶ Systematic SAR analysis aims at extracting available SAR information from large compound sets.^{6,7} Accordingly, this approach heavily depends on computational data mining.

Despite the interest in activity cliffs, little has been done thus far to generalize activity cliff descriptions.² For any assessment of activity cliffs, molecular similarity and potency difference criteria for cliff formation must be defined, but there currently are no general rules.² In fact, activity cliffs might be defined in

rather different ways, using a variety of molecular representations and similarity metrics,⁹ discrete potency ranges,^{10,11} or a continuum of potency differences.¹² Thus far, activity cliffs have mostly been described on the basis of fingerprint representations and calculation of Tanimoto similarity.² In medicinal chemistry, calculated similarity relationships are often critically viewed because they might not be readily interpretable in chemical terms.^{2,6} Therefore, efforts have also been made to replace similarity calculations with well-defined structural relationships in activity cliff analysis. As long as one only focuses on individual analog series, cliffs might be assessed on the basis of R-group decomposition, by considering replacements at single sites.¹³ Following the matched molecular pair (MMP) concept,¹⁴ a generally applicable structure-based definition of activity cliffs has been formulated, leading to the introduction of so-called MMP-cliffs.¹⁵ An MMP is defined as a pair of compounds that only differ at a single site. By restricting the size of substructure differences between compounds forming MMPs, activity cliffs can be generalized, without the need to calculate similarity values.¹⁵

Another thus far only little investigated aspect of activity cliffs is their global distribution in bioactive compounds, beyond individual compound series or data sets. Only a few studies, often with a rather special focus, have been reported that survey activity cliffs. In one of these studies, a search was carried out for coordinated activity cliffs, i.e., overlapping cliffs involving active compounds having multiple partners.¹⁶ This inves-

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tigation led to the identification of so-called activity ridges. Such ridgelike activity cliff arrangements were detected in different data sets.¹⁶ In another recent study, X-ray structures available in the Protein Data Bank¹⁷ were searched for three-dimensional (3D) activity cliffs.¹⁸ For this purpose, the similarity of binding modes of active compounds in complex X-ray structures of given targets was compared using a 3D similarity metric. Pairs of ligands with similar binding modes but significant differences in reported potency values were identified, yielding a collection of 3D activity cliffs for different targets.¹⁸ In addition to these analyses focusing on special types of activity cliffs, a more general activity cliff survey has been reported.¹⁹ In this study, a search for large-magnitude activity cliffs was conducted in the BindingDB²⁰ database. Fingerprint similarity was calculated as a cliff criterion, and only compound pairs were considered in which the strongly active compound had a reported potency of more than 100 nM and the weakly active counterpart a potency of 10 μ M or lower. Thus, for this purpose, the intermittent potency range was excluded from cliff considerations. On the basis of this analysis, ~12% of all compounds active against human targets were involved in the formation of activity cliffs.¹⁹ Furthermore, we have recently introduced a structural categorization of activity cliffs that differs from the MMP-based cliff definition.²¹ Five different types of activity cliffs were defined on the basis of distinguishing structural criteria including chirality cliffs, topology cliffs, R-group cliffs, scaffold cliffs, and scaffold/topology cliffs. A survey of these structural categories of activity cliffs was carried out²¹ in the ChEMBL database.^{22,23}

Herein, we present a detailed analysis of conventional activity cliffs formed by ChEMBL^{22,23} compounds on the basis of high-confidence activity data. We compare fingerprint- and MMP-based cliff populations, consider the entire experimentally observed potency range and determine the rates at which activity cliffs are formed. Furthermore, special emphasis has been put on analyzing the potency range distribution of cliffs. Taken together, the results reveal the probability of activity cliff formation and provide a comprehensive view of their distribution across bioactive compounds.

MATERIALS AND METHODS

Compounds and Activity Data. From ChEMBL,²³ compounds with direct interactions (i.e., target relationship type “D”) with human targets at the highest confidence level (i.e., target confidence score 9) and available K_i measurements were assembled. On the basis of these criteria, 27 610 compounds were selected that were active against 414 targets (with a total of 45 139 potency records). The selected compounds were assembled into individual target sets.

Fingerprints and Similarity Calculations. Activity cliffs were calculated for two different 2D fingerprints including MACCS structural keys²⁴ and the Extended Connectivity Fingerprint with Bond Diameter 4 (ECFP4).²⁵ MACCS keys represent a compendium of 166 structural fragments and ECFP4 is a topology fingerprint monitoring layered atom environments up to a diameter of four bonds around each atom in a test compound. In terms of their structural resolution and relative complexity, these representations essentially mark opposite ends of the current spectrum of 2D fingerprints.²⁶ In addition, these fingerprint descriptors have thus far been mostly used to represent activity cliffs.² Fingerprint overlap was quantified as a measure of similarity by calculating the Tanimoto coefficient (Tc).²⁷

Transformation Size-Restricted MMPs. MMPs were generated using a previously described¹⁵ in-house implementation of the Hussain and Rea algorithm.²⁸ For molecular fragmentation, single, double, and triple cuts of bonds²⁸ were permitted. As a structural criterion for cliff formation, the maximal size of a fragment exchanged in a chemical transformation was set to 13 non-hydrogen atoms and the difference in the size of exchanged fragments was limited to maximally 8 non-hydrogen atoms.¹⁵ As an upper limit, these restrictions permitted, for example, the replacement of differently substituted five- or six-membered rings or the replacement of such a ring with a condensed two-ring system. Thus, compounds in MMP-cliffs had well-defined but only limited structural differences.¹⁵

RESULTS AND DISCUSSION

Activity Cliff Criteria. For a nonambiguous assessment of activity cliffs, similarity and potency difference criteria must be clearly specified. In addition, it must be decided which types of experimental measurements to consider. For comparison, we selected three molecular representations including the MACCS and ECFP4 fingerprints and MMPs. For MACCS and ECFP4, Tc values of 0.85 and 0.55 were set as similarity thresholds for cliff formation, respectively, in accord with previously applied cliff definitions.² A MACCS Tc value of 0.85 approximately corresponds to an ECFP4 Tc of 0.55 (i.e., many compound pairs yield these corresponding values).^{2,19} These criteria have thus far been most widely applied in activity cliff analysis.

A conceptual drawback is that activity cliffs defined on the basis of whole-molecule similarity calculations might often be difficult to interpret from a medicinal chemistry perspective. Therefore, structural classification schemes for activity cliffs have also been introduced.^{15,21} Activity cliffs defined on the basis of substructure relationships or other structural criteria might often provide advantages for medicinal chemistry analysis. Cliff categories can be introduced in which cliff forming compounds are only distinguished by well-defined and subtle structural changes. For example, we have determined that ChEMBL currently contains a limited number of ~160 activity cliffs where cliff partners only differ by stereochemistry.²¹ Furthermore, we have shown that the majority of all activity cliffs in ChEMBL (i.e., nearly 9600) are distinguished by different R-groups attached to the same scaffold.²¹ R-group replacements leading to the formation of activity cliffs might of course be of rather different chemical nature. They might range from subtle changes such as the replacement of hydrophobic substituents of similar size (which nevertheless might affect critical ligand–receptor interactions) to more drastic changes such as the introduction of a charged R-group (altering the global charge state of a molecule and its biophysical properties). However, such changes in individual substituents are generally meaningful activity cliff criteria. Differences in their chemical nature reflect differences in the types of critically important binding determinants.

For our current analysis, activity cliffs defined on the basis of calculated whole-molecule similarity and MMP-cliffs were considered. MMP-cliffs were defined based on transformation size and size difference restrictions detailed in the Materials and Methods section. We intended to compare the distributions of these different types of activity cliffs.

Furthermore, we consistently required a potency difference of at least two orders of magnitude as a cliff criterion. An at least 100-fold difference in potency between cliff partners

essentially excluded the influence of typical variations in potency measurements²⁹ and focused the analysis on cliffs of relatively large magnitude. Importantly, the formation of activity cliffs was considered over the entire potency range observed for bioactive compounds and the potency range distribution of cliffs was another focal point of our analysis. As experimental measurements, we exclusively considered equilibrium constants. These K_i values represent high-confidence measurements that are comparable across different assays and generally yield most reliable activity cliff assignments.²⁹ Table 1 summarizes all activity cliff criteria applied in our analysis.

Table 1. Activity Cliff Definitions^a

representation	cliff criteria
ECFP4	ECFP4 Tanimoto coefficient (Tc) value of ≥ 0.55 potency difference ≥ 2 orders of magnitude
MMP	size difference for exchanged fragment ≤ 8 non-hydrogen atoms size of exchanged fragment ≤ 13 non-hydrogen atoms potency difference ≥ 2 orders of magnitude
MACCS	MACCS Tanimoto coefficient (Tc) value of ≥ 0.85 potency difference ≥ 2 orders of magnitude

^aAll criteria applied to define activity cliffs for three different molecular representations are specified.

Potency Values. The compound pool meeting our data selection criteria contained $\sim 28\,000$ molecules with $\sim 45\,000$ available potency records (active against more than 400 targets). We initially determined the global potency range and the potency value distribution, as reported in Table 2. The

Table 2. Potency Range and Value Distributions^a

	potency subranges		potency values	%
pM	$> 11 - \leq 14$	$> 10 \text{ pM} - \leq 0.01 \text{ pM}$	31	0.07
nM	$> 8 - \leq 11$	$> 10 \text{ nM} - \leq 0.01 \text{ nM}$	10 560	23.39
μM	$> 5 - \leq 8$	$> 10 \mu\text{M} - \leq 0.01 \mu\text{M}$	31 047	68.78
mM	$> 2 - \leq 5$	$> 10 \text{ mM} - \leq 0.01 \text{ mM}$	3370	7.47
M	$> -1 - \leq 2$	$> 10 \text{ M} - \leq 0.01 \text{ M}$	131	0.29

^aSubranges spanning the entire potency range of all qualifying compounds are delineated using pK_i (left) and K_i (right) values. The total number and percentage of potency values falling into each subrange are reported.

observed potency range spanned 12 orders of magnitude, from molar to picomolar potency. Accordingly, the overall potency range was divided into five standard subranges. The extremely low (M) and high (pM) potency ranges contained only a small number of values (131 and 31, respectively). The bulk of the data fell into the micromolar (with 31 047 values corresponding to $\sim 68.8\%$ of all data points) and nanomolar range (10 560 values corresponding to $\sim 23.4\%$). However, more than 3000 mM values were also obtained, which represented $\sim 7.5\%$ of all measurements. Low potency values falling into this range should only be considered if high-confidence measurements are available, consistent with our data selection strategy.

Activity Cliff Statistics. Activity cliffs were determined on a per-target basis, and statistics were calculated across all target sets. All possible compound pairs were assembled that met the similarity criteria for cliff formation. Then pairs were identified that displayed the required potency difference and thus qualified as activity cliffs. The results are reported in Table 3.

Table 3. Activity Cliff Statistics^a

	ECFP4	MMP	MACCS
no. activity cliffs	16 941	9481	17 886
no. qualifying pairs	316 034	198 569	314 665
% cliff pairs	5.36%	4.77%	5.68%
no. cliff partners	9159	6286	9520
% compounds	31.70%	22.77%	34.48%

^a“Activity cliffs” reports the total number of activity cliffs for each molecular representation, and “qualifying pairs”, the total number of compound pairs that qualified for cliff formation on the basis of Tc values or MMP transformation size limits. “Cliff partners” gives the number of compounds involved in the formation of activity cliffs. “% Cliff pairs” reports the percentage of all qualifying compound pairs that formed activity cliffs with an at least 100-fold difference in potency, and “% compounds”, the percentage of all compounds involved in the formation of these cliffs.

For MACCS, ECFP4, and MMP representations, a total of 17 886, 16 941, and 9481 activity cliffs were identified, respectively. Hence, many more fingerprint-based activity cliffs than MMP-cliffs were detected. However, the number of observed activity cliffs should be related to the total number of qualifying compound pairs meeting the similarity criteria. On the basis of fingerprint similarity, significantly larger numbers of qualifying compound pairs were obtained (314 665–316 034) than on the basis of MMPs (198 569). Thus, the relative frequency of cliff formation among all qualifying compound pairs was similar, ranging from $\sim 4.8\%$ for MMPs to $\sim 5.4\%$ and $\sim 5.7\%$ for ECFP4 and MACCS, respectively. These activity cliffs involved a total of $\sim 22.8\%$, $\sim 33.2\%$, and $\sim 34.5\%$ of all bioactive compounds for the MMP, ECFP4, and MACCS representations, respectively.

Table 4 reports the top 10 target sets containing the largest number of ECFP4-based activity cliffs and their MMP-cliffs. Among these targets were different types of G protein coupled receptors and two proteases. Seven of these sets contained more than 1000 compounds. For both ECFP4 and MMPs, the

Table 4. Target Sets Containing Most Activity Cliffs^a

target set ^b	no. Cpd	ECFP4			MMP		
		no. Cliffs	no. QP	% CP	no. Cliffs	no. QP	% CP
CFX	1052	1414	11999	11.78	904	8220	11.00
KOR	1102	1120	12756	8.78	454	6749	6.73
MR4	1183	1011	18284	5.53	459	12687	3.62
AA3	1318	908	11097	8.18	297	5698	5.21
P2Y	433	557	34943	1.59	43	14836	0.29
THR	658	548	3845	14.25	229	2247	10.19
CB2	1130	527	8020	6.57	392	5489	7.14
MHR	805	501	8032	6.24	311	4901	6.35
MOR	1207	499	8933	5.59	238	6176	3.85
DOR	1042	474	8639	5.49	170	5094	3.34

^aThe top 10 target sets containing the largest number of activity cliffs are reported in the order of decreasing numbers of ECFP4-based cliffs. For each target set, the number of compounds, activity cliffs, qualifying compound pairs (QP), and the percentage of cliff pairs (CP) are reported for the ECFP4 and MMP representations. ^bTarget abbreviations: CFX, coagulation factor X; KOR, kappa opioid receptor; MR4, melanocortin receptor 4; AA3, adenosine A3 receptor; P2Y, purinergic receptor P2Y12; THR, thrombin; CB2, cannabinoid CB2 receptor; MHR, melanin-concentrating hormone receptor 1; MOR, mu opioid receptor; DOR, delta opioid receptor.

largest number of activity cliffs was found for coagulation factor X inhibitors (with 1414 and 904 cliffs, respectively). Among these sets, the percentage of all qualifying compound pairs forming activity cliffs varied significantly. For ECFP4 and MMPs, the largest fraction was observed for thrombin (14.3%) and factor X inhibitors (10.2%), respectively.

Taken together, the findings indicated that MMP-cliffs provided an overall more conservative representation of activity cliffs than those identified on the basis of Tanimoto similarity. In the latter case, approximately a third of all bioactive compounds were involved in the formation of at least one activity cliff. However, only ~5% of all qualifying compound pairs also formed cliffs. Thus, on the basis of relative compound pair frequency analysis, the formation of activity cliffs has consistently been a rather rare event.

Activity Cliff Overlap. The different activity cliff populations displayed only limited overlap, as reported in Table 5, which clearly reflected the molecular representation

Table 5. Overlap Between Activity Cliff Populations

	ECFP4	MMP	MACCS
no. activity cliffs	16 936	9481	17 886
no. overlapping cliffs			
ECFP4–MMP	6963		
MMP–MACCS		4413	
ECFP4–MACCS		8804	
consensus cliffs		3909	

For the three molecular representations, the overlap between their activity cliff populations is reported. “Consensus cliffs” are shared by all three representations.

dependence. Importantly, although ECFP4 and MACCS produced comparable numbers of ~17 000 to ~18 000 activity cliffs, only roughly half of these cliffs (8804) were conserved. Of the 9481 MMP-cliffs, 6963 (73.4%) and 4413 (46.5%) were also detected with ECFP4 and MACCS, respectively. Hence, although the absolute number of MMP-cliffs was much lower than fingerprint-based cliffs, many structure-based cliffs were not identified on the basis of Tanimoto similarity, especially for MACCS. However, there were also 3909 activity cliffs (41.2% of all MMP-cliffs) that were consistently detected for all three molecular representations. These cliffs, termed consensus cliffs, are reported in Table 5, and representative examples are shown in Figure 1. Consensus cliffs we inspected were generally chemically intuitive.

Potency Range Distribution. We then determined the distribution of activity cliffs over the entire potency range and subranges. Figures 2a and 2b show the results for all detected cliffs and consensus cliffs, respectively. For all three molecular representations, the by far largest number of cliffs involved a compound with nanomolar potency and a cliff partner with micromolar potency (nM/ μ M cliff). For ECFP4, MACCS, and MMP, these nM/ μ M cliffs represented 58.4%, 43.7%, and 50.5% of all activity cliffs, respectively. Cliffs formed among compounds with μ M potency followed with ~12% to ~17% of all cliffs. The dominance of nM/ μ M and μ M/ μ M cliffs was consistent with the fact that most activity measurements fell into the μ M and nM range, as reported in Table 2. By contrast, only 258 (ECFP4), 157 (MACCS), and 139 (MMP) cliffs were exclusively formed by compounds with nanomolar potency (nM/nM cliffs). In addition, for all three representations, 41–77 activity cliffs were detected that were formed by compounds

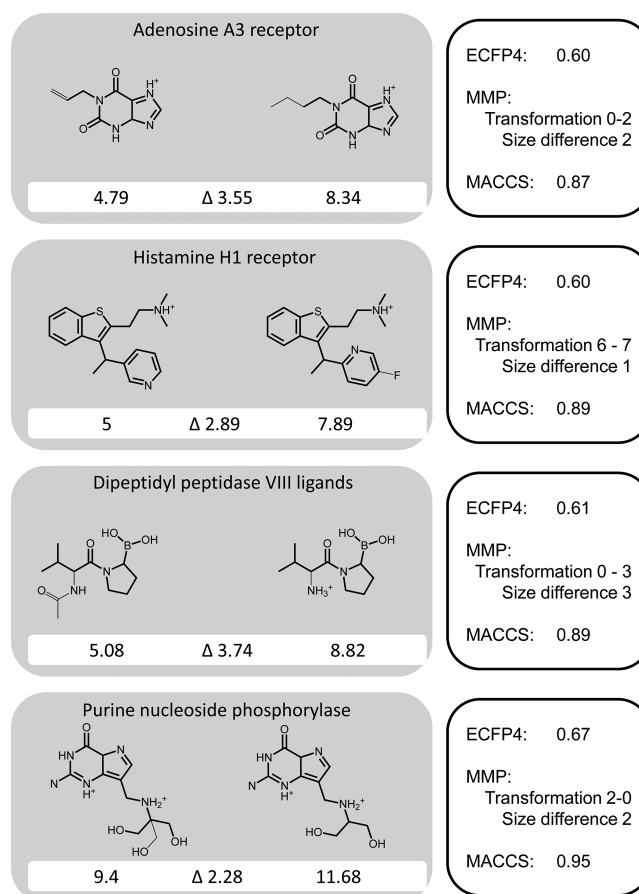


Figure 1. Consensus activity cliffs. Examples of consensus activity cliffs are shown that consistently met all criteria for cliff formation. For each consensus cliff, the target, ECFP4, and MACCS Tc values and the MMP transformation size and size difference are reported. In addition, the pK_i value of each cliff partner and their potency difference are given. For example, “Transformation 6-7” means that a fragment of size 6 in one cliff partner was replaced with a fragment of size 7 in the other, corresponding to “Size difference 1”.

with picomolar and micromolar potency. These pM/ μ M cliffs had the largest magnitude we observed. For SAR analysis, an important observation has been that many more nM/ μ M than μ M/ μ M cliffs were found. These cliffs cover the potency range that is most relevant for medicinal chemistry efforts. In addition, ~7% to ~12% of all cliffs were “weak” μ M/mM, which again illustrated the need to exclusively utilize high-confidence potency data. With approximate potency measurements, many false-positive cliffs would likely be detected within this low potency range. Numbers of cliffs detected within or across other potency subranges were significantly lower than found for the three major categories of (nM/ μ M, μ M/ μ M, and μ M/mM) cliffs. Analogous trends were observed for the consensus cliffs in Figure 2b. Here, 2492 nM/ μ M consensus cliffs were detected (25.7% of all activity cliffs), followed by 769 μ M/ μ M, and 496 μ M/mM cliffs. In addition, 44 nM/nM and 10 pM/ μ M consensus cliffs were identified. The subset of 2492 nM/ μ M consensus cliffs should also be of particular interest for SAR exploration.

CONCLUDING REMARKS

We have systematically identified activity cliffs in ChEMBL compounds and determined their frequency of occurrence and

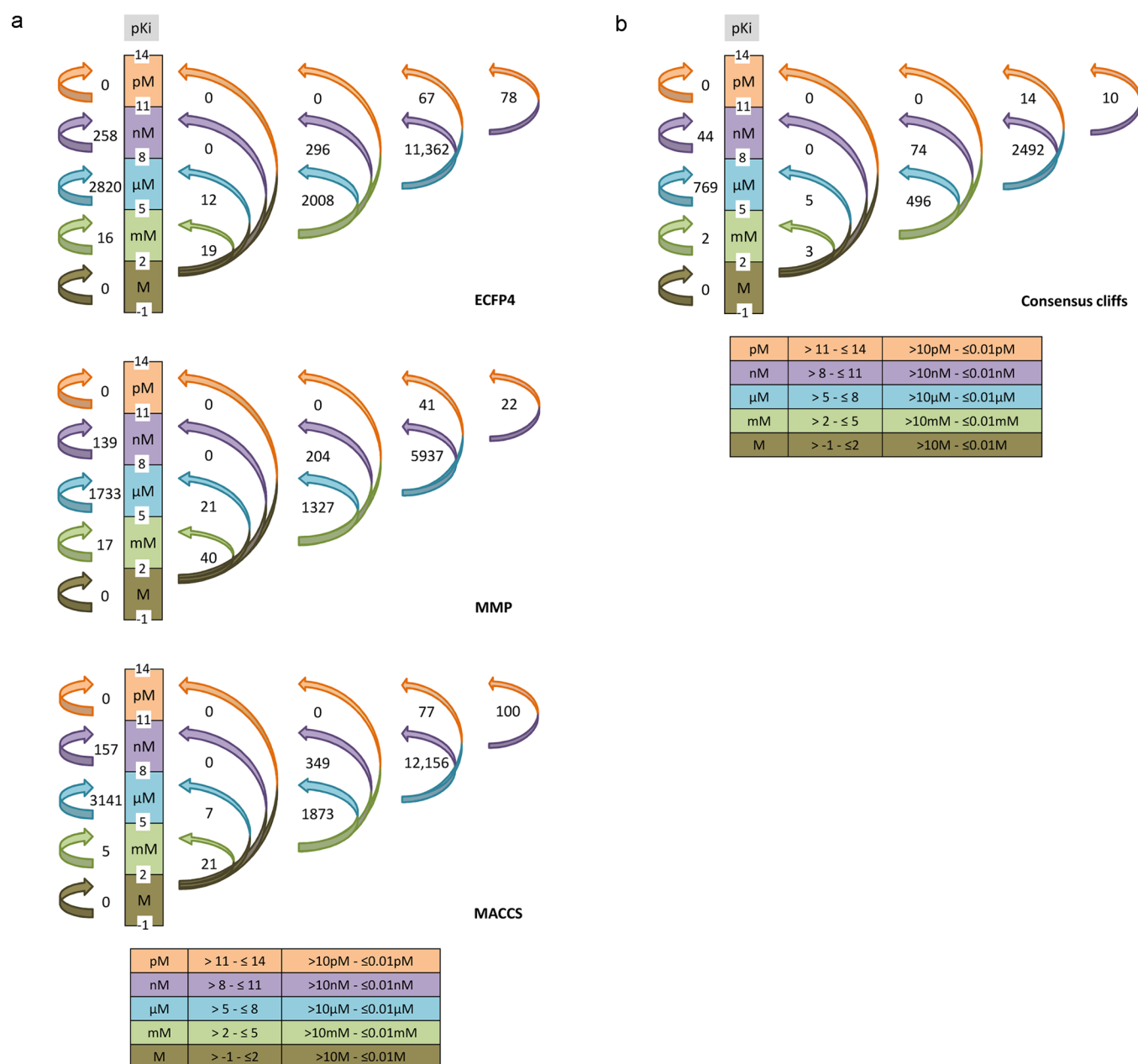


Figure 2. Potency range distribution of activity cliffs. Colored bars define five potency subranges (from molar to picomolar potency) on the basis of pK_i values. Color-coded arrows indicate the formation of activity cliffs within or across these subranges. Numbers next to arrows report activity cliffs falling into each subrange or pair of subranges. Shown are the distributions of (a) all identified activity cliffs and (b) the subset of consensus cliffs.

potency range distribution. Compounds active against more than 400 targets were analyzed. In addition, alternative molecular representations and similarity criteria were used to identify compound pairs that qualified for cliff formation. Furthermore, only high-confidence potency data were taken into account and activity cliff criteria were clearly specified. Also, only cliffs were considered that spanned a potency difference of at least two orders of magnitude. The applied data selection and activity cliff criteria ensured that the probability of false-positive or false-negative cliff assignments was low. Accordingly, the results of our analysis are expected to provide a meaningful account of activity cliff distributions in bioactive compounds.

On the basis of our analysis, approximately one-third of all active compounds were involved in the formation of at least

one high-confidence activity cliff when ECFP4 or MACCS fingerprints were used as a molecular representation. For MMP-cliffs, the frequency of occurrence was lower. In this case, only ~20% of the compounds formed activity cliff(s). Given the well-defined transformation and size restrictions of MMP-cliffs, this representation was more conservative than whole-molecule Tanimoto similarity assessment. However, by compound pair frequency analysis, we determined that only ~5% of all qualifying compound pairs represented activity cliffs, regardless of the chosen molecular representation. Hence, from this point of view, activity cliff formation is a rare event. In addition, the potency range distribution of activity cliffs revealed that the majority of cliffs were formed between compounds with micromolar and nanomolar potency, consistent with the overall potency data distribution. Thus, for the purpose of SAR

analysis, it should be readily possible to identify activity cliffs in many data sets by considering the structural neighborhood of compounds with nanomolar potency.

In light of the findings reported herein, we are also able to formulate a preferred general definition of activity cliffs, at least for compound data mining. Given the strong representation dependence of cliffs illustrated by the comparison of ECFP4- and MACCS-based similarity calculations, we give preference to MMP-cliffs, which are structurally more conservative and often also more intuitive. Given the frequency-of-occurrence of activity cliffs and the potency range distribution we observed, a two order of magnitude difference in potency is considered appropriate as a cliff criterion. Moreover, as long as equilibrium constants are used as potency measurements, cliffs should be monitored over all available potency ranges. Hence, taken together, we favor the general MMP-based definition of activity cliffs according to Table 1 for data mining applications.

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Notes

The authors declare no competing financial interest.

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