

Automated Property Profiling for Cellular Drug Delivery

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1. Introduction

Drug discovery research has been primarily focused on structure-activity relationships (SAR). Compounds from libraries are screened for activity and the “hits” are used as the starting point for the synthesis of analogs that explore the chemical space around the core structure in order to increase activity. Thus, the relationship of structure to function is mapped using various bio-assays of increasing sophistication. Molecular modeling assists in the planning of structural modifications by visualizing the active site of the target protein and modeling small-molecule structures that would have higher binding affinity for the target.

An increasing interest has arisen in an alternate means of increasing activity: the improved delivery of drug to the therapeutic target (1–12). Many barriers oppose the access of drug molecules to the target. In living systems, access to the target can be estimated via pharmacokinetics (PK). Thus, PK is used as a tool during drug discovery to monitor drug exposure after dosing. When PK characteristics are acceptable, then delivery is not considered a hurdle. However, in a large number of cases, PK is a hurdle and improvement must be undertaken for the compound series to be improved to reach the goal of discovering an effective development candidate. In order to diagnose the cause(s) of inadequate PK (1,3–5,13,14), discovery researchers recognized the role of physicochemical and structural (“drug-like”) properties. Some of the physicochemical properties most responsible for systemic exposure are solubility, permeability, lipophilicity, pKa, and stability. Screening of these properties is often termed “pharmaceutical profiling.” Measurement of properties *in vitro* was initiated in order to diagnose or predict PK properties.

The availability of data from the measurement from pharmaceutical profiling has opened new opportunities in other areas of pharmaceutical research beyond *in vivo* animal experiments. Properties also affect *in vitro* experiments using enzymes/receptors and cell-based assays (9,15). In the past, the lack of compound activity in an *in vitro* assay led to its removal from further consideration. However, binding to the active site of an enzyme or receptor target is not the only variable in an *in vitro* assay. Activity is also affected by barriers that attenuate compound delivery to the protein target. As with *in vivo* systems, physicochemical properties affect how the compound performs

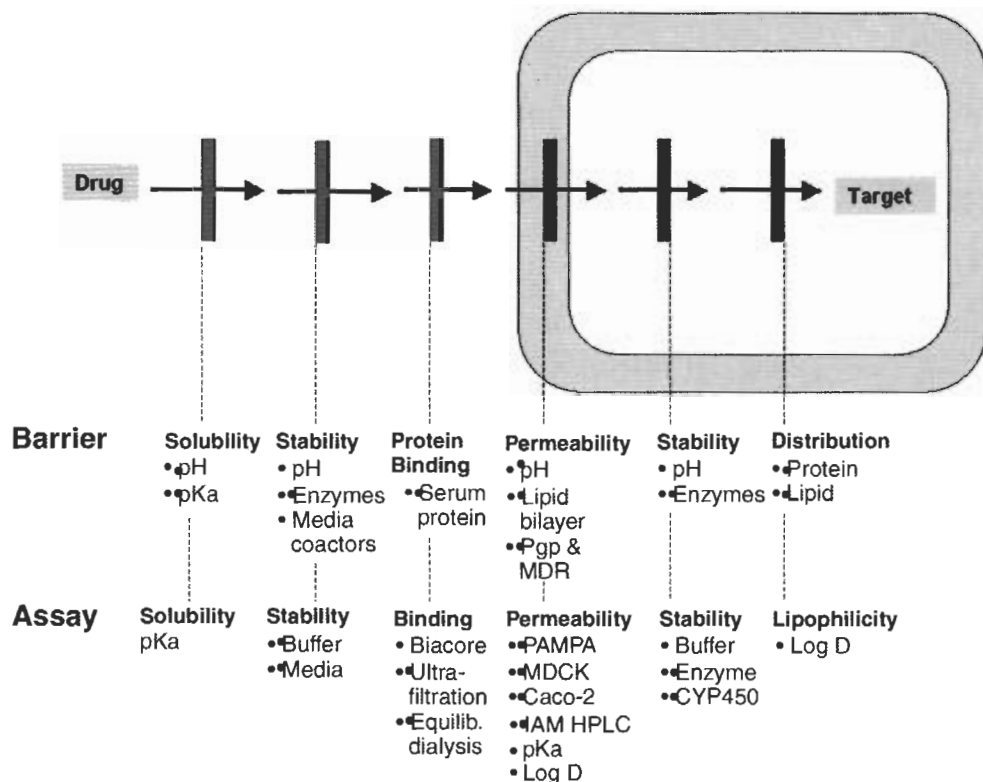


Fig. 1. Barriers to cellular drug delivery. Barriers are shown in bold. Detailed aspects of a barrier are listed as bullets. Pharmaceutical profiling assays that provide information on performance of compounds at the barriers are shown.

at these barriers and determine the ultimate concentration of compound reaching the target protein.

In this chapter, physicochemical and biochemical properties that affect cellular drug delivery will be discussed, along with automated assays that are used to measure them, and how this information may be used effectively.

2. Barriers to Cellular Drug Delivery

Several barriers exist between the drug that is in solution around the cell (in the interstitial fluid or cell-culture medium) and the target protein. These barriers are diagrammed in Fig. 1. The drug candidate first encounters the aqueous environment and its solubility at the pH of the solution depends on its intrinsic solubility and its pKa(s). For some compounds, poor solubility limits the concentration of compound surrounding the cells. Next the compound encounters the possibility of chemical conversion owing to chemical reactions. Its stability against hydrolysis by water, reactions with a substance in the solution, or catalysis by an enzyme (e.g., esterase) determines the dosed compound concentration available for cell penetration. Binding to proteins also limits the free compound in solution. Permeability of the cell membrane determines

how much compound is accessible to intracellular targets. The total rate of permeability is a composite of several permeability mechanisms. The compound pK_a and the pH of the solution determines the fraction of compound that is in the neutral form, that penetrates the lipid bilayer membrane. Passive diffusion through the lipid membrane is typically the predominant route of permeation. Active transport by membrane proteins can be a factor in the uptake of some compounds. Conversely, efflux by other membrane proteins (e.g., p-glycoprotein [P-gp]), removes drug candidate after it has gotten into the cell. Inside the cell, the compound may encounter other stability barriers, such as the intracellular pH and enzymes (e.g., esterase and cytochrome P450). Binding to other intracellular components owing to high lipophilicity distributes the compound and can limit the free movement of the compound to the target protein. Knowledge of these barriers and how the compound's properties perform at the barriers can help to interpret the net observed activity in cell culture experiments relative to noncellular *in vitro* enzyme and receptor-binding studies.

3. Pharmaceutical Profiling Assays for Cellular Delivery

As we have seen, compound properties can greatly affect cellular activity assays and should be taken into consideration in planning and interpreting experiments. In a later section, tactics for applying property information to cell-based research will be discussed. In this section, individual properties and how they can be screened for a large number of compounds is discussed (1,4,9). Property screening can begin as soon as compounds are first studied, such as "hits" from high throughput screening, and carried through during the synthesis of analogs in a chemical series.

3.1. Integrity

For any compound that is new to a research project, it is first important to establish the integrity (identity and purity) of the material. A compound may have been synthesized several years before and could have been mis-identified or could have degraded during storage owing to oxidation or hydrolysis. Sometimes compounds are mislabeled. If an activity or property is attributed to the wrong structure, any subsequent SAR or structure-property relationships (SPR) will be incorrect. Liquid chromatography/ultra-violet/mass spectrometry (LC/UV/MS) methodology typically is used to check integrity. Recent methods (16) use a rapid (<5 min) high-performance liquid chromatography (HPLC) analysis with a wide solvent-polarity gradient to separate sample components, detection, and quantitation by UV, and alternating positive and negative ion mass spectrometry for the molecular weight of each component (Fig. 2). Typically, only compounds with identity verified by molecular weight and having high purity should be studied. Integrity profiling should be performed for all compounds tested in cell-based activity assays.

3.2. Solubility

Sufficient solubility is necessary for most experiments performed in drug discovery and development (2,10,11). The maximum concentration at which a compound can be studied is determined by its solubility. Formulations can be developed to solubilize compounds at higher levels, if necessary, for *in vivo* dosing experiments. In drug discovery it is increasingly common for compounds to have low solubility. Solubility can affect activity as well as property assays (10,11). Solubility experiments traditionally have been conducted by placing solid material into an aqueous buffer, followed by

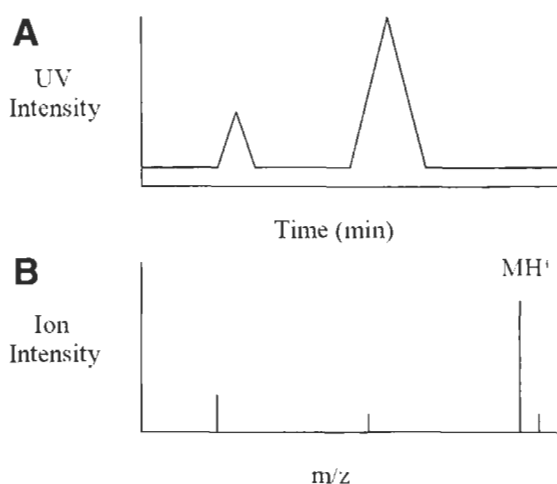


Fig. 2. Integrity profiling involves the estimation of sample purity from an HPLC chromatogram (A) and confirmation of identity from a mass spectrum (B).

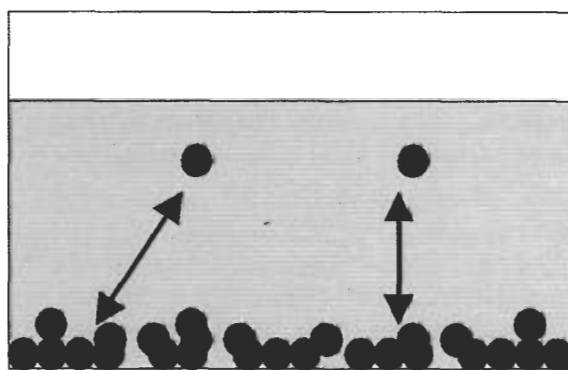


Fig. 3. Solubility profiling involves measurement of compound in aqueous buffer after a small volume of DMSO solution is added. Insoluble compound precipitates.

stirring and assaying using HPLC. This is typically called thermodynamic or equilibrium solubility measurement. However, for *in vitro* research it is more relevant to measure solubility when a dimethyl sulfoxide (DMSO) solution of the compound is added to an aqueous buffer (Fig. 3). This is because *in vitro* experiments are usually conducted by adding compound that has already been dissolved in organic solvent. This is a very different experiment and can produce very different solubility estimates. The experiment is typically called kinetic solubility measurement. Several automated assays for solubility have been described. These include turbidimetry (2), nephelometry (17), and direct UV (18) analysis. The turbidimetric and nephelometric methods involve the addition of DMSO compound solution to aqueous buffer. The solubility is determined from the appearance of precipitate, which is detected by light scattering. In the direct UV method, a 20 mg/mL solution of compound in DMSO is added to aqueous buffer in a 96-well plate. The final DMSO concentration is critical in any solubility assay because DMSO enhances aqueous solubility of organics. In this assay the final DMSO concen-

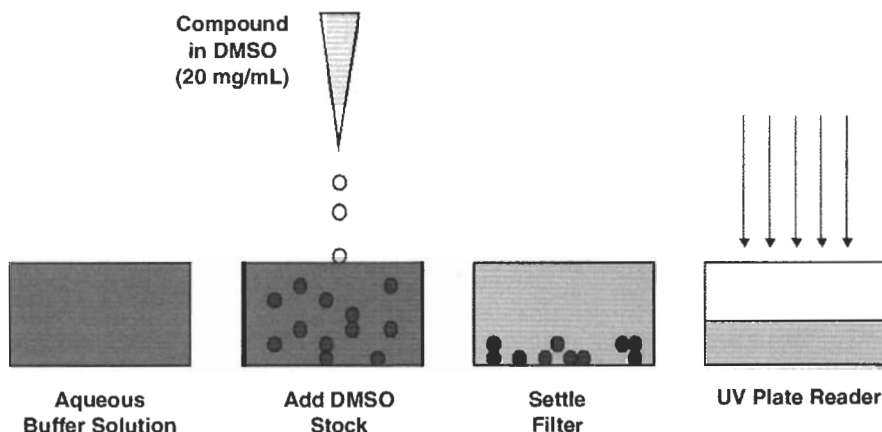


Fig. 4. Diagram of direct UV solubility assay. A DMSO solution of test compound is added to an aqueous buffer and allowed to equilibrate. The buffer is filtered to remove precipitate and the UV absorbance is obtained on a plate reader to measure concentration.

tration is less than 0.5% volume/volume. The solution equilibrates for a period of time (e.g., 18 h), the solution is filtered to remove precipitate, and the concentration of compound in solution is measured using a UV plate reader (*see* Fig. 4). Solubility measurement at the pH of the *in vitro* experiment is most valuable, because the compound's pK_a and the solution pH can dramatically affect solubility. This is because compounds are most soluble in their charged form.

Lipinski (10) discussed how Caco-2 cellular experiments are affected by the compound solubility. For prediction of gastrointestinal (GI) drug absorption, Caco-2 should be run at a concentration of 100 μM , because this is the approximate GI concentration for a 1 mg/kg orally administered drug. However, many labs perform the experiment at 10 μM . A concentration of 100 μM can saturate transporter proteins, which is not as likely at 10 μM . Moreover, even at 10 μM , up to one-third of compounds are not fully soluble, leading to inaccurate results.

Solubility should be screened as early as possible, owing to its great effect on activity assays. Currently, *in silico* methods for solubility are improving, but a measurement will assure greatest confidence for planning and interpreting experiments.

3.3. Permeability

In order for a compound to be active at intracellular therapeutic targets, it must permeate cellular lipid bilayer membranes. There are several major mechanisms of permeation into cells: passive diffusion, active uptake by membrane transporters, and active efflux by other membrane transporters. If the concentration of drug is high enough, protein transporters can become saturated, making passive diffusion the predominant permeation mechanism (19). Currently, it is very difficult to predict by software whether or not a compound will be a substrate for an active transport protein.

Several assays have been described for predicting permeability by passive diffusion through lipid membranes (*see* Fig. 5). Early methods used liposomes (20). Immobilized artificial membrane (IAM) chromatography has been used to simulate interactions of compounds with lipid membranes in a convenient, high-throughput format (21–23). Cel-

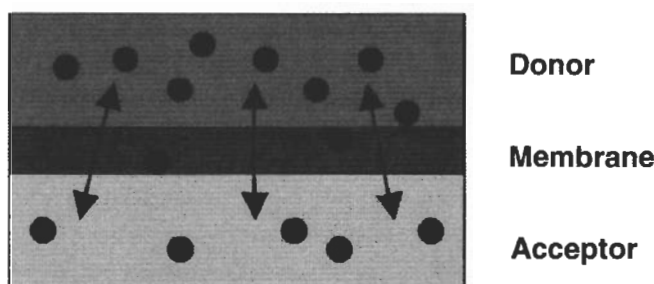


Fig. 5. Passive diffusion permeability profiling measures the rate of compound movement from the donor aqueous compound solution into the acceptor after passing through a membrane consisting of a cell monolayer or artificial lipid membrane.

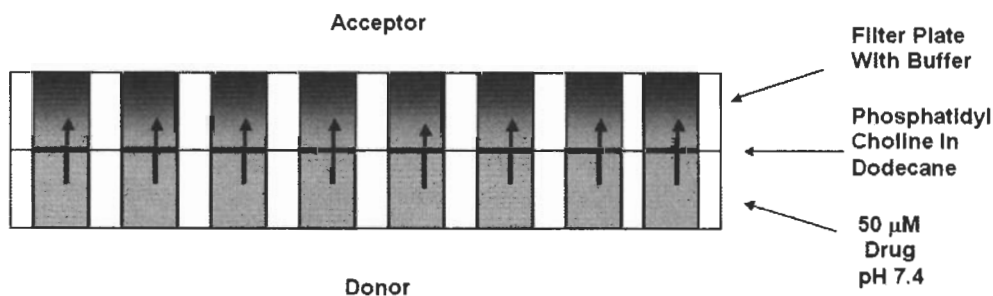


Fig. 6. Diagram of PAMPA assay. Test compound is placed in an aqueous buffer at 50 μM concentration in the donor well of a 96-well plate. A porous filter plate is placed on top in contact with the water and the pours are filled with a solution of phosphatidyl choline in dodecane. The test compound diffuses through the lipid and penetrates into the buffer in the acceptor wells.

lular-based models for permeability have been widely implemented using Caco-2 (24,25) and Madin-Darby canine kidney (MDCK) (26) cells. A monolayer of cells is grown to confluence on a porous filter surface and the test compound is placed in the buffer on one side of the monolayer (e.g., apical side). The rate of appearance of the compound on the other side of the monolayer (e.g., basolateral side) is measured using HPLC or LC/MS to calculate the permeability of the compound. Cellular models combine many mechanisms of permeability, so diagnosis of each mechanism for structural optimization requires additional studies. Expression of transporters varies with the lab or passage and should be monitored. Higher-throughput 96-well versions of Caco-2 have been reported (27). Caco-2 cells are polarized, in that the transport proteins are typically found on the apical side. Thus, they may not accurately predict the intracellular concentration and may be most appropriate for studying mechanisms of GI absorption for a compound.

Parallel artificial membrane permeability assay (PAMPA) is receiving increasing attention in drug discovery (28–31) (see Fig. 6). In place of a cellular monolayer of

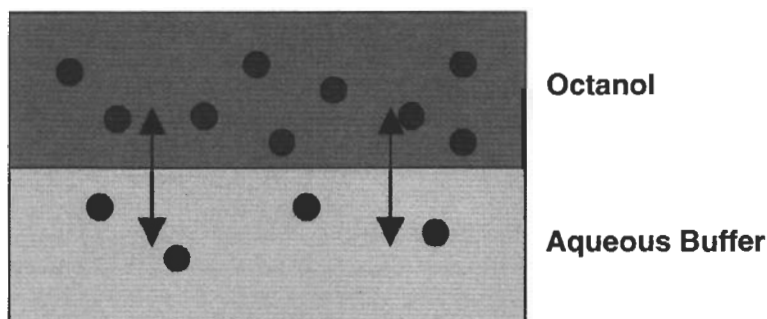


Fig. 7. Lipophilicity profiling measures the ratio of compound in the octanol and aqueous buffer layers after equilibration.

Caco-2, an artificial membrane is created using lipid in an organic diluent and supported within the pores of a 96-well filter plate. Because of the simpler aqueous matrix, a 96-well UV plate reader can be used for accelerated quantitation. The PAMPA assay is typically higher-throughput than Caco-2 and uses as little as 5% of the resources. A useful strategy for permeability is to use PAMPA for high-throughput assessment of passive diffusion for all compounds, then to use Caco-2 or another assay to probe other permeability mechanisms for a limited set of compounds. Permeability data has been used to correlate permeability to activity in cell-based activity assays and to diagnose mechanisms of permeability (32).

Permeability through specialized *in vivo* membranes, such as the blood-brain barrier (BBB), may be predicted using specialized cell-culture models (33), or with PAMPA using modified assay conditions (34). Some *in silico* methods for permeability have been developed, including QMPRplus (Simulations Plus Inc.) for MDCK cell permeability.

3.4. Lipophilicity

Many properties are affected by the fundamental molecular property lipophilicity, the tendency of a compound to partition into nonpolar vs aqueous environments (see Fig. 7). Increasing lipophilicity generally has the following effects on barriers: increasing permeability, decreasing solubility, decreasing metabolic stability, increasing protein binding, and increasing distribution to lipophilic sites. The typical measure of lipophilicity is the log of the coefficient of partitioning between octanol and water, termed "Log P." Log P is used for the partitioning when all of the compound is in the neutral state. A related term, "Log D," is used to describe the distribution when the aqueous phase is at a pH where part or all of the solute is charged. Log P and Log D values have been compiled from the literature by Hansch (35). For automation, the traditional "shake flask" method for Log P (36–38) was scaled down to 96-deep-well plate format (39). In this assay, a sample in DMSO is added to a plate well containing octanol and aqueous buffer, equilibrium is established between the phases, and each phase is sampled and quantitated. Other methods for lipophilicity include the pH-metric method (40,41), reversed-phase (RP)-HPLC (16,42–48) and microemulsion electrokinetic chromatography (49). In the chromatographic methods, retention time is calibrated to Log P or Log D using standards and the lipophilicity of test compounds is

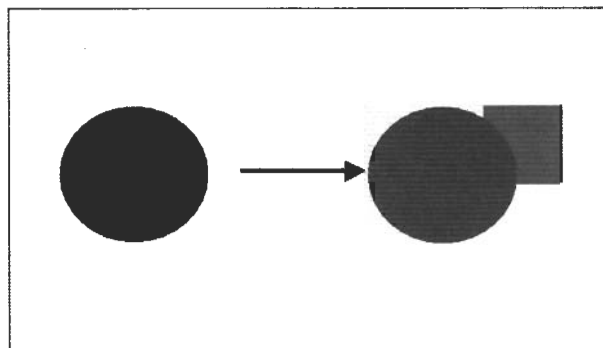


Fig. 8. Physicochemical stability measures the ratio of conversion product to unchanged compound after incubation in a physicochemical environment.

estimated from their retention time. Good calculations of Log D can be obtained from *in silico* programs such as Prolog D (CompuDrug Chemistry Ltd.).

3.5. Physicochemical Stability

Compounds can be chemically converted in aqueous solutions (*see* Fig. 8). These reactions can produce permanent products or reversible products in equilibrium with the starting material. Chemical conversion can cause variability in assay results. Conversion can be screened by incubating the compounds with buffer and testing for the percent of starting compound compared to an unincubated control. Components in some assay buffers, such as dithiothreitol (DTT), can react directly with compounds. Physical conditions in the laboratory can cause compound reactions also. These include basicity of some glassware, buffer pH, laboratory light exposure, solution temperature, and air oxidation or hydrolysis (50–52). If compound degradation is a potential problem, compounds can be incubated under various pHs, high-intensity light, or elevated temperature to measure their rate of degradation. Quantitation typically uses LC/MS or HPLC.

3.6. *pKa*

Many compounds of interest in biomedical research have one or more *pKas* (the negative exponent of the equilibrium constant between the charged and uncharged forms of a particular functional group of the compound). The *pKa* and pH of the solution dictate the degree of compound ionization in solution (*see* Fig. 9). The ionized form is more soluble in water, because it is more polar. The neutral form is more permeable through lipid bilayer membranes. The *pKa* can also affect binding energies of a compound to the active site of the target protein. The *pKa* of a typical amine is in the range of 9–10; weaker bases have lower *pKas*. When the pH is below the *pKa*, the base will have more of its population in the protonated form. The *pKa* of a carboxylic acid is in the range of 3–4; weaker acids (e.g., phenols) have higher *pKas*. When the pH is below the *pKa*, the acid will have more of its molecules in the neutral form. A useful rule of thumb is that an equal concentration of the neutral and charged species is found in solution when the pH equals the *pKa*.

pKa has been measured in automated mode using techniques involving potentiometric titration (40), capillary electrophoresis (CE) (53), and spectral gradient analysis

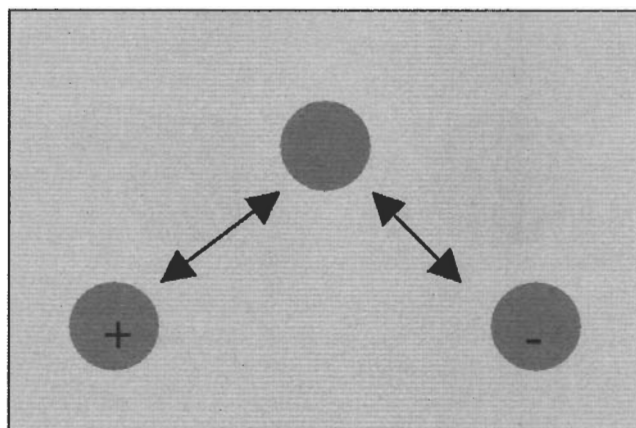


Fig. 9. pKa profiling measures the ratio of neutral compound to charged compound at a range of pHs.

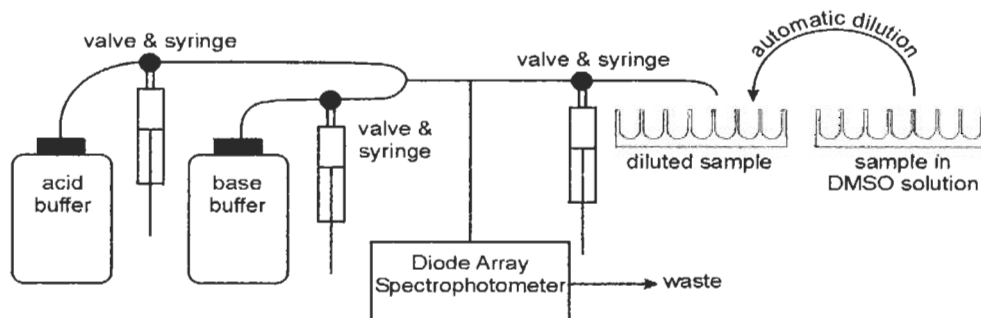


Fig. 10. Diagram of the SGA pKa analysis method. Sample in DMSO is diluted in aqueous buffer and injected into a flowing stream created by gradient mixing of acid and base buffers. pKa is determined from the change in UV absorbance across the pH range.

(SGA) (54) (*see* Fig. 10). The SGA method creates a pH gradient in a flowing liquid stream over a 2 min period; the compound is injected continually into the stream and the UV absorbance of any chromophore in the near vicinity of the ionizable group changes with the ionization. This change is used to determine the pKa. pKa is calculated as the pH at which equal concentrations of charged and uncharged species are present. When the pKa is known, chemists can modify the ionizable functional groups on a molecule to change the solubility and permeability characteristics. Good calculations of pKa can be obtained from some *in silico* programs, such as Advanced Chemistry Development Inc.

3.7. Biochemical Stability

Many compounds in biomedical research are susceptible to biochemical conversion (*i.e.*, metabolism) (*see* Fig. 11). Conversions can be caused by cytochrome P450 oxidations within the cell or other enzymatic reactions (*e.g.*, phosphorylation, hydrolysis). Biochemical reactions reduce the concentration of compound. Significant instability

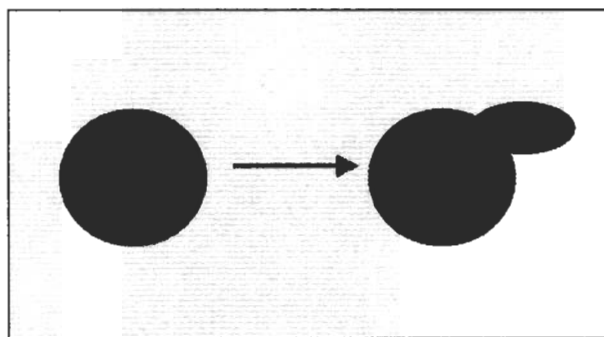


Fig. 11. Biochemical stability measures the ratio of metabolic product to unchanged compound after incubation in a biochemical environment.

compromises the proper interpretation of experiments and indicates a poor compound for *in vitro* studies. Metabolic stability assays are often conducted with liver microsomes containing the cytochrome P450 enzymes (55–57), plasma (58), or specific enzymes. Various animal species are often tested. It is useful to test stability in the same species as is being used in the cell culture or *in vivo* experiment. Stability assays are readily automated using standard laboratory robots (e.g., Packard, Tecan). Following incubation, the samples are typically quantitated using LC/MS.

Stability assays require high analytical sensitivity (owing to the low concentrations used to ensure that the enzymes are not saturated [e.g., 1 μ M]) and selectivity to resolve the test compound signal from interfering signals of sample matrix components. Often high-throughput stability assays can only be achieved using mass spectrometry-based methods. Janizewski (59) described a customized LC/MS method having throughput on the order of 30 s/sample. Recently introduced instruments and software make implementation of high-throughput LC/MS/MS analysis easier for the average scientist (60).

As with other profiling methods, the assay seems uncomplicated and easy to implement. However, experience has shown that results can vary widely, depending on the conditions and their day-to-day control. Factors such as DMSO concentration, sample concentration, species, and biochemical reagent (e.g., microsome) preparation can greatly affect the rate of biochemical reactions (57). The data can be misleading if the assay is performed improperly.

3.8. Conversion Product Identification

When significant instability is indicated for a compound, useful information can be obtained by identifying the conversion products (*see* Fig. 12). This information allows redesign of structures in order to reduce reactions at the labile site of the molecule and reduce the conversion barrier. By identifying and isolating or synthesizing the conversion products, they can be tested for activity and/or toxicity. The use of LC/MS/MS for rapid structure elucidation has been described (61–64). By using the MS/MS fragmentation of the parent compound as a template, the structures of major metabolites can be determined. MS techniques have the advantage of high throughput and low sample consumption (1–100 ng). Modern instrumentation and software allow the trained analytical chemist to rapidly identify the major conversion products of several compounds each day (*see* Fig. 13). When MS techniques do not indicate sufficient structural detail, nuclear magnetic resonance (NMR) or LC/NMR techniques (65) are

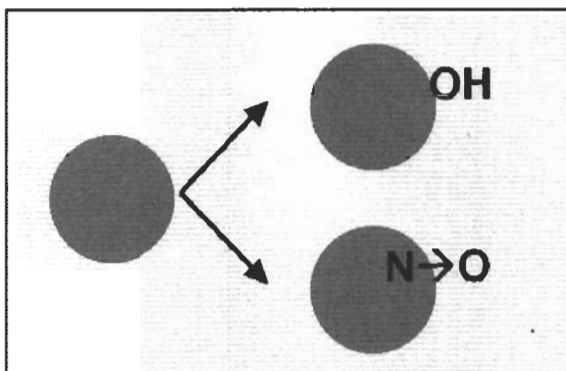


Fig. 12. Conversion product identification uses spectroscopic methods to rapidly identify the structure of the conversion product(s) from stability profiling.

used. This technique has made strides over the past 5 yr for trace compounds, but significantly higher amount of sample (0.1–10 μg) is needed and throughput is low compared to LC/MS/MS.

3.9. Active Transporters

The effect of active transport (*see* Fig. 14) on compound permeability is being studied more frequently in biomedical research. Cell-based assays can provide deeper understanding of transporter effects. Unknown affinity for transporters can cause errant prediction of membrane permeability if passive diffusion alone is considered. One of the most studied transporters, P-gp, is active in the intestine, BBB, and drug-resistant cancer cells. Polli (66) described and compared three methods for P-gp affinity: ATPase, Calcein AM, and MDR1-MDCK cell permeation. Many groups also use Caco-2 to study transport by use of apical to basolateral ($A \rightarrow B$), basolateral to apical ($B \rightarrow A$), and inhibitor studies (e.g., verapamil inhibits P-gp). Reduced P-gp affinity will likely improve performance in bioavailability, brain penetration, or activity in cancer cells.

Uptake transporters (e.g., OCT1, OATP1, OATP2, MOAT) and efflux transporters (MRPs) are under intensive study (67). Not only will this research help to explain compound behavior in cell-culture experiments, but it will provide opportunities to enhance intracellular concentrations and permeation rates by reducing efflux or increasing uptake.

3.10. Cell Uptake

Because intracellular concentration often correlates with compound activity, assays for cell concentration are sometimes used (*see* Fig. 15). If the cellular activity is lower than expected from cell-free *in vitro* studies, the cellular concentration may be the cause, not the intrinsic activity. Low cellular concentration of drug may be owing to P-gp efflux, poor passive membrane permeation, or intracellular conversion/metabolism. In some cases, metabolic conversion may be necessary for activity and formation of the active species can be monitored. Cellular-exposure experiments have often utilized radiolabeled compounds for sensitive and rapid analysis. However, radiolabeling can be expensive and is not feasible in research involving the study of hundreds to thousands of compounds per year. Increasingly, LC/MS/MS techniques are being used to measure the

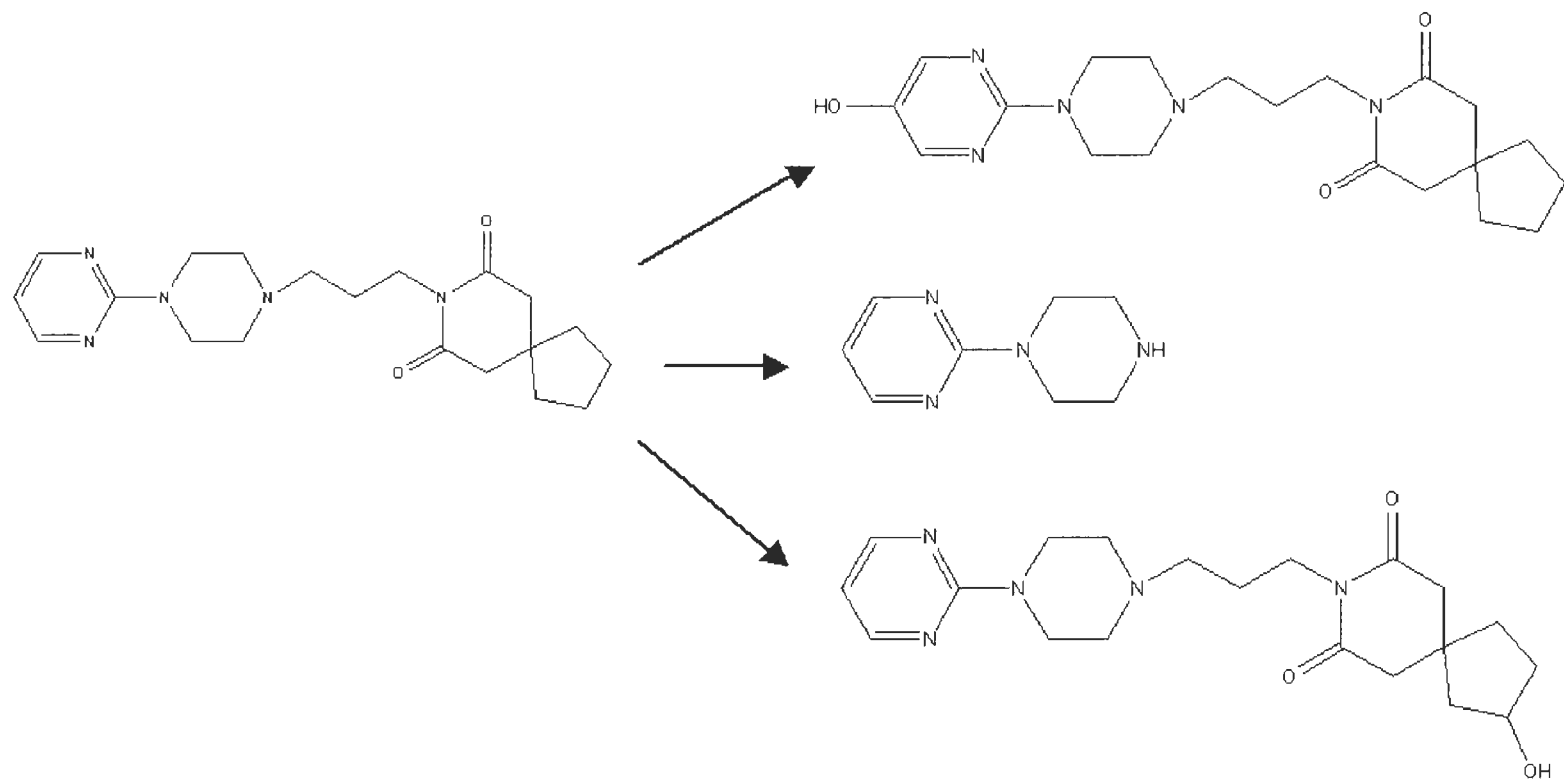


Fig. 13. Some common routes of metabolic degradation of busiprone catalyzed by cytochrome P450 enzymes in liver microsomes.

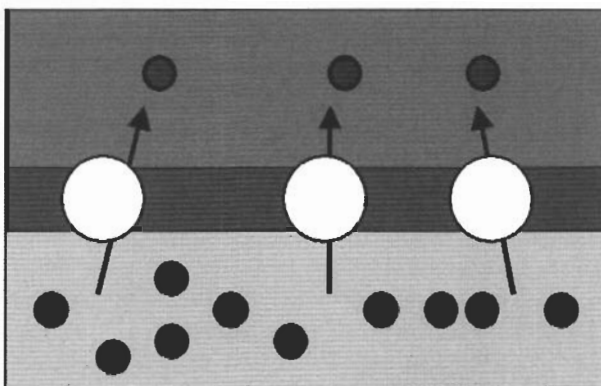


Fig. 14. Active transporter profiling indicates if cellular-membrane permeation is enhanced by active uptake or reduced by membrane efflux-transporter proteins.

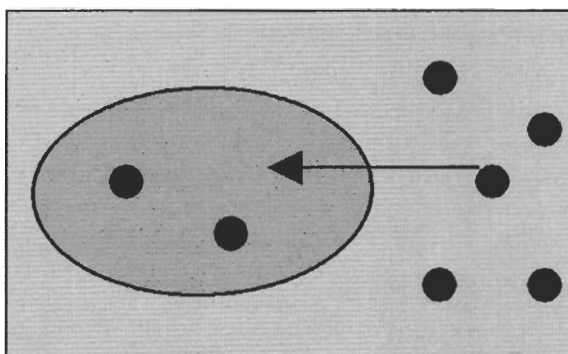


Fig. 15. Cell-uptake profiling measures the intracellular concentration of compound after it is added to the culture medium.

intracellular concentration of compounds, following incubation, washing, and harvesting of cells in culture plates (68,69) (*see* Fig. 16). Sensitive and selective LC/MS/MS methods can be rapidly and inexpensively developed, compared to radiolabeled detection schemes. The measured cellular concentration is the sum of compound bound to the cell membranes and proteins, as well as that dissolved in the cytoplasm.

3.11. Plasma Protein Binding

Binding of drug molecules to proteins (PPB) in plasma, cell-culture medium (e.g., serum albumin, α -acid glycoprotein), on the cell surface, or inside cells limits their free motion to the target protein (*see* Fig. 17). Several of the effects of binding offset each other, with regard to compound concentration at the therapeutic target, so application of PPB information can be confusing for chemists and pharmacologists and can be misinterpreted as a negative characteristic. PPB can be estimated using higher-throughput techniques, such as HPLC with HSA columns (70), 96-well equilibrium dialysis (71), Biacore (72), or ultrafiltration (Millipore Microcon[®]).

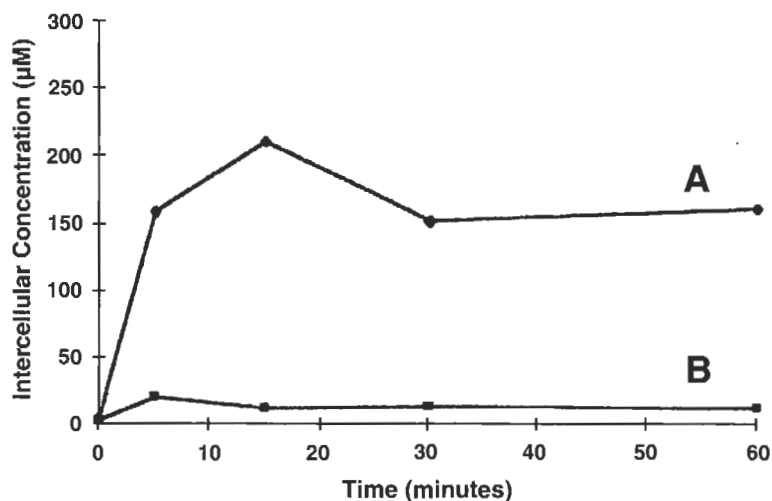


Fig. 16. Intracellular concentration of paclitaxel in normal cells (A) and multidrug resistant cells (B) in culture after dosing paclitaxel in cell-culture medium.

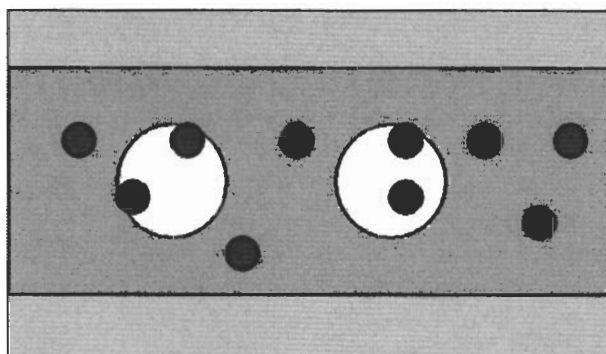


Fig. 17. Plasma protein-binding measures the ratio of compound that is bound to plasma proteins vs unbound in aqueous buffer solution.

4. Applying Pharmaceutical Profiling Data

In drug discovery, favorable properties are often termed “drug-like” and are predictive of potential drug-candidate success. This is because these properties are necessary for the delivery of compound to the target protein, which is often within the cell. Early knowledge of compound properties will aid any biomedical research because property information can be applied in several important ways.

There is a tradeoff between properties and potency. Lipinski (2) discussed the close relationship between properties and potency; poorer properties (solubility and permeability) can be tolerated for drugs with high potency (low mg/kg dose), but more favorable properties are necessary for drugs with lower potency. Van de Waterbeemd (1) provided a very informative discussion on “property-based design.” This looks at optimizing the properties of compounds by structural modification, in order to improve the delivery of compound to the target protein. This can be done in parallel with structure-based design, in which SARs are a primary focus.

A key opportunity for the application of property information is better-informed research, by having more information to use in experimental design and interpretation. For example, solubility has a major effect on cellular experiments, so experiments can be planned at concentrations that are consistent with compound solubility. Unfavorable properties limit delivery of compound to the target protein, so properties greatly affect the perceived activity of compounds. Observed activity is a function of intrinsic target-protein binding, as well as delivery of compound to the intracellular target. Without property information, the cellular activity experiment may be incorrectly interpreted and limit research progress.

Complex processes, such as activity in cell-based assays, are functions of several properties. Thus, measurement of the individual properties that are components of a complex process allows the researcher to diagnose the limiting properties. Gan and Thakker (12) discussed how poor compound performance in complex systems can be diagnosed by examining individual properties. When this screening reveals the property responsible for the poor performance, then this property can be targeted for synthetic improvement. As new compounds are synthesized, the automated property profiling assays can be used to monitor improvement.

The dependence of a given biological activity assay on properties can be correlated using mathematical analysis techniques such as multivariate analysis (MVA). In one example (9), MVA revealed that a cell-based potency assay correlated well with solubility and permeability, but not with stability. Once such correlations are recognized, then the properties most favorable to enhanced activity can be improved.

Early warning of major faults with a compound can save significant resources, time, and emotional investment. Research scientists can redirect their energies into more promising directions. If research focuses solely on SAR, time and resources can be wasted on compounds that have unfavorable properties. SAR is often favored by higher lipophilicity to promote better binding to the target protein, but such compounds can have low solubility and instability. An early alert to poor properties can help to avoid costly mistakes.

Often cellular experiments are performed in advance of *in vivo* experiments with sophisticated models such as transgenics or models that require weeks to complete. These models provide critical information, but are expensive and/or time-consuming. Property information can be included in the multifaceted information used to select compounds for advancement from cellular to sophisticated *in vivo* models.

If properties are well-characterized, they can also be used to guide optimization of compounds to improve cellular delivery. Sometimes the most promising leads have poor properties and this information helps to highlight the resources that will be needed to improve the properties. Drug-likeness has received increasing attention as an important predictor of success. The lack of drug-likeness reaches the attention of decision-makers who insure that a particular deficiency is addressed as the project proceeds. The SAR approach modifies the compound structure to make it bind better to the therapeutic enzyme or receptor. The SPR approach modifies the structure to improve properties that increase the delivery of the compound to the therapeutic target and sustain its concentration there. Thus, properties can help to guide synthetic chemistry efforts aimed at optimizing compounds during drug discovery. Examples of successful synthetic modifications include molecular size, hydrogen bonding, pKa, adding an ionizable group, and blocking substructures that are unstable. The optimum candidates are now optimized for both activity and properties.

At the same time, one must be aware that structural modifications that improve one property may diminish another property (13). For example, increasing metabolic stability at one site may increase metabolism at another site, reduce permeability, or increase renal or biliary extraction. Also, in some cases, a property cannot be improved through structural modification without significantly reducing the activity. A rule of thumb is that if the poor properties result from a substructure that is critical for the activity, it will be difficult to both improve properties and maintain activity. Medicinal chemists can initiate property improvements by focusing on the modification of substructures that are shown, through SAR studies, to have little effect on activity.

Improvement of properties such as permeability through prodrugs is an established strategy. Profiling assays can be used to compare prodrug conversion rates. Specialized assays for the targeted conversion mechanism can be developed.

Activity response at the cellular level follows a dose-response relationship. Thus, a compound that is very active in an enzyme/receptor assay may not be active in cell culture if sufficient concentration is not established in the cells. A less potent compound may provide a better activity if its properties permit enhanced intracellular concentration over a longer time.

Lipinski discussed how solubility greatly affects activity assays (10,11). In addition, if a compound has poor permeability it will not perform well in cell-based assays. Thus, the perceived activity of the compound in cells would be low, and the actual mechanism of the low activity, whether biochemical or physicochemical, would not be understood. In the past, property information was not available, but now researchers can take advantage of the improved methods for obtaining this information to enhance their research.

5. Method Selection Considerations

Several methods are available to researchers for assessing properties. The researcher should carefully examine how a method was developed and validated, such as the quality of the data set used and the quality of the correlation. Many methods are developed using a diverse set of compounds, but the compounds used may not be relevant to the researchers compound class and thus produce poor results. It is generally agreed that data and predictions are most accurate within a chemical series.

Rules and filters, such as the "rule of 5" (2), rotatable bonds (73), and polar surface area (74) provide a quick and easy guide for whether compounds are likely to have favorable properties for penetration into cellular targets (10).

In silico models (75) are fast to use. They are inexpensive if the software cost can be spread over many compounds or research projects. They can be used in planning prior to chemical synthesis and to estimate the properties of compounds for which there is insufficient material for analysis, or when a particular property assay is not available.

Automated assays provide actual measurements of properties. The assays are rapidly performed and little material (mg level) is required. These assays are most efficient when a large number of compounds or several projects share the expense. Measurements provide an increased level of data assurance over in silico methods, but the assays need to be carefully developed, validated, and controlled for quality.

In-depth analyses provide the most definitive and reliable information. These assays require large quantities of material (10–100 mg), often require weeks to complete, and can only be performed on a limited number of compounds. Careful quality control (QC) must be performed.

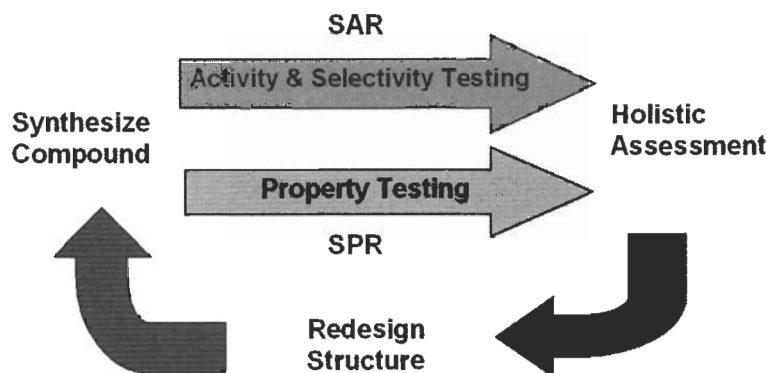


Fig. 18. Strategy for optimization involving parallel SAR and SPR studies, leading to holistic understanding of the compound and redesign of the structure for the next cycle of synthesis and testing.

The predictivity of *in silico* methods is rapidly improving as academic and commercial groups focus on their improvement. They rely on high-quality data from diverse compound structures. As *in silico* methods improve, they will provide sufficient information for early studies and measurement resources can be repositioned to assess other critical properties. Combination of *in silico* and measurement methods appears to provide the solution for obtaining the optimum property information with the available resources at this time.

Automated assays are often applied in a stepwise approach. Clearly, certain assays should be performed as early in research as possible: integrity to insure that the SAR is correct, and solubility and permeability to insure that biological activity measurements are not compromised by inappropriate assay conditions. Without this knowledge, active pharmacophores that were studied at levels above their actual solubility, may be eliminated prematurely from consideration. As research projects progress, additional property information can be obtained, or “full” profiles can be done on all new series compounds.

The effective application of pharmaceutical properties in drug discovery requires acceptance by discovery chemists and biologists. Several elements are necessary to gain buy-in. Pharmaceutical profiling must be as rapid as biological assays. Measurement of properties can occur in parallel with activity (Fig. 18) so that all the information is considered in redesigning structures for series optimization. Rapid and effective data communication to the researcher is critical. Tables of data that allow compound comparison are useful. Color coding of results, according to “bins” of favorable, moderate, and unfavorable property values allow researchers to rapidly review the data to prioritize compounds (9). Visualization tools such as Spotfire (76) and multivariate analysis tools such as Simca (77) can provide additional insight that is not discerned by inspection of tables or single dimensional data. Lipinski (11) has discussed how simple graphical presentations are most effective for data communication. The research leader and scientists must be trained to understand the properties and their effects on the barriers encountered by the compound. Property data must be relevant to chemical structure, so that medicinal chemists can design new structures that improve lipophilicity, pKa, molecular weight (MW), hydrogen bonding, and stability. Effective automation of pharmaceutical profiling assays is necessary for efficiency, accuracy, and precision.

6. Conclusions

Pharmaceutical profiling is an emerging field in drug discovery. It complements the current SAR paradigm by providing SPR information. This is useful because properties have a major effect on the delivery of compounds to cellular therapeutic targets. Automated property assays have been developed for the critical properties so that data can be provided rapidly for researchers. The information allows researchers to plan and interpret their experiments in greater depth. It also allows the diagnosis of compound performance at barriers that limit cellular delivery. Compound properties can be improved via structural modifications guided by the assay data.

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