Kinomics: characterizing the therapeutically validated kinase space

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The annotation and visualization of medicinally relevant kinase space revealed that kinase inhibitors in the clinic are, on average, of higher molecular weight and more lipophilic than all other clinically investigated drugs. Tyrosine kinases from the vascular endothelial growth factor and epidermal growth factor receptor families are the most pursued targets. Furthermore, oncological indications account for 75% of all kinase-related clinical interest. In addition, analysis of the similarity between kinase targets with respect to sequence, selectivity and structure has revealed that kinases with ≥60% sequence identity are most likely to be inhibited by the same classes of compounds and have similar ATP-binding sites. The identification of this threshold, together with the widely accepted representation of the sequence-based kinase space, is expanding our understanding of the clinical and structural space of the kinome.

The protein kinases [1], responsible for the phosphorylation of tyrosine, threonine and serine residues in other proteins, are among the most extensively studied gene families [2,3]. The seminal work of Manning *et al.* [1] has defined and categorized the human 'kinome' space, consisting of 518 kinases. Multidisciplinary research involving biology, medicine, chemistry and informatics is rapidly advancing our understanding of the function, disease relevance, drugability and inter-relationships of the kinases [4–9].

The analysis of the human kinome by Manning *et al.* [1] has provided important foundations for understanding kinase signaling pathways and their role in various diseases. Similarity of protein sequences has been used to establish the relationship of kinases within the super-family, which has facilitated the understanding of kinase properties using an intuitive graphical representation. Beyond sequence-based comparisons, other methods for establishing links between kinases include structural comparisons, classification using selectivity profiles, biological pathways and disease indications, as well as clinical and preclinical interest and activities. Here, connections are drawn between multiple representations of the kinome space with the objective of attaining a better understanding of the therapeutically relevant subspace.

Our analysis of the therapeutically validated kinase space has potential implications for target selection, homology modeling [10], use of X-ray structures [11], anticipation of adverse effects and aspects of chemogenomics [12,13]. In chemogenomics, a lead molecule developed against one target in a gene family provides a potential opportunity for a related target. In many cases, structurally related targets might be involved in the pathology of different diseases and/or belong to separate pathways. This lack of selectivity could be advantageous in early stages of drug discovery, but detrimental in later stages, particularly if it is linked to adverse effects. However, *in vitro* selectivity data are, at best, guidelines in decision-making processes leading to the identification of molecules with functional *in vivo* activity and efficacy linked to target inhibition. That

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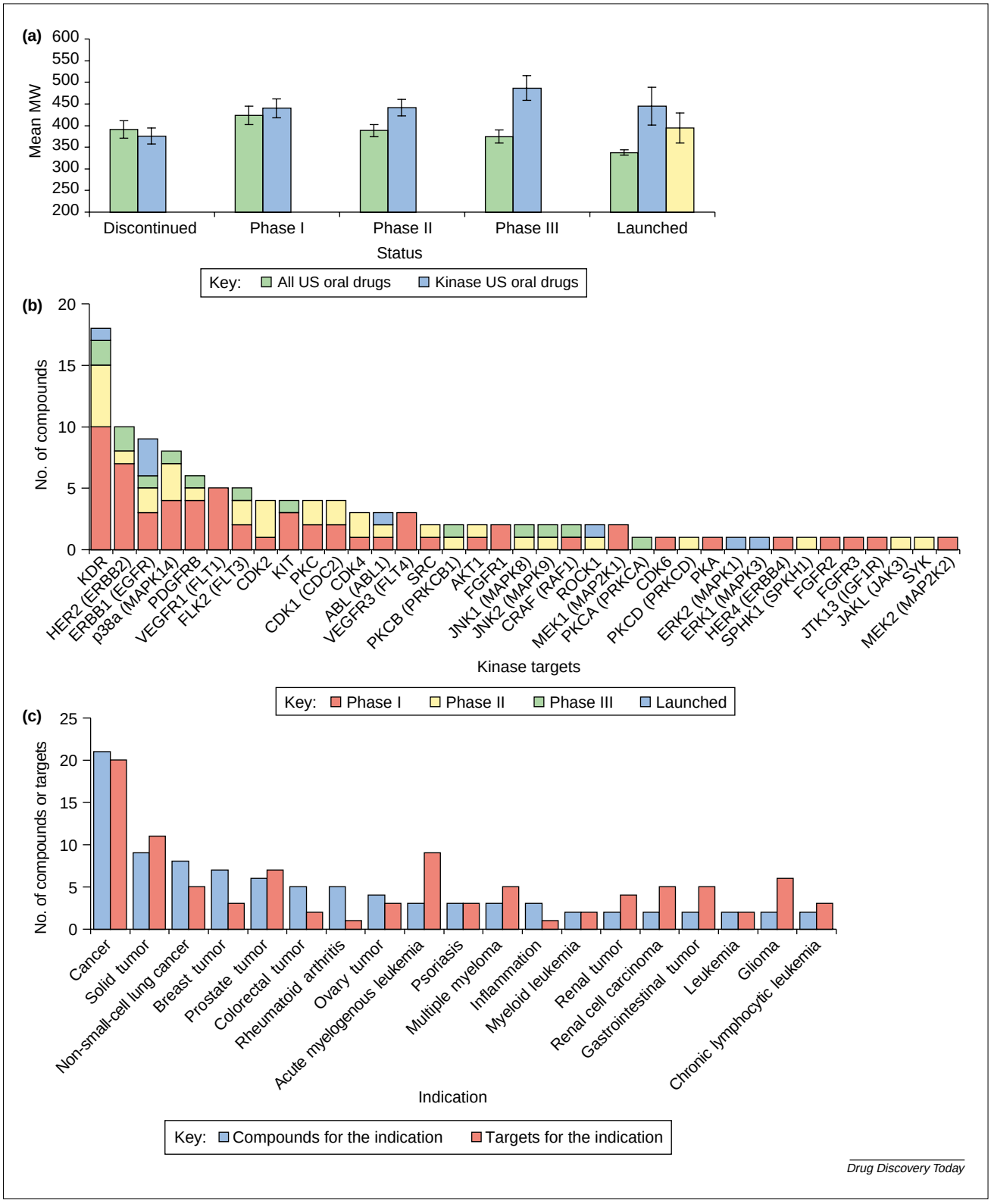


is to say, a lack of *in vitro* selectivity might or might not become apparent in the functional or organism setting [8].

Kinase clinical space: compound properties, targets and indications

Fabian *et al.* [14] recently presented an elegant study of interaction maps of 16 clinical kinase inhibitors, which indicated that differences in selectivity profiles might not be correlated with chemical structure, and suggested potential new targets for clinical compounds. However, it should be noted that these kinase selectivity data were generated using phage display and represent the binding of inhibitors, not necessarily kinase activity. In addition, it is unclear whether the kinase produced in phage presents the same conformation and phosphorylation and activation state of a native mammalian kinase in its cellular milieu [15].

FIGURE 1

Distribution of clinical kinase space according to the mean molecular weight of small molecule inhibitors, the kinase targets in various stages of development and the clinical indication pursued by two or more small molecule and large molecule compounds. (a) Mean MW of oral small molecule kinase inhibitors versus all oral drugs [16]. Compounds are categorized by highest clinical phase reached (Phase I, Phase II or Phase III), launched drugs or discontinued development. The oral drugs are only those approved in the USA; for kinases, drugs launched worldwide, in addition to those approved in the USA, are shown. Non-small molecule inhibitors were excluded from the analysis. **(b)** Kinase targets pursued by 76 active clinical compounds. PKC indicates typical isoforms of protein kinase C (α, β1, β2 and γ). The number of compounds in each development stage is shown for each kinase target. The Sugen [1] and/or HUGO (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene; [30]) nomenclature is used for the targets (see supplementary table online). **(c)** The number of targeted protein kinases for each indication is shown. Only the indications associated with two or more molecules are displayed. Abbreviations: Abl, v-abl Abelson murine leukemia viral oncogene homolog; AKT, v-akt murine thymoma viral oncogene homolog; Erbb, erystoblastic; ERK, extracellular signal-regulated kinase; FGFR, fibroblast growth factor receptor; FLT, fms-related tyrosine kinase; HER, human epidermal growth factor receptor; JNK, Janus kinase; JTK, Janus tyrosine kinase; KIT, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; MEK, meiosis-specific serine/threonine protein kinase; PDGFRB, platelet-derived growth factor receptor beta; PKC, protein kinase C; PRKC, protein kinase C; RAF, raf murine leukemia viral oncogene homolog; ROCK, Rho-associated, coiled-coil containing protein kinase; SPHK, sphingosine kinase; SRC, sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; SYK, spleen tyrosine kinase.

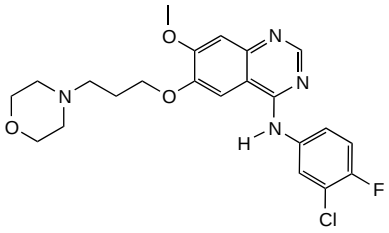
The information on clinical trials of 101 kinase inhibitors was extracted by a combination of text processing, manual curation and annotation [14] using information available on public electronic sources (www.clinicaltrials.gov), patent literature (www.cas.org/SCIFINDER/scicover2.html) and small molecule databases (www.gvkbio.com; www.mdli.com). Although every attempt was made to perform a thorough evaluation, the analysis in this review, by necessity, contains only a representation of the clinical space. Clinical data for US trials are comprehensive, thanks to the information provided by the US National Library of Medicine (www.clinicaltrials.gov). Compounds pursued in the clinic that target kinases were extracted, together with their associated indications. A set of 101 molecule entries with a mechanism of action that involves a specific kinase and reported *in vitro* inhibition data was combined. Each of the 101 entries was annotated with: (i) development stage or phase; (ii) specific kinases targeted; (iii) reported selectivity data for other kinases; (iv) clinically pursued indications; (v) the company developing the compound; and (vi) the chemical structure, when available. Of these compounds, 76 were reported to be active in the clinic, whereas 25 were no longer in active development, 12 entries are antibody–oligonucleotide molecules and 87 are small molecule inhibitors, 60 of which have disclosed structures. Kinase inhibition was identified as the mechanism of action in a total of 36 targets associated with 48 distinct clinical indications. An additional 16 kinase targets that were not identified as the intended target are significantly inhibited *in vitro*.

For each development phase, the properties of kinase-targeted small molecule compounds were examined and compared with those of all oral drugs approved in the USA (Figure 1a). Oral drugs show a marked decline in mean molecular weight (MW) from Phase I to launch [16,17], which is sometimes linked with the high attrition rate of clinical compounds [16,18]. Despite the paucity of data for kinase inhibitors in the clinic, their properties differ slightly from those of 'non-kinase-targeted' oral drugs. Only the discontinued and Phase I kinase compounds have similar MW to other orally targeted compounds in the same development phase [16]. There is no significant change in MW noted in the progression from Phase I to marketed drugs. Thus, the trends observed for small molecule drugs targeting kinases are different from all other drugs and the failure rate in the clinic for kinase-targeted small molecule compounds cannot be linked to their MW. This observation is supported by the finding that the discontinued kinase inhibitors have lower average MW than those in other development phases. In summary, the small molecule clinical compounds targeting kinases have significantly higher MW (486 for Phase III and 445 for US-launched kinase inhibitors) and calculated logP (clogP of 5.1 for Phase III and 4.7 for US-launched kinase inhibitors) than other compounds in the same phases of development.

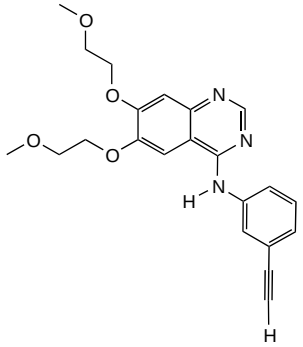
The clinically relevant kinase target space consists of 37 proteins (Figure 1b). The reported selectivity profiles of clinical compounds reveal that an additional 16 kinase targets are inhibited at a similar potency to those already targeted. Because small molecules are typically profiled against a subset of existing kinases (<200), it is entirely possible that these compounds could inhibit even more kinases. The highest level of clinical interest is devoted to the vascular endothelial growth factor (VEGF) family (in particular, kinase insert domain receptor/VEGF receptor 2 with 18 clinical compounds) and the epidermal growth factor receptor (EGFR) family from the tyrosine kinase (TK) group. The third most targeted kinase with eight compounds is p38a/mitogen-activated protein kinase 14 (p38a/MAPK14). This attention is not entirely surprising given the level of target validation for these kinases in cancer (KDR [19], EGFR [20,21] and p38a/MAPK14 [22]) and inflammation (p38a/MAPK14) [23].

An interesting perspective of the clinical kinase space emerges when examining the distribution of compounds across therapeutic indications and the number of targets associated with each indication (Figure 1c). Generic cancer indications, pursued with the largest number of clinical compounds (21), are associated with 20 different kinase targets (18 typical kinases, protein kinase A regulatory subunit and Sphingosine kinase). Oncological indications account for almost 75% of all clinical entries (59 of 76 compounds are still in the clinic). These are also spread across a large number of targets and target groups. Rheumatoid arthritis and inflammatory indications grouped together account for 15–20% of kinase clinical interest (12 of 76 compounds are still in the clinic). Some compounds are pursued for inflammatory as well as oncological indications (three compounds are still in the clinic). Given the growing body of evidence demonstrating colocalization of inflammation, angiogenesis and cancer [24], in addition

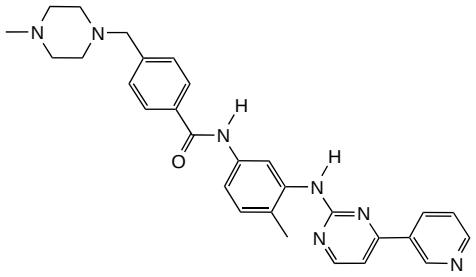
(a)



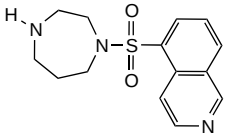
Gefitinib
EGFR,
Non-small-cell lung cancer
Launched in USA in 2003



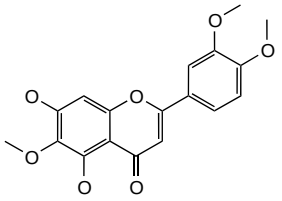
Erlotinib
EGFR
Non-small-cell lung cancer
Launched in USA in 2004



Imatinib
ABL1, KIT and PDFRB
Myeloid leukemia and gastrointestinal tumor
Launched in USA in 2001

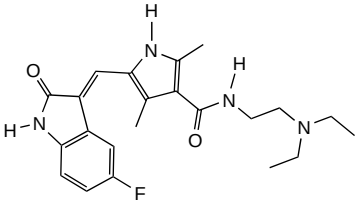


Fasudil
ROCK1
IV for brain hemorrhage
Launched in Japan in 1995

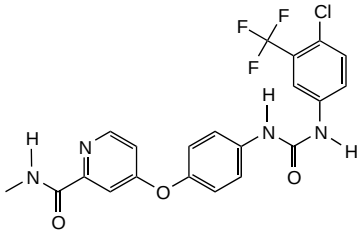


Eupatilin
ERK1, ERK2 and CDKs
Launched in Korea for gastritis in 2003

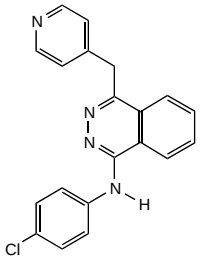
(b)



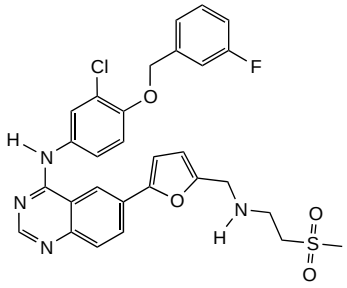
Su11248
FLT3, KIT, KDR and PDGFRB
Renal cell cancer and gastrointestinal neoplasms



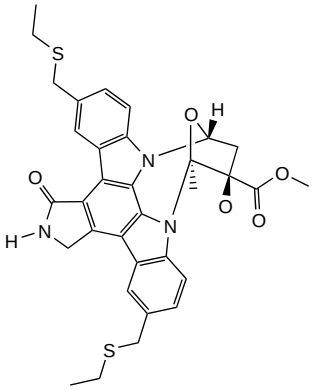
Sorafenib
RAF1, KDR and PDGFRB,
Carcinoma and renal cell



Vatalanib
KDR
Colorectal neoplasms, colonic neoplasms,
rectal neoplasms and NLR



Lapatinib
EGFR and ERBB2
Metastatic breast cancer



CEP1347
MAPK8 and MAPK9
Parkinson's disease and NLR

Drug Discovery Today

FIGURE 2

Selected launched kinase-targeted small molecule drugs and Phase III compounds. Drugs launched in the USA are shown in **(a)**, those at Phase III of clinical trials are shown in **(b)**. Indications in the highest phase in the USA and kinases targeted by the compounds are listed. NLR indicates the trials in the USA that are no longer registering patients. Two monoclonal antibodies targeted at EGFR (cetuximab) and VEGF (bevacizumab), not shown here, were also approved by the FDA in 2004.

to the link between cytokines and chemokines and tumor anchorage, growth and metastasis [25], it is expected that these dual indications (inflammation and cancer) will increase in frequency.

Five launched and five Phase III kinase small molecule drugs, together with their primary targets and indications, are presented in Figures 2a and 2b, respectively.

The relationship between selectivity and sequence similarity

Kinases can be compared using selectivity profiles arising from differences in the binding affinity of known inhibitors [7]. As an alternative to sequence-based similarity, a SAR-based similarity metric that ranges from 0 to 1, which increases with increasing similarity of kinase pIC_{50} values [$-\log(IC_{50})$] for a series of inhibitors, has been described [7]. Our previous analysis has been significantly expanded with 1479 kinase pairs representing 78 kinases with IC_{50} values for four or more compounds.

Dendrograms based on selectivity data differ in some cases from the traditional sequence-based similarity classifications [7]. However, the selectivity-based similarity is dependent on the chemotypes used for profiling across multiple targets, and the absence of a sufficient quantity of data for many kinases limits the scope of possible comparisons. To increase the applicability of SAR similarity beyond the kinase space represented by the available biochemical *in vitro* enzymatic selectivity data, the relationship between sequence similarity (which can be established for any pair of kinases) and SAR similarity was examined (Figure 3a). Statistical analysis demonstrates that kinase pairs with sequence identity between 90 and 100%, which are expected to be inhibited to a similar degree, have, on average, statistically indistinguishable SAR similarity from kinase pairs with a sequence identity of >60%. Kinase pairs with a sequence identity of <60% have a lower average SAR similarity distributed over a considerable range. As a general guideline, kinases with ≥60% sequence identity compared with a kinase of interest have a high probability of being inhibited by the same groups of compounds. However, kinases with <60% identity might or might not be inhibited by the same compounds (Figure 3a). This observation is useful for assembling selectivity panels that are complementary to targets of interest and for projecting the extent of selectivity based on limited, but diverse, profiling data. It also shows that it should be possible to cover the complete kinome with a representative set of targets [8].

Structural distance

The similarity of the binding sites of proteins can be quantified using a generalization of the fingerprint approach described by Mason *et al.* [26]. This method is a derivative of fingerprint-encoding of small organic molecules [26], where the presence of binding site atom pairs at various distances is encoded by a string of integers. The fingerprint is organized in consecutive segments representing pairs of non-hydrogen atoms differentiated by Sybyl atom types (www.tripos.com) and each segment is further subdivided into several bins for recording the distance between the atoms. As of 14 January 2005, 59 typical human kinase sequences map to an entry in the Protein Data Bank (PDB) [27] when applying the criteria of ≥95% sequence identity calculated over 150 or more alignment positions (excluding continuous insertions that are the result of introns or alternative splicing). When counting multiple instances of the same kinase (e.g. when it is complexed with multiple inhibitors), the number of structures in the PDB rises to 284. The similarity of 45,451 pairs of kinase catalytic domains was calculated using the Tanimoto coefficient (in the form that is applicable to integer strings) applied to the ATP-binding site fingerprints and computed using 24 active-site residues that have been defined previously [7].

The relationship between SAR and sequence similarity can be understood by examining the correlation between the sequence similarity of kinases and the corresponding ATP-binding site similarity (Figure 3b). Kinases with high sequence identity also have high ATP site similarity, whereas kinases with low sequence identity exhibit a range of structural similarities. For example, 1QD3 (LCK) and 1QPJ (LCK) share 15% sequence identity and 0.991 structural similarity. By contrast, 1UWH (BRAF) and 1P4F (DAPK1) share 17% sequence identity and 0.775 structural similarity, which is one of the lowest values. Using 4429 pairs of catalytic domains corresponding to the same kinase (e.g. different cyclin-dependent kinase 2 structures), it was possible to estimate the intrakinase variability of the structure-based fingerprints. Statistical analysis was performed to determine the level of sequence identity at which ATP-binding site similarity significantly differs from the mean intrakinase structural similarity. Interestingly, the threshold of 60% sequence identity differentiated between binding sites of related kinases and variations in the binding sites of the same kinase. This observation lends credence to the hypothesis that similarity in the selectivity profiles of ATP-competitive inhibitors results from the structural similarity of the corresponding ATP pockets. In addition, this threshold is consistent with the accepted value at which homology modeling becomes reliable [10]. This observation significantly increases the applicability of structure-based fingerprints for understanding the properties of kinases in structural space; at ≥60% identity, the total number of human kinases that are represented in the PDB, directly or indirectly, rises from 59 to 138.

Kinase selectivity: interpretation of caveats

In general, most kinase data reported in *in vitro* biochemical enzyme assays are IC_{50} values, because routine determination of K_i values is not feasible for large compound sets. It is important to note that, whereas K_i values are essentially 'immutable' properties of the compound, IC_{50} values are not (i.e. they are dependent on the concentration of ATP, substrate and/or enzyme, depending on the mode of action of the compound). For example, with ATP-competitive compounds, Cheng–Prusoff [28] (Equation 1) dictates that the concentration of ATP in the assay relative to K_m can alter the IC_{50}, particularly if the concentration of ATP in the assay exceeds the K_m ATP concentration.

$$IC_{50} = K_i\left(1 + \frac{[ATP]}{K_m}\right) \qquad \text{[Equation 1]}$$

When comparing IC_{50} values, care should be taken to note the concentration of ATP relative to the K_m ATP concentration. If a given biochemical assay is performed at ATP concentrations significantly higher than the K_m concentration, then true comparisons of enzyme selectivity can only be made using apparent K_i values (corrected for assay concentration of ATP relative to K_m ATP concentration). Assays performed at or below K_m are generally not a problem, because the IC_{50} changes minimally (approximately twofold or less) relative to the error of most assays. In many cases, the IC_{50} values become the default because of a lack of the necessary characterization. Although ATP-competitive compounds are predominant and, in many cases, are assumed to be ATP-competitive, the mode of action for many kinase inhibitors could differ. One example involves 'slow binding' inhibitors (e.g. those that bind allosterically but then cause a perturbation in the ATP-binding site), which often require preincubation with the enzyme to reach an 'equilibrium IC_{50}' [29]. In these cases, determination of on–off rates is valuable. This highlights potential caveats in the use of annotated kinase IC_{50} values in the analysis. Although the data are extremely useful for establishing correlations in large datasets of kinase inhibitors, it can be even more useful (and perhaps better correlated and with lower error) when care is taken to include characterization of kinase inhibitors.

Cell-based data for kinase activity are of particular importance because such data combine the effects of enzyme and substrate(s) localization in a more 'physiological' context. Cell-based inhibition represents a higher hurdle because cell permeability, stability and greater solubility (where the cells do not tolerate solvent) are required. These data are of great value in determining whether the *in vitro* enzyme data translate to inhibition of substrate phosphorylation *in vivo*. In addition, the selectivity of the kinase inhibitor can be assessed when measuring multiple phosphorylated substrates and/or downstream effectors of kinase pathways. Unfortunately, there is a paucity of cell-based kinase data relative to the *in vitro* data available for isolated enzymes. Given new techniques for multiplexed measurements of phosphorylated substrates and pathways endpoints, it is hoped that this dataset could become more enriched in the near future [3,6]. Cell-based data must also be well annotated because the conditions can dramatically affect the interpretation (e.g. selection of substrate, phosphorylation site, stimuli and time course).

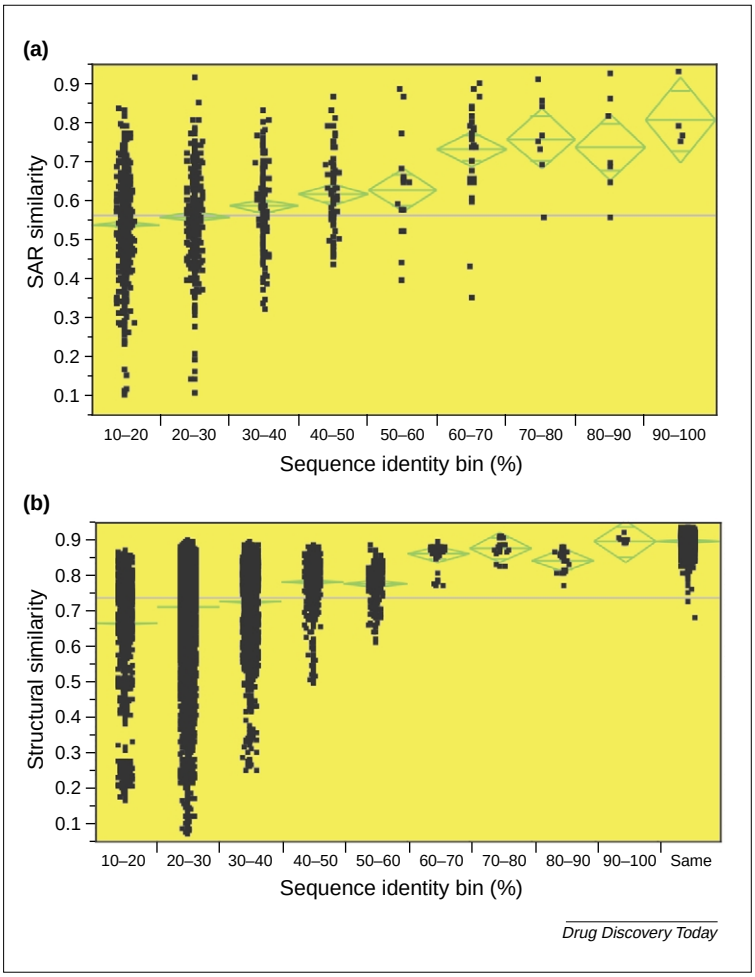


FIGURE 3

Relationship between sequence identity of kinase catalytic domains and SAR similarity or ATP-binding site structural similarity. SAR similarity approaches unity when kinases are inhibited to a similar extent by different groups of inhibitors. ATP-binding site similarity approaches unity as kinase binding site structures become identical. Kinase pairs were grouped into ten evenly separated sequence identity bins. For SAR similarity **(a)**, 1479 pairs arising from 78 kinases are plotted; for ATP-binding site similarity **(b)**, 45,451 pairs arising from all catalytic domains (including multiple chains) in 225 PDB entries are plotted. The average SAR similarity or ATP-binding site structural similarity for each sequence bin is shown at the center of the diamonds, with the vertices corresponding to 95% confidence limits. An all-pairs Tukey HSD (honestly significant difference) test indicates that differences in intrakinase ATP-binding site variations becomes significant below 60% sequence identity; a similar test for SAR similarity, when comparing the 90–100% bin with all other bins, becomes significant below 60% identity. The SAR similarity analysis used public domain data distributed by GVK Biosciences (www.gvkbio.com) and was supplemented by selected data from internal projects to improve statistics.

Visualizing medicinally relevant kinase space

Multiple approaches for visualizing the human kinome have been proposed. In our research, we have used the

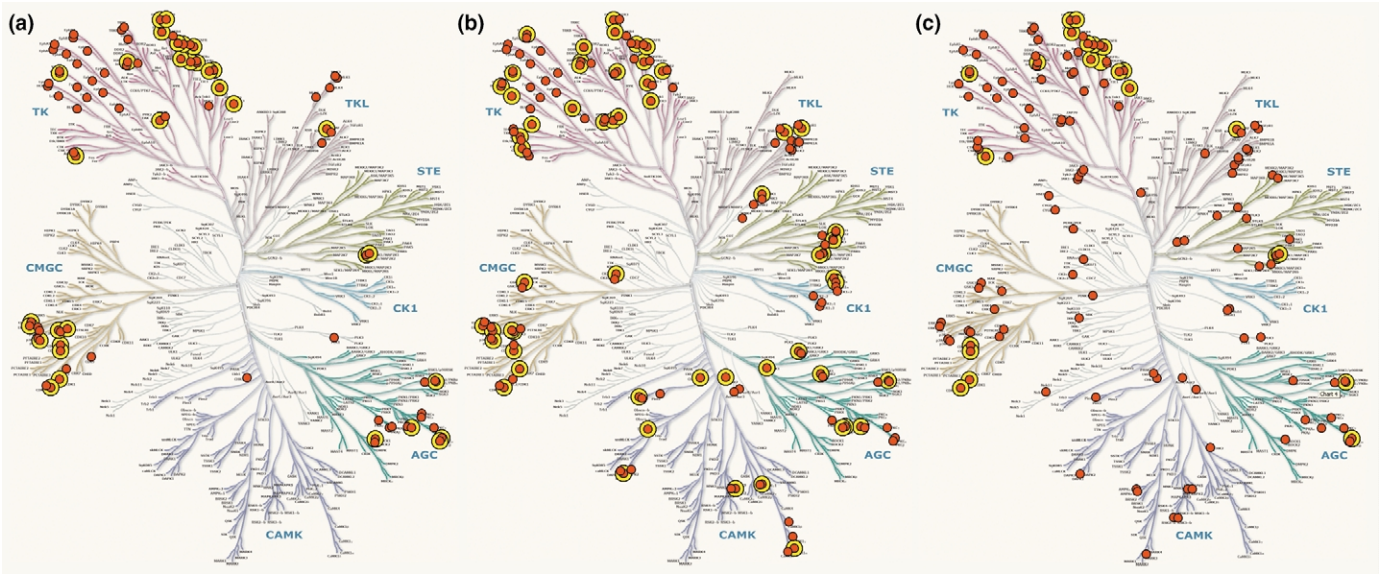


FIGURE 4

Sequence-based kinase space. These diagrams were originally presented by Manning *et al.* [1]. **(a)** Kinase clinical interest space (34 typical kinases shown in yellow); red dots show the 34 primary targets, 15 other targets reported to be inhibited by the clinical compounds (total of 49 kinases with reported data) and 39 other kinases with ≥60% identity to the 49 kinases. The total number of yellow dots is 88 – 49 with reported data and 39 with a high probability of inhibition based on the 60% rule. **(b)** Kinases with structures available on the PDB (59 shown in yellow) and other kinases with ≥60% sequence identity with a structure available on the PDB (138 shown in red). **(c)** Human cancer map showing typical protein kinases associated with cancer (132 shown in red) (www.proteome.com) and clinically pursued targets for cancer and tumor indications (23 shown in yellow). The kinase dendrogram is adapted from www.sciencemag.org/cgi/data/298/5600/1912/DC2/1 and is reproduced with the kind permission of Cell Signaling Technology and Science. Abbreviations: CAMK, calcium/calmodulin-dependent protein kinase; CK, casein kinase; CMGC, containing CDK, MAPK, GSK3, CLK families; STE, homologs of sterile 11 and sterile 20 kinases; TKL, tyrosine kinase-like.

work of Fabian *et al.* [14], who adopted the dendrogram established by Manning *et al.* [1], as the basis for visualizing selectivity profiles. Here, clinical and structural space of the human kinome have been depicted, together with the corresponding extensions determined using the 60% sequence-identity rule established in this work (Figures 4a,b). From this representation, it is evident that the currently characterized clinical and structural kinome spaces overlap significantly. The characterization of key kinases by X-ray crystallography would significantly increase the scope of structure-based kinase comparisons. In addition, a comparison of kinases associated with various cancers in the literature with those targets pursued for oncological indications with clinical compounds suggests that our present exploration of the drugable human kinome remains limited in scope (Figure 4c). A more complete understanding of *in vitro* and animal data for kinase selectivity, as it translates to human clinical safety and efficacy, will be crucial for successfully developing therapeutically relevant kinase inhibitors.

Conclusions

Despite the association of kinases with many disease states, kinase inhibitors account for a small fraction of marketed drugs (<1%) and compounds entering clinical trials (<3% in Phase I). In recent years, there has been a rapid growth of biochemical *in vitro* profiling of kinase inhibitors. However, the data generated have not been fully translated into understanding compound evaluation in cellular and clinical settings. By assembling profiling panels of representative kinases, an earlier assessment of relevant selectivity could be achieved. Nonetheless, it has not been shown that lack of selectivity *in vitro* will translate into a lack of selectivity in the clinic. Kinases that are not targeted by late-stage clinical compounds, but are found to be inhibited by them in biochemical assays, could be classified as posing lower selectivity risks in early stages of kinase inhibitor development. Because the vast majority of kinase clinical compounds target oncological indications, the identification of compounds that inhibit multiple clinically pursued targets could be a significant milestone in kinase research.

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