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A Systematic Analysis of Physicochemical and ADME Properties of All Small Molecule Kinase Inhibitors Approved by US FDA from January 2001 to October 2015



Zhihong O'Brien¹ and Mehran F. Moghaddam^{2,*}

¹Nitto Biopharma, Inc., 10628 Science Center Dr., San Diego, CA 92121, USA; ²OROX BioSciences, Inc., 4971 Sterling Grove Lane, San Diego, CA 92130, USA

Abstract: Background: During lead identification and optimization, the advancement criteria may be driven based on scientific principles, prior experiences, and/or by examining the path paved by approved drugs. However, accessing the discovery data on physicochemical and ADME properties of the approved kinase inhibitors is a monumental task as these are either scattered in the literature or have not been published.

Objective: Our goals were: 1) To compile the relevant data on all kinase inhibitors approved prior to 2016 for easy access by the biopharmaceutical community, 2) To provide a retrospective analysis to highlight trends and attributes which may have contributed to the "developability" of these drugs, and 3) To ignite focused debates on what constitutes "actionable", "nice-to-have", and unnecessary data. Such debates bring about more clarity on stage appropriateness of different types of information and prevent confusion due to abundance of unnecessary data, leading to more efficient and less costly drug discovery programs.

Methods: A careful and thorough analysis of different bodies of data such as published manuscripts, and available regulatory documents were employed.

Results: We were able to assemble a large body of data on the first thirty kinase inhibitors approved by US FDA since 2001.

Conclusion: In conclusion, we have compiled physicochemical and ADME data on the first 30 approved kinase inhibitors and provided our retrospective analysis, which we hope is helpful in constructing advancement criteria in discovery programs. The examination of this data provides an opportunity to develop an opinion on data prioritization and stage appropriateness of assays.

Keywords: Kinase, inhibitor, drug, ADME, physicochemical, discovery, approved, FDA, druggability.

1. INTRODUCTION

Protein kinases play pivotal roles in regulating all aspects of cellular function, including differentiation, metabolism, survival, programmed cell death and signal transduction [1]. Protein kinase pioneering research can be traced back to the 1950s [2]. Burnett and Kennedy first characterized protein kinase activities by using isolated rat mitochondria, in 1954 [3]. In 1955,

Fischer and Krebs, Sutherland and Wosilai first discovered and characterized a specific type of protein kinase, phosphorylase kinase catalyzing ATP-phosphorylation reaction [4-6]. The role of c-AMP, as the second messenger of hormonal signaling, in leading to the activation of phosphorylase was first reported by Sutherland's group in 1958 [7, 8]. Their research findings led to the discovery of protein kinase A (c-AMP dependent protein kinase), a protein-serine/threonine kinase in late 1960s [9]. As kinase research further advanced, emerging pharmacological research interests were focused on exploiting kinase malfunctions at the genetic level as well as connections between dysregulation of kinase

*Address correspondence to this author at the OROX BioSciences, Inc., 4971 Sterling Grove Lane, San Diego, CA 92130, USA; Tel: (860) 235-5260; E-mails: memo@oroxbios.com; memo1227@yahoo.com

pathways and various types of diseases. In 1960, an abnormal minute chromosome, called Philadelphia Chromosome, was discovered by Peter Nowell and David Hungerford [10, 11]. The Philadelphia Chromosome was the result of reciprocal translocation of chromosomes 9 and 22, generating an elongated chromosome 9 and a truncated chromosome 22. The translocation juxtaposes the Abl1 gene on chromosome 9 to a part of the BCR on chromosome 22 and leads to CML [10,12]. The translocated Abl1 gene, which encodes a tyrosine kinase, causes deregulated and continual overexpression of kinase activity resulting in tumor development. From this landmark discovery, it became evident that many human malignant diseases were associated with mutations, chromosomal rearrangements and/or overexpression of protein kinases [11, 13-16]. This discovery quickly led to protein kinases becoming well accepted targets for anticancer drug development [17-24]. During late 1980s and early 1990s, tremendous efforts were made to unfold the intracellular signal transduction pathways and aberrations of signaling pathways leading to variety of diseases at the genetic and molecular levels [25-30]. Many extra- and intracellularly associated kinases, such as MAPK, ERK, JAK and PI3K, were reported to regulate normal cellular functions [31, 32]. So far, a total of 518 human kinases and 900 human genes encoding for kinase proteins have been revealed [33]. In the meantime, it has been discovered that deregulation and/or overexpression of certain types of kinases lead to changes in the normal cellular functions which further advance to disease states. As evident by a number of marketed drugs and a substantial number of publications, kinase inhibitors can be used for the treatment of various types of cancers and inflammatory diseases (Table 1).

Imatinib was the first kinase inhibitor approved to treat chronic myelogenous leukemia [34, 35]. It was approved in 2001, by the FDA and was marketed by Novartis as Gleevec (USA). Imatinib competes with ATP for the ATP binding site of TK (selective inhibitor of the BCR-Abl TK domain, also inhibits proto-oncogene c-kit and PDGF-R) and has since become the model drug for protein kinase-targeted drug discovery and development [36, 37]. As the understanding of the role of protein kinases in signaling pathways continues to grow, the uses of protein kinase inhibitors are also expanding to include the treatment of inflammation, diabetes, infectious and cardiovascular disease [38]. At present, approximately 150 kinase-targeted compounds are in clinical development and many more are in various stages of preclinical discovery and development [39]. The FDA has approved a total of 30 kinase-

targeted drugs between the years 2001 and October 2015 (Table 1 and Fig. 1). Among the 30 approved kinase inhibitors, 27 are used for the treatment of malignancies and 3, pirfenidone (p38 mitogen-activated protein kinase inhibitor), tofacitinib (selective JAK1/JAK3 kinase inhibitor) and nintedanib (multi-kinase inhibitor), are used for the treatment of inflammatory diseases. FDA approval rate for kinase inhibitors was approximately one per year from 2001 to 2010. This increased significantly to approximately five per year from 2011 to 2015. The current trend in kinase-targeted drug development suggests that 1) more efforts will be spent in further exploration of the kinase inhibitor space, 2) the therapeutic indications will expand to cover a broad spectrum of diseases ranging from cancer and inflammation to metabolic, immune, cardiac and CNS disorders, 3) the therapeutic use of combinations of kinase inhibitors and other targeted agents will become the focus for disease treatment and management, 4) there is an urgent need to explore new pharmacophores to diversify the currently available chemical scaffolds for kinase inhibition, 5) multi-kinase inhibitors and single kinase inhibitors with polypharmacology will be needed to produce more efficacious treatment regimens, and 6) major challenges reside in the development of kinase inhibitors with better selectivity, more specificity to driver mutations in diseases, better control of resistance development and less side effects [38-41].

In today's fast-paced and competitive environment, there is an ever-increasing need for more effective drug design by fully balancing potency, selectivity, molecular properties and appropriate ADME properties early in drug discovery. However, incorporating drug-like properties at the onset is often a challenging task. Understanding the molecular and ADME properties of successful drugs provides helpful insight in defining parameters to facilitate this process. In 2013, we published our detailed analysis on molecular and ADME properties of the first 14 kinase inhibitors approved by FDA between 2001-2011 [42]. Herein, we add the next 16 approved kinase inhibitors and summarize our systematic analysis of the common physicochemical and ADME properties of all 30 marketed kinase inhibitors. Although the dataset is still limited, we believe this retrospective analysis provides a valuable insight into what attributes are more crucial to the success of kinase inhibitor discovery and development programs. Furthermore, this work allows us to determine whether the new generation of kinase inhibitors is significantly different than the first set.

Table 1. Kinase-targeted inhibitors approved by the FDA from January 2001 to October 2015.

No.	Drug	Trade Name	Company	Approval Date	Targeted Kinases	Therapeutic Area	Human Efficacious Dose (mg/day)
1	Imatinib	Gleevec	Novartis	2001	Bcr-Abl tyrosine kinase, PDGRR	Chronic Myeloid Leukemia (CML)	400-800, QD
2	Gefitinib	Iressa	Astra-Zeneca	2003	Selective EGFR tyrosine kinase (EGFR-TK1)	Non-small-cell lung cancer (NSCLC)	250, QD
3	Erlotinib	Tarceva	OSI	2004	EGFR tyrosine kinase (EGFR-TK1)	Non-small-cell-lung cancer (NSCLC)	150, QD
4	Sorafenib	Nexavar	Bayer	2005	C-RAF, B-RAF, c-KIT, FLT3, VEGFR2, -3, PDGFR- β	Advanced renal cell carcinoma (RCC) and unresectable hepatocellular carcinoma (HCC)	400, BID – 800, QD
5	Dasatinib	Sprycel	BMS	2006	Bcr-Abl, SCR-family kinases, PDGFR β , c-KIT, ephrin (EPH) receptor kinases	Chronic myeloid leukemia (CML) and Philadelphia Chromosome Positive (Ph+)	140, BID
6	Sunitinib	Sutent	Sugen (Pfizer)	2006	PDGFR α , - β , VEGFR1, -2, -3, c-KIT, RET, CSF-1R, FLT3	Gastrointestinal stromal tumor (GIST) and advanced renal cell carcinoma (RCC)	50, QD
7	Lapatinib	Tykerb	GSK	2007	ErbB2 (HER2) and Erb1 (EGFR) tyrosine kinases	Advanced or metastatic breast cancer	1250, QD
8	Nilotinib	Tasigna	Novartis	2007	Bcr-Abl, c-KIT, PDGFR α , - β	Chronic Myeloid Leukemia (CML) and Philadelphia Chromosome positive (Ph+)	600 – 800, QD
9	Pirfenidone	Glaspira	Marnac	2008	Antifibrotic P38 MAP kinase and TGF β and TNF α synthesis	Idiopathic pulmonary fibrosis (IPF)	2403, TID
10	Pazopanib	Votrient	GSK	2009	VEGF receptor tyrosine kinase, PDGFR/c-Kit	Advanced cell carcinoma	200 – 400, QD
11	Crizotinib	Xalkori	Pfizer	2011	Anaplastic lymphoma kinase receptor; Ros1 tyrosine kinase receptor	Non-small-cell lung cancer; anaplastic large cell lymphoma	250, BID
12	Vemurafenib	Zelboraf	Plexxikon (Daiichi Sankyo)	2011	Raf B protein kinase	Thyroid tumor; melanoma	960, BID
13	Vandetanib	Zactima	Astra-Zeneca	2011	EGFR family tyrosine kinase receptor, VEGF-2-receptor	Thyroid tumor; Small-cell lung cancer; Multiple myeloma	300, QD
14	Ruxolitinib	Jakafi	Incyte	2011	Jak1 and 2 tyrosine kinases	Multiple myeloma; inflammatory disease	15 25, BID
15	Axitinib	INLYTA	Pfizer	2012	Selective VEGFR tyrosine kinase, PDGFR, c-Kit	Advanced Renal Cell Carcinoma	5-10, BID
16	Bosutinib	Bosulif	Pfizer	2012	Tyrosine kinase, specifically an inhibitor of Bcr-Abl and Src-family kinases	Chronic, accelerated or blast phase Ph+ chronic Myelogenous Leukemia	500, QD
17	Regorafenib	Stivarga	Bayer	2012	EVEGFR, EGF, KIT, RET, RAF, KIT, RET, RAF1, BRAF and BRAFV600E and FGFR1	Metastatic colorectal cancer	160, QD

(Table 1) contd....

No.	Drug	Trade Name	Company	Approval Date	Targeted Kinases	Therapeutic Area	Human Efficacious Dose (mg/day)
18	Tofacitinib	Xeljanz	Pfizer	2012	JAK1/JAK3 kinases	Rheumatoid Arthritis (RA)	5-10, BID
19	Cabozantinib	Cometriq	Exelixis	2012	Multi-targeted inhibitor of receptor tyrosine kinases (RTKs)	Thyroid cancer (MTC)	140, QD
20	Ponatinib	Iclusig	Ariad	2012	Tyrosine kinase (BCR-ABL), FLT3	Chronic phase, accelerated phase or blast phase chronic myeloid leukemia (CML) or Ph+ALL acute lymphoblastic leukemia	45, QD
21	Trametinib	Mekinist	GSK	2013	Mitogen-activated extracellular signal regulated kinase ½ (MEK1 and MEK2)	Metastatic Melanoma	2, QD
22	Dabrafenib	Tafinlar	GSK	2013	B-raf kinase (BRAF)	Metastatic Melanoma	150, BID
23	Afatinib	Gilotrif	Boehringer Ingelheim	2013	EGFR, HER2 and HER4 tyrosine kinases	Non-Small Cell Lung Cancer	40, QD
24	Ibrutinib	Imbruvica	Janssen	2013	Bruton's tyrosine kinase (BTK)	Non-Hodgkin's Lymphoma Leukemia, Chronic Lymphatic Leukemia	560, QD
25	Ceritinib	Zykadia	Novartis	2014	Anaplastic Lymphoma Kinase (ALK)	Non-Small Cell Lung Cancer	750, QD
26	Idelalisib	Zydelig	Gilead Sciences	2014	Phosphoinositide 3-kinase (PI3Kδ)	Refractory indolent non-Hodgkin's Lymphoma, Relapsed Chronic Lymphocytic Leukemia, Follicular Lymphoma	150, BID
27	Nintedanib	Ofev	Boehringer Ingelheim	2014	Tyrosine kinase, Flt-3, Lck, Lyn and Src kinases	Idiopathic Pulmonary Fibrosis	100/150, BID
28	Palbociclib	Ibrance	Pfizer	2015	Cyclin-dependent kinases 4 and 6 (CDK4 and CDK6)	Breast Cancer	125, QD
29	Lenvatinib	Lenvima	Eisai	2015	Multiple receptor tyrosine kinase (RTK)	Thyroid Cancer	24, QD
30	Co-bimetinib	Cotellic	Genentech	2015	MEK tyrosine/serine/threonine protein kinase	Metastatic Melanoma	60, QD

2. RETROSPECTIVE ANALYSIS OF AVAILABLE DATA ON THIRTY MARKETED KINASE INHIBITORS

2.1. Structural and Physicochemical Properties

2.1.1. MW, Log P, H-bond Acceptors/Donors/ Rotations, and PSA

Physicochemical properties considered for lead optimization include hydrogen bonding, lipophilicity, molecular weight, pKa, PSA, shape and reactivity. Fig. (2) depicts the relationship and interplay of physicochemical/biochemical properties and *in vivo* drug pharmacokinetic and dynamic processes. One way to isolate the more impactful physicochemical factors in drug

discovery is to examine the marketed drugs and their attributes.

Christopher Lipinski *et al.* published the analysis of 2245 marketed drugs and drug candidates in clinical trials and their guidelines in 1997 [43]. This guideline, commonly referred to as the Lipinski "Rule of Five" (RO5), states that ideally an orally active drug has a MW<500 daltons, Log P≤5, H-bond donors ≤5, and H-bond acceptors≤10. Lipinski RO5 was used to enable the selection of compounds more likely to become orally bioavailable drugs based on early discovery data. Daniel Veber *et al.* examined over 1100 drug candidates at SmithKline Beecham Pharmaceuticals (now GlaxoSmithKline) and analyzed the relationship

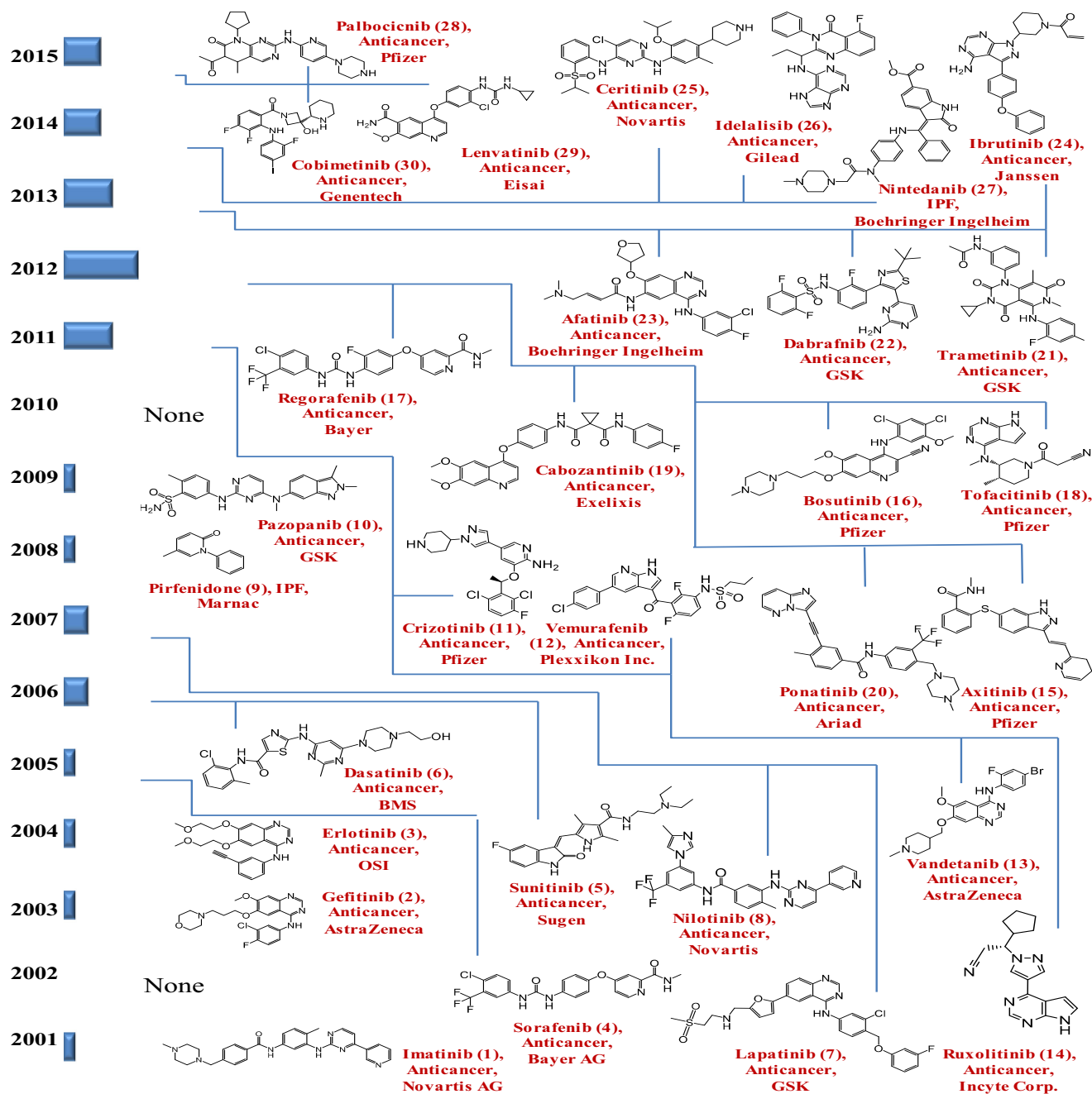


Fig. (1). Thirty FDA approved kinase inhibitors from January 2001 to October 2015.

between physicochemical properties and rat bioavailabilities [44]. Veber found that compounds with total hydrogen bonds ≤ 12 , rotatable bonds ≤ 10 and PSA ≤ 140 tend to have oral bioavailability $\geq 20\%$ in rats. This analysis gave rise to Veber's rules, which complement Lipinski's RO5 and enhance drug discovery efficiency. Verber's analysis was based only on compounds with rat bioavailability data. Mark Wenlock [45] reported on the limitation inherent in the compound collections used to give rise to Lipinski's and Veber's rules and suggested that following these rules was likely to lead to lead-like molecules rather than

drug-like molecules. Furthermore, they concluded that the mean MW and lipophilicity of orally administered compounds decreased with NCE's that progressed further down discovery/development path and eventually converge towards the mean values of marketed drugs.

We performed a trend analysis on the physicochemical properties of 30 FDA approved kinase inhibitors and identified the commonality of their structural properties. For the 30 FDA approved kinase inhibitors, structural properties were either measured or predicted using ACD software (Advanced Chemistry Development, Inc., Toronto, Ontario, Canada) and GastroPlus

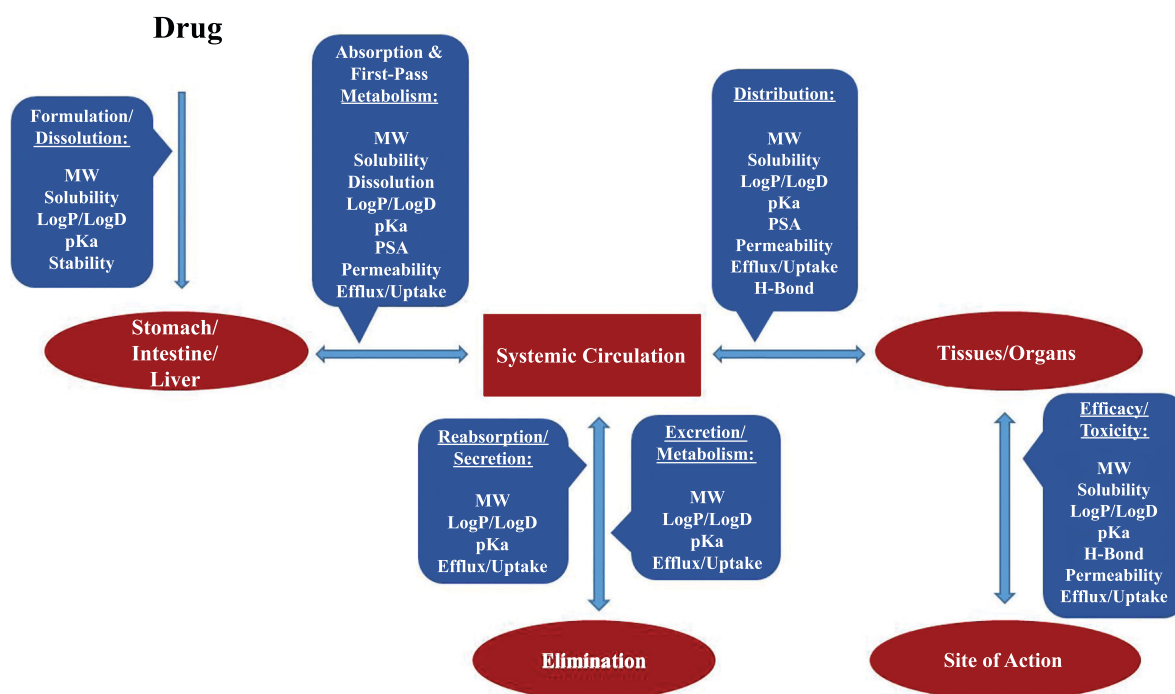


Fig. (2). Relationship and interplay of physicochemical properties and *in vivo* drug kinetic/dynamic processes following oral administration.

ADMET Predictor software (Simulation Plus Inc., Lancaster, CA).

2.1.1.1. Analysis

Of the 30 kinase inhibitors ~30% violated Lipinski's RO5 with molecular weights slightly over 500 daltons (Fig. 3A). The measured lipophilicity (LogP values) was not available for eight of the drugs. For the remaining ones, ~20% violated RO5 with LogP>5 (Fig. 3B). The same level of violation was observed when using predicted LogP for all 30 drugs (Fig. 3C). Overall, ~80% of the drugs had LogP values between 1-5. While all thirty followed RO5 by having ≤ 5 H-bond donors, ~97% actually had ≤ 3 H-bond donors (Fig. 3D). While all 30 followed RO5 and had ≤ 10 H-bond acceptors, ~85% actually had ≤ 8 H-bond acceptors (Fig. 3E).

As shown in (Fig. 4), all of the drugs followed Veber's rule by having ≤ 12 total hydrogen bonds. With one exception, all drugs had ≤ 10 rotatable bonds. With regards to PSA, 29 drugs followed Veber's rule and had PSA ≤ 140 , however, ~73% of them actually had PSA ≤ 100 (Fig. 4C).

2.1.2. pKa

pKa is an important parameter, which has not received adequate attention from Lipinski and Weber. pKa is a measurement of the ionization potential of a compound and depends on ionizability of different

functional groups within a molecule. Based on the pKa values on the strongest ionizable groups, NCEs can be classified into acidic, neutral and basic compounds. *In vivo*, whether NCEs remain in their neutral state or ionized form is influenced by the physiological pH in a given environment. For a given compound, solubility consists of both intrinsic solubility in the neutral state and apparent solubility of its ionized form at the local physiological pH. Oral absorption is affected by dissolution rate, apparent solubility in gastrointestinal tract, and permeability and stability throughout different regions of GI tract. Because pKa determines the ionization state of an NCE, it is a critical factor affecting the rate and extent of oral absorption from the GI tract therefore influencing its bioavailability. A highly-ionized molecule tends to have higher solubility and dissolution, but because ions cannot easily pass through membrane lipid bilayer, that same trait might compromise the ability of that molecule to pass through gut cells and into circulation; resulting in low bioavailability.

Tissue distribution of basic molecules has been in part related to their pKa values. Since the mid-1970s, many researchers have investigated the *in vivo* distribution profiles of basic molecules, including their whole body and intracellular distributions, and their interactions with factors leading to pharmacology and toxicity [46-50]. Their findings suggest that strongly basic amines tend to be sequestered in acidic organelles such

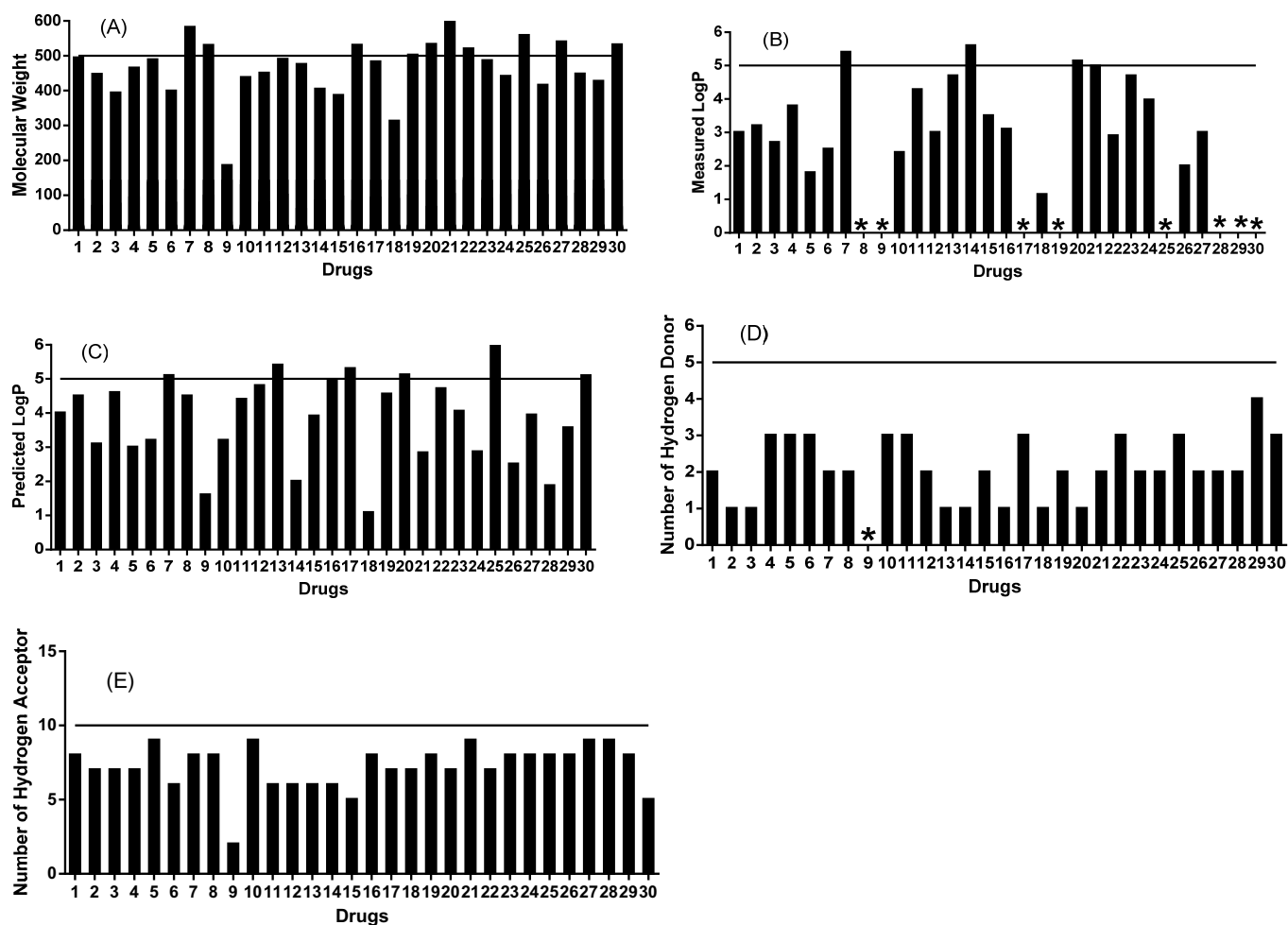


Fig. (3). Structural properties of the 30 approved kinase inhibitors follow Lipinski's Rule of Five. Each kinase inhibitor is shown on the X-axis with the numbering system used in table 1. **(A)** 30 out of 30 had molecular weight (MW) <600 Daltons while 20 out 30 had MW \leq 500 Daltons; **(B)** 19 out of 22 kinase inhibitors had measured lipophilicity LogP values \leq 5.0; **(C)** 17 out of 22 kinase inhibitors had measured lipophilicity LogP values \leq 5.0; **(D)** 29 out 29 had total number of hydrogen donor \leq 5 while 28 out of 29 had \leq 3.0 ; **(E)** all 30 kinase inhibitors had a total number of hydrogen acceptor \leq 10. * symbolize the kinase inhibitors without reported values.

as lysosomes and bind to tissue membrane phospholipids. These result in high volumes of distribution, highly localized intracellular concentrations, and possible alterations of PK behavior and potential toxicity [46, 47, 50]. In addition, molecules with strong basicity tend to dissolve in gastric fluid but precipitate under intestinal environment with more basic pH; thereby resulting in low absorption owing to low solubility and slow dissolution rates at the site of absorption.

Together with LogP, plasma protein binding and blood-to-plasma partition ratio, the pKa value can be a predictor of intracellular distribution profiles of NCEs. These factors directly affect absorption, distribution, metabolism, elimination and toxicity (ADMET) properties and, in turn, systemic and target tissue exposures (Fig. 2) [46-51].

2.1.2.1. Analysis

A wide range of weak to strong basicity was observed for the approved kinase inhibitors. We summarized the measured and predicted pKa values for the 30 kinase inhibitors (Fig. 5). Measured pKa values were not available in 30% of compounds. GastroPlus software was used to predict the most basic pKa values for all 30 drugs. The most basic pKa of pirfenidone and trametinib could not be determined because they lacked basic nitrogens. The predicted pKa's were in good agreement with the measured values when available. Examination of the predicted values revealed that ~64% of these drugs had a pKa \leq 8, ~29% had pKa values within 8-9, and ~ 7% with pKa values >9 (Fig. 5).

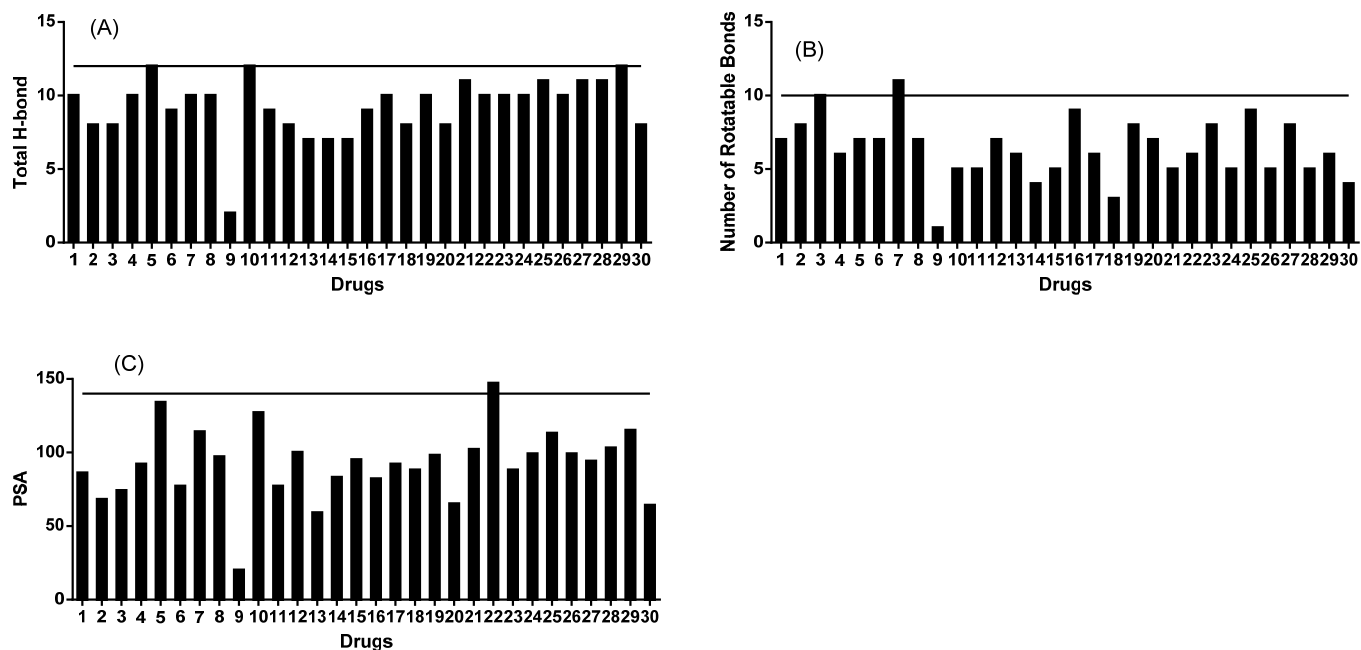


Fig. (4). Structural properties of 30 approved kinase inhibitors follow Veber's Rule. Each kinase inhibitor is shown on the X-axis with the numbering system used in table 1. **(A)** all kinase inhibitors had a total H-bond ≤ 12 while 23 out of 30 ≤ 10 ; **(B)** 30 out of 30 had a total number of rotatable bonds ≤ 12 while 29 out of 30 ≤ 10 ; **(C)** Twenty-nine kinase inhibitors had polar surface area (PSA) values ≤ 140 while 22 out of 30 ≤ 100 .

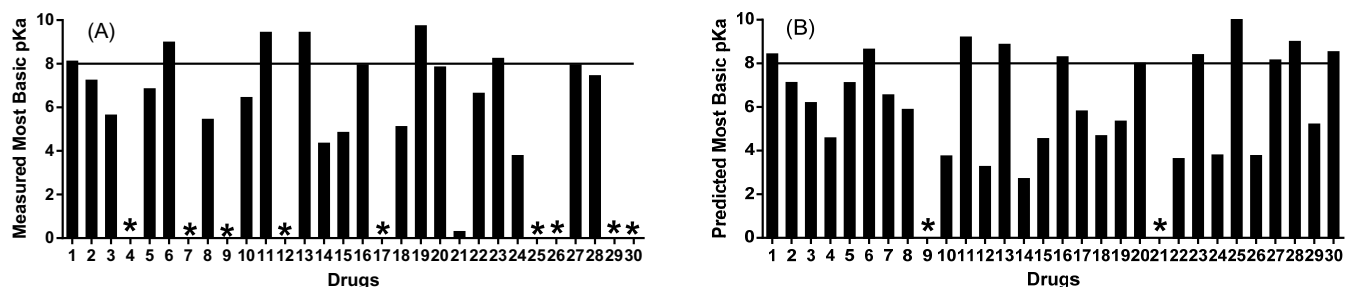


Fig. (5). Structural properties of 30 approved kinase inhibitors (predicted using GastroPlus software and measured most basic pKa values). Each kinase inhibitor is shown on the X-axis with the numbering system used in table 1. **(A)** 15 out of 21 kinase inhibitors had measured most basic pKa values ≤ 8.0 while 18 out of 21 ≤ 9.0 **(B)** 18 out of 28 kinase inhibitors had predicted most basic pKa values ≤ 8.0 while 26 out of 28 ≤ 9.0 . * symbolize the inhibitors without reported values

2.1.3. Solubility, Permeability, and Efflux

Following oral administration, successful delivery of a drug to the site of action requires adequate absorption from the GI tract into the systemic blood circulation. Solubility, permeability, and efflux potential are the primary properties which impact the rate and the extent of oral absorption. These parameters have been used to categorize NCE's according to BCS guidelines provided by US FDA [52]. Chemical and metabolic stability are also important factors which are beyond the scope of this review.

In brief, BCS classification is based primarily on solubility and permeability. Drugs can be grouped into

four categories. Class I and II refer to high permeability drugs with high and low solubility, respectively. Class III and IV refer to low permeability drugs with high and low solubility.

2.1.3.1. Analysis

Twenty-nine drugs had reported values for *in vitro* Caco-2 permeability (Papp A-B) and solubility (Table 2). For these, BCS characterization data revealed that $\sim 10\%$ fell into BCS Class I and exhibited high solubility and high permeability, $\sim 52\%$ fell into BCS Class II and exhibited low solubility but high permeability, $\sim 7\%$ fell into BCS Class III with high solubility but low permeability, while $\sim 27\%$ had low solubility and

Table 2. Measured permeability and solubility of approved kinase-targeted inhibitors.

No.	Drug	Papp A-B ^a (x 10 ⁻⁶ cm/sec)	Solubility ^b (mg/mL at pH)	BCS Class	References
1	Imatinib	0.95 at 1 μM, 7.9 at 50 μM	Very soluble	I	[53]
2	Gefitinib	15.5 at 3 μM	1 at pH 1.2 - 4.0, 0.43-0.01 at pH 5.0 - 6.0, < 0.01 at pH 6.8-8.0	II	[54]
3	Erlotinib	34 at 10 μM	0.4 at pH 2.0, pH dependent solubility	II	[55]
4	Sorafenib	16.4 at 0.1 μM, 33.5 at 1 μM	Low solubility	II	[56, 127]
5	Dasatinib	10.2 at 50 μM	~18 at pH 2.6, <0.001 at pH 7.0	II	[57]
6	Sunitinib	2.20 at 1 μM, 3.81 at 10 μM	25 at pH 1.0-6.8, low solubility at pH > 6.8	IV	[58]
7	Lapatinib	Low permeability	0.007 in water, 0.001 at pH 1.1	IV	[59, 60]
8	Nilotinib	Moderate permeability	Insoluble in buffer solutions of pH ≥ 4.5, pH dependent solubility	IV	[61]
9	Pirfenidone	NA	Sparingly soluble in water at any pH	NA	[62]
10	Pazopanib	17.6 at 1 μM, 14.9 at 3 μM, 18.1 at 10 μM	Slightly soluble at pH 1.0, Insoluble above pH 4.0	II	[63, 64]
11	Crizotinib	Low permeability, <LOQ at 1-10 μM	Low solubility, pH dependent solubility	IV	[65]
12	Vemurafenib	2.9	Insoluble in aq. media (<0.0005) across the pH range of 1 to 7.5	IV	[69]
13	Vandetanib	17.9 at 1 μM, 23.8 at 10 μM	Highly soluble at pH ≤ 6.0, Low solubility at pH > 6.0	II	[68]
14	Ruxolitinib	21.5 at 10 μM	0.52 at pH ≤ 3.3, 0.15 at pH=7.5	I	[66, 67]
15	Axitinib	13.8	1.8 at pH 1.1, 0.0002 at pH ≥ 6.0	II	[70]
16	Bosutinib	2.08 at 1 μM, 2.96 at 10 μM	11.03 at pH 1.0, 0.02 at pH 6.8	IV	[71]
17	Regorafenib	12.4	Low solubility across the pH range of 1 to 7.5	II	[72]
18	Tofacitinib	Moderate permeability	3.48 - >28 at pH 1.0-3.9, 0.20 - 0.59 at pH 4.53 - 8.0	III	[73, 74]
19	Cabozantinib	Highly permeable	Low solubility	II	[75, 109]
20	Ponatinib	4.4	Highly soluble at pH < 1.7, Slightly soluble at pH 1.7-2.7, Insoluble > pH 2.7	IV	[76]
21	Trametinib	16.2-59.5 at pH 7.4, MDCKII-MDR1	Low solubility at pH 1.0 - 8.0	II	[77]
22	Dabrafenib	High permeability - MDR1-MDCK	0.043 at pH 1.2, 0.068 at pH 4.9 FeSSIF, 0.0062 at pH 6.3 FaSSIF	II	[78]
23	Afatinib	Higher permeability	>50 at pH less than 6.0, >1 at pH 6-7, 0.04 at pH > 7.0	I	[79]
24	Ibrutinib	57.9	2 at pH 1.2, 0.003 at pH 6.0	II	[80]
25	Ceritinib	Low permeability	11.9 at pH 1.2, 0.01 at pH 6.8, pH dependent solubility	IV	[81, 82]
26	Idelalisib	High permeability	at pH 1.2, <0.1 at pH 7.7	II	[83]
27	Nintedanib	High permeability	>1 at acidic media, <0.001 at pH ≥ 7.0	II	[84, 85]
28	Palbociclib	High permeability	High solubility at pH < 4.0, Solubility significantly reduced at pH > 4.0	II	[86, 87]
29	Lenvatinib	NA	pH dependent solubility	II, IV	[88]
30	Cobimetinib	18.5-5.1, MDCK moderate-high permeability	48 at pH 1.9, 0.78 at pH 6.8	III	[89]

^a Apparent permeability was determined using Caco-2 assays at pH 7.4. Permeability was categorized in comparison to the permeability values obtained from low and high permeability standards recommended by FDA, which were run in parallel with the corresponding drugs in each study to calibrate permeability boundary of each drug substance.

^b Solubility was categorized based on FDA guideline: a drug substance is considered highly soluble when the highest dose strength is soluble in 250 mL or less aqueous media at pH range of 1 to 7.5.

Table 3. Enzymes and transporters involved in pharmacokinetics of kinase inhibitors.

No.	Drug	Major CYP450 enzymes responsible for kinase inhibitor metabolism	Major transporters responsible for efflux transport	References
1	Imatinib	CYP3A4	P-gp and BCRP	[53, 138]
2	Gefitinib	CYP3A4 and CYP2D6	P-gp and BCRP	[54]
3	Erlotinib	CYP3A4/5 and CYP1A1	P-gp and BCRP	[55]
4	Sorafenib	CYP3A4	BCRP	[56, 90, 140]
5	Dasatinib	CYP3A4	P-gp and BCRP	[57]
6	Sunitinib	CYP3A4	P-gp and BCRP	[58]
7	Lapatinib	CYP3A4/5	P-gp and BCRP	[59, 60]
8	Nilotinib	CYP3A4 and CYP2C8	P-gp and BCRP	[61]
9	Pirfenidone	CYP1A2	None	[62]
10	Pazopanib	CYP3A4	P-gp	[63, 64]
11	Crizotinib	CYP3A4/5	P-gp	[65]
12	Vemurafenib	CYP3A4	P-gp and BCRP	[69]
13	Vandetanib	CYP3A4	BCRP	[68]
14	Ruxolitinib	CYP3A4	None	[67]
15	Axitinib	CYP3A4	P-gp and BCRP	[70]
16	Bosutinib	CYP3A4	P-gp, BCRP and MRPs	[71]
17	Regorafenib	CYP3A4 and UGT1A19	None	[72]
18	Tofacitinib	CYP3A4 and CYP2C9	P-gp	[73, 74]
19	Cabozantinib	CYP3A4, CYP2C9, UGT, ST and amidase	None	[75, 109]
20	Ponatinib	CYP3A4, CYP2C8, CYP2D6 and CYP3A5	P-gp and BCRP	[76]
21	Trametinib	Primarily non-CYP mediated metabolism, CYP3A4 plays minor role	None	[77]
22	Dabrafenib	CYPs 2C8, 3A4 and 2C9	P-gp and BCRP	[78]
23	Afatinib	Mainly CYP3A4, FMO and UGT	P-gp	[79]
24	Ibrutinib	CYP3A4	None	[80]
25	Ceritinib	CYP3A4/5	P-gp and BCRP	[81, 82]
26	Idelalisib	CYP3A4 and UGT1A4	P-gp and BCRP	[83, 125]
27	Nintedanib	Esterase, CYP3A4 and UGT	P-gp and OCT-1	[84, 85, 116]
28	Palbociclib	CYP3A and SUL1A1	P-gp and BCRP	[86, 87]
29	Lenvatinib	AO, CYP3A and GST	P-gp, MDR1 and BCRP	[88]
30	Cobimetinib	CYP3A4 and UGT2B7	None	[89]

low to moderate permeability (BCS Class IV). Only one drug, lenvatinib, had inclusive classification and could fall into either Class II or IV category [53-89, 109, 127].

Uptake and efflux transporters can also play a role. Six major uptake and efflux transporters are P-gp, BCRP, BSEP, OATP, OAT and OCT. The interaction of these transporters with the approved kinase inhibitors was evaluated. In general, it was determined that

~80% of these drugs were substrates for one or more efflux transporters with the majority interacting with P-gp and BCRP efflux transporters (Table 3) [53-89, 90, 109, 116, 125, 140].

The above data can be summarized as follows: 1) ~65% were Class I and II drugs with high permeability, 2) ~83% showed poor solubility, and 3) 80% were substrates of P-gp and/or BCRP efflux transporters. It is noteworthy that 30% of these drugs had poor solubility

and poor permeability. While formulation attempts to combat poor solubility/dissolution to improve exposure levels have had some success, similar attempts aimed at improving permeability without damaging intestinal epithelial membranes have proven challenging. It is also important to point out that *in vitro* Caco-2 and MDCK system may underestimate *in vivo* permeability through membranes due to their intrinsic flaws of tighter junction and lack of adequate expression of uptake transporters. Finally, efflux transporters can be saturated in the GI tract at higher dose level or with high apparent intestinal solubility. These factors might explain satisfactory exposure levels of these drugs despite their low solubility and poor permeability data from *in vitro* assays.

3. PHARMACOKINETICS

3.1. Absorption

As eluded to previously, the overall oral exposures usually reflect an interplay of systemic CL (metabolism in gastrointestinal tract and liver, and other organs in addition to excretion), permeability, solubility, efflux/uptake transporters, and dissolution rate in small intestine as well as chemical stability. During lead optimization, systemic CL is the most important guides for compound selection industry-wide [91,128]. This parameter is always judged against liver blood flow (LBF) to be binned as rapid, moderate, or slow CL. It is customary to characterize CL values as rapid if >70% LBF. LBF values have been reported to be 90 mL/min/kg in the mouse, 60 mL/min/kg in the rat, 31 mL/min/kg in the dog, 44 mL/min/kg in the monkey [92-94]. As a general trend, kinase inhibitors with acceptable F% tend to have slow to moderate CL (Tables 4 and 5). However, ~26% of these drugs were cleared rapidly in one or more preclinical species, nonetheless the oral plasma exposures for the majority of these drugs appeared to be reasonable with F% values $\geq 20\%$ (Tables 4 and 5). Nevertheless, it is critical to emphasize that CL is still a critical factor in advancement of compounds in discovery as it is ultimately a determinant of dose.

In general, these kinase inhibitors reached C_{max} relatively rapidly (T_{max} between 0.5 to 4 hr) across all species (Table 4). About 76% of these inhibitors had F% values greater than 20% across all reported species. High solubility and/or high permeability in GI tract might overwhelm intestinal and even hepatic first-pass clearance mechanisms. This might off-set the negative impact of rapid clearance to oral exposures and salvage bioavailability normally impaired by the rapid clear-

ance. This could be the case for kinase inhibitors in BCS class I (high permeability and high solubility) and II (high permeability and low solubility) (Table 2). The available data cannot offer a good explanation for bosutinib (BCS Class IV), which exhibited poor permeability, poor solubility, and moderate to rapid CL (Tables 2, 4 and 5).

3.2. Distribution

Distribution of drugs into the body can be studied by various methods. It can be estimated by calculating volume of distribution at steady state (V_{ss}), during PK modeling, tissue extraction followed by quantitation of the drug, or by using tissue imaging techniques such as MALDI-TOF using non-labeled drugs, or whole body autoradiography using radiolabeled drugs. V_{ss} is a commonly used theoretical parameter to reflect the extent of drug distribution throughout the body. It is devoid of actual physiological meaning, but provides an overall understanding of distribution. In relation to total body water volume (~ 0.7 L/kg), V_{ss} can be classified into three categories: small (<0.7 L/kg), moderate (0.7 – 3.5 L/kg) and large (> 3.5 L/kg) [95].

All 30 kinase inhibitors were well distributed into various tissues, including liver, kidney, lung, gastrointestinal tract and glandular tissues. Also, the majority exhibited limited CNS penetration [53-63,65-69, 90, 96, 97, 99,100-118]. Their V_{ss} values ranged from 0.28 L/kg to 43.6 L/kg (Table 5). In rodents, majority of these drugs fell into small to large V_{ss} category, ranging from less than total body water volume (~ 0.7 L/kg) to greater than 10 L/kg. Our limited data analysis (Tables 4 and 5) suggested that basic kinase inhibitors (~33%) with the most basic pK_a > 9 tend to have V_{ss} > 10 L/kg, which may pose a risk for undesirable effects due to higher tendency for tissue accumulation, ie. in the heart. Lipophilicity (logP) and plasma protein binding may also be indicators of large V_{ss}. However, in the overall evaluation of pK_a, logP and PPB and their relationship to V_{ss} it was observed that pK_a was a greater correlate to V_{ss}.

MRT describes the sojourn of drug in the body and correlates with t_{1/2}. The MRT values reported here were estimated using clearance and steady-state volumes of distribution. MRT values ranged from 0.11 to 42 hr with majority of the drugs having MRTs around 2-5 hr in mice, rats, dogs and monkeys (Table 5). The trend analysis on V_{ss} and MRT for these kinase inhibitors revealed that V_{ss} ≤ 10 L/kg and MRT ≥ 2 hr in mice, rats, dogs and monkeys were prevalent in this class of drugs. However, because ~30% of these approved

Table 4. Oral pharmacokinetic parameters of the kinase inhibitors.

Drug (Dose (mg/kg) in Mouse/Rat/ Dog/Monkey)	Cmax (ng/mL)				Tmax (hr)				AUC (ng.hr/mL)				Bioavailability (%)				Reference	
	Ms	Rt	Dg	Mnk	Ms	Rt	Dg	Mnk	Ms	Rt	Dg	Mnk	Ms	Rt	Dg	Mnk		
Imatinib (50/10/10/3)	6990	870	60- 1410	40	NA	2.0	2-4	2.5	12820	4030	250- 12400	216	28	53	29- 68	27	[96]	
Gefitinib (NA/5/5/NA)	NA	164	357- 510	NA	NA	2.0	2-4	NA	NA	1440	3380- 3882	NA	NA	44	49- 64	NA	[142]	
Erlotinib (NA/NA/10/NA)	NA	NA	650	NA	NA	NA	1.0	NA	NA	NA	4792	NA	NA	NA	45 -	88	NA	[90]
Sorafenib (NA)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79	79	60	NA	[146]	
Dasatinib (15/10/3/5)	156	239	146	166	2.0	2.3	0.75	0.60	586	1854	683	366	14	27	34	15	[103]	
Sunitinib (NA)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	100	NA	41	[104]	
Lapatinib (10/10/10/NA)	504	288	555	NA	0.5	0.5	4.0	NA	1735	861	5916	NA	50	24	42	NA	[143]	
Nilotinib (25/20/10/NA)	7910	1740	518	NA	0.5	4.0	NA	2.7	36100	26100	3880	NA	43	34	NA	24	[144]	
Pirfenidone (NA/50/18/NA)	NA	45100	11700	NA	NA	0.35	0.25	NA	NA	1706000	711000	NA	NA	52	81	NA	[62]	
Pazopanib (10/10/1/50)	19500	17267	810	30310	6.0	0.8	3.5	0.9	220200	70429	5332	141017	NA	72	47	30	[145]	
Crizotinib (NA/10/10/NA)	NA	220	938	NA	NA	8.0	6.0	NA	NA	3160	17600	NA	NA	26- 63	38- 66	44	[99, 100]	
Vemurafenib (NA/30/ 24.5/NA)	NA	NA	NA	NA	NA	NA	NA	NA	NA	70.5-172	62.1	NA	NA	18- 43	40	NA	[102]	
Vandetanib (NA/10/20/NA)	NA	326	267	NA	NA	2.0	3.3	NA	NA	5298	4930	NA	NA	55	56	NA	[101]	
Ruxolitinib (NA/50/10/NA)	NA	970	3522	NA	NA	NA	2.0	NA	NA	2184	15730	NA	NA	29	57	NA	[67]	
Axitinib (NA)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	16	3- 31	10- 59	3	[105]	
Bosutinib (50/50/5/NA)	1509	224	206	NA	4.0	3.0	1.3	NA	11677	1507	3091	NA	53	23	50	NA	[106]	
Regorafenib (NA/0.5/1.0/NA)	NA	277	369	NA	NA	6.0	2.67	NA	NA	2830	2460	NA	NA	85	67	NA	[107, 141]	
Tofacitinib (NA/10/5/5)	NA	261- 670	1020	791	NA	0.5	0.5	1.1	NA	462- 1138	2330	2280	NA	12- 17	43	48	[108]	
Cabozantinib (NA/5/3/3)	NA	4584- 7623	4278- 6118	20.2- 491	NA	4.0	4.0	2.0- 3.0	NA	76731- 144435	51154- 69208	547- 3175	NA	66- 90	51- 55	13- 73	[109]	
Ponatinib (NA/15/NA/2-3)	NA	453	NA	96	NA	6.0	NA	4.0	NA	8320	NA	942	NA	54	NA	21	[110]	

(Table 4) contd....

Drug (Dose (mg/kg) in Mouse/Rat/ Dog/Monkey)	Cmax (ng/mL)				Tmax (hr)				AUC (ng.hr/mL)				Bioavailability (%)				Reference
	Ms	Rt	Dg	Mnk	Ms	Rt	Dg	Mnk	Ms	Rt	Dg	Mnk	Ms	Rt	Dg	Mnk	
Trametinib (3/3/0.3/NA)	1662	289	80	NA	NA	NA	NA	NA	14462	3754	1723	NA	111	42	86	NA	[111]
Debrafenib (10/4.0/0.7/1.0)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	70	77	82	46	[131]
Afatinib (NA)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	38	81	42	[113]
Ibrutinib (NA)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	9.4	11	0.33	[113]
Ceritinib (20/25/NA/60)	388	363	NA	947	7.0	12	NA	13	6864	8390	NA	45300	55	48	NA	56	[114]
Idelalisib (NA/3/1/NA)	NA	129	209	NA	NA	3.0	1.0	NA	NA	141	671	NA	NA	39	48	NA	[115]
Nintedanib (50/50/NA/40)	547	105	NA	175	NA	NA	NA	NA	2720	375	NA	2390	NA	12	NA	13	[116]
Palbociclib (NA/5/20/2.66)	NA	178	664	86	NA	3.5	8.7	2.7	NA	1140	17400	768	NA	53	37	23	[117]
Lenvatinib (3/3/3/3)	1965	6167	1272	2501	0.5	0.5	2.0	2.0	5596	20697	5481	10272	64	69	70	78	[118]
Cobimetinib (3/3/3/3)	0.184	0.193	0.843	0.050	0.25	3.5	2.7	2.0	1.51	1.59	11.9	0.64	54	69	69	20	[132]

Ms = Mouse, Rt = Rat, Dg = Dog, Mnk = Monkey, Hmn = Human, NA = Not Available.

drugs have $V_{ss} > 10$ L/kg, it should be considered that in absence of preclinical adverse events large V_{ss} should be tolerated. In our view, V_{ss} cannot constitute a selection criteria in compound advancement in discovery.

Reviewing the PPB levels across mice, rats, dogs, monkeys, and human, revealed that about 30% of these drugs had extremely high PPB values ($\geq 99\%$) and less than 10% of them exhibited low PPB ($<70\%$). In between the two extremes, roughly 50% of these drugs had high PPB (90-99%) and less than 17% of them had moderate PPB (70-90%) (Table 6). Overall, $\sim 80\%$ of approved kinase inhibitors are highly protein bound with PPB values $\geq 90\%$. Therefore, in our view PPB should not be used as a screening criteria during lead optimization or as a determinant for drug candidacy.

3.3. Potential Drug-drug Interactions (DDI) for Transporters and CYP450 Enzymes

Inhibition of transporters and metabolizing enzymes by drugs could pose potential DDI effects leading to altered PK for co-administered drugs (or standalone). The reason why DDI has attracted so much attention is

that patients may be treated with multiple drugs simultaneously (polypharmacy) and their interactions might result in adverse drug reaction (ADR). In a study done in United States outpatient setting, rates of ADRs due to DDI ranges from 2% to 50% [119, 120].

Majority of oncology drugs are cleared *via* CYP450 metabolism pathways. Therefore, it is critical to understand DDI potential for kinase inhibitors, particularly in oncology, to better manage polypharmacy. While gathering the data, it became evident that a variety of formats were used to report transporter inhibition and CYP inhibition/induction. This posed a challenge in organizing the data. As a result, we have simplified the available information into Tables 7 and 8 for ease of discussion. More detailed information is available in the references cited in these tables.

3.3.1. Transporter Inhibition

Transporter inhibition data were reported on ABC and SLC transporter proteins, such as P-gp, BCRP, OATP, OCT and OAT (Table 7). About 53% of the kinase inhibitors inhibited P-gp at some level. Other transporters such as BCRP, OATP,

Table 5. Disposition parameters of kinase-targeted inhibitors in mouse, rat, dog and monkey.

Drug	Most Basic pKa (Predicted/measured)	Plasma Clearance ^a (mL/min/kg)				Vss ^b (L/kg)				MRT (hr)				References
		Ms	Rt	Dg	Mnk	Ms	Rt	Dg	Mnk	Ms	Rt	Dg	Mnk	
Imatinib	8.4/8.1	18	9.5	52	38	5.2	3.3	10.4	11	4.9	5.8	3.3	4.8	[96]
Gefitinib	7.1/7.2	NA	34	13	NA	NA	9.4	2.1	NA	NA	5.3	2.5	NA	[142]
Erlotinib	6.5/5.6	NA	~70	34	NA	NA	NA	2.6	NA	NA	NA	1.3	NA	[90]
Sorafenib	4.5/NA	2.5	0.67	2.2	NA	0.68	0.65	0.74	NA	4.5	16	5.7	NA	[146]
Dasatinib	7.1/6.8	62	26	25	34	4.2	6.3	4.7	3.5	1.1	4.1	3.2	1.7	[103]
Sunitinib	8.6/8.9	77	30	41	31	8.1	5.6	21	17	1.8	4.7	8.5	14	[104]
Lapatinib	6.5/NA	48	23	13	NA	9.6	1.8	5.5	NA	3.3	2.1	7.8	NA	[143]
Nilotinib	5.9/5.4	5.0	4.3	NA	11	0.52	7.9	NA	0.67	1.7	31	NA	1.0	[144]
Pirfenidone	NA/NA	100	29	20	NA	0.71	NA	NA	NA	0.11	NA	NA	NA	[62]
Pazopanib	3.7/6.4	NA	1.8	1.4	1.5	NA	0.45	0.30	0.28	NA	4.7	3.5	2.9	[145]
Crizotinib	9.2/9.4	NA	35	7.9	18	NA	24	12	13	NA	11	25	12	[99, 100]
Vemurafenib	3.2/NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	[102]
Vandetanib	8.8/9.4	NA	17.1	34.7	NA	NA	27.2	43.6	NA	NA	27	21	NA	[101]
Ruxolitinib	2.7/4.3	NA	157	8.0	NA	NA	3.8	1.1	NA	NA	0.40	2.3	NA	[67]
Axitinib	4.5/4.8	25	397	12	11.2	1.67	32.3	1.17	0.8	1.1	1.4	1.6	1.2	[105]
Bosutinib	8.3/7.9	37.5	128	15.2	NA	11.5	15.2	13.5	NA	5.1	2.0	15	NA	[106]
Regorafenib	5.8/NA	NA	2.42	4.53	NA	NA	0.88	1.89	NA	NA	6.1	7.0	NA	[107, 141]
Tofacitinib	4.6/5.1	NA	62	19.4	18.2	NA	2.6	1.8	1.7	NA	0.7	1.5	1.6	[108]
Cabozantinib	5.3/NA	NA	0.50	7.18	1.07	NA	0.41	NA	2.67	NA	13-16	NA	42	[109]
Ponatinib	8.0/7.8	NA	26.5	NA	8.7	NA	17.7	NA	2.6	NA	11.1	NA	5.0	[110]
Trametinib	NA/0.25	3.44	5.41	2.41	NA	0.9	2.9	3.0	NA	4.4	8.9	21	NA	[111]
Debrafenib	3.6/6.6	43.5	17.6	3.6	22.5	1.0	1.0	0.4	0.5	0.38	0.95	1.9	0.37	[131]
Afatinib	8.4/8.2	NA	90	64	25	NA	NA	NA	NA	NA	NA	NA	NA	[113]
Ibrutinib	3.8/3.7	NA	34	54	28	NA	NA	NA	NA	NA	NA	NA	NA	[113]
Ceritinib	10/9.7	26.6	24.8	NA	13	9.7	19.9	NA	13.5	6.1	13	NA	17	[114]
Idelalisib	3.7/3.4	NA	47.8	11.6	NA	NA	2.49	1.23	NA	NA	0.87	1.8	NA	[115]
Nintedanib	8.1/7.9	NA	202	NA	37.5	NA	41.2	NA	8.64	NA	3.4	NA	3.8	[116]
Palbociclib	9.0/7.4	NA	38	7.22	13.4	NA	5.7	6.2	5.1	NA	2.5	14	6.3	[117]
Lenvatinib	5.2/NA	5.76	1.67	6.14	3.96	0.714	0.391	1.61	0.794	2.1	3.9	4.4	3.3	[118]
Cobimetinib	8.5/NA	33.5	37.8	5.53	29.6	9.4	34.6	3.99	29.6	4.7	15	12	17	[132]

^a LBF of mouse, rat, dog and monkey are: 90, 60, 31 and 44 mL/min/kg, respectively.

^b Total body water volume of mouse, rat, dog and monkey are: 0.725, 0.668, 0.604 and 0.693 L/kg, respectively.

OCT, OAT, and BSEP were inhibited by about 27%, 20%, 17%, 13%, and 3% of these approved drugs, respectively. As can be observed, transporter inhibition is prevalent among kinase inhibitors.

3.3.2. Metabolic Enzyme Profiling

After examining metabolic pathways and enzymes responsible for the clearance of these 30 kinase inhibitors,

Table 6. Plasma protein binding of the kinase inhibitors.

Drug	Plasma Protein Binding (%)					References
	Ms	Rt	Dg	Mnk	Hmn	
Imatinib	98	95	81	90	93	[96]
Gefitinib	91	87	91	NA	90	[142]
Erlotinib	89	85	85	NA	94	[90]
Sorafenib	>99	>99	99	NA	>99	[146]
Dasatinib	92	97	96	97	94	[103]
Sunitinib	94	98	95	95	95	[104]
Lapatinib	>99	>99	>99	>99	>99	[143]
Nilotinib	97	99	98	99	98	[144]
Pirfenidone	30	30	30	NA	50	[62]
Pazopanib	>99	>99	>99	>99	>99	[145]
Crizotinib	96	94	96	93	91	[99, 100]
Vemurafenib	NA	NA	NA	NA	NA	[102]
Vandetanib	>99	>99	>99	>99	>99	[101]
Ruxolitinib	97	82	90	NA	97	[67]
Axitinib	97	98	98	96-99	99	[105]
Bosutinib	93-95	93-94	95-96	NA	93-94	[106]
Regorafenib	>99	>99	>99	>99	>99	[141]
Tofacitinib	33	6-31	20	35	39	[108]
Cabozantinib	>99	>99	>99	>99	>99	[109]
Ponatinib	>99	>99	NA	>99	>99	[110]
Trametinib	95	96	97	98	97	[111]
Debrafenib	93	99	95	NA	98-99	[131]
Afatinib	94	91-93	93	92	90-95	[113]
Ibrutinib	99	97 to >99	96-98	97	97-98	[113]
Ceritinib	NA	98	98	95	97	[114]
Idelalisib	80	81	79	NA	86	[115]
Nintedanib	97	98	NA	93	98	[116]
Palbociclib	81-87	87-88	55-61	NA	84-86	[117]
Lenvatinib	96-97	98	90-92	96	98-99	[118]
Cobimetinib	96-97	97	99	95-96	94-95	[132]

Ms = Mouse, Rt = Rat, Dg = Dog, Mnk = Monkey, Hmn = Human, NA = Not Available.

tors, our analysis suggested that ~90% of kinase inhibitors were cleared by CYP3A4 (Table 3). Also, CYP2D6, CYP1A1, CYP2C8, UGT1A19, CYP2C9, UGT, SULT1A1 as well as hydrolytic enzymes play a role in metabolizing ~30% of these drugs.

3.3.3. CYP450 Inhibition and Induction

The *in vitro* CYP450 inhibition and induction data are summarized in Table 8. Some clinical DDI data are

also briefly touched on here for simple comparison between *in vitro* and *in vivo* results.

K_i, IC₅₀ and % inhibition at defined inhibitor concentrations are commonly used to describe the inhibitory potentials of drugs toward an enzyme [39, 53-69, 90, 96-102, 121, 122], while k_{inact}, K_i and k_{inact}/K_i ratio are often used to describe the potentials for the time-dependent inhibition (TDI) or mechanism based inhibi-

Table 7. Transporter inhibition potential for kinase inhibitors.

Drug	Transporters						References
	P-gp	BCRP	BSEP	OAT	OATP	OCT	
Imatinib	Y	Y	NA	NA	NA	NA	[39, 53, 96, 133, 139]
Gefitinib	NA	NA	NA	NA	NA	NA	[39, 54, 134]
Erlotinib	NA	NA	NA	NA	NA	NA	[39, 55, 90, 136]
Sorafenib	Y	NA	NA	NA	NA	NA	[39, 56, 127, 134, 140]
Dasatinib	N	NA	NA	NA	NA	NA	[39, 57, 97, 103, 134, 137]
Sunitinib	NA	NA	NA	NA	NA	NA	[39, 58, 98, 104, 134]
Lapatinib	Y	Y	NA	Y	Y	NA	[39, 59, 134]
Nilotinib	Y	NA	NA	NA	NA	NA	[39, 61, 134]
Pirfenidone	N	NA	NA	NA	NA	NA	[62]
Pazopanib	N	NA	NA	NA	NA	NA	[39, 63, 134]
Crizotinib	Y	Y	NA	NA	NA	NA	[39, 65, 99, 100, 134]
Vemurafenib	Y	Y	NA	NA	NA	NA	[39, 69, 102, 134, 135]
Vandetanib	N	NA	NA	NA	NA	NA	[39, 68, 101, 134]
Ruxolitinib	N	N	NA	NA	NA	NA	[39, 66, 67, 134]
Axitinib	Y	NA	NA	NA	NA	NA	[70, 77]
Bosutinib	Y	NA	NA	NA	NA	NA	[71]
Regorafenib	Y	Y	NA	NA	NA	NA	[72]
Tofacitinib	Y	NA	NA	NA	Y	Y	[73, 74]
Cabozantinib	Y	NA	NA	NA	NA	NA	[75]
Ponatinib	Y	NA	NA	NA	NA	NA	[76]
Trametinib	N	N	NA	NA	N	NA	[77]
Debrafenib	N	Y	NA	NA	Y	NA	[78]
Afatinib	Y	NA	NA	NA	NA	NA	[79]
Ibrutinib	Y	NA	NA	NA	NA	NA	[80]
Ceritinib	N	N	NA	Y	Y	Y	[81, 82]
Idelalisib	N	N	NA	N	N	N	[83]
Nintedanib	Y	Y	NA	NA	NA	Y	[85]
Palbociclib	Y	Y	NA	Y	Y	Y	[86, 87]
Lenvatinib	N	N	Y	Y	Y	Y	[88]
Cobimetinib	N	N	NA	NA	NA	NA	[89]

Y = Yes, N = No, NA = Not Available

Efflux transporter inhibition: A decrease in net flux ratio of probe substrate of P-gp/BCRP/BSEP in presence of investigational kinase drug.

Uptake transporter inhibition: A decrease in uptake of model substrate of OATP/OAT/OCT in presence of investigational kinase drug.

tion of an enzyme. TDI is due to reactive metabolites of a drug irreversibly or quasi-irreversibly binding to an enzyme or the heme iron of the CYP's in a manner that deactivates that enzyme. In general, the impact of TDI could be more significant compared to reversible inhibition due to its irreversible nature of deactivation of the enzymes. Therefore, TDI effect continues even

after cessation of dosing and elimination of the drugs, until newly expressed enzymes are available. We have organized the data according to each drug's ability to inhibit enzymes. More detailed discussions can be found in the references cited in Table 8.

Over 66% of the kinase inhibitors inhibited CYP3A4 with a third of them falling in the "Moderate"

Table 8. CYP450 inhibition and induction potentials for kinase inhibitors.

Drug	CYP450 Inhibition								CYP450 Induction			References
	1A2	2A6	2B6	2C8	2C9	2C19	2D6	3A4	1A2	2B6	3A4	
Imatinib	N	N	NA	NA	N	N	L	L and TDI	NA	NA	NA	[39, 53, 96, 123, 134]
Gefitinib	L	NA	NA	NA	L	L	L	L and TDI	N	N	N	[39, 54, 123, 134]
Erlotinib	L	NA	NA	L	L	L	L	L and TDI	NA	NA	Y	[39, 55, 90, 123, 136]
Sorafenib	NA	NA	M	M	L	L	L	L	N	NA	N	[39, 56, 123, 134]
Dasatinib	L	L	N	M	N	N	N	L and TDI	N	N	N	[39, 57, 97, 123, 134]
Sunitinib	M	L	N	N	N	N	M	L and TDI	Y	Y	Y	[39, 58, 98, 104, 123, 134]
Lapatinib	NA	NA	NA	M	NA	NA	NA	M and TDI	N	N	N	[39, 59, 123, 134]
Nilotinib	NA	NA	NA	H	H	M	M	H and TDI	Y	Y	Y	[39, 61, 123, 134]
Pirfenidone	N	N	N	N	N	N	N	N	N	N	N	[62]
Pazopanib	M	NA	M	M	M	M	M	M and TDI	N	Y	Y	[39, 63, 123, 134]
Crizotinib	L	NA	L	L	L	L	L	M and TDI	NA	NA	Y (mRNA)	[39, 65, 99, 100, 134]
Vemurafenib	L	N	NA	NA	M	L	L	N	NA	NA	Y (mRNA)	[39, 69, 102, 134, 135]
Vandetanib	N	N	N	N	N	N	N	N	Y	NA	Y	[39, 68, 101, 134]
Ruxolitinib	L	NA	L	L	L	L	L	L	N	N	N	[39, 66, 67, 123, 134]
Axitinib	M	NA	NA	NA	M	NA	NA	NA	N	NA	N	[70, 105]
Bosutinib	N	N	N	N	N	N	N	N	N	N	N	[71]
Regorafenib	NA	NA	L	M	L	L	NA	L	N	N	N	[72]
Tofacitinib	N	N	N	N	N	N	N	N	N	N	N	[73, 74]
Cabozantinib	L	NA	NA	M	M	M	L	L	Y	N	N	[75]
Ponatinib	L	NA	M	M	NA	M	L	L to M	N	N	N	[76]
Trametinib	L	L	L	H	M	M	L	L	N	N	Y	[77]
Debrafenib	N	N	N	M	M	L	N	L	N	Y (mRNA)	Y (mRNA)	[78]
Afatinib	N	N	N	N	N	N	N	N	N	N	N	[79]
Ibrutinib	N	NA	M	M	M	M	M	M	N	N	N	[80]
Ceritinib	N	M	M	L	M	N	L	H	N	N	Y (mRNA)	[81, 82]
Idelalisib	N	NA	L	L	N	N	N	N	N	Y (mRNA)	Y (mRNA)	[83]
Nintedanib	N	N	N	N	N	N	N	N	N	N	N	[84, 85]
Palbociclib	L	L	L	L	L	L	L	L and TDI	N	N	N	[86, 87]
Lenvatinib	N	N	N	M	N	N	N	N and TDI	N	N	Y (mRNA)	[88]
Cobimetinib	N	NA	N	N	N	N	M	L to M and TDI	N	N	Y (mRNA)	[89]

For CYP450 inhibition: L = Low Risk Potential (IC₅₀: 10-50 μ M), M = Moderate Risk (IC₅₀: 1-10 μ M), H = High Risk (IC₅₀<1 μ M), N = No Risk Potential (IC₅₀>50 μ M), NA = Not available, TDI = Time Dependent Inhibition, For Ki values: High Risk Potential (<0.5 μ M), Moderate Risk Potential (0.5-5 μ M), Low Risk Potential (5-25 μ M), No Risk Potential (>25 μ M)

For CYP450 induction: Y = Yes, N = No, \geq 40% positive control response is considered as inductive potential.

to "High" risk categories. Additionally, 40% of these drugs inhibited other CYP450's tested in "Moderate" to "High" risk categories (Table 8).

Kenny *et al.* published *in vitro* time-dependent CYP3A4 inhibition profile of 26 marketed oncology drugs, of which, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, sorafenib and sunitinib were kinase inhibitors [123]. It was indicated that all nine kinase inhibitors were moderate to strong inhibitors of CYP3A4 capable of time-dependent inhibition. This information alerted the investigators to the possibility of DDI in the clinic. Surprisingly, the available clinical DDI data did not validate this concern (dasatinib, erlotinib, gefitinib, imatinib, nilotinib, pazopanib and sorafenib as perpetrators [123]), bringing preclinical prediction of DDI into question. According to FDA guidance [124], perpetrators can be classified into weak, moderate and strong inhibitors based on the AUC ratio of victim drug in the presence and absence of perpetrator drug (CYP inhibitor) after oral administration of the two interacting drugs. The DDI is considered weak, moderate, or strong if the ratio of victim drug's exposure with and without co-administered perpetrator is ≥ 5 , 5-2, 2-1.25-fold, respectively [125]. The clinical data showed that with the exception of imatinib, the observed AUC ratios for 6 victim drugs in the presence and absence of these 7 kinase drugs with potential for TDI to be less than 1.5-fold; suggesting an overestimation of clinical DDI by *in vitro* inhibition assays used in preclinical setting.

3.3.4. CYP450 Induction

CYP450 expression is inducible by certain compounds after multiple doses and continuous exposure. This results in increased levels of CYP's leading to enhanced drug metabolism and reduced exposure levels *in vivo*. Metabolizing enzyme induction is of concern because it might lead to loss of efficacy. It has become increasingly attractive for discovery scientists to assess induction potential of NCEs early in the process in order to alleviate potential DDI risks. The most commonly used *in vitro* systems are gene reporter (PXR, AhR and CAR) and cultured human hepatocyte assays. *In vivo*, transgenic mouse and monkey models are also known as appropriate to recapitulate human enzyme induction potential of interested drugs. Herein, we simply report the *in vitro* CYP450 induction potential of approved kinase inhibitors as "Yes" and "No" based on criteria set by FDA ($\geq 40\%$ positive control response is considered inductive potential). Due to lack of availability of a full data packages, a comprehensive list of could not be assembled. Available information on *in*

vitro hepatocyte induction potential of kinase inhibitors for CYP1A2, CYP2B6, and CYP3A4 was organized in Table 8. This data set suggested that at least 40% of the approved kinase inhibitors were *in vitro* inducers of one or more of the three CYP's mentioned above. It is important to point out that to this date a correlation between the *in vitro* and clinical CYP450 induction data has not been observed. This may in part be due to the dual inhibitory and inductive effects observed in many drugs which results in net lessening of these effects and manifestation of a minor inhibition or induction in the clinic.

4. DISCUSSION

It has been 15 years since the discovery and launch of the first kinase drug, imatinib. Imatinib marked a significant breakthrough in cancer treatment. This opened a new era for exploring targeted therapy for oncology indications with better therapeutic effectiveness and reduced adverse effects compared to conventional cancer therapy. From 2001 to October 2015, a total of 30 kinase inhibitors have been approved by FDA and numerous others have entered in various stages of clinical development. An extraordinary level of attention has been paid to the discovery and development of tyrosine kinase inhibitors, which covers the majority of the approved drugs. Despite all the efforts to advance high quality kinase inhibitors into the clinic, the clinical attrition rate is still rising [126]. Determination of what constitutes the right balance of attributes for an inhibitor in early discovery is crucial, yet challenging. This manuscript summarizes the physicochemical and ADME properties of the first 30 kinase inhibitor drugs that have entered the market. Retrospective analysis of the attributes for these kinase inhibitors can provide insights as to what the minimally acceptable criteria might be to advance the candidate molecules through lead optimization efforts in discovery. Furthermore, availability of this dataset can better guide discussions between medicinal chemists and DMPK scientists in deciding the significance of different parameters during lead optimization. In absence of such information, an overwhelming body of low impact and expensive data is generated at the expense of productivity and efficiency.

With the advent of automation, high throughput screening processes have been devised to rapidly and indiscriminately generate huge volumes of data in *in vitro* ADME assays for an excessively large number of compounds. Often, the misconception that more data provides better clarity rules the philosophy of drug dis-

covery groups, in particular in organizations with the means to capitalize such efforts. The net result in the creation of large sets of data which might not be necessary or stage appropriate is that it leads to confusion and distraction in teams.

We have offered our opinion on efficiency in drug discovery elsewhere [91, 128-130]. Our analysis suggests that not all ADME data are equally critical in early discovery. One conclusion might be that by using solubility, plasma protein binding, CYP450 inhibition and induction, permeability and efflux data as selection criteria in early discovery one could have inadvertently excluded some of the approved kinase inhibitors from advancement into the clinic. These data can only be valuable when put in the context of targetted disease, *in vivo* efficacy, efficacious exposure, and dosing regimen, which are all late stage considerations. Therefore, we recommend reserving judgment based on such criteria until early development. Based on this work and our internal experience, we propose focusing on *in vitro* metabolic stability and *in vivo* rodent disposition properties as primary screening assays. Moreover, we recommend against using permeability/efflux data as gate keeper in early screening stage. Instead, this data can be effective as a diagnostic assay to explain poor oral exposure or as a way to salvage compounds with rapid rodent plasma clearance, if they exhibit other redeeming qualities.

Oral systemic exposure of an NCE is a result of the interplay of structural features, apparent solubility/dissolution rate, permeability and efflux in the GI tract, disposition properties and food effects. Based on our analysis, Lipinski's and Veber's rules should be followed closely with regards to the number of H-acceptors, total number of H-bonds, and rotatable bonds. The recommendations on the number of H-donors (5) and PSA (140) can be followed also, however, it is noteworthy that most kinase inhibitors seem to follow a stricter trend of H-donor ≤ 3 and PSA ≤ 100 , respectively (Fig. 5). There seems to be some tolerance for violating the recommendations on MW and LogP. Kinase inhibitors with MW and logP as high as 600 and 6, respectively, have reached the market (Fig. 4). One parameter for which a guideline has not been reported is the most basic pKa. Our recommendation is to maintain this parameter below 9 in order to improve PK behavior. If rat IV PK is a primary screen in early discovery, we recommend prioritizing compounds with plasma CL $\leq 70\%$ liver blood flow. However, our analysis revealed that plasma CL $> 70\%$ LBF criteria may be tolerated only if the higher species plasma CL

is in line with LBF and there are other exquisite and compelling attributes such as potency (Table 5). The majority of kinase inhibitors had bioavailability above 20% in all preclinical species (Table 4), and therefore, prioritizing compounds with that level of bioavailability is warranted. The exception to that recommendations may apply to compounds with exquisite potency and selectivity, treatment rationale for unmet medical needs, and other positive attributes.

Based on the current information, *in vitro* data such as solubility, PPB, Caco-2 (or MDCK) permeability/efflux, transporter (uptake/efflux), CYP450 inhibition/induction, preclinical DDI do not appear to be gating, and therefore, one should carefully consider where and when to place these on the critical path for compound optimization. As shown, many negative attributes related to these data have been tolerated in the first 30 approved kinase inhibitors. Going forward these negative attributes may be minimized or eliminated to provide a competitive edge and yield higher quality drugs with better safety/efficacy profiles for patients; particularly as investigators move into areas beyond oncology.

An example of an ongoing challenge regarding the relevance of a preclinical assay to the clinic might be *in vitro* DDI. The *in vitro* DDI data are often over-interpreted likely because the *in vitro* systems designed for investigating potential for DDI are over-simplified and devoid of all the proper *in vivo* interactions. In the closed *in vitro* system, the interaction between the drug and CYPs can be exaggerated due to proximity and lack of compartmentalization. Also, it is nearly impossible to conduct the *in vitro* DDI studies at clinically relevant drug concentrations while in discovery mode, simply due to lack of accurate information on human efficacious dose/exposure levels early in the process. Moreover, the extent of decline in circulating concentrations of drugs over time due to *in vivo* clearance mechanisms can be more extensive than in isolated *in vitro* systems. Finally, *in vivo* DDI is a dynamic process and can be an interplay of multiple factors such as inhibition and induction, compensating metabolic pathways, contribution of CYPs to the entire metabolic pathways, etc. *In vitro* inhibition and induction studies are by nature closed system and more static. Therefore, *in vitro* DDI data can only become meaningful when combined with factors such as profiles of co-administered drugs, dosing route, dosing regimen, elimination pathways, enzyme phenotyping profile, efficacious exposure concentrations, concentration-time profile, and therapeutic target in the clinic.

A glance at the attributes of the approved kinase inhibitors between 2001-2011 compared to the ones approved from 2012-2015 reveals the following about the new generation kinase inhibitors: 1) more diverse types of kinases have been targeted, expanding beyond oncology, 2) metabolism of these new drugs is no longer predominantly through CYP3A4, 3) while rat CL has become more rapid, this parameter has been better optimized in the higher species, 4) Pgp/BCRP inhibition is more prevalent, 5) CYP450 induction and inhibition appears to be less of a concern.

CONCLUSION

Herein, we have organized a large body of physico-chemical and ADME data on the first 30 approved kinase inhibitors. A detailed examination of this data can reveal what criteria may be acceptable/tolerable or in need of further optimization. It is clear that discovery teams should continue to strive for continuous improvement of NCE's based on rigorous analysis of what is proven to add value, and not necessarily the conventional thinking. We hope that our analysis offers a baseline for attributes of kinase inhibitors and constitutes a platform for improving quality and provides insights into what "actionable" and "stage-appropriate" data might be. This can help to minimize generation of unnecessary data; thereby preventing "data pollution" and confusion during lead optimization.

LIST OF ABBREVIATIONS

ABC	=	ATP-Binding Cassette	CNS	=	Central Nervous System
ADME	=	Absorption, Distribution, Metabolism and Elimination	CYP450	=	Cytochrome P450
ADR	=	Adverse Drug Reaction	DDI	=	Drug-Drug Interaction
AUC	=	Area Under the Curve	DMPK	=	Drug Metabolism and Pharmacokinetics
BCR-Abl	=	Breakpoint Cluster Region-Abelson murine leukemia viral oncogene homolog 1	ERK	=	Extracellular Signal-regulated Kinase
BCRP	=	Breast Cancer Resistance Protein	F%	=	Bioavailability
BCS	=	Biopharmaceutics Classification System	FaSSIF	=	Fasted-state Simulated Small Intestinal Fluid
BSEP	=	Bile Salt Export Pump	FDA	=	Food and Drug Administration
Caco-2	=	Human colorectal adenocarcinoma	FeSSIF	=	Fed-state Simulated Small Intestinal Fluid
c-AMP	=	Cyclic AMP	GI	=	Gastro-Intestinal
CL	=	Clearance	IV	=	Intravenous
Cmax	=	Maximum Concentration	Ki	=	Inhibitory Constant
CML	=	Chronic Myelogenous Leukemia	Kinact	=	Maximal Rate of Enzyme Inactivation
			LBF	=	Liver Blood Flow
			MDCK	=	Madin-Darby Canine Kidney
			MDR	=	Multi-Drug Resistant
			MRP	=	Multidrug resistance-associated protein
			MRT	=	Mean Residence Time
			MW	=	Molecular Weight
			NA	=	Not Available
			NCE	=	New Chemical Entities
			OAT	=	Organic Anion Transporter
			OATP	=	Organic Anion-Transporting Polypeptide
			OCT	=	Organic Cation Transporter
			Papp A-B	=	Apparent Permeability from A-B
			PDGF-R	=	Proto-oncogen c-kit and platelet-derived Growth Factor Receptor
			Pgp	=	P-glycoprotein
			PI3K	=	Phosphoinositide 3-Kinase
			PK	=	Pharmacokinetics
			PO	=	Per Os (Oral)
			PPB	=	Plasma Protein Binding
			PS	=	Phosphatidylserine
			PSA	=	Polar Surface Area
			SGF	=	Simulated Gastric Fluid
			SLC	=	Solute Carrier

SULT	=	Sulfotransferase
TDI	=	Time Dependent Inhibition
TK	=	Tyrosine Kinases
Tmax	=	Time at maximum concentration
UGT	=	UDP Glucuronosyltransferases
Vss	=	Steady State Volume of Distribution

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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