Getting physical in drug discovery II: the impact of chromatographic hydrophobicity measurements and aromaticity

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Here, we review the performance of chromatographic hydrophobicity measurements in a data set of 100 000 GlaxoSmithKline compounds, demonstrating the advantages of the method over octanol–water partitioning and highlighting new insights for drug discovery. The value of chromatographic measurements, versus other hydrophobicity estimates, was supported by improved relationships with solubility, permeation, cytochrome P450s, intrinsic clearance, hERG binding and promiscuity. We also observed marked differentiation of the relative influence of intrinsic and effective hydrophobicity. The summing of hydrophobicity values plus aromatic ring count [log $D_{pH7.4}$ (or log *P*) + #Ar], indicated a wide relevance for simplistic 'property forecast indices' in developability assays, clearly enhanced by chromatographic values; therefore establishing new foundations for enriching property-based drug design.

Introduction

The optimisation of physical properties is fundamental to the drug discovery process [1,2] and central to this is the measurement and prediction of hydrophobicity [3]. Hydrophobicity (or its etymological synonym, lipophilicity) is an appraisal of the preference for a compound to reside in a hydrophobic versus aqueous environment. Investigations into the dispersion of molecules between aqueous buffers and organic solvents led to the establishment of OW (see Glossary) as the gold standard for measuring the partition (usually expressed as log *P*) or distribution (log *D* at a given pH) of molecules [4,5].

Log *P* represents the intrinsic hydrophobicity of a compound and is a constant for a given solvent system. The log *P* of a molecule with an ionisable centre will only be measurable when the compound bears no charge. Log *D* is the effective hydrophobicity of a molecule and relates to the distribution of all species present at a given pH and, thus, is not a constant. The partitioning of a given molecule is readily predictable by summation of the incremental contributions of component fragments, originally derived from measured values [5,6]; inclusion of pK_a values enables the prediction of distribution at any pH.

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Many computational packages are now available to predict log *P* and log D_{pH} for any given molecule and these values have been widely used in medicinal chemistry to rationalise structure–property relationships, in predictive design and in the generation of predictive models [7]. Hydrophobicity is almost invariably at the very core of these predictive processes [5] and an optimum range for drug molecules is apparent from analyses of various developability parameters [7]. Nonetheless, contemporary reviews indicate an ongoing tendency towards increased hydrophobicity values in drug candidates [8], despite the demonstrable risks and lower probability of success associated with such molecules [9]. These inflated values, termed 'molecular obesity' [10], have been attributed to misguided pursuits of *in vitro* potency, often at the cost of poorer pharmacokinetic profiles [11]. Such reviews have focused on predicted physical data, most usually clog *P*, which have established trends and enabled the formulation of predictive models and rules. These parameters have been combined into visualisation tools, which aid medicinal chemistry optimisation by identifying preferred regions of chemical space; for example, the Golden Triangle of Johnson *et al.* [12], the 3/75 rule of Hughes *et al.* [13] or the 4/400 of Gleeson [14].

We recently highlighted shortcomings of the OW model in contemporary drug discovery in a set of compounds with

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GLOSSARY

AMP artificial membrane permeability
c calculated
CCP comprehensive compound profiling
CHI chromatographic hydrophobicity index
Chrom chromatographic
Cl_{int} intrinsic clearance
CLND ChemiLuminescent nitrogen detection
GSK GlaxoSmithKline
HSA human serum albumin
hERG human Ether-a-go-go Related Gene
iPFI intrinsic Property Forecast Index (i.e. Chrom log P + #Ar)
log D_{pH} $\log_{10}$ (distribution coefficient at the given aqueous buffer pH)
log *P* $\log_{10}$ (partition coefficient)
m measured
OW octan-1-ol/water (implicitly octan-1-ol/aqueous buffer)
Pgp permeability glycoprotein
PFI Property Forecast Index (i.e. Chrom log $D_{pH7.4}$ + #Ar)
SFI Solubility Forecast Index

measured OW hydrophobicity plus solubility data [15]. Therein, the key observation was that, for poorly soluble compounds (measured solubility <30 μM), measured hydrophobicity values in the OW system are effectively meaningless. Implicit in this analysis was that more hydrophobic molecules are likely to be less soluble and would thus have unreliable OW log *D* values. Additionally, an upward trend in the numbers of aromatic rings in drug candidates has been noted [16] and this further reduces solubility. The detrimental effect of aromatic ring count on solubility, over and above its contribution to increased hydrophobicity, is demonstrated by our proposed 'Solubility Forecast Index' (SFI) [15]. Further questions have been raised as to why OW remains the standard method in contemporary drug discovery [17], despite the widespread availability of alternative higher throughput methods for hydrophobicity determination [18,19].

The automated CHI measure of hydrophobicity [20] has been used alongside OW shake flask determinations for several years at GSK. CHI log *D* values [21], where CHI log D_{pH} = CHI_{pH} × 0.0525 − 1.467, show significant correlation with both measured (OW) and predicted log $D_{pH7.4}$ values (e.g. ACD software, version 11.0, which is used in this review), albeit with a compressed scale and cross over between the values.[a] The utility and impact of this parameter has been demonstrated in several programmes at GSK [22–24]; however, exploitation of these data has been sporadic, perhaps owing to disconnects between actual values and smaller incremental changes versus the octanol scale. Consequently, CHI data were reanalysed, leading to the establishment of a modified conversion factor with a rescaled output. This new parameter, reported as chromatographic log *D* (mChrom log D_{pH} = CHI_{pH} × 0.0857 − 2) gave both similar linear increments and a normally distributed dynamic range comparable with those observed with OW predictions.[a] A disconnect between measured Chrom log $D_{pH7.4}$ and measured OW log $D_{pH7.4}$ values is illustrated in Fig. 1a, where the skewed distribution and narrow range for OW values is clear, producing a poor correlation at the more hydrophobic end of the scale, consistent with our previous observations with calculated versus measured OW data [15]. A consequence of the scaling used was a positive offset of approximately two log units, making Chrom log $D_{pH7.4}$ values higher than traditional OW values. In practice, this offset was retained to highlight the different origins of the data. Subsequently, an in-house predictor, cChrom log $D_{pH7.4}$, has been developed, which provides enhanced hydrophobicity predictions (Fig. 1b).

The determination of chromatographic partition coefficients has been achieved by additional measurements at pH 2 and pH 10.5. It is reasonable to assume that the maximum hydrophobicity value obtained at these pH extremes is that of the unionised form of the compound and provides a measure of log *P* [21]. Chrom log *P* measurements on approximately 8000 compounds (including ionisable examples) gave a better correlation (R^2 = 0.51) and alignment with Daylight clog *P* (using Daylight software v4.9),[a] than that observed for OW measurements of unionised compounds in this set (R^2 = 0.30).

To explore the utility of chromatographic measurements, their impact across a range of developability assays where hydrophobicity is known to have a strong influence, was investigated. The set of 100 000 compounds used previously [15] was further annotated, so all had measured CLND solubility in addition to Chrom log $D_{pH7.4}$, although varying proportions had been through all of the developability assays. An analysis of whether effective hydrophobicity (log D_{pH}), or intrinsic hydrophobicity (log *P*) had the greater influence in a particular assay, was a secondary objective. Third, the summations of log $D_{pH7.4}$ + #Ar or log *P* + #Ar were investigated to explore the potential wider impact of our proposed 'Forecast Indices' beyond that observed with solubility.

Presentation of data

The distributions of values and trends therein were conveniently conveyed through normalised bar graphs, effectively showing the probability of achieving a particular outcome in each bin; these are used in the main text and the numbers above the bars indicate the number of values in each bin. Additional forms of analysis are included in the supplementary data available online[a]; box–whisker plots were used to demonstrate statistical significance in observed trends; categorised multiple pie charts, using binned hydrophobicity and/or other descriptors, gave a clear indication of where parameters showed independent effects over and above any correlation between them. These plots gave impactful and visually appealing representations of the data, highlighting clear trends in a form readily interpretable by medicinal chemists, without recourse to multivariate data analysis.

Solubility

When comparing the differentiation between kinetic solubility classes achieved with Chrom log $D_{pH7.4}$ and OW data, a statistically significant improvement was observed with the former, as would be expected by extension of previous findings [15], Indeed, the categorised multiple pie representation,[a] incorporating Chrom log $D_{pH7.4}$ values and the number of aromatic rings, gives a better differentiation in comparison with the same plot using calculated ACD log $D_{pH7.4}$ [15]. The latter was the basis of the proposed SFI (clog $D_{pH7.4}$ + #Ar), which is enhanced by using the

[a] Further supporting/illustrative data are presented in the supplementary material online.

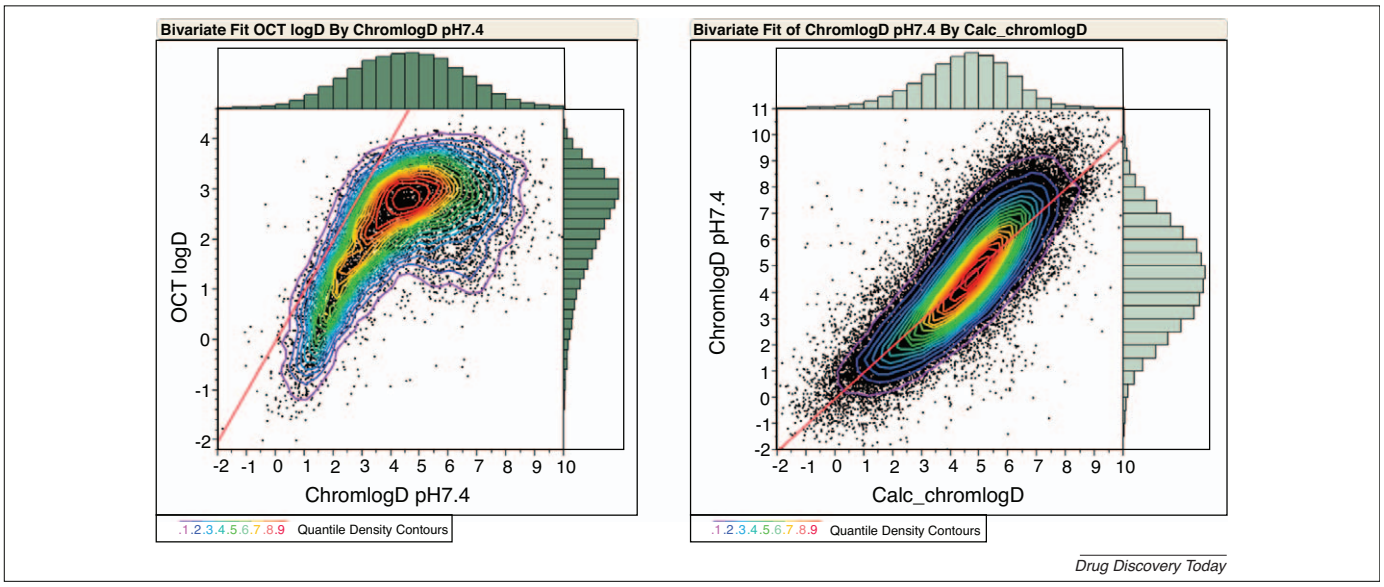

FIGURE 1

Bivariate fit between **(a)** measured OW log $D_{pH7.4}$ and measured Chrom log $D_{pH7.4}$ and **(b)** measured and calculated Chrom log $D_{pH7.4}$ showing the line of unity.

chromatographic parameter, albeit with the implicit two-unit shift arising from the way in which Chrom log $D_{pH7.4}$ is derived (the diagonal differentiation is now represented by the line of Chrom log $D_{pH7.4}$ + #Ar = 7). This is in contrast to a categorised multiple pie plot of binned SFI and molecular weight as variables[a]; whereby in a given SFI bin there is little or no variation in solubility distribution with molecular weight changes. These data support the notion that both hydrophobicity and aromaticity profoundly influence solubility beyond their interdependence, but that molecular weight correlates with solubility only owing to its relationship with these two parameters.

Human serum albumin binding

The binding of molecules to plasma proteins, such as serum albumin, is frequently a concern in drug discovery [25,26]. Although it might not be an attrition risk *per se* [27], high percentage binding is a characteristic of lipophilic compounds and contributes to reductions in efficacy and drug efficiency [28], owing to a lower free fraction of available drug. High throughput %HSA binding data [29] were available on 43 700 compounds in the data set and these showed clear trends with higher levels of binding observed [either by %bound or expressed as log K_{HSA}, where K = (%bound/%unbound)] as Chrom log $D_{pH7.4}$ increased (Fig. 2a). Interestingly, both Chrom log P and Chrom log $D_{pH7.4}$ gave effective resolution of increasing binding[a]; each produced clearly enhanced resolution compared with that achieved using measured or calculated OW log $D_{pH7.4}$. Given the strong correlation between the two, it was not surprising that mChrom log P and clog P gave comparable outcomes. It might be that these observations are reflective of the multiple types of interaction involved with HSA binding, whereby both intrinsic and effective hydrophobicity can have a role. In particular, log K_{HSA} correlates with Chrom log $D_{pH7.4}$; however, for acids, the affinity is over and above that expected owing to hydrophobicity alone[a] because of the known presence of binding sites for acids on HSA. The summation of Chrom log $D_{pH7.4}$ plus #Ar, gave further enhanced resolution of these data, both in box plots[a] and categorised bar graphs (Fig. 2b), in a similar manner to solubility. The impact of #Ar in binding over and above correlation with hydrophobicity was again obvious in a categorised multiple pie plot (Fig. 3c), where a clear diagonal split (again, as observed with solubility) was apparent, showing the contribution of aromaticity to HSA binding above and beyond its contribution to hydrophobicity. Remarkably, the line of Chrom log $D_{pH7.4}$ + Ar = 7 (PFI) again splits the regions of high and low binding; (when PFI >7, log K_{HSA} plots indicate that >95% binding is likely).

Permeability

Investigations aiming to establish a better understanding of the permeability of experimental drug molecules have received extensive attention of late [30,31]. Permeation of biological membranes has importance in various scenarios, such as absorption processes (key to oral bioavailability, kidney re-absorption and brain penetration) and cell penetration to enable access to intracellular drug targets. The reported relationships between hydrophobicity and permeability were recently summarised as being, in different analyses, either linear, hyperbolic, sigmoidal, parabolic or bi-linear [32]. However, chromatographic measurements gave compelling indications that the relationship is bi-linear and dependent on effective hydrophobicity. This dependency was previously described for a set of drug molecules [31,33]; but the message has perhaps been lost as further data have been generated over the years, probably distorted by the trend towards increasingly more hydrophobic and aromatic molecules. Given that the hydrophobicity range of the set of actual drug molecules investigated [33] is likely to engender good solubility, then it is implicit [15] that the measured OW log $D_{7.4}$ values would probably be reliable. The observed trend was rationalised by recognising that permeation is effectively controlled by both the partition of the molecule into the lipid membrane and from there into the aqueous environment on the other side. For hydrophilic compounds, the partitioning into the hydrophobic layer is the rate-limiting step and, conversely, for hydrophobic compounds,

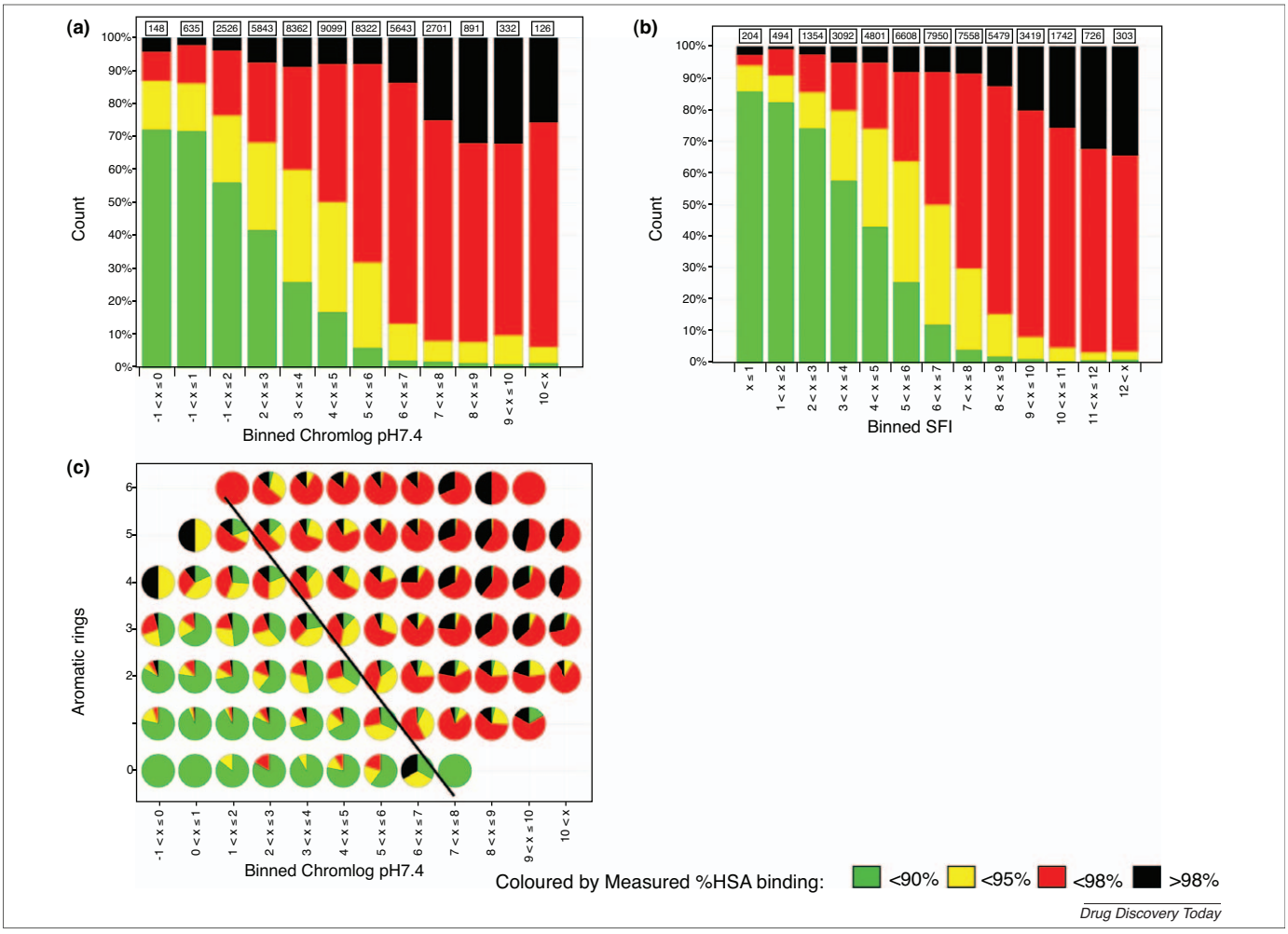

FIGURE 2

Distribution of HSA binding by **(a)** Chrom log $D_{pH7.4}$ or **(b)** Chrom log $D_{pH7.4}$ + #Ar bins and a categorised multiple pie plot **(c)** of binned #Ar versus binned Chrom log $D_{pH7.4}$ (with a diagonal line of Chrom log $D_{pH7.4}$ + #Ar = 7). **(c)** Illustrates the diagonal split between values, supporting the notion that aromatic ring count has an impact over and above its correlation with hydrophobicity.

partitioning back out would be limiting; the combination of which gives the observed bi-linear distribution.

Permeability (P_{app}) data were investigated for 46 300 compounds measured in the AMP assay [34] and 1050 passing through MDCK cells [35]. The latter data set comprised measurements in the baso-apical direction under conditions with added GF120918 to inhibit Pgp efflux mechanisms [36], such that measurements gave a best representation of passive diffusion. Plots of P_{app} in both assays gave fairly clear bi-linear distributions (Fig. 3a[a]), irrespective of solubility class,[a] suggesting that, as hydrophobicity increases to a Chrom log $D_{pH7.4}$ of 5, then permeation increases to a maximum before tailing off again.

Molecular size is implicated as a factor in permeation rates [32], and a clear decrease in permeation as size increases was apparent in this data set,[a] although the change in distribution was not as marked as the hydrophobicity relationship. In the MDCK assay, the potential for penetration by the paracellular route [37] for smaller, hydrophilic compounds was evident. Interestingly, there was a bi-linear response for P_{app} versus #Ar, with two being the optimum for good permeation. Remarkably, the summation of Chrom log $D_{pH7.4}$ + #Ar gives a statistically significant bi-linear response,[a] shifting the maximum by two units to seven (possibly the optimum Chrom log $D_{pH7.4}$ noted above, plus two aromatic rings). It is notable that the most favourable average permeation rates were observed for compounds in the Chrom log $D_{pH7.4}$ + #Ar range of 6–8, which is the region where other parameters reviewed herein show increasingly higher risks.

Cytochrome P450s

The propensity for a compound to interfere with cytochrome P450 metabolism is another widely used developability benchmark. Rates of metabolism for known substrates of particular cytochrome P450 isoforms are monitored in the presence of the test substance; activity in these assays is undesirable, indicative of potential drug–drug interactions with other substrates or inhibitors of particular isoforms. Data were interrogated across the five P450 isoforms regularly screened at GSK in bactosome assays [38], with 50 000–70 000 data points available. In addition to hydrophobicity data [39], particular focus was paid to size, charge and aromaticity, reflecting established structure–activity relationships in such assays [40]. Table 1 summarises where particular relevance of the descriptors showed impact; this was generally consistent with published data [39,40].

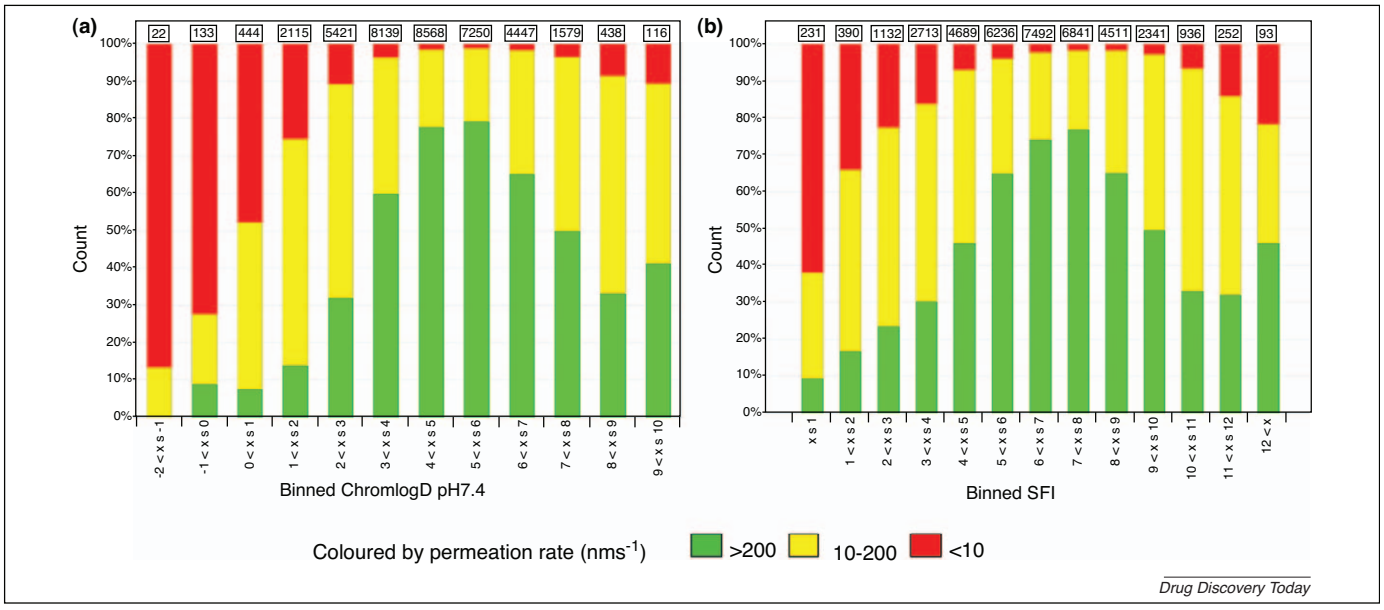

FIGURE 3

Distribution by permeation classes in the AMP assay, binned by **(a)** Chrom log $D_{pH7.4}$ and **(b)** Chrom log $D_{pH7.4}$ + #Ar bins, showing the bi-linear relationships.

For example, the 1A2 isoform, which showed relatively few active compounds, only interacted with smaller, flatter, molecules (those with a higher proportion of aromatic rings rather than #Ar *per se*). The effect of increasing activity with increased size was apparent for the 2D6, 2C9, 2C19 and 3A4 isoforms; evidence was also observed for activity increased with particular charge states, as expected [39,40]. However, the nature of the impact of measured Chrom log $D_{pH7.4}$ was of particular interest with 2D6, 2C9, 2C19 and 3A4; clear bi-linear responses were observed, in both distribution graphs (2C19 shown in Fig. 4a) and box plots.[a] These trends were not immediately apparent with intrinsic hydrophobicity values or measured and predicted OW log $D_{pH7.4}$, although, with hindsight, tentative evidence for bi-linear relationships could be observed for neutral compounds using clog *P* with 2C9, 2C19 and 3A4 data.[a] Together, these observations support the influence of effective hydrophobicity (log *D*) on P450 activity. This can be rationalised, in part, by the effect of the bactosome preparations used in the assay, whereby permeation into the bactosome, which itself is an artificial membrane, is a prerequisite event ahead of any particular binding to the P450 enzyme itself. There have been reports of apparent bi-linear relationships based on small data sets [41], although findings in broader reviews [39,40] have been more indicative of a linear relationship between activity and hydrophobicity.

Aromatic ring count had a clear influence on the activity of 2C9, 2C19 and 3A4 [16] and, interestingly, there appears to be an effect for the Chrom log $D_{pH7.4}$ + #Ar addition; the bi-linear relationship remains (Fig. 4b) with a two-unit shift in the maxima, as was observed with permeation.

Intrinsic clearance

The Cl_{int} of molecules, measured by the rate of disappearance of a given compound in a human liver microsome preparation, although being a multi-functional process, is also known to be hydrophobicity dependant [42] in addition to particular structural liabilities. Analyses of these data (8700 records) also showed that Chrom log $D_{pH7.4}$ gave the best differentiation between bins; the small subset with measured Chrom log *P* vales and clog *P* (on all) showed no significant variation within the key window of clog *P* 3–7.[a] Interestingly, there was no apparent effect on intrinsic clearance as #Ar varied; although the composite Chrom log $D_{pH7.4}$ + #Ar (PFI) value accentuated a bi-linear shape between variables, perhaps consistent with a key role for cytochrome P450-mediated metabolism. Remarkably, the Chrom log $D_{pH7.4}$ + #Ar value of seven again indicated a statistically significant differentiation point where marked increase in clearance occurred; when PFI >7 more than 50% have Cl_{int} of 5 ml/min/kg.[a]

TABLE 1

Influence of descriptors on P450 binding activity[a]

P450 isoform	Chrom log $D_{pH7.4}$	Size (CMR)	#Ar	Recognition factors
1A2	–	–++–	%Ar not #Ar	Highly aromatic/flat structures, smaller/hydrophobic
2D6	–+++–	–++–	+	Hydrophobic, optimum size, basic
2C9	–+++–	–++–	+++	Hydrophobic, optimum size, aromatic, acidic
2C19	–+++–	–++–	+++	Hydrophobic, optimum size aromatic, basic
3A4	–+++–	+++	+++	Hydrophobic, large, aromatic, basic

[a] +, ++, +++ present increasing impact of the parameter; minus signs at either end (e.g. –+++–) are indicative of a bi-linear relationship.

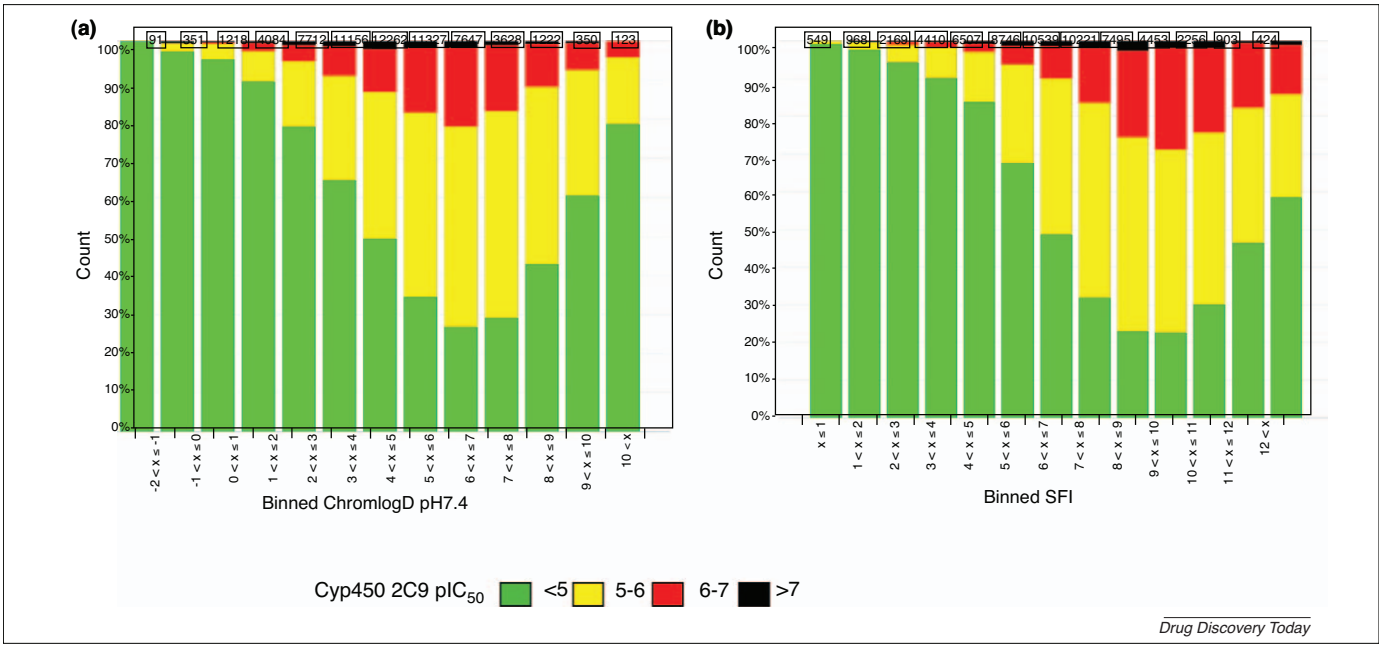

FIGURE 4

Distribution plot of cytochrome P450 2C9 pIC_{50} versus **(a)** Chrom log $D_{pH7.4}$and **(b)** Chrom log $D_{pH7.4}$ + #Ar bins, showing the bi-linear distribution.

hERG binding

Interactions with hERG, causing increases in the cardiac QT interval, have provided a bench mark for identifying cardiovascular risk in drug development [43]. It is known to be a particular issue for positively charged lipophilic compounds [44], although not all compounds are susceptible and homology models have been successfully exploited in structure-based design to reduce activity [45]. This analysis of 18 600 compounds clearly showed that stronger trends were observed with Chrom log *P* and clog *P*,[a] consistent with the intrinsic hydrophobicity of the molecules being more important. Furthermore, as expected, positively charged compounds (indicated as basic by the Chrom log *D* reduction between measurements at pH 7.4 and 11) showed increased propensity for hERG inhibition.

An increase in #Ar is implicated as driver of hERG activity [16], which was observed in this data set, most notably for positively charged compounds. In this charged subset, in particular, the summation of Chrom log *P* or clog *P* and #Ar (iPFI) produced a significant upward trend of increased risk. Again, a significant differentiation was observed for Chrom log *P* + #Ar >7 or clog *P* + #Ar >5 (Fig. 5); such that when iPFI >7 more than 50% have hERG pIC_{50} >5.

Promiscuity

A GSK initiative, CCP, sought to determine the utility of large-scale compound profiling in drug discovery. Over 2500 compounds, including exemplars from GSK lead optimisation projects, marketed drugs, legacy leads and failed development candidates, were screened in >490 assays incorporating a diverse range of >380 protein targets and phenotypic end points. The promiscuity of a compound was expressed as the frequency of it showing a pXC_{50} of >5 (this enables a valid comparison with the study by Leeson and Springthorpe [8], who took >30% inhibition at 10 μM as the definition of an 'active'). Hydrophobicity is known to be a key driver [8,46], but whether this can be attributed to intrinsic or effective hydrophobicity was unclear, probably owing to the shortcomings in the OW model. Of this set, approximately 800 compounds had measured Chrom log *P* and Chrom log $D_{pH7.4}$ data, which correlated well with the predicted values (clog *P* and cChrom log $D_{pH7.4}$, respectively) and the frequency distribution clearly showed the impact of hydrophobicity in increasing promiscuity [expressed as log(# hits)]. Most notably, the trend appeared better differentiated using Chrom log *P* rather than Chrom log $D_{pH7.4}$[a]; calculated OW log *P* and measured Chrom log *P* values gave a similar outcome across the whole data set.[a] This is consistent with intrinsic hydrophobicity (i.e. log *P*) being the more important parameter governing binding affinity (as observed with hERG). Leeson and colleagues [8,9] and Peters *et al.* [46] reported particularly increased

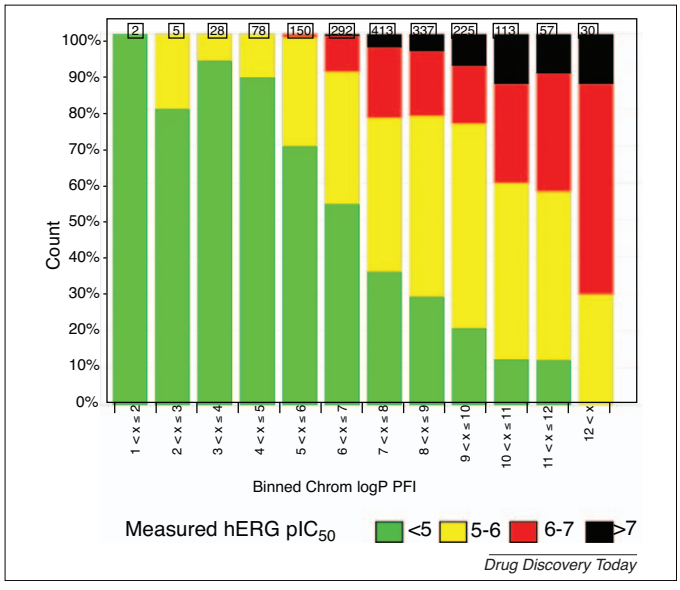

FIGURE 5

Distribution of hERG pIC_{50} values versus binned measured Chrom log *P* + #Ar (the iPFI) for basic compounds.

promiscuity for lipophilic bases; however, although we observed a raised overall promiscuity for basic compounds compared with other charge classes in this data set, it appears that log *P* was the key driver regardless of ionisation class. Within measured and calculated intrinsic hydrophobicity bins, little charge-implicated variation in hit rates was observed.[a] It was notable that the average log *P* values of charged compounds were markedly higher than those for neutral compounds, which in itself would be indicative of increased promiscuity.

Aromatic ring count indicated a further clear risk signal in this analysis[a]; a statistically significant increase in the number of hits was observed for aromatic ring count increments between one and four. The summation of #Ar together with clog *P* (iPFI) in this scenario also indicated another application of the forecast index, with clearly better differentiation than for log *P* alone (Fig. 6). A categorised multiple pie plot again supports the notion of aromatic ring count having an effect over and above its contribution to the hydrophobicity of the molecule.[a] Fascinatingly, it appears that values of Chrom log *P* + #Ar <7 (or <5 on the OW scale using clog *P*) are commensurate with the indication of probable low promiscuity; above these values, there is more than a 50% chance of inhibiting more than five assays with pIC_{50} >5.

Aromatic rings: benzenoids versus heteroaromatics

Additional evaluation of this data set [47] indicated that heteroaromatic rings, in comparison with their benzenoid analogues, generally reduce the propensity for compounds to show undesirable activity in the developability assays described herein. Yet, the 'PFI' summation of log $D_{pH7.4}$ + #Ar appears to have wide applicability; thus, by implication, the impact of the flat structures on, for example, crystal packing, π-stacking or reduced degree of freedom (entropic) contributions to the free energy of binding processes must still hold. It was apparent that the beneficial effects of heterocycles versus carbocyclic aromatics could be explained by their generally lowered hydrophobicity. Indeed, inspection of these data indicated that, within particular 'PFI' bins (i.e. narrow ranges of log $D_{pH7.4}$ + #Ar), the median solubility, % HSA binding and other developability parameters showed little or no statistically significant variation regardless of the types of ring present in the molecule. Of course, the PFI summation for most heteroaromatics would be correspondingly less than the analogous benzenoid structures owing to their lesser hydrophobicity.

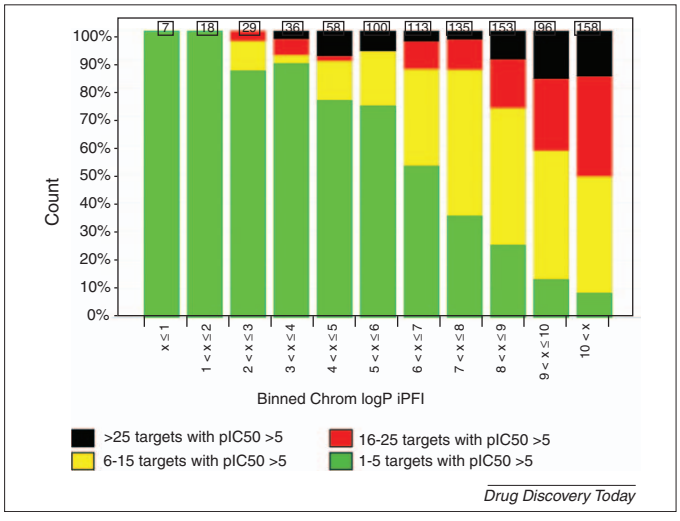

FIGURE 6

Promiscuity data. Distribution of number of targets inhibited with a $pIC_{50} > 5$ by 2500 compounds, categorised by binned log *P* + #Ar values (the iPFI).

Conclusions

Chromatographic hydrophobicity determination has been shown to be linear, non-solubility dependent, predictable and, above all, relevant. Although this review might not signal the end of the story for the measurement and prediction of hydrophobicity with reliability and precision, or even be the beginning of the end, it might hasten the end for the more time-consuming and, as demonstrated [15], less reliable OW system as the standard model in contemporary drug discovery, especially if more hydrophobic (implicitly less soluble) molecules need to be measured.

Important new insights into the hydrophobicity-dependent behaviour of molecules have been established, confirming where effective or intrinsic hydrophobicity is the key parameter and bringing a sharper focus to relationships that were previously less well resolved. The enduring quality of the log *P* predictions derived from the MASTERFILE database of fragment values [48] was a notable feature within these data, these calculated values being more realistic appraisals of the true intrinsic hydrophobicity than are measured OW values. However, the key parameter was often shown to be effective hydrophobicity; whereby measured or predicted Chrom log $D_{pH7.4}$ showed much better differentiation than did measured or calculated OW log $D_{pH7.4}$ values. A wider relevance for simplistic PFIs has established the impact of aromaticity, over and above its contribution to hydrophobicity, as a risk in solubility and promiscuity in its various forms relevant to attrition. That the 'PFI' guide of OW log *P* (or log $D_{pH7.4}$) + #Ar should be less than 5 is no coincidence, given the average clog *P* of 3 and #Ar of 1.8 in oral drugs [8,14]; the two-unit shift to Chrom log $D_{pH7.4}$ + #Ar <7 is also entirely consistent with this. Whereas the structure–property relationships in this analysis are clearly more complex than being just due to the hydrophobicity and aromaticity of that molecule, it is clear that chromatographic measurements give a better refined and more relevant hydrophobicity assessment; OW values, polar surface area or molecular weight simply do not differentiate as effectively. Although the analyses by binned Chrom log *P*/log $D_{pH7.4}$ demonstrate simplistic, yet sound, rules of thumb, further refined and more complex predictive models will surely benefit from their inclusion as the hydrophobicity parameters. This will not only be due to enhanced precision, but also through the establishment of linear or bi-linear dependencies of the property and when intrinsic or effective hydrophobicity is the more relevant parameter. The binning by hydrophobicity or PFI/iPFI classes expresses the chances of a property-related risk in that narrow range, which is more indicative and informative than defining a particular cut off such as 3/75 [13] or 4/400 [14]. Nevertheless, risks for the developability parameters investigated are clearly exacerbated above a PFI/iPFI of 7 on the chromatographic hydrophobicity scale; indeed a value of <5 would appear desirable (Table 2), although there is a potential need for concessions to enable more effective permeation.

There is a growing discussion on implications of cross correlations in molecular properties and physical properties [3,49] and how they relate to the concepts of bulk and cohesiveness proposed by Cramer

TABLE 2

Percentages of compounds achieving defined target values in the various developability assays categorised by PFI or iPFI bins[a]

	PFI = mChrom log $D_{pH7.4}$ + #Ar								
Assay / target value	<3	3-4	4-5	5-6	6-7	7- 8	8-9	9-10	>10
Solubility >200 μM	89	83	72	58	33	13	5	3	2
%HSA <95%	88	80	74	64	50	30	17	8	4
2C9 pIC_{50} <5	97	90	83	68	48	32	23	22	38
2C19 pIC_{50} <5	97	95	91	82	67	52	42	42	56
3A4 pIC_{50} <5	92	83	80	75	67	60	58	61	66
CI_{int} <3 ml/min/kg	79	76	68	61	54	42	41	39	52
Papp >200 nm/s	20	30	46	65	74	77	65	50	33
	iPFI = mChrom log P + #Ar								
hERG pIC_{50} <5 (+1 charge)	86	93	88	70	54	36	29	21	11
Promiscuity <5 hits with pIC_{50} >5	85	78	74	65	49	30	20	13	7

[a] Colouring refers to the % chance of achieving benchmark value in that PFI bin: green, ≥67%; yellow, 34–67%; and red, <33%.

[50]; this analysis suggests that hydrophobicity should be the pre-eminent parameter in medicinal chemistry, rather than size, weight or polar surface area. Additionally, a shape characteristic with orthogonality to hydrophobicity, which is demonstrated to be well represented by #Ar in this analysis, is a second key molecular descriptor. Together, as their sum (PFI), these form a sound and relevant foundation for property-based design that should facilitate better predictions and, thus, decision making in future [51]. The impact of low PFI figures reflects the trends noted by Leeson *et al.* [49], whereby the proposed shape parameter of Ar-sp^3 is considerably lower in given hydrophobicity bins for oral drugs than for compounds in pharmaceutical patents. Of course, a drug can and will lie outside of the chemical space defined by these and other guidelines [52]; but the probability of success, defined by minimising the chance of undesirable effects, is much greater within them. Lipinski's ground-breaking Rule of 5 [53], which was proposed as a guide to solubility and permeability, has been the established benchmark for drug discovery over the past decade; recent reviews [9,10,14] suggest much stricter property values should be adhered too. Perhaps optimal PFI values, underpinned by improved hydrophobicity determination, represent the aspiration for quality, non-obese and shapely candidates over the next decade, which should contribute to a reduction in attrition; by paying attention to PFI, these data suggest that many issues will take care of themselves.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.drudis.2011.06.001.

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