# Part 2
# Physicochemical Properties

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# Rules for Rapid Property Profiling from Structure

*Overview*

- *Lipinski and Veber rules are guidelines for structural properties of drug-like compounds.*
- *Rules are effective and efficient means of rapidly assessing structural properties.*

The fastest method for evaluating the drug-like properties of a compound is to apply "rules." Rules are a set of guidelines for the structural properties of compounds that have a higher probability of being well absorbed after oral dosing. The values for the properties associated with rules are quickly counted from examination of the structure or calculated using software that is widely available. These guidelines are not absolute, nor are they intended to form strict cutoff values for which property values are drug-like and which are not drug-like. Nevertheless, they can be quite effective and efficient.

## 4.1 Lipinski Rules

Although medicinal chemists and pharmaceutical scientists had used structural properties in various ways for many years, rules became more prominent and defined in the field with the report by Lipinski et al.[1] of the "rule of 5," or what has become known as the "Lipinski rules." These rules are a set of property values that were derived from classifying the key physicochemical properties of drug-like compounds. The rules were used at Pfizer for a few years prior to their publication and since then have become widely used. The impact of these rules in the field has been very high. This acceptance can be attributed to many factors:

- The rules are easy, fast, and have no cost to use.
- The "5" mnemonic makes the rules easy to remember.
- The rules are intuitively evident to medicinal chemists.
- The rules are a widely used standard benchmark.
- The rules are based on solid research, documentation, and rationale.
- The rules work effectively.

It is important to keep in mind the intended purpose of the rule of 5. The article[1] states: poor absorption or permeation are more likely when:

- >5 H-bond donors (expressed as the sum of all OHs and NHs)
- MW > 500

- logP > 5 (or MlogP > 4.15)
- >10 H-bond acceptors (expressed as the sum of all Ns and Os)
- Substrates for biological transporters are exceptions to the rule

Examples of counting hydrogen bond donors and acceptors are given in Table 4.1. For example, an R-OH counts as both one H-bond donor (HBD) and one H-bond acceptor (HBA). Although "violation" of one rule may not result in poor absorption, the likelihood of poor absorption increases with the number of rules broken and the extent to which they are exceeded.

**TABLE 4.1 ► Examples of Counting Hydrogen Bonds for Lipinski Rules**

| Functional group | H-bond donor | H-bond acceptors |
|---|---|---|
| Hydroxyl | 1 (OH) | 1 (O) |
| Carboxylic acid | 1 (OH) | 2 (2 Os) |
| –C(O)-N-$R_2$ | 0 | 2 (N, O) |
| Primary amine | 2 ($NH_2$) | 1 (N) |
| Secondary amine | 1 (NH) | 1 (N) |
| Aldehyde | 0 | 1 (O) |
| Ester | 0 | 2 (O) |
| Ether | 0 | 1 (O) |
| Nitrile | 0 | 1 (N) |
| Pyridine | 0 | 1 (N) |

The rules were derived by examining the structural properties of compounds that, by examination of the United States Adopted Names (USAN) Directory, had survived phase I clinical trials and had moved on to phase II studies. Phase I studies involve human dosing to determine the toxicity and pharmacokinetics. The fact that the compounds had moved on to phase II studies indicates that the compounds had sufficient absorption in humans for pharmaceutical companies to continue investment in their development. The structural properties of this set of 2,200 compounds were examined and clear trends were observed, which became the rules. The rules were set at the 90th percentile of the compound set; thus, 90% of the compounds that had sufficient absorption after oral dosing had molecular property values within the Lipinski rules. Compounds that approach or exceed these values have a higher risk of poor absorption after oral dosing.

The rules are based on a strong physicochemical rationale. Hydrogen bonds increase solubility in water and must be broken in order for the compound to permeate into and through the lipid bilayer membrane. Thus, an increasing number of hydrogen bonds reduces partitioning from the aqueous phase into the lipid bilayer membrane for permeation by passive diffusion. Molecular weight (MW) is related to the size of the molecule. As molecular size increases, a larger cavity must be formed in water in order to solubilize the compound, and solubility decreases. Increasing MW reduces the compound concentration at the surface of the intestinal epithelium, thus reducing absorption. Increasing size also impedes passive diffusion through the tightly packed aliphatic side chains of the bilayer membrane. Increasing Log P also decreases aqueous solubility, which reduces absorption. Finally, membrane transporters can either enhance or reduce compound absorption by either active uptake transport or efflux, respectively. Thus, transporters can have a strong impact on increasing or decreasing absorption.

Lipinski et al. discussed important implications of these rules in light of current drug discovery strategies. The discovery lead optimization stage often increases target binding by

adding hydrogen bonds and lipophilicity. Thus, activity optimization can reduce the drug-like properties of a compound series. Combinatorial chemistry and parallel array synthesis tend to be more facile with more lipophilic groups; thus, analog series often increase in lipophilicity. In biology, high-throughput screening (HTS) tends to favor more lipophilic compounds than screening strategies in previous decades because compounds are first dissolved in DMSO and not in aqueous media, as in the past. Therefore, to obtain favorable biological data from modern in vitro biology techniques, a compound need not have significant aqueous solubility. Compounds previously were tested initially by dissolution in aqueous media for in vivo testing and, thus, were required to have aqueous solubility in order to be successful in biological testing. The use of screening libraries having good drug-like properties was recommended. This concept has been extended in recent years with the concept of "lead-like" compounds (see Chapter 20).

## 4.2 Veber Rules

Additional rules were proposed by Veber et al.[2] They studied structural properties that increase oral bioavailability in rats. They concluded that molecular flexibility, polar surface area (PSA), and hydrogen bond count are important determinants of oral bioavailability. Rotatable bonds can be counted manually or using software. PSA is calculated using software and is closely related to hydrogen bonding.

Veber rules for good oral bioavailability in rats are as follows:

- $\leq$10 rotatable bonds
- $\leq$140 Å$^2$ PSA, or $\leq$12 total hydrogen bonds (acceptors plus donors)

## 4.3 Other Rules

Other researchers have proposed other rule sets. Pardridge[3] proposed rules for blood–brain barrier permeability. These rules are used for predicting compounds that have a greater likelihood of permeating the blood–brain barrier (see Chapter 10). Oprea et al.[4–7] proposed the "rule of 3" for lead-like compounds. These rules are used to guide the selection of leads for the lead optimization stage of discovery (see Chapter 20).

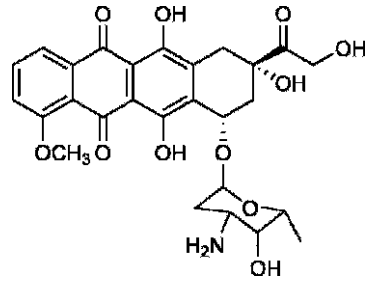
## 4.4 Application of Rules for Compound Assessment

Rules are typically used for the following purposes:

- Anticipating the drug-like properties of potential compounds when planning synthesis
- Using the drug-like properties of "hits" from HTS as one of the selection criteria
- Evaluating the drug-like properties of compounds being considered for purchase from a compound vendor

An example of the counting and calculation of rules the compound doxorubicin is shown in Figure 4.1. Doxorubicin has a very low oral bioavailability, as would be anticipated from the structural properties covered by the rules.

Figure 4.2 shows an example of how rules could help anticipate absorption in a drug discovery project.[8] Structural modifications of the compound on the left were made to optimize



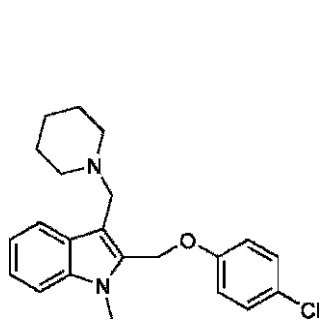
Lipinski Rules

- H-bond donors = 7
- MW = 543
- ClogP = –1.7
- H-bond acceptors = 12

Veber Rules

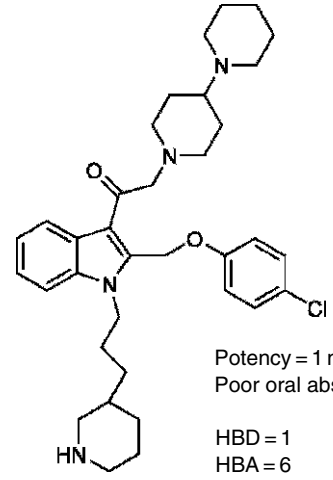
- Rotatable bonds = 11
- PSA = 206
- Total H-bonds = 19

**Figure 4.1** ► Example of counting and calculations for the Lipinski and Veber rules for doxorubicin, which has an oral bioavailability of approximately 5%. Guidelines are exceeded for all rules except ClogP.



Potency = 2 μM

HBD = 0
HBA = 3
MW = 369
Log P = 5.7
PSA = 17
Rotatable bonds = 6



Potency = 1 nM
Poor oral absorption

HBD = 1
HBA = 6
MW = 591
Log P = 7.3
PSA = 50
Rotatable bonds = 14
Total HB = 6

**Figure 4.2** ► Structural optimization for activity in this neuropeptide Y Y1 antagonist discovery project modified the lead on the **left** to the compound on the **right**. Although a 2,000-fold increase in potency was achieved, the resulting compound had poor absorption properties after oral dosing, as anticipated from the structural rules.

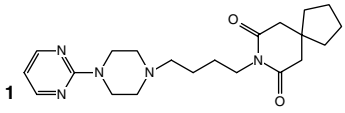
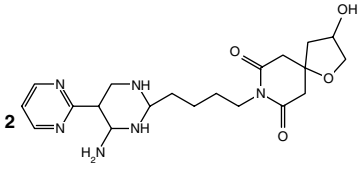
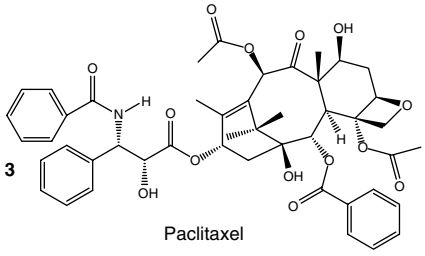
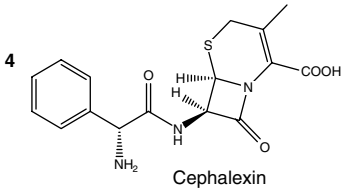
activity, resulting in the compound on the right. Unfortunately, the binding-optimized compound had poor absorption after oral dosing, as could be predicted from the structural properties prior to synthesis.

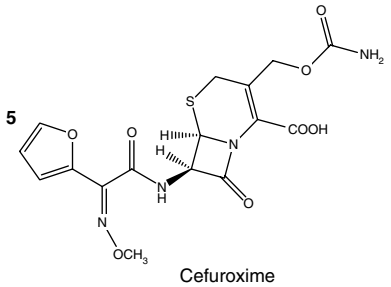
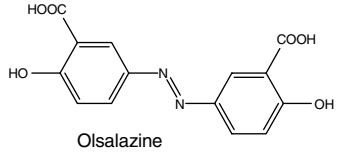
Exceeding the rules often reduces absorption after oral dosing. Poor absorption properties result in low bioavailability or the need for dosing via an alternate route, either of which limits the potential scope of the drug product.

## Problems

(Answers can be found in Appendix I at the end of the book.)

1. Low absorption is more likely for a compound that has which of the following?: (a) 7 H-bond donors, (b) 2 H-bond donors, (c) MW 350, (d) MW 580, (e) ClogP 7.2, (f) ClogP 2.7, (g) 5 H-bond acceptors, (h) 13 H-bond acceptors, (i) high permeability by uptake transporter, (j) PSA 155, (k) PSA 35.
2. Why are H-bonds important in absorption?
3. Why is high Log P unfavorable in absorption?
4. Rules are best used for: (a) strict guidelines for compound rejection, (b) assessing compounds for which no in vitro property data are available, (c) sole basis for selecting compounds for in vivo studies, (d) anticipating the metabolism of compounds?
5. Count the number of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) in the following using the Lipinski rule: (a) $-COOCH_3$, (b) $R_1$–NH-C(O)-$R_2$.
6. Determine the rule of 5 values for the following compounds. Indicate which structural properties exceed the rules and are a problem.

| Structure | #HBD | #HBA | MW | cLogP | PSA | Problem |
|---|---|---|---|---|---|---|
| 1  Buspirone | | | 385 | 1.7 | 7.0 | |
| 2  | | | 418 | −3.3 | 143 | |
| 3  Paclitaxel | | | 852 | 4.5 | 209 | |
| 4  Cephalexin | | | 347 | 0.5 | 138 | |

| Structure | #HBD | #HBA | MW | cLogP | PSA | Problem |
|---|---|---|---|---|---|---|
| 5  Cefuroxime | | | 424 | −1.5 | 199 | |
| 6  Olsalazine | | | 302 | 3.2 | 141 | |

7. Which of the following characteristics puts a compound at risk for poor absorption?: (a) MW 527, (b) 5 H-bond acceptors, (c) ClogP 6.1, (d) is a substrate for an uptake transporter, (e) 7-H-bond donors, (f) PSA 152.
8. Which of the following is an effect of H-bonding?: (a) H-bonds increase lipid solubility, (b) H-bonds increase water solubility, (c) H-bonds decrease water solubility, (d) H-bonds must be broken for the molecule to partition into the bilayer membrane.
9. Which of the following is a positive effect of a lower MW?: (a) water solubility increases, (b) acid decomposition decreases, (c) passive diffusion increases.

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# Lipophilicity

*Overview*

- *The lipophilicity of a compound is commonly estimated using Log P from octanol/water partitioning.*
- *Lipophilicity is a major determinant of many ADME/Tox properties.*

Lipophilicity is a property that has a major effect on absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) properties as well as pharmacological activity. Lipophilicity has been studied and applied as an important drug property for decades. It can be quickly measured or calculated. Lipophilicity has been correlated to many other properties, such as solubility, permeability, metabolism, toxicity, protein binding, and distribution.

## 5.1 Lipophilicity Fundamentals

Lipophilicity is the tendency of a compound to partition into a nonpolar lipid matrix versus an aqueous matrix. It is an important determinant of most other drug properties. Lipophilicity is readily calculated, thanks to the work of Hansch and Leo.[1] It is a rapid and effective tool for initial compound property assessment, as indicated by its inclusion in the "rule of 5."

One traditional approach for assessing lipophilicity is to partition the compound between immiscible nonpolar and polar liquid phases. Traditionally, octanol has been widely used as the nonpolar phase and aqueous buffer as the polar phase. The partitioning values that are measured are termed Log P and Log D. It is important to recognize that these terms are different.

**Log P**: Log of the partition coefficient of the compound between an organic phase (e.g., octanol) and an aqueous phase (e.g., buffer) at a pH where all of the compound molecules are in the neutral form.

$$\text{Log P} = \log([\text{Compound}_{\text{organic}}]/[\text{Compound}_{\text{aqueous}}]).$$

**Log D**: Log of the distribution coefficient of the compound between an organic phase (e.g., octanol) and an aqueous phase (e.g., buffer) at a specified pH ($x$). A portion of the compound molecules may be in the ionic form and a portion may be in the neutral form.

$$\text{Log D}_{\text{pH}x} = \log([\text{Compound}_{\text{organic}}]/[\text{Compound}_{\text{aqueous}}]).$$

Log P depends on the partitioning of the neutral molecules between the two matrices. Log D depends on the partitioning of the neutral portion of the molecule population plus the partitioning of the ionized portion of the molecule population. Ions have greater affinity for the polar aqueous phase than for the nonpolar organic phase. The fraction of the molecule population that is ionized depends on the pH of the aqueous solution, the p$K_a$ of the compound, and whether the compound is an acid or base. The effects of p$K_a$ and pH are discussed in Chapter 6. For acids, the neutral/anion ratio of molecules in solution decreases with increasing pH; therefore, log D decreases with increasing pH. Conversely, for bases, the neutral/cation ratio of molecules in solution increases with increasing pH; therefore, log D increases with increasing pH.

Abraham et al.[2,3] have shown that Log P is affected by several fundamental structural properties of the compound:

- Molecular volume
- Dipolarity
- Hydrogen bond acidity
- Hydrogen bond basicity

Molecular volume is related to molecular weight and affects the size of the cavity that must be formed in the solvent to solubilize the molecule. Dipolarity affects the polar alignment of the molecule with the solvent. Hydrogen bond acidity is related to hydrogen bond donation, and hydrogen bond basicity is related to hydrogen bond acceptance. They affect hydrogen bonding with the solvent. In-depth study of these effects by Abraham et al. resulted in linear free energy equations from which partitioning behavior can be predicted. Researchers frequently apply this approach to evaluating the predictability of methods for partition-based properties and enhancing the predictability of assay methods by adding calculations in order to better model particular properties.[4]

Remember that lipophilicity changes with the conditions of the phases, including the following:

- Partitioning solvents/phases
- pH
- Ionic strength
- Buffer
- Co-solutes or co-solvents

For example, partitioning between octanol and water is different than between cyclohexane and water. This is due to the differences in the molecular properties of the phases, which lead to different interactions of the solvent and solute molecules. pH affects the degree of ionization, as discussed previously. Increasing ionic strength results in increasing polarity of the aqueous phase. The buffer also affects the polarity, molecular interactions and formation of in situ salts (as counter ions) with drug molecules. Co-solvents, such as dimethylsulfoxide (DMSO), can interact with solutes and change their partitioning behavior, even at low percentage compared to the phases. Thus, the effect of solution conditions should be considered when predicting the effect of lipophilicity and reporting assay data.

## 5.2 Lipophilicity Effects

Lipophilicity has been correlated to various models of drug properties affecting ADME/Tox.[5,6] They include permeability, absorption, distribution, plasma protein binding, metabolism, elimination, and toxicity. Lombardo et al.[5,6] correlated pharmacokinetic volume of distribution ($V_d$) to lipophilicity.

A general guide for optimal gastrointestinal absorption by passive diffusion permeability after oral dosing is to have a moderate Log P (range 0–3), as suggested in Figure 5.1. In this range, a good balance of permeability and solubility exists. Compounds with a lower Log P are more polar and have poorer lipid bilayer permeability. Compounds with a higher Log P are more nonpolar and have poor aqueous solubility.

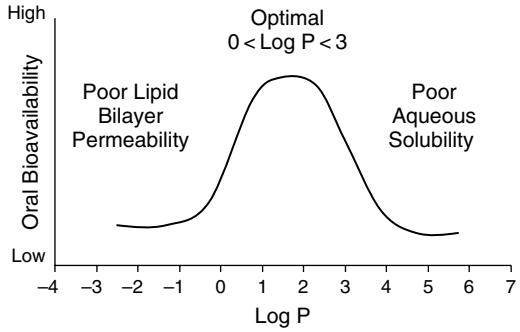



**Figure 5.1** ► Hypothetical example of how Log P can affect oral bioavailability for a compound series. Absorption by passive diffusion permeation after oral dosing is generally considered optimal for compounds having a moderate Log P and decreases for compounds having higher and lower Log P values.

Table 5.1 is useful for estimating the impact of log $D_{7.4}$ on drug-like properties in discovery[7]:

- Log $D_{7.4} < 1$: There is good solubility but low absorption and brain penetration, owing to low passive diffusion permeability. These compounds tend to have high clearance by the kidney, owing to their polarity. These compounds may exhibit paracellular permeation if the molecular weight is low.
- $1 <$ Log $D_{7.4} < 3$: This is an ideal range. These compounds generally have good intestinal absorption, owing to a good balance of solubility and passive diffusion permeability. Metabolism is minimized, owing to lower binding to metabolic enzymes.
- $3 <$ Log $D_{7.4} < 5$: These compounds have good permeability but absorption is lower, owing to lower solubility. Metabolism is increased in this range, owing to increased binding to metabolic enzymes.
- Log $D_{7.4} > 5$: Compounds in this range tend to have low absorption and bioavailability, owing to low solubility. Metabolic clearance is high because of high affinity for metabolic enzymes. $V_d$ and half-life (see Chapter 19) are high because compounds partition into and stay in tissues.

**TABLE 5.1 ► Impact of Log $D_{7.4}$ on Drug-like Properties**[7]

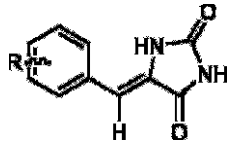
| Log $D_{7.4}$ | Common Impact on Drug-like Properties | Common Impact *In Vivo* |
|---|---|---|
| < 1 | Solubility high<br>Permeability low by passive transcellular diffusion<br>Permeability possible via paracellular if MW < 200<br>Metabolism low | Volume of distribution low<br>Oral absorption and BBB penetration unfavorable<br>Renal clearance may be high |
| 1 to 3 | Solubility moderate<br>Permeability moderate<br>Metabolism low | Balanced volume of distribution<br>Oral absorption and BBB penetration favorable |
| 3 to 5 | Solubility low<br>Permeability high<br>Metabolism moderate to high | Oral bioavailability moderate to low<br>Oral absorption variable |
| > 5 | Solubility low<br>Permeability high<br>Metabolism high | High volume of distribution (especially amines)<br>Oral absorption unfavorable and variable |

## 5.3 Lipophilicity Case Studies and Structure Modification

Lipophilicity is an underlying structural property that affects higher-level physicochemical and biochemical properties. It often is an effective guide for modifying the structure of a lead series to improve a property. The effects of lipophilicity on specific properties and structure modification strategies are discussed in most of the subsequent chapters on properties.

ΔLog P has been used to predict permeation of the blood–brain barrier (BBB).[8] ΔLog P is the Log P from partitioning between octanol and aqueous phases minus the Log P from partitioning between cyclohexane and aqueous phases. The difference is attributed to the contribution of hydrogen bonding to Log $P_{ow}$ (octanol/water) compared to Log $P_{cw}$ (cyclohexane/water). As ΔLog P increases, BBB permeability generally decreases. Its correlation to BBB permeation has been interpreted in terms of the negative effect of hydrogen bonding on BBB permeability (see Chapter 10 and Section 28.2.1.4).

Lipophilicity also has been correlated to activity. One example is shown in Figure 5.2 [9] for a series of 11 compounds with anticonvulsant activity. Log P correlated with –Log ED with an $R^2$ of 0.83. Thus, activity increased (ED decreased) as Log P increased.




$$-\text{Log ED} = -1.247 + 0.795\ \text{LUMO} + 0.150\ \text{Log P}$$
$$n = 11,\ r^2 = 0.834,\ r^2_{cv} = 0.793,\ \text{SE} = 0.063$$

**Figure 5.2 ►** Correlation between anticonvulsant activity and Log P.[9] For this series, activity increased with Log P.

## Problems

(Answers can be found in Appendix I at the end of the book.)

1. What is the major difference between Log P and Log D?
2. What factors affect Log P?
3. What is the most favorable Log $D_{7.4}$ range for drugs?
4. Why is a low Log P unfavorable for absorption? Why is a high Log P unfavorable for absorption?
5. Which is measured for the neutral form of a compound?: (a) Log D, (b) Log P.
6. At a Log $D_{7.4}$ of 2, which of the following can be predicted?: (a) high intestinal absorption, (b) low solubility, (c) high permeability, (d) high metabolism, (e) high central nervous system penetration.
7. At a Log $D_{7.4}$ greater than 5, which of the following can be predicted?: (a) high intestinal absorption, (b) low solubility, (c) high metabolism, (d) low bioavailability.

## References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# p$K_a$

*Overview*

- *The ionizability of a compound is indicated by* p$K_a$.
- *Ionizability is a major determinant of solubility and permeability.*
- *When* pH = p$K_a$, *the concentrations of ionized and neutral molecules in solution are equal.*
- *Basicity of bases increases as* p$K_a$ *increases; acidity of acids increases at* p$K_a$ *decreases.*

The great majority of drugs contain ionizable groups (Figure 6.1). Most are basic, and some are acidic. Only 5% are not ionizable. p$K_a$ indicates a compound's ionizability. It is a function of the acidity or basicity of group(s) in the molecule. Medicinal chemists can modify the acidic or basic substructures on the scaffold in order to obtain the desired p$K_a$, which affects solubility and permeability.

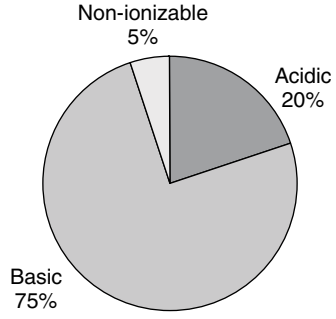



**Figure 6.1** ► Most drugs are ionizable.

## 6.1 p$K_a$ Fundamentals

p$K_a$ is the negative log of the ionization constant $K_a$. It is common to use p$K_a$ for both acids and bases.

For acids:

$$HA = H^+ + A^-$$

$$pK_a = -\log([H^+] \bullet [A^-]/[HA]).$$

For bases:

$$HB^+ = H^+ + B$$
$$pK_a = -\log([H^+] \bullet [B]/[HB^+]).$$

Useful aspects of the behavior of acids and bases can be derived from the above relationships.

For acids:

- As pH decreases, there is a greater concentration of neutral acid molecules (HA) and a lower concentration of anionic acid molecules ($A^-$) in solution.
- Acids with a lower p$K_a$ are stronger (greater tendency to form $A^-$).

For bases:

- As pH decreases, there is a lower concentration of neutral base molecules (B) and a higher concentration of cationic base molecules ($HB^+$) in solution.
- Bases with lower p$K_a$ are weaker (lower tendency to form $HB^+$).

The Henderson-Hasselbach equation is a useful relationship for discovery.

For acids:

$$pH = pK_a + \log([A^-]/[HA]) \quad \text{or} \quad [HA]/[A^-] = 10^{(pK_a - pH)}.$$

For bases:

$$pH = pK_a + \log([B]/[HB^+]) \quad \text{or} \quad [BH^+]/[B] = 10^{(pK_a - pH).}$$

These relationships provide a means of calculating the concentration of ionic and neutral species at any pH, if p$K_a$ is known. Moreover, it is useful to note that when pH is the same as p$K_a$, then there is an equal concentration of ionic and neutral species in solution. This relationship as well as the change in concentration of each species is shown in Figure 6.2.

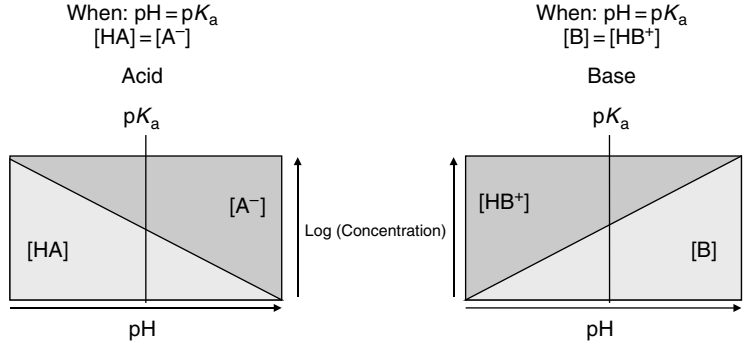



**Figure 6.2** ► Concentration of neutral and ionic species of acids and bases at pHs above and below their p$K_a$.

## 6.2 $pK_a$ Effects

Ionized molecules are more soluble in aqueous media than neutral molecules because they are more polar. Solubility is determined by both the intrinsic solubility of the neutral molecule and the solubility of the ionized species, which is much greater.

Conversely, ionized molecules are less permeable than neutral molecules. The neutral molecules are much more lipophilic than the ionized molecules and are considered to be the dominant form that permeates by passive diffusion.

Because $pK_a$ determines the degree of ionization, it has a major effect on solubility and permeability. These, in turn, determine intestinal absorption after oral dosing. The effects of ionization suggest a relationship frequently encountered by medicinal chemists: highly permeable compounds often have low solubility and vice versa. Thus, there is a tradeoff between solubility and permeability because of the opposite effects of ionization on these properties.

An example of this effect is shown in Figure 6.3. An acidic compound with a $pK_a$ of 5 exhibits decreasing permeability as the pH of the solution increases. Conversely, the solubility increases at increasing solution pH.

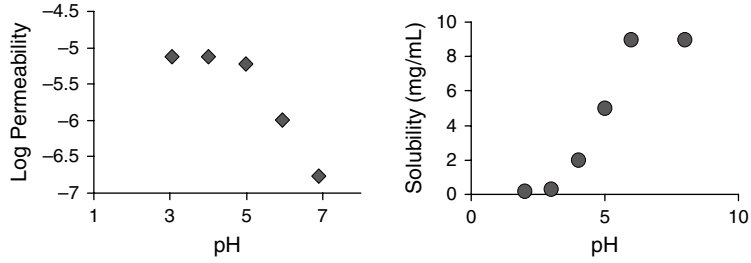



**Figure 6.3** ► Permeability and solubility profiles for an acidic compound with a $pK_a$ of 5. Permeability and solubility are pH dependent for ionizable compounds. The properties exhibit opposite effects with pH because of the effects of ionization.

$pK_a$ also affects the activity of a structural series. Presumably, this is due to changes in interactions at the active site of the target protein.

## 6.3 $pK_a$ Case Studies

Examples of the $pK_a$ values of a number of substructures that commonly appear in drug molecules are listed in Table 6.1. A more extensive listing is available.[1] The $pK_a$ values of some drugs are listed in Table 6.2.

An example of the effect of $pK_a$ and molecular size on the activity of a structural series is shown in Figure 6.4.[2] The piperidine with a $pK_a$ around 10 was modified with aliphatic groups that have little effect on $pK_a$. Their increasing size appears to be responsible for some loss of activity. However, when the aromatic ring was added, the basicity of the amine greatly decreased and caused the activity to be much lower than would be expected from the size of the aromatic ring compared to changes in size from other added moieties.

An example of the effect of $pK_a$ on activity is shown in Figure 6.5.[3] $IC_{50}$ decreased as $pK_a$ decreased (increasing acidity).

Basic drugs tend to penetrate the blood–brain barrier more effectively than acids do. An example is shown in Figure 6.6, where the basic trifluoroperizine ($pK_a$ 7.8) permeates the blood–brain barrier, whereas the acidic indomethacin ($pK_a$ 4.2) does not.[4]

**TABLE 6.1 ► Examples of Acidic and Basic Substructures and Respective $pK_a$**

| Acids | $pK_a$ |
|---|---|
| $CF_3COOH$ | 0.23 |
| $CCl_3COOH$ | 0.9 |
| $CCl_2HCOOH$ | 1.3 |
| $CClH_2COOH$ | 2.9 |
| HCOOH | 3.8 |
| $C_6H_5COOH$ | 4.2 |
| Succinic acid | 4.2, 5.6 |
| $H_3COOH$ | 4.8 |
| Thiophenol | 6.5 |
| p-Nitrophenol | 7.2 |
| m-Nitrophenol | 9.3 |
| $C_6H_5OH$ | 10.0 |
| **Bases** | **$pK_a$** |
| Guanidine | 13.6 |
| Acetamide | 12.4 |
| Pyrrolidine | 11.3 |
| Piperidine | 11.1 |
| Methyl amine | 10.6 |
| Piperazine | 9.8, 5.3 |
| Trimethyl amine | 9.8 |
| Glycine | 9.8 |
| Morpholine | 8.4 |
| Imidazole | 6.8 |
| Pyridine | 5.2 |
| Quinoline | 4.9 |
| Aniline | 4.9 |
| Triazole | 2.5 |
| Purine | 2.4 |
| Pyrimidine | 1.2 |
| Diphenylamine | 0.8 |

**TABLE 6.2 ► Example Drugs and $pK_a$**

| Acids | $pK_a$ |
|---|---|
| Penicillin V | 2.7 |
| Salicylic acid | 3.0, 13.8 |
| Acetylsalicylic acid | 3.5 |
| Diclofenac | 4.1 |
| Sulfathiazole | 7.1 |
| Phenobarbital | 7.4, 11.8 |
| Phenytoin | 8.3 |
| Acetaminophen | 9.9 |
| Caffeine | 14 |
| **Bases** | **$pK_a$** |
| Caffeine | 0.6 |
| Quinidine | 4.1, 8.0 |

***Continued***

**TABLE 6.2 ►** *Continued*

| Bases | $pK_a$ |
|---|---|
| Tolbutamide | 5.3 |
| Cocaine | 8.4 |
| Ephedrine | 9.4 |
| Imipramine | 9.5 |
| Atropine | 9.7 |

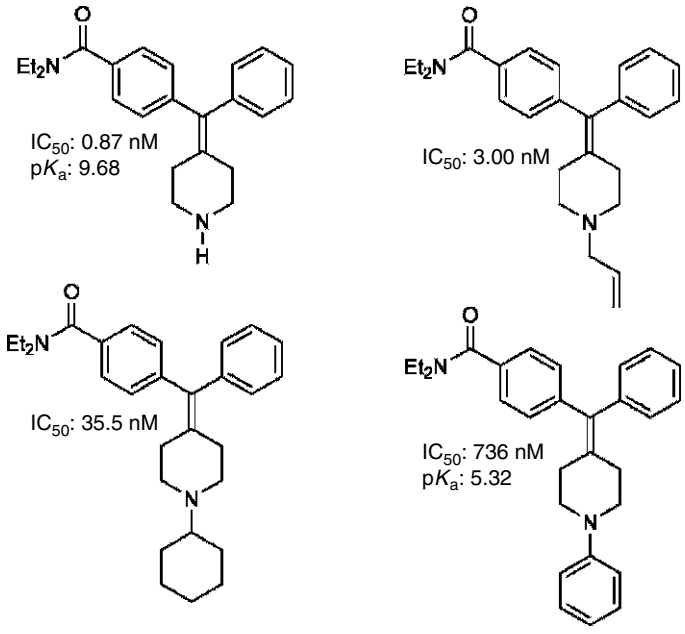
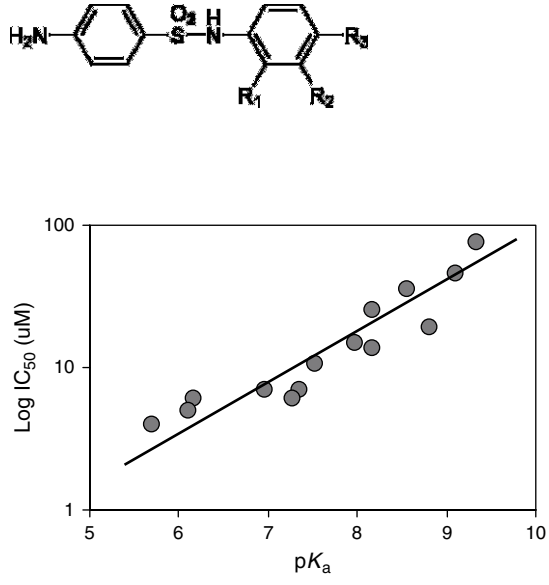



**Figure 6.4 ►** Effect of $pK_a$ and size on activity.[2]




| Compounds | $IC_{50}$ (uM) | $pK_a$ |
|---|---|---|
| 4-$OCH_3$ | 75 | 9.34 |
| H | 45 | 9.10 |
| 4-Cl | 35 | 8.56 |
| 4-I | 25 | 8.17 |
| 2-Cl, 4-$OCH_3$ | 19 | 8.81 |
| 3-$CF_3$ | 15 | 7.98 |
| 2-Cl | 14 | 8.18 |
| 4-$COCH_3$ | 11 | 7.52 |
| 4-CN | 7 | 7.36 |
| 4-$NO_2$ | 7 | 6.97 |
| 2-$OCH_3$, 4-$NO_2$ | 6 | 7.27 |
| 2-Cl, 4-$NO_2$ | 6 | 6.17 |
| 2-$NO_2$, 4-$CF_3$ | 5 | 6.10 |
| 2-Br, 4-$NO_2$ | 4 | 5.70 |

**Figure 6.5 ►** Effect of $pK_a$ on activity of a structural series.[3]

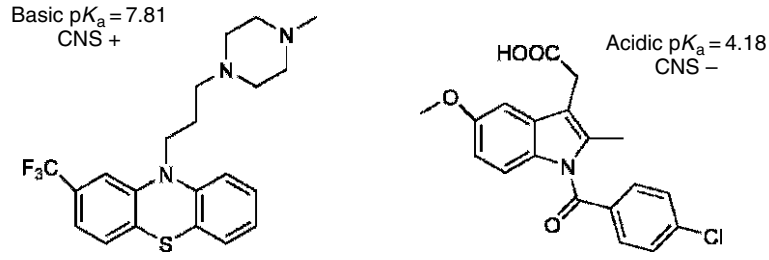



**Figure 6.6** ► Basic drugs tend to permeate the blood–brain barrier, whereas acidic drug generally do not.[4]

The effect of p$K_a$ on water solubility for a set of bile acids is shown in Figure 6.7.[5] As the acidity of the bile acids increased, water solubility increased. Figure 6.8 shows two SRC kinase inhibitors.[6] Increasing basicity increased solubility due to ionization.

Wohnsland and Faller[7] reported that the parallel artificial membrane permeability assay (PAMPA) permeability of diclofenac (acidic p$K_a$ 5.6) was higher at lower pH than at higher pH, whereas desipramine (basic p$K_a$ 6.5) had the opposite behavior. Avdeef[8] showed acid

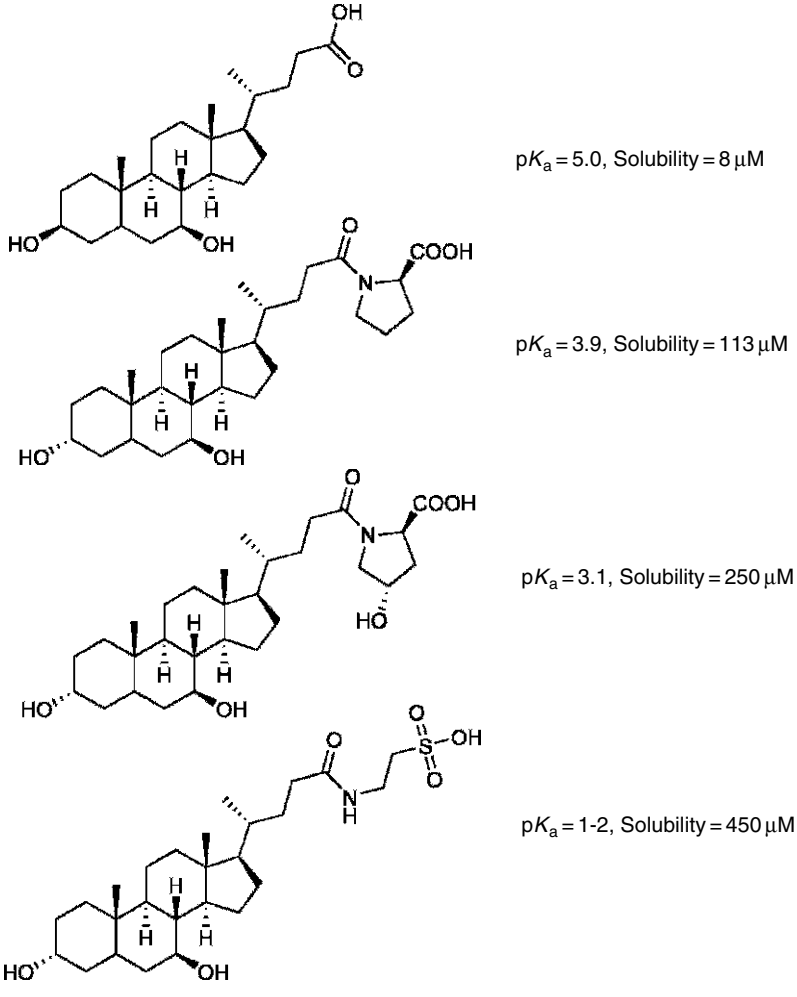



**Figure 6.7** ► Effect of p$K_a$ on water solubility of bile acids.[5]

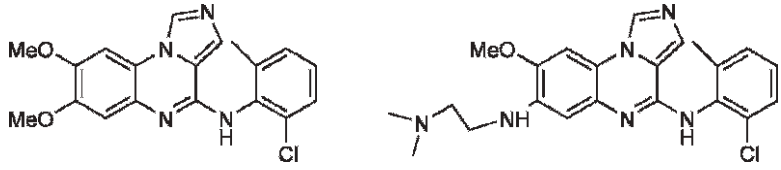



**Figure 6.8** ► Effect of $pK_a$ on solubility for SRC kinase inhibitors.[6]

(ketoprofen, $pK_a$ 3.98), base (verapamil, $pK_a$ 9.07), and ampholyte (piroxicam, $pK_a$ 5.07, 2.33) had unique permeability profiles.

## 6.4 Structure Modification Strategies for $pK_a$

When synthetic modifications are planned for the purpose of improving the water solubility or permeability of a structural series, a wide selection of substructures can be used. It is important to remember that structural modifications that increase solubility will also decrease permeability.

By modifying the substructures of a molecule to introduce groups with differing $pK_a$ values, medicinal chemists can modify the solubility and permeability of the compound. Examples of $pK_a$ modifications to enhance solubility and permeability can be found in Chapters 7 and 8, respectively. Electron donating and withdrawing groups can be added or removed to add electron density at the acid or base, depending on the desired effect. For example, the strength of an acid can be increased by adding α-halogen(s) or other electron withdrawing groups (e.g., carboxy, cyano, nitro). Addition of aliphatic groups has little effect. Removing electron-withdrawing groups can decrease acid strength.

The strength of a base is decreased by attachment an aromatic group (e.g., aniline). The lone pair is delocalized into the aromatic ring. It is also decreased slightly with addition of an aliphatic group. The basicity of an aniline can be increased with attachment of a methoxy group, which donates electrons, or it can be decreased with the attachment of a nitro group, which withdraws electrons.

## Problems

(Answers can be found in Appendix I at the end of the book.)

1. For acids, as pH decreases, are there: (a) more anions, (b) more neutral molecules, (c) higher solubility, (d) lower solubility, (e) higher permeability, (f) lower permeability?
2. For bases, as pH decreases, are there: (a) more cations, (b) more neutral molecules, (c) higher solubility, (d) lower solubility, (e) higher permeability, (f) lower permeability?
3. At pH 6.8, a basic compound of $pK_a$ 9.5 is mostly in what form?: (a) ionized, (b) neutral.
4. For benzoic acid ($pK_a$ 4.2), estimate the degree of ionization in the fasted state for the stomach, duodenum, and blood. For $HA = H^+ + A^-$, use the relationship: $[HA]/[A^-] = 10^{(pK_a - pH)}$

| **Location** | **pH** | $\mathbf{[HA]/[A^-] = 10^{(pK_a - pH)}}$ | **Ionization** |
|---|---|---|---|
| Stomach | 1.5 | | |
| Duodenum | 5.5 | | |
| Blood | 7.4 | | |

5. For piperazine (p$K_a$ 9.8), estimate the degree of ionization in the fasted state for the stomach, duodenum, and blood. For $BH^+ = H^+ + B$, use the relationship: $[BH^+]/[B] = 10^{(pK_a - pH)}$

| **Location** | **pH** | $\mathbf{[BH^+]/[B] = 10^{(pK_a - pH)}}$ | **Ionization** |
|---|---|---|---|
| Stomach | 1.5 | | |
| Duodenum | 5.5 | | |
| Blood | 7.4 | | |

6. If the pH is 2 units above the p$K_a$ of an acid, the predominant species is: (a) neutral, (b) anion. If the pH is 2 units below the p$K_a$ of a base, the predominant species is: (c) neutral, (d) cation.

## References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# Solubility

*Overview*

- *Solubility is the maximum dissolved concentration under given solution conditions.*
- *Solubility is a determinant of intestinal absorption and oral bioavailability.*
- *Solubility is increased by adding ionizable groups or reducing Log P and MW.*
- *Salt forms increase dissolution rate.*

Solubility is one of the most important properties in drug discovery. Insoluble compounds can plague discovery. Many negative effects can occur for low-solubility compounds, including the following:

- Poor absorption and bioavailability after oral dosing
- Insufficient solubility for IV dosing
- Artificially low activity values from bioassays
- Erratic assay results (biological and property methods)
- Development challenges (expensive formulations and increased development time)
- Burden shifted to patient (frequent high-dose administrations)

These are major hurdles and deserve serious consideration by discovery project teams. Chapter 40 is devoted to solving solubility problems in biological assays. Lipinski et al.[1] caution that solubility is a much larger issue for drug discovery than is permeability.

Solubility problems can intensify during discovery because the molecular characteristics needed for strong binding to the target protein can be deleterious to solubility. For example, lipophilic structures may seem to be the best available leads for a discovery project, or lipophilic groups are often added during optimization to enhance target binding. Unfortunately, these examples, while helping to meet discovery's primary goal of finding active compounds, can reduce solubility and have a negative impact on other discovery and development goals.

Under short time lines and high expectations, discovery scientists can make the mistake of placing too high of a reliance on advanced formulation and delivery technologies to improve the pharmacokinetics and bioavailability of insoluble compounds. This reliance can lead a discovery project team toward a clinical candidate that cannot achieve sufficiently high absorbed doses to produce an effective therapeutic treatment. It is wise to solve solubility insufficiencies during discovery with structural modifications.

# 7.1 Solubility Fundamentals

## 7.1.1 Solubility Varies with Structure and Physical Conditions

Solubility is the maximum concentration that a compound reaches in a solvent matrix at equilibrium with solid compound. It is important to remember that there is no single solubility value for a compound. Solubility is determined by many factors:

- Compound structure
- Physical state of compound that is introduced into solution
  - Solid: Amorphous, crystalline, polymorphic form
  - Liquid: Predissolved in solvent (e.g., dimethylsulfoxide [DMSO])
- Composition and physical conditions of solvent(s)
  - Types of solvents
  - Amount (%) of co-solvents
  - Solution components (e.g., salts, ions, proteins, lipids, surfactants)
  - pH
  - Temperature
- Methods of measurement
  - Equilibration time
  - Separation techniques (e.g., filter, centrifuge)
  - Detection (e.g., ultraviolet, mass spectrometry, turbidity)

For example, the solubility of a compound can be very different in pH 7.4 buffer, simulated intestinal fluid, blood, and biological assay media containing 1% DMSO. Therefore, it is necessary to specify the conditions of the compound and solution for proper use of the solubility data. In drug discovery, various solubility experiments are performed to estimate the effect of solubility in different systems to better mimic the actual in vitro and in vivo conditions.

## 7.1.2 Dissolution Rate

Dissolution rate is the speed at which a compound dissolves from a solid form into a solvent. Solid forms vary among the neutral form (free acid or base), salt form, or formulated dosage forms. Modification of the solid form to achieve different rates of dissolution is discussed in Sections 7.5 and 7.6. Such modifications allow control of the rate of absorption and pharmacokinetics of a compound.

## 7.1.3 Structural Properties Affect Solubility

Solubility is affected by physicochemical properties, which can be estimated using in vitro assays or software calculations. Each of these is determined by underlying structural properties.

- Lipophilicity: Determined by van der Waals, dipolar, hydrogen bonds, ionic interactions
- Size: Molecular weight, shape
- p$K_a$: Determined by functional group ionizability
- Crystal lattice energy: Determined by crystal stacking, melting point

Medicinal chemists have the ability to change solubility by modifying the structure, which affects these physicochemical properties. Structural modification of polarity, hydrogen bonding, molecular size, ionizability, and crystal stacking are discussed in a later section of this chapter. The crystalline forms can be modified by crystallization conditions and salt form.

A demonstration of the effect of lipophilicity and crystal lattice energy on solubility is the empirically derived general solubility equation of Yalkowsky and Banerjee[2] for estimating the aqueous solubility of a compound based on measurable or calculable properties:

$$\text{Log S} = 0.8 - \text{Log P}_{\text{ow}} - 0.01(\text{MP} - 25),$$

here S is solubility, Log $P_{ow}$ is the octanol/water partition coefficient (a measure of lipophilicity), and MP is the melting point (a measure of crystal lattice strength). This relationship shows that solubility decreases 10-fold as:

- Log P increases by 1 unit
- Melting point increases by 100°C

p$K_a$ and solution pH are important because the charged form of a drug compound is more soluble than the neutral form. At a particular pH, there is a distribution of molecules between the neutral and ionized state. Therefore, the solubility of a compound at a particular pH is the sum of the "intrinsic solubility" (solubility of the neutral compound) of the neutral species portion of molecules in solution, plus the solubility of the charged species portion of molecules in solution. This has implications for the solubility of compounds in various physiological fluids and solutions that have different pHs in drug discovery.

This phenomenon also can be described mathematically, as follows. Drugs ionize according to the reactions:

$$\text{HA} + \text{H}_2\text{O} = \text{H}_3\text{O}^+ + \text{A}^- \quad \text{(Acid)}$$
$$\text{B} + \text{H}_2\text{O} = \text{OH}^- + \text{HB}^+ \quad \text{(Base)}.$$

At equilibrium, the solubility of a mono-acid or mono-base can be described as follows:

$$\text{S} = [\text{HA}] + [\text{A}^-] \quad \text{(Acid)}$$
$$\text{S} = [\text{B}] + [\text{HB}^+] \quad \text{(Base)},$$

where S = solubility.

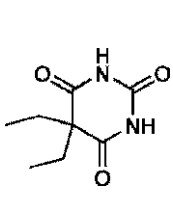
A mathematical derivation of the Henderson-Hasselbalch equation provides insights for solubility:

$$S = S_0(1 + 10^{(pH - pKa)}) \quad \text{(Acid)}$$
$$S = S_0(1 + 10^{(pKa - pH)}) \quad \text{(Base)},$$

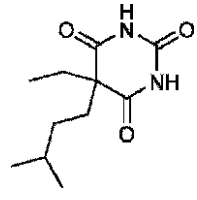
where $S_0$ = intrinsic solubility (solubility of the neutral compound). Solubility changes linearly with $S_0$ and exponentially with the difference between pH and p$K_a$. Examples of the effects of intrinsic solubility and p$K_a$ of acids are listed in Table 7.1.[3] Barbital and amobarbital have the same p$K_a$ (7.9, weak acid), but barbital has higher intrinsic solubility than amobarbital (7.0 mg/mL vs. 1.2 mg/mL). Therefore, barbital has higher total solubility than amobarbital at all pH values, including pH 9 (95 mg/mL vs. 15 mg/mL). Naproxen and phenytoin have similar intrinsic solubility but different p$K_a$ values. This results in dramatically different solubility at pH 9. Naproxen is much more acidic (p$K_a$ 4.6) than phenytoin (p$K_a$ 8.3) and therefore is much more soluble because solubility increases exponentially with the difference in pH and p$K_a$. This example demonstrates that introducing an ionization center is an effective structure modification for increasing solubility.

**TABLE 7.1 ► Solubility at a Given pH is a Function of the Intrinsic Solubility of the Neutral Portion of Molecules and Solubility of the Ionized Portion of Molecules[3]**

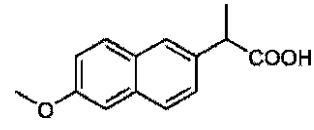
| | p$K_a$ | Intrinsic Solubility (mg/mL) | Solubility @ pH 9 (mg/mL) |
|---|---|---|---|
| Barbital | 7.9 | 7.0 | 95 |
| Amobarbital | 7.9 | 1.2 | 15 |
| Naproxen | 4.6 | 0.016 | 430 |
| Phenytoin | 8.3 | 0.02 | 0.12 |



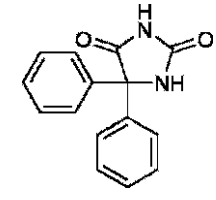
Barbital



Amobarbital



Naproxen



Phenytoin

One mistake that discovery scientists should avoid is confusing the pH solubility curve for a p$K_a$ titration curve. The p$K_a$ titration curve (Figure 7.1) plots the change in ionization with pH. The sharp rise occurs in the region of p$K_a$, and the inflection point occurs where pH equals p$K_a$. Scientists may think that the sharp rise in solubility should correspond to where pH equals p$K_a$. In fact, the pH region of the sharp rise in solubility is dependent on the intrinsic solubility ($S_0$), as shown in Figure 7.2. This figure plots the pH solubility profiles of four acidic compounds with the same p$K_a$ of 4.5 but with different intrinsic solubilities (0.1, 1, 10, and 100 mg/mL). The inflection point does not correspond to the p$K_a$ of the compound. The p$K_a$ of the compound is at the pH when total solubility is two times the intrinsic solubility because solubility and p$K_a$ follow a log-linear correlation. A plot of solubility on a log scale versus pH on a linear scale (Figure 7.2, inset) shows that the turning point pH is the p$K_a$ of the compound.

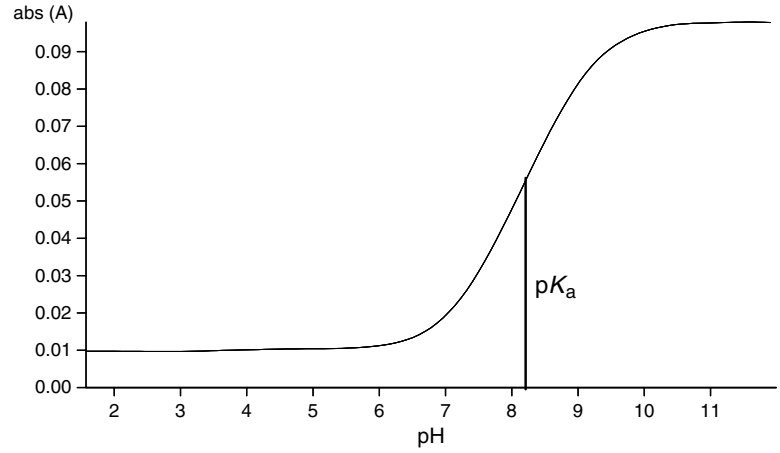



**Figure 7.1** ► $pK_a$ titration curve for a compound with $pK_a$ of 8.2.

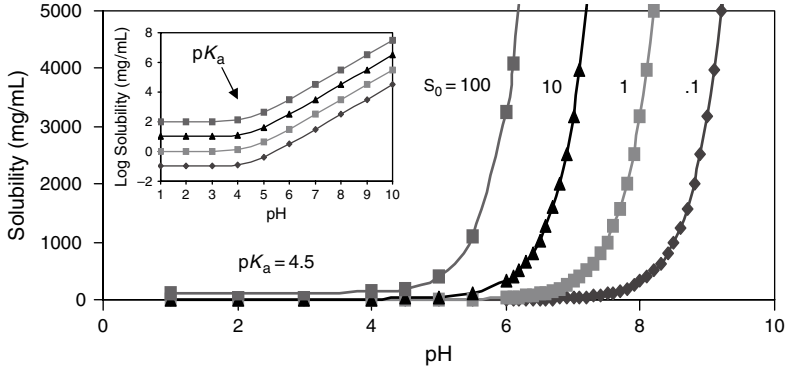



**Figure 7.2** ► pH solubility profiles for compounds all having $pK_a = 4.5$ but different $S_0$ values. When $pH = pK_a$, then total solubility equals $2S_0$.

### 7.1.4 Kinetic and Thermodynamic Solubility

It is important to distinguish between "kinetic" and "thermodynamic" solubility. Discovery scientists should know how solubility was measured to properly interpret the data or to apply the data to project decision-making.

*Kinetic solubility* has two distinguishing characteristics: (a) the compound initially is fully dissolved in an organic solvent (e.g., DMSO) then added to the aqueous buffer, and (b) equilibrium is not reached between dissolved compound and solid compound. If the compound precipitates from solution, it can be in a "metastable" crystalline form, such as amorphous or mixture of crystal forms. The kinetic solubility conditions mimic the conditions of discovery biological and property assays, in which compounds are predissolved in DMSO prior to addition into an in vitro assay solution, exposure times vary, equilibrium usually is not established, and assay concentrations are comparable (μM). Kinetic solubility data are useful in drug discovery to:

- ► Alert teams to potential absorption or bioassay liabilities
- ► Diagnose erratic bioassay results
- ► Develop structure–solubility relationships

- Select compounds for NMR-binding and x-ray co-crystallization experiments
- Develop generic formulations for animal dosing

*Thermodynamic solubility* is distinguished by (a) the addition of aqueous solvent directly to solid crystalline material and (b) establishment of equilibrium between the dissolved and solid material. Excess solid is used, so solid crystalline material is always present in the original solid form to which the aqueous solution was added. Long mixing time is applied to ensure equilibrium (e.g., 24–72 hours). Higher target concentrations (mg/mL) are used to guide formulation development. The thermodynamic solubility value varies with the crystal form of the solid (amorphous, crystalline, different polymorphs, hydrates, and solvates). High-energy crystal forms (less stable forms) tend to have higher solubility than low-energy forms. For example, amorphous material has higher solubility than crystalline material. Bioassays in drug discovery do not start with solid material (DMSO stock solution is used), nor do they generally reach equilibrium or have solid present, so the relevance of thermodynamic solubility to bioassays in drug discovery is limited. Using thermodynamic solubility data in early drug discovery can be counterproductive because (a) it can vary among different synthetic batches of the same compound due to different crystal forms, and (b) it is not relevant to amorphous solids typically made in discovery. Thermodynamic solubility is most useful in late discovery and early development, where a large batch has been synthesized and its crystal form has been characterized to:

- Guide formulation development
- Diagnose in vivo results
- Plan development strategy
- File regulatory submissions

Kinetic solubility in general tends to be higher than thermodynamic solubility. This is because the presence of DMSO helps enhance solubility, metastable crystal forms with higher crystal packing energy, and supersaturation of the solution occurs due to a nonequilibrium state.

Discovery and development scientists have very different viewpoints on solubility.[4,5] The goal of discovery scientists is to dissolve the compound into solution in any way possible to enable biological assays and demonstrate proof of concept. Amorphous and metastable crystal forms are okay, and discovery scientists love to use DMSO. Kinetic solubility is consistent with this viewpoint. In development, the goal is to develop a human dosage form and perform the detailed technical studies required for regulatory approval. Solubilization options are constrained, and unrealistically solubilized systems can be misleading. Crystal forms are well characterized, and development scientists never use DMSO. In development, all that matters is thermodynamic solubility.

#### 7.1.4.1 Consequences of Chirality on Solubility

Chirality affects solubility because of the crystal form. The two enantiomers crystallize in different forms from each other and from the racemate. The Wallach rule states that racemate crystals are more stable and dense than their chiral counterparts. For example, *S*-ketoprofen has a melting point of 72°C, whereas *RS*-ketoprofen has a melting point of 94°C. The increased stability of the crystal results in a higher melting point. This affects aqueous thermodynamic solubility values, which are 2.3 mg/mL for *S*-ketoprofen and 1.4 mg/mL for *RS*-ketoprofen. Kinetic solubility values are not affected by crystal stability.

## 7.2 Effects of Solubility

### 7.2.1 Low Solubility Limits Absorption and Causes Low Oral Bioavailability

In order for a compound to be absorbed in the intestine after oral dosing, the solid dosage form or suspension must disintegrate, dissolve, and diffuse to the surface of the intestinal epithelium to be absorbed into systemic circulation. As the concentration of compound increases when it dissolves, more drug molecules are present at the surface of the epithelial cells and a greater amount of drug per unit time per surface area (flux) is absorbed. This is the reason why solubility is so important for absorption. Insoluble compounds tend to have incomplete absorption and, therefore, low oral bioavailability.

Optimization of oral bioavailability is a goal of the discovery stage. Oral bioavailability incorporates both the extent of intestinal absorption, which is affected by solubility and permeability, and the presystemic metabolism (Figure 7.3). Insoluble compounds, such as the compound YH439 (Figure 7.4) have low oral bioavailability.[6] YH439 has low oral bioavailability (0.9%–4.0% in rat) due to poor aqueous solubility. When the compound was formulated in a mixed-micelle formulation, the oral bioavailability increased to 21%. Formulation helped solubilization of the compound and improved absorption and oral bioavailability. When the compound was tested in a dose-escalating toxicity study, area under the curve $AUC_{0-t}$ remained unchanged when the dose increased from 100 mg/kg (AUC = 32 μg min/mL) to 500 mg/kg (AUC = 37 μg min/mL), indicating solubility limits its absorption.[6] Low solubility hampered toxicity study at higher doses.

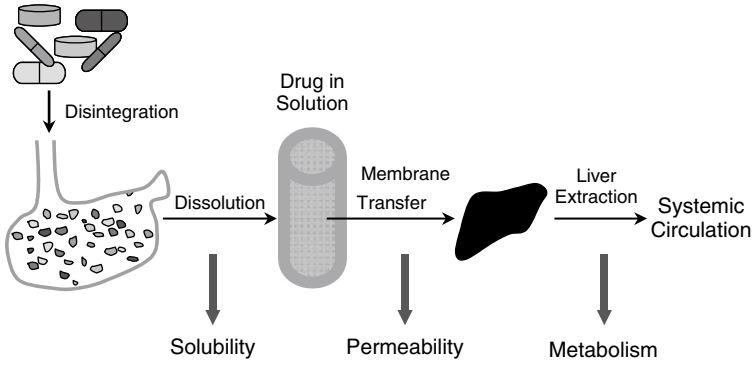



**Figure 7.3** ► Solubility, permeability, and metabolic stability affect oral absorption and bioavailability following oral dosing.

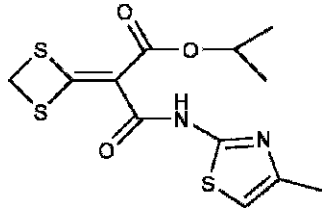



**Figure 7.4** ► Structure of YH439.[6] Insoluble compounds have low bioavailability after oral dosing.

Another example of how solubility affects oral bioavailability is shown in Figure 7.5 for two protease inhibitors.[7] The early lead compound L-685,434 had good potency in vitro in both enzyme and cell-based assays but was completely inactive in vivo after oral dosing due to poor solubility. The compound was modified by introducing ionizable centers into the

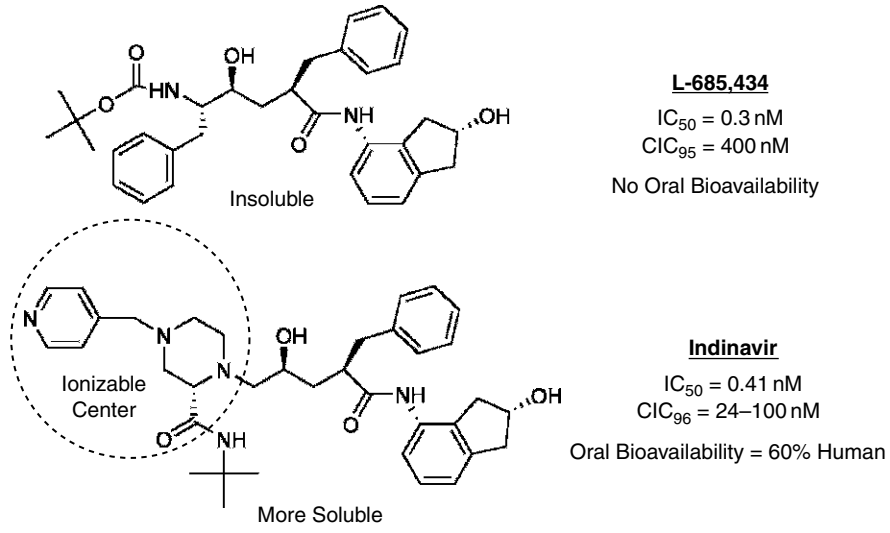



**Figure 7.5** ► Effect of solubility on oral bioavailability. (Reprinted with permission from [39].)

molecule to increase solubility. The modified compound (indinavir) is much more soluble, maintains good potency, and has oral bioavailability of 60% in human.

## 7.2.2 Good Solubility is Essential for IV Formulation

To successfully develop an IV formulation, compounds must have sufficient solubility in the vehicle to deliver the expected dose in a restricted volume. Insoluble compounds can be a challenge to formulate, and the process is not always successful. For example, it was reported that high-dose rat and monkey IV studies could not proceed for LY299501 (Figure 7.6) because the solubility of the compound was too low for the targeted IV dose of 30 mg/kg.[8] In many cases, a high organic solvent content must be used to solubilize insoluble compounds for IV administration in laboratory animals. This can cause homolysis (nonisotonic), dissolution of tissues, artificially higher oral absorption, and brain penetration due to damage of membrane integrity. This can generate erroneous data and mislead project teams. Compounds with low aqueous solubility can precipitate at the site of injection and result in nonlinear pharmacokinetics, such as second peak phenomena, which has been observed for lidocaine, disopyramide, and YH439 as a result of redissolution of the precipitated material.[6]

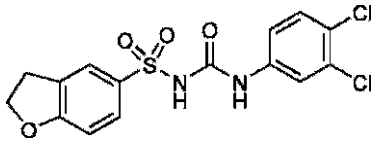


**Figure 7.6** ► Drug discovery compound LY295501 for oncology.

## 7.2.3 Acceptance Criteria and Classifications for Solubility

One question commonly asked by medicinal chemist is: "What is the minimum solubility required for a compound?". This really depends on permeability and dose of a compound.

The maximum absorbable dose (MAD) is the maximum amount of a drug that can be absorbed at a certain dose. MAD is defined as follows[9,10]:

$$\mathrm{MAD} = \mathrm{S}^{*}K_{\mathrm{a}}^{*}\mathrm{SIWV}^{*}\mathrm{SITT},$$

where S = solubility (mg/mL, pH 6.5), $K_a$ = intestinal absorption rate constant ($min^{-1}$; permeability in rat intestinal perfusion experiment, quantitatively similar to human $K_a$), SIWV = small intestine water volume (~250 mL), and SITT = small intestine transit time (min; ~270 min).

Thus, solubility and permeability are two major factors in achieving maximum absorption. Table 7.2 shows the minimum acceptable solubility of a drug for humans at a given dose and permeability in order to achieve maximum absorption. This is graphed in Figure 7.7. The more potent (i.e., dose producing the pharmacological effect if fully absorbed) and the more permeable the compound, the lower the solubility required to achieve complete absorption. On the contrary, the less potent and the less permeable the compound is, the higher the solubility required to achieve complete absorption. For example, if the compound has low permeability (0.003 $min^{-1}$) and low potency (10 mg/kg), a solubility of 3.46 mg/mL would be required to achieve maximum absorption of the dose. In drug discovery, solubility less than 0.1 mg/mL is quite common. Formulation often can help to increase the solubility of insoluble compounds. Therefore, maximum absorption of a compound with minimal solubility depends on high permeability and potency.

**TABLE 7.2 ► Minimum Acceptable (Target) Solubility for Human Dosing at a Given Dose and Permeability to Achieve Maximum Absorption[9]**

| | | | | | | |
|---|---|---|---|---|---|---|
| Human dose (mg) (MAD) | 7 | 7 | 70 | 70 | 700 | 700 |
| Human dose (mg/kg) | 0.1 | 0.1 | 1 | 1 | 10 | 10 |
| Permeability ($K_a$, $min^{-1}$) | 0.003 (low) | 0.03 (high) | 0.003 (low) | 0.03 (high) | 0.003 (low) | 0.03 (high) |
| Minimum acceptable solubility (mg/mL) | 0.035 | 0.0035 | 0.35 | 0.035 | 3.5 | 0.35 |

MAD, maximum absorbable dose.

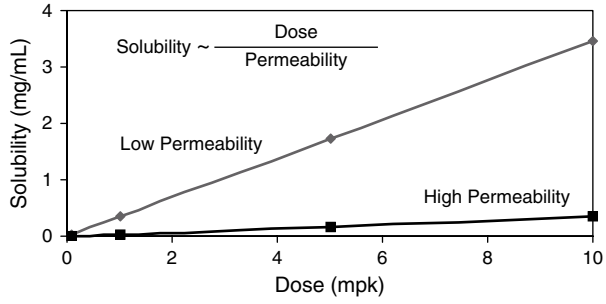



**Figure 7.7 ►** Relationship of solubility, permeability, and maximum absorbable dose. High-permeable compounds require lower solubility than low-permeability compounds to achieve maximum oral absorption. (Reprinted with permission from [38].)

Lipinski[11] has developed a useful graphical representation for the correlation of solubility, permeability, and dose (Figure 7.8). For example, if the compound has average permeability (shown as "avg $K_a$") and average potency (shown as "1.0" mg/kg dose if fully

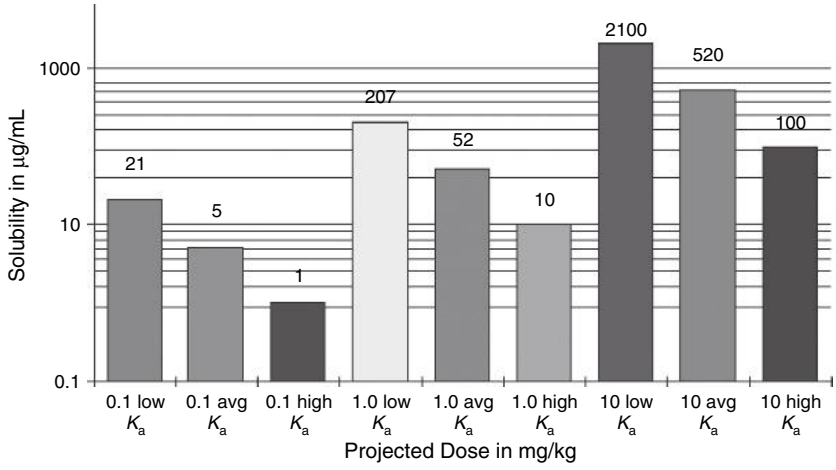



**Figure 7.8 ►** Graph for estimating the solubility (µg/mL) of discovery compounds needed to reach targeted dose levels (mg/kg), depending on the permeability (low, avg, high $K_a$). (Reprinted with permission from [11].)

absorbed), the compound will need to have a minimum solubility of 52 µg/mL to be completely absorbed. If the compound is not very potent, with a dose of 10 mg/kg and average permeability, then the solubility must be 10 times higher (520 µg/mL). Such estimates provide useful guidelines for optimizing solubility during discovery. In rating the solubility of compounds for discovery project teams, the following solubility classification ranges are suggested for medicinal chemists:

- ► <10 µg/mL Low solubility
- ► 10–60 µg/mL Moderate solubility
- ► >60 µg/mL High solubility

These classification ranges are intended to provide general guidelines on potential solubility issues for *human oral absorption*. However, these criteria usually are too low for *animal dosing* in solution formulation. Table 7.3 gives estimates of the solubility needed for dosing a rat with a solution in discovery in vivo studies. The solubility requirement typically is much higher than 60 µg/mL, which is considered "high" in drug discovery for predicting human absorption.

**TABLE 7.3 ► Target Solubility of Dosing Solution for Dosing a 250-g Rat at Ideal Dosing Volumes**

| | Target solubility (mg/mL) | |
|---|---|---|
| Dose (mg/kg) | PO | IV |
| 1 | 0.1–0.2 | 0.2–1 |
| 5 | 0.5–1 | 1–5 |
| 10 | 1–2 | 2–10 |
| Ideal volume (mL/kg) | 5–10 | 1–5 |

Different solubility classification systems are used in different stages of drug discovery and development. They were developed to provide general guidelines on how to use solubility information to guide compound selection and advancement.

### 7.2.3.1 Biopharmaceutics Classification System

The solubility classifications used in drug discovery are quite different than those used in drug development. The Biopharmaceutics Classification System (BCS) is widely used in drug development. It divides compounds into four classes based on solubility and permeability (Figure 7.9).[12]

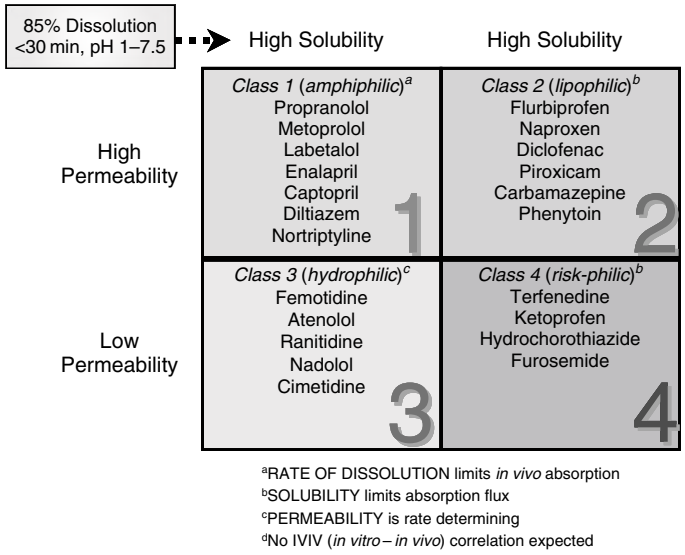



**Figure 7.9** ► Biopharmaceutics Classification System (BCS) for in vitro/in vivo (IVIV) correlation.[12]

As development colleagues become involved in projects during the late discovery and predevelopment stages, it is often useful for discovery scientists to understand the BCS classifications. This facilitates experiments that address development issues, in order to promote the optimum candidate to development and streamline the transition to development.

- ► Class I is high solubility and high permeability. This is an ideal class for oral absorption.
- ► Class II is low solubility and high permeability. Formulation typically is used to enhance solubility of compounds in this class.
- ► Class III is high solubility but low permeability. Prodrug strategies typically are used for these compounds.
- ► Class IV is low solubility and low permeability. Development of this class of compounds can be risky and costly. No in vitro/in vivo correlations are expected.

The purpose of the BCS is to indicate the similarities and differences between compounds with regard to solubility and permeability. Compounds in the same BCS class tend to behave in a similar manner with regard to absorption and can follow the same regulatory approval process with regard to in vitro/in vivo correlation experiments. The Food and Drug Administration (FDA) can grant a waiver of bioavailability/bioequivalence studies for

immediate-release orally administered formulations if the compound is class I and does not have a narrow therapeutic index. This saves a lot of resources and time for the development of new drugs, formulations, and generics.

The "high" solubility classification here is much more strict than the "high" solubility used in drug discovery (>60 μg/mL). High solubility in BCS is defined as (a) 85% dissolution of the dose within 30 minutes at all pH values from 1 to 7.5 and (b) dose/solubility (D/S) $\leq$ 250 mL (e.g., theophylline).[12,13]

For discovery scientists, the BCS emphasizes the critical roles and balance of solubility, permeability, and fully absorbed dose (i.e., potency), which challenge the development of a successful drug product. Beyond the role of solubility, permeability, and potency, discovery scientists must consider additional properties not addressed by the BCS (e.g., metabolic, plasma and solution stability, plasma protein binding, renal and biliary clearance, transporters). Wu and Benet[14] suggested a Biopharmaceutics Drug Disposition Classification System (BDDCS), which includes considerations of routes of elimination.

### 7.2.4 Molecular Properties for Solubility and Permeability Often are Opposed

Structural properties that determine solubility and permeability are shown in Figure 7.10.[15] All the physicochemical properties are intercorrelated. Changing one property can affect several others. The figure shows how structural features that enhance solubility often reduce permeability. For example, increasing charge, ionization, or hydrogen bonding capacity will increase solubility but will decrease permeability. Increasing lipophilicity and size to some extent will increase permeability but will decrease solubility. Medicinal chemists must balance the different structural features to find a balance between solubility and permeability for the clinical candidate in order to achieve optimal absorption.

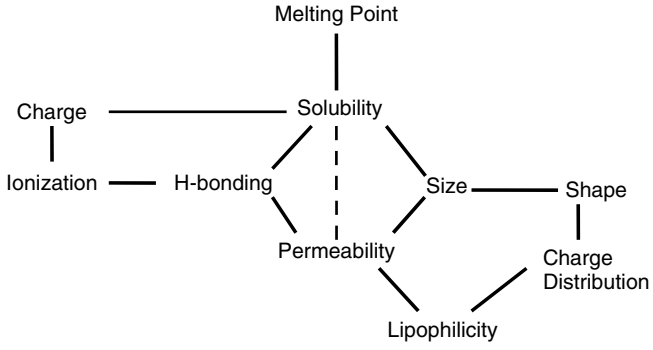



**Figure 7.10** ► Effects of structural properties on solubility and permeability. (Reprinted with permission from [15].)

Permeability tends to vary over a more narrow range than does solubility.[9] The difference between a high-permeability and a low-permeability compound can be 50-fold (0.001–0.05 $min^{-1}$). The difference between a high-solubility and low-solubility compound can be one million–fold (0.1 μg/mL–100 mg/mL). Therefore, if a structural modification improves solubility by 1,000 fold while it reduces permeability by 10-fold, then there will still be a 100-fold improvement in absorption.

## 7.3 Effects of Physiology on Solubility and Absorption

The gastrointestinal (GI) tract is a dynamic environment with changing conditions that affect compound solubility. Differences exist between species, and it is important to know what these differences are in order to extend the results of animal species dosing experiments to humans.

### 7.3.1 Physiology of the Gastrointestinal Tract

Some of the important characteristics of the GI tract are shown in Figure 7.11.[16] The GI tract has a pH gradient throughout its length, from acidic pH at the stomach to acidic–neutral pH in the small intestine to basic pH in colon. The wide pH range, long transit time, and high surface area of the small intestine allow for much higher absorption of drugs than in the stomach and colon. Acidic and basic drugs have different solubilities throughout the GI tract. Bases are more soluble in the stomach and upper small intestine due to ionization at acidic pHs. Acids are more soluble in later sections of the small intestine because the region is more basic.

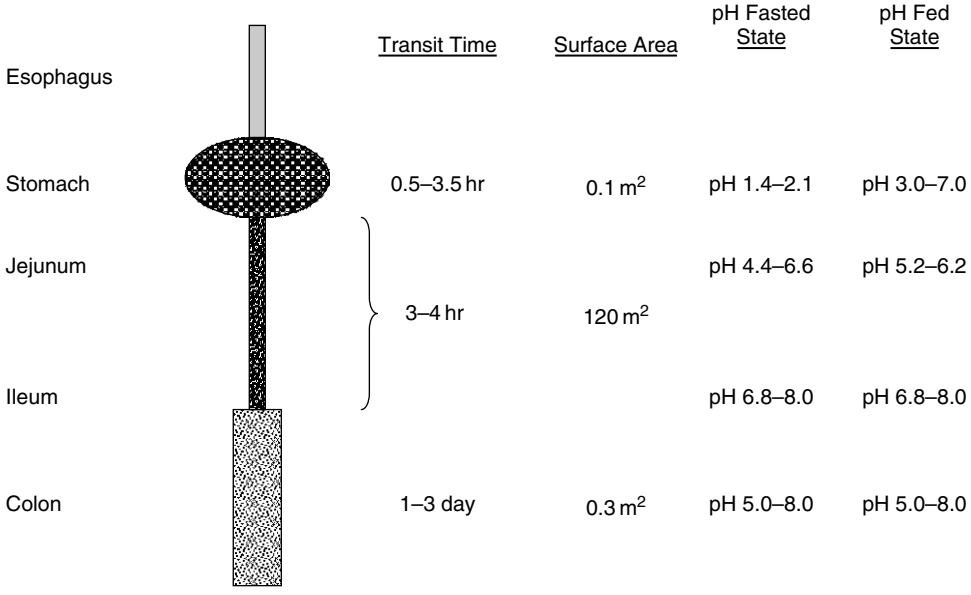



**Figure 7.11** ► Physiology and biophysics of the gastrointestinal tract. (Reprinted with permission from [40].)

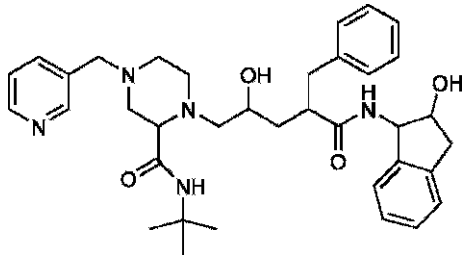
### 7.3.2 Species Differences in Gastrointestinal Tract

Gastric emptying time varies among species (Table 7.4).[17] If a drug is primarily absorbed in the intestine, it should reach plasma concentrations faster in the rat than in the human as a result of the earlier entry into the gastric lumen. Thus, rapid emptying results in earlier absorption.

The GI tract pH can be different among species. Rats and humans are good acid secretors. However, cats and dogs secrete less acid. Therefore, if the solubility of the discovery compound is pH dependent, differences in solubility between species will result in differences in absorption. An example is shown in Figure 7.12.[18] The compound L-735,524, an HIV

**TABLE 7.4 ► Species Differences In Gastric Emptying Time**

| Species | Gastric-emptying time (min) |
|---|---|
| Rat | ~10 |
| Rabbit | 30 |
| Dog | 40–50 |
| Human | 60 |




Solubility is pH dependent
- 60 mg/mL at pH 3.5
- <0.03 mg/mL at pH 5

| Vehicle | Species | Oral %F, 10 mpk |
|---|---|---|
| 0.5% Methylcellulose, pH 6.5 Suspension | Rat | 16% |
| | Dog | 16% |
| 0.05 M Citric Acid, pH 2.5 Solution | Rat | 23% |
| | Dog | 72% |

**Figure 7.12 ►** Species dependence of solubility and oral absorption for L-735,524. Dog is a poor acid secretor and has a gastrointestinal tract pH of 7. First-pass metabolism in dog is less than in rat.[18]

protease inhibitor analog of indinavir, shows steep changes in solubility with pH. It is much more soluble at low pH because of the presence of three basic amines as ionization centers. First-pass metabolism was higher for rat than dog. However, when the compound was dosed in a methylcellulose suspension formulation at pH 6.5 (owing to low solubility), the two species had the same oral bioavailability (16%). Rat is a good acid secretor but dog is not; thus, the compound is more soluble in rat stomach than in dog stomach. Even though the metabolism is faster in rat, the higher solubility in rat, due to lower acidity, results in the same oral bioavailability as in dog. When the compound is formulated in acidic buffer (citric acid), the compound is soluble and the oral bioavailability in dog increases to 72%, whereas the oral bioavailability in rat remains about the same as when a suspension formulation is used. This suggests that when solubility is not limited, first-pass metabolism is the dominant factor affecting oral bioavailability.

### 7.3.3 Food Effect

It is commonly thought that a high-fat diet will increase the solubility of lipophilic compounds and, therefore, enhance absorption. Actually, food can affect oral bioavailability in many different ways.[19,20] Food can either increase or decrease oral bioavailability by delaying

gastric emptying (delays absorption), slowing input into the intestine (delays absorption), stimulating bile salt secretions (increases solubility of lipophilic compounds), altering the pH of the GI fluid (changes solubility), increasing blood flow (improves "sink condition" that enhances absorption and faster metabolism), and increasing competition for metabolic enzymes (slows metabolism). Different buffers have been developed to simulate gastric fluid conditions in fasted and fed states (Table 7.5).[16] It has been found that solubility measured in gastric fluid gave better prediction for oral bioavailability than solubility measured in aqueous buffer alone, when permeability is considered.[21]

**TABLE 7.5 ► Buffer Composition that Simulates Fasted and Fed States[16]**

| | Simulated fasted state | Simulated fed state |
|---|---|---|
| Sodium taurocholate | 5 mM | 15 mM |
| Lecithin | 1.5 mM (0.1%) | 4 mM (0.3%) |
| pH | 6.8 | 6.0 |

## 7.4 Structure Modification Strategies to Improve Solubility

Through the years, many cutting-edge technologies have been developed to formulate insoluble compounds (Figure 7.13).[22] These are discussed in Chapter 41. In drug discovery, medicinal chemists would like to solve drug delivery problems "with covalent bonds,"[9] to improve solubility through structure modification. The strategies are listed in Table 7.6.

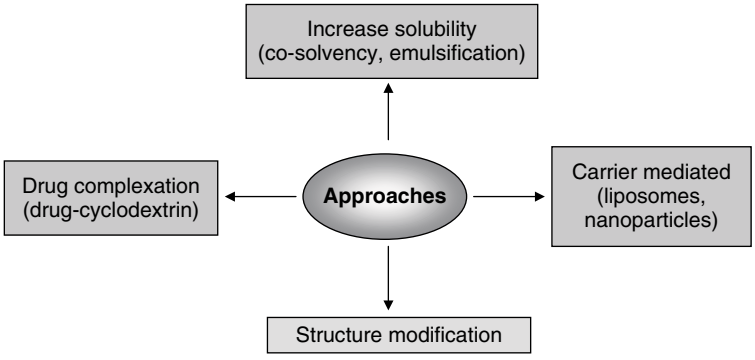



**Figure 7.13 ►** Approaches to improve solubility. The first choice is to modify the structure.

**TABLE 7.6 ► Structure Modifications Strategies for Solubility Improvement**

| Structure modification | Section |
|---|---|
| Add ionizable group | 7.4.1 |
| Reduce Log P | 7.4.2 |
| Add hydrogen bonding | 7.4.3 |
| Add polar group | 7.4.4 |
| Reduce molecular weight | 7.4.5 |
| Out-of-plane substitution to reduce crystal packing | 7.4.6 |
| Construct a prodrug | 7.4.7 |

### 7.4.1 Add Ionizable Groups

Addition of ionizable groups is commonly used for enhancing solubility. It is one of the most effective structural modifications to increase solubility. Typically, a basic *amine* or a *carboxylic acid* is introduced to the structure. Compounds with an ionizable functional group will be charged in pH buffers and have increased solubility.

One example is solubility enhancement of artemisinin (Figure 7.14), which is an antimalarial agent.[23] The sodium salt of a *carboxylic acid* analog achieved higher solubility, but, in this case, the compound was unstable. Ultimately, the *amine* analogs attained better solubility and stability and were active after oral dosing.[23]

For the series shown in Figure 7.15, the analogs with ether-containing side chains had good potency against a panel of tumor cell lines but had low solubility. Upon incorporation

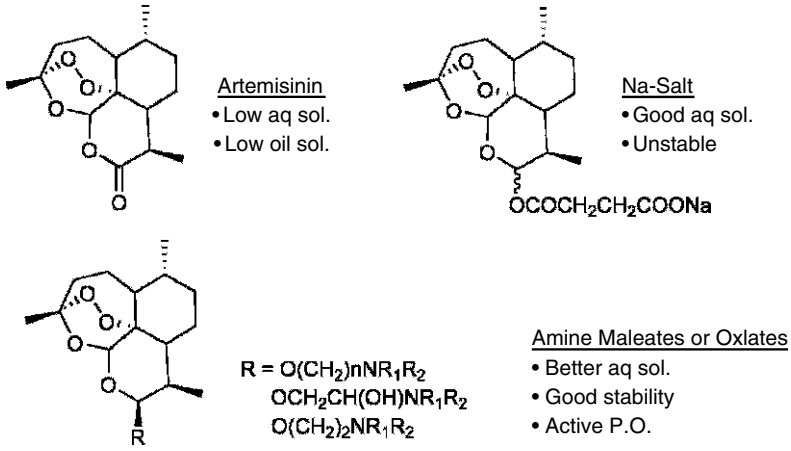
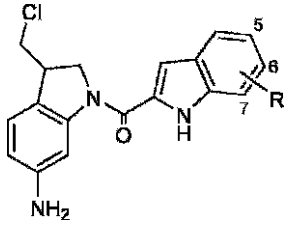



**Figure 7.14** ► Introduction of a side chain with a carboxylic acid or amine enhances the solubility of artemisinin.




| | | IC50 (μM) | | | |
|---|---|---|---|---|---|
| R | Solubility (μM) | AA8 | UV4 | EMT6 | SKOV3 |
| 5,6,7-triOMe | 32 | 0.35 | 0.055 | 0.27 | 0.63 |
| 5-OMe | 23 | 0.31 | 0.047 | 0.23 | 0.67 |
| 5-$O(CH_2)_2NMe_2$ | 700 | 0.16 | 0.044 | 0.12 | 0.26 |
| 5-OMe, 6-$O(CH_2)_2NMe_2$ | >1200 | 0.22 | 0.039 | 0.11 | 0.15 |
| 5-OMe, 7-$O(CH_2)_2NMe_2$ | 47 | 0.14 | 0.029 | 0.09 | 0.16 |

**Figure 7.15** ► Improved solubility of antitumor agents without loss of activity. (Reprinted with permission from [38].)

of a basic *amine*-containing side chain at position 5 or 6, solubility was greatly enhanced.[24] The last compound differed only at the position of substitution (position 7 vs. 5 or 6) but had lower solubility. This could be due to difference in crystal packing.

The compounds shown in Figure 7.16 were tested for solubility in simulated gastric fluid (pH 1.2) and phosphate buffer (pH 7.4).[25] Solubility was much higher for the compound that had a basic amine, especially at acidic pH.

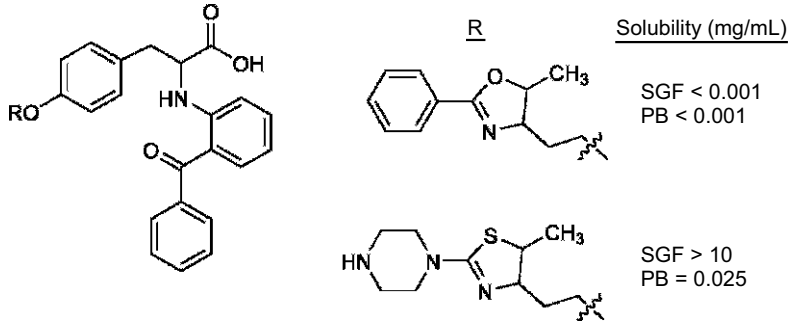



**Figure 7.16** ► Solubility improves with increasing basicity. PB, phosphate buffer (pH 7.4); SGF, simulated gastric fluid (pH 1.2).

The most active compound in vitro is not necessarily the most active compound in vivo. The compounds shown in Figure 7.17 are an example.[26] The first compound has an $IC_{50}$ of 0.004 nM; however, it is not active in vivo because of its low solubility. The second compound is 5-fold less active in vitro, but it is active in vivo because of its higher solubility. Adding a basic nitrogen to the molecule as an ionization center enhances solubility. A successful drug possesses a balance of potency and drug-like properties.

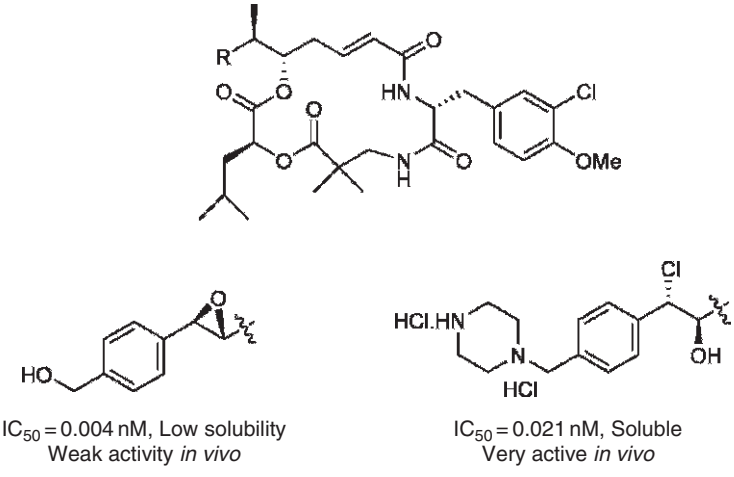
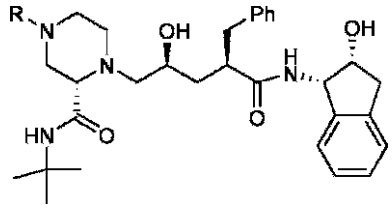



**Figure 7.17** ► Series compounds that are more active in vitro and have low solubility may not be as active in vivo as series analogs that have lower in vitro activity but are soluble and, thus, better absorbed. (Reprinted with permission from [38].)

### 7.4.2 Reduce Log P

Several protease inhibitors are shown in Figure 7.18.[17] Reducing Log P increased solubility and led to higher systemic exposure, as indicated by enhanced maximum concentrations in the blood, $C_{max}$. Reducing Log P and increasing solubility enhances the in vivo exposure.




| # | R | Cmax (uM) | Solubility (mg/mL) at pH 7.4 | Log P |
|---|---|---|---|---|
| 1 | benzyloxycarbonyl | <0.10 | <0.001 | 4.67 |
| 2 | 8-quinolinylsulfonyl | <0.10 | <0.001 | 3.7 |
| 3 | 2,4-difluorophenylmethyl | 0.73 | 0.0012 | 3.69 |
| 4 | 3-pyridylmethyl | 11.4 | 0.07 | 2.92 |

**Figure 7.18** ► For a series of protease inhibitors, absorption increased (as indicated by $C_{max}$) as solubility increased. Compound 4 in the chart was developed into the commercial drug indinavir.

### 7.4.3 Add Hydrogen Bonding

Introducing hydrogen bond donors and acceptors, such as OH and $NH_2$, can enhance aqueous solubility. Two anti-AIDS agents are shown in Figure 7.19.[27] The first compound has poor aqueous solubility and poor oral bioavailability, which limited its further development. Introducing a hydroxyl group into the molecule increased solubility and oral bioavailability.

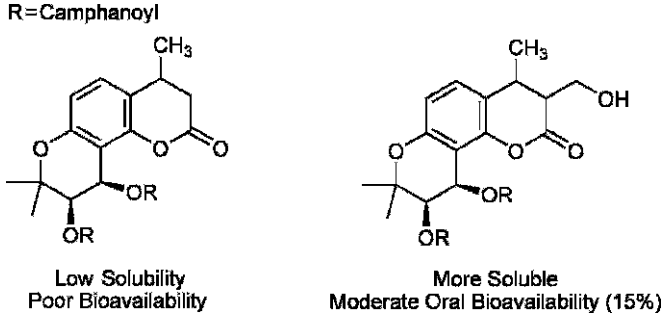



**Figure 7.19** ► Effects of H-bonds on solubility for anti-AIDS agents.

An example of antifungal agents is shown in Figure 7.20. Although nystatin, a polyene macrolide, is an effective antifungal agent, its use in medical practice is problematic because of its low solubility and significant human toxicity.[28] Structural modification by introducing hydroxyl groups at positions C31 and C33 increased the solubility by more than 2,000-fold. This dramatic increase in solubility is due, in part, to disruption of aggregate formation.[28]

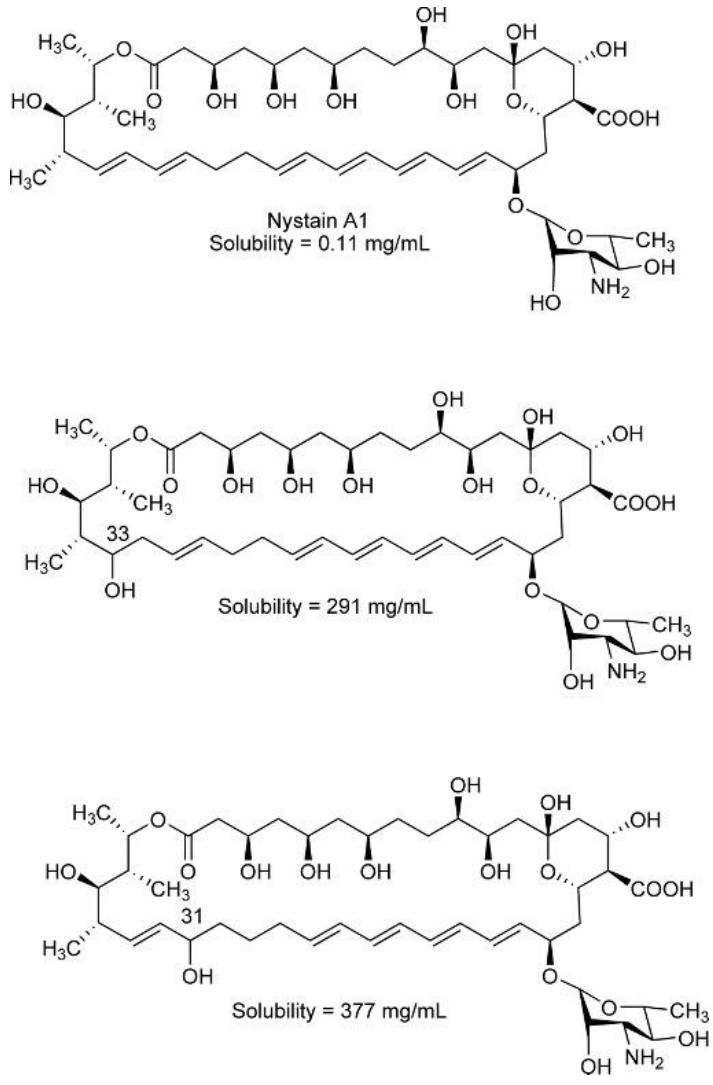



**Figure 7.20** ► Addition of hydrogen bond increased aqueous solubility.

### 7.4.4 Add Polar Group

Water solubility usually increases with the addition of a polar group. Figure 7.21 shows a series of epoxide hydrolase inhibitors.[29] Solubility increased with the introduction of the ester group (more polar) and carboxylic acid group (more polar and ionizable).

### 7.4.5 Reduce Molecular Weight

Reduction in molecular weight is another useful approach for increasing solubility. An example of CDK2 inhibitors is shown in Figure 7.22.[30] The lower molecular weight increased solubility and metabolic stability, while maintaining in vitro activity. In vivo potency was improved because of the increased solubility and stability.

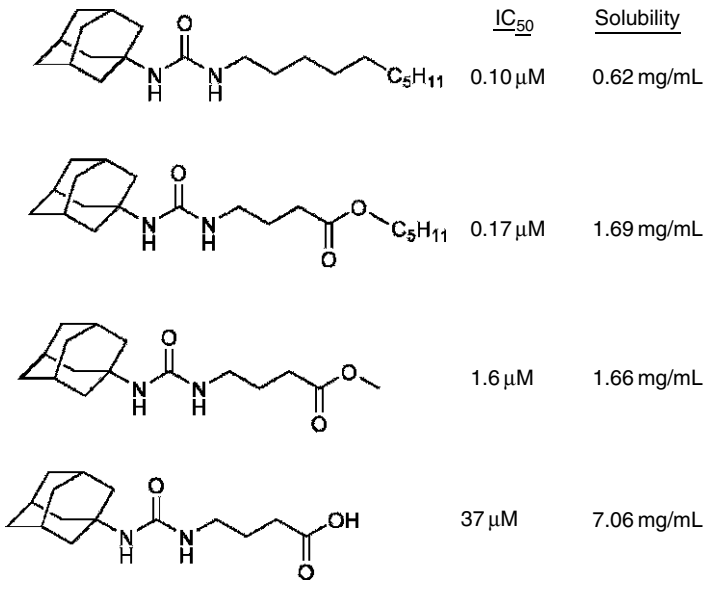



**Figure 7.21** ► Water solubility increased with addition of polar and ionizable groups in these epoxide hydrolase inhibitors.

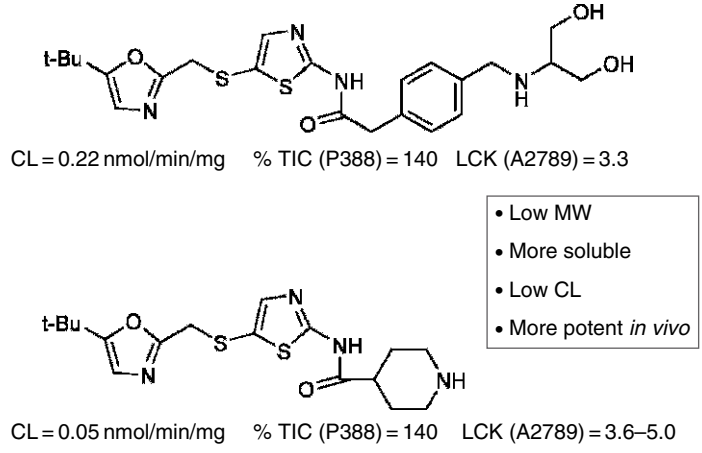



**Figure 7.22** ► Reduction in molecular weight for these CDK2 inhibitors resulted in increased solubility, improved metabolic stability, and increased in vivo potency.

## 7.4.6 Out-of-Plane Substitution

Out-of-plane substitutions are illustrated in Figure 7.23.[31] Addition of the ethyl group shifts the planarity of the molecule, resulting in a disruption of the crystal packing to form a higher-energy crystal that is more soluble.

Figure 7.24 shows two AMPA/$Gly_N$ receptor antagonists. Although PNQX is very potent in both in vitro and in vivo models, the major disadvantage is its poor solubility (8.6 μg/mL at pH 7.4), which leads to the potential of crystallization in the kidney. Introducing out-of-plane substitutions enhanced solubility to 150 μg/mL.[32]

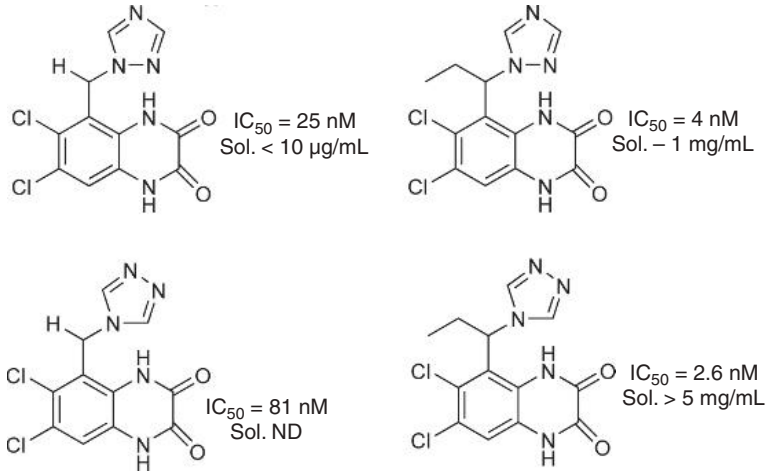



**Figure 7.23** ► Addition of the ethyl group causes an out-of-plane conformation, which disrupts the crystal packing and increases the solubility. (Reprinted with permission from [38].)

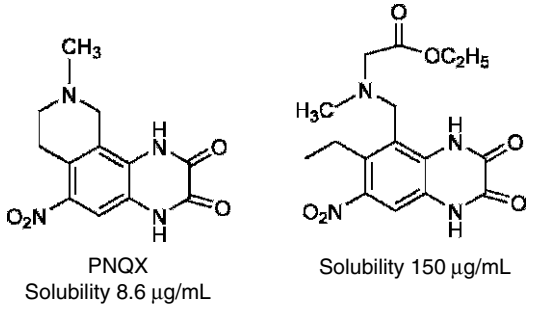



**Figure 7.24** ► Introducing out-of-plane substitutions increases solubility.

## 7.4.7 Construct a Prodrug

Charged or polar groups can be added to make prodrugs with increased aqueous solubility. Figure 7.25 shows fosphenytoin, which is a prodrug of phenytoin.[33] The phosphate group greatly increases the solubility, making it much easier to formulate for clinical dosing. Enzymatic hydrolysis in the intestine releases phenytoin for absorption. Prodrugs are discussed in Chapter 39.

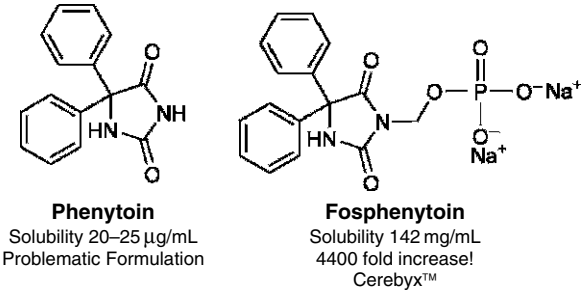



**Figure 7.25** ► Fosphenytoin was prepared as a prodrug of phenytoin to increase solubility.

## 7.5 Strategies for Improving Dissolution Rate

Solubility is *how much* of a compound can dissolve in solution. Dissolution rate is *how fast* a compound can dissolve into solution. Increasing the dissolution rate will make a drug dissolve faster so that it can be absorbed within the GI transit time, even though solubility remains the same. Several approaches for increasing dissolution rate are listed in Table 7.7. These strategies are especially worthwhile for discovery animal dosing experiments to study in vivo efficacy and pharmacokinetics. Formulation strategies for discovery are discussed in Chapter 41.

**TABLE 7.7 ► Strategies for Increasing Dissolution Rate**

| Goal | Change | Section |
|---|---|---|
| Increase surface area of solid | Reduce particle size | 7.5.1 |
| Predissolve in solution | Oral solution | 7.5.2 |
| Improve wetting of solid | Formulate with surfactants | 7.5.3 |
| | Prepare a salt form | 7.5.4 |

### 7.5.1 Reduce Particle Size

Milling the solid material to a smaller particle size increases the surface area so that more molecules will be exposed to solvent at the same time. This results in an increased dissolution rate and increased oral absorption. Newer technologies allow the preparation of "nanoparticles" for even higher surface area. Figure 7.26 shows the effect of particle size reduction on oral exposure of MK-0869 in Beagle dogs. Exposure increases with decreasing particle size.

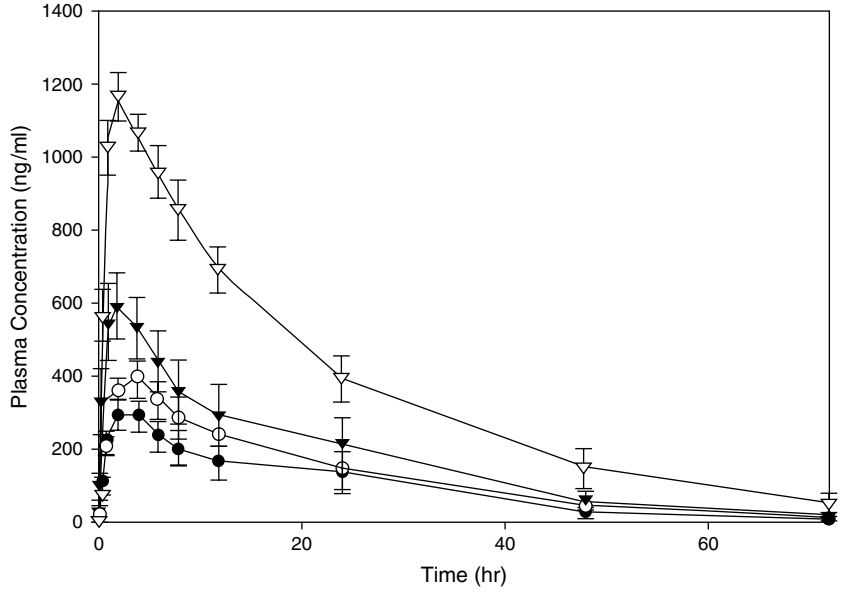



**Figure 7.26 ►** Effects of particle sizes from different milling processes on oral absorption of MK-0869 in Beagle dogs. Oral absorption of MK-0869 increased in Beagle dogs with decreasing particle size. (Reprinted with permission from [41].)

### 7.5.2 Prepare an Oral Solution

Solid material can be dosed orally as a solid dosage form, suspension (dispersed in a solution), or solution. If a compound is dosed as a solution rather than a suspension or solid dosage form, it has a better chance of being absorbed because it no longer must dissolve throughout the GI tract. Especially in drug discovery, a compound that can be dissolved in solution is the preferred formulation because it has a better chance of being absorbed and demonstrating proof of concept.

### 7.5.3 Formulate with Surfactants

Surfactants improve the wetting of the solid and increase the rate of disintegration of the solid material to finer particles. This increases dissolution rate and absorption.

### 7.5.4 Prepare a Salt Form

A salt of an acid or a base typically has a higher dissolution rate than the corresponding free acid or base. This produces increased absorption. The salt form does not change the intrinsic solubility of a free acid or a free base but does increase the overall solubility through ionization. Salt forms are discussed in greater detail in Section 7.6.

## 7.6 Salt Form

Salt forms typically are selected to modify physicochemical properties (e.g., dissolution rate, crystallinity, hygroscopicity, etc.) and mechanical properties (hardness, elasticity, etc.), leading to increased bioavailability, stability, and manufacturability.[34,35]

Examples of commercial drugs and their salt forms are listed in Table 7.8.[36] The solubility of the salt in pure water is much greater than the intrinsic solubility of the corresponding free acid or base. (Its solubility in buffer is affected by the solution pH, see Section 7.6.2.)

**TABLE 7.8 ► Example Salts of Commercial Drugs[36]**

| Name | Solubility in water[a] (mg/mL) |
|---|---|
| Codeine | 8.3 |
| Sulfate | 33 |
| Phosphate | 44 |
| Atropine | 1.1 |
| Sulfate | 2,600 |
| Pseudoephedrine | 0.02 |
| Hydrochloride | 2,000 |
| Cetirizine | 0.03 |
| Dihydrochloride | 300 |

[a] Final pH of water after salt dissolves differs with the salt.

### 7.6.1 Solubility of Salts

Three equilibria govern the relationship between a free base (or acid) and its corresponding salt (Figure 7.27). First is the equilibrium between the salt in the solid state and the salt

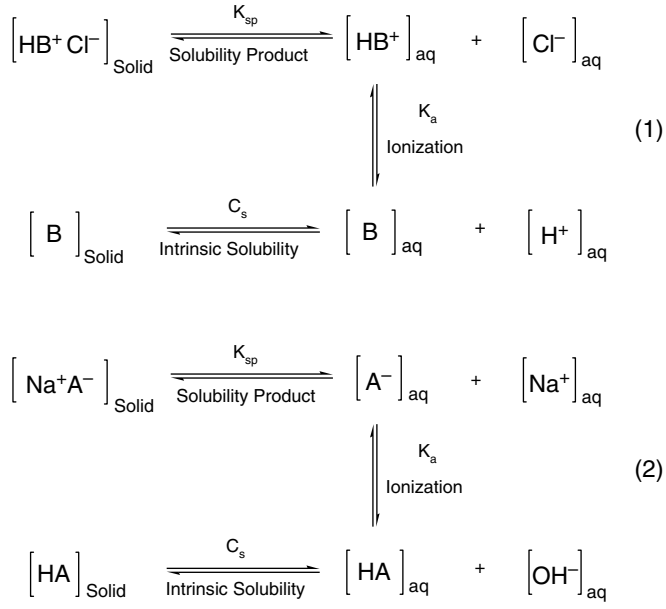



**Figure 7.27** ► Equilibrium of (**1**) free base and its salts and (**2**) free acid and its salts.

in solution ($K_{sp}$, solubility product constant). Second is the equilibrium between the free base (or acid) in solid state and free base (or acid) in solution ($C_s$, intrinsic solubility of base or acid). Third is the equilibrium between the free base (or acid) in solution and the corresponding salt in solution ($K_a$, ionization constant).

The solubility of a salt form at different pH values follows the curves that are generalized in Figure 7.28. The solubility at lower pH values for the salt of a base (Figure 7.28A) is determined by the $K_{sp}$ of the salt. Different salts have different maximum solubilities. Before reaching maximum solubility, the solubility is determined by the pH, ionization constant $K_a$, and intrinsic solubility of the free base. At higher pH values, the solubility is the intrinsic solubility of the free base. The concentration behavior of the salt of an acid works in the opposite manner with regard to pH.

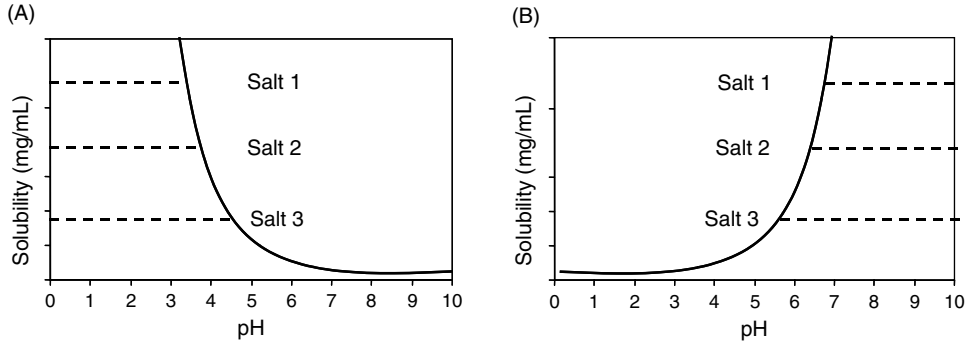



**Figure 7.28** ► Solubility of hypothetical salts with pH. **A**, Three salts of a basic compound. **B**, Three salts of an acidic compound.

The solubility of a salt is not initially governed by the pH but by the solubility product of the salt ($K_{sp}$, solubility of the salt):

$$\text{Acid: } K_{sp} = [\text{Counterion}^+][\text{Anion}^-]$$
$$\text{e.g.: NaA} = \text{Na}^+ + \text{A}^- \qquad K_{sp} = [\text{Na}^+][\text{A}^-]$$
$$\text{Base: } K_{sp} = [\text{Counterion}^-][\text{Cation}^+]$$
$$\text{e.g.: BHCl} = BH^+ + \text{Cl}^- \qquad K_{sp} = [\text{Cl}^-][\text{BH}^+].$$

The high initial solubility of a salt puts a high concentration of the compound into solution for absorption. Once the compound is in solution, its concentration is governed by the ionization constant $K_a$ and by the pH of the solution. Under these conditions, the compound may precipitate in the GI system, as governed by the solubility of free base or acid.

When the salt of a base (Figure 7.28A) is orally administered, its initial solubility is determined by its $K_{sp}$, which is independent of the pH. Once it is dissolved, pH and intrinsic solubility of the free base play a role. The acidic pH in the stomach favors the ionized form of the free base, keeping the base in solution, but neutral and basic pH values in the intestine and colon favor the free base, which can shift the equilibrium and lead to precipitation.

When the salt of an acid (Figure 7.28B) is orally administered, its initial solubility is also determined by its $K_{sp}$, which is independent of pH. Once it is dissolved in the acidic pH of the stomach, the free acid is favored, and its solubility is determined by its intrinsic solubility, which is low compared to the anion, so much of the material precipitates.

### 7.6.2 Effect of Salt Form on Absorption and Oral Bioavailability

In an aqueous medium at a specific pH with sufficient buffer capacity, a compound will have the same solubility regardless of whether it presents as a salt form or a free acid or base. Salts increase absorption by increasing dissolution rate. When the salt first encounters the aqueous phase, the high dissolution rate rapidly puts a lot of the compound in solution, which enhances absorption. Salts can stay in solution in a supersaturated state and do not precipitate immediately, even if the pH in the GI tract favors free acid (or base) formation and the free acid (or base) has low solubility. Supersaturation leaves a wider time window for compound to be absorbed. Furthermore, if the compound precipitates as a free acid or free base as a result of pH changes, it tends to precipitate as amorphous material and fine particles, which have higher solubility and a higher chance of being absorbed than crystalline material with a large particle size. As a result, the salt has higher absorption than the free acid (or free base) has on its own.

An example of this effect of salt form on increasing absorption is shown in Figure 7.29.[37] The absorption of the free acid p-amino-salicylic acid (PAS) was incomplete. Only 77% of the dose was absorbed because of low solubility and dissolution rate. However, absorption of the salts (Na, K, and Ca) was complete and had faster absorption, as indicated by shorter pharmacokinetic $T_{max}$ (time of maximum concentration) and higher $C_{max}$ (highest concentration). The salt form of PAS has higher oral exposure than the free acid.

For the protease inhibitor indinavir (Figure 7.5), the intrinsic solubility of the free base has very low solubility, and the solubility showed steep dependence on pH. HIV/AIDS patients tend to lack hydrochloric acid in their stomachs. Dosing of the free base caused unpredictable variable blood levels and resulted in rapid development of drug resistance by

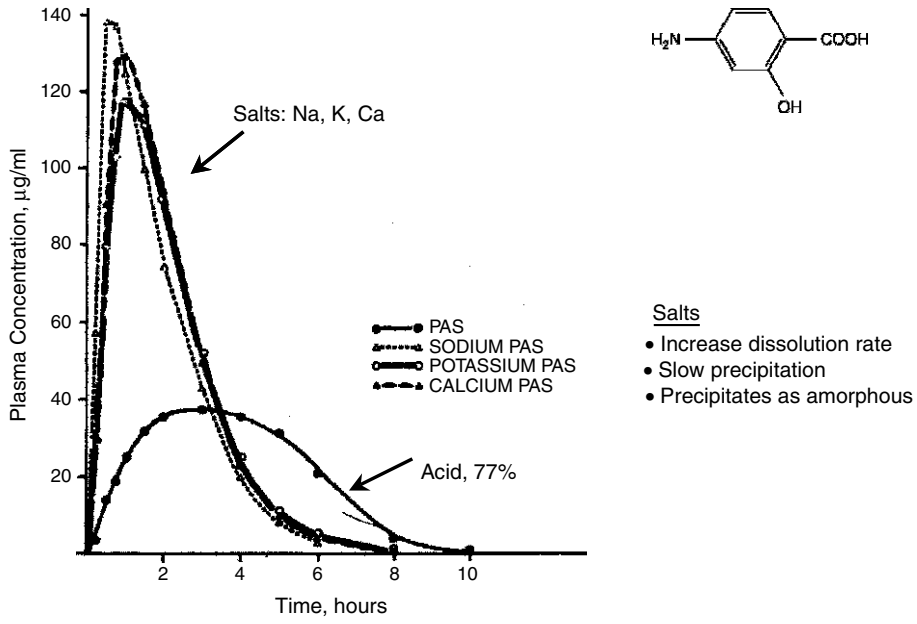



**Figure 7.29 ►** Salt forms have a higher initial solubility and absorption rate than the free acid and precipitate as a fine amorphous solid, resulting in higher initial dissolution and absorption rates. (Reprinted with permission from [37].)

viruses. A sulfate salt of indinavir was developed, which produced more consistent exposure. Indinavir is marketed as the sulfate ethanolate Crixivan.

### 7.6.3 Salt Selection

The counter ion for a salt should have a p$K_a$ that differs from the drug by 2–3. For human studies, FDA-approved counter ions should be used. If not, enough toxicological data supporting selection of the counter ion must be provided. Approximately 70% of the counter ions used in commercial drugs are anions and 30% are cations. The 10 most commonly used anions and cations for salt formation are shown in Table 7.9.[35,38] The most common anion is $Cl^-$ and the most common cation is $Na^+$.

**TABLE 7.9 ► Commonly Used Counter Anions and Cations for Salt Formation[38]**

| Counter anions | Percent |
|---|---|
| Chloride | 48 |
| Sulfate | 5.8 |
| Bromide | 5.2 |
| Mesylate | 3.2 |
| Maleate | 3.1 |
| Citrate | 2.8 |
| Tartrate | 2.7 |
| Phosphate | 2.5 |
| Acetate | 2.1 |
| Iodide | 1.2 |

***Continued***

**TABLE 7.9 ►** *Continued*

| Counter cations | Percent |
|---|---|
| Sodium | 58 |
| Calcium | 12 |
| Potassium | 9.8 |
| Magnesium | 4.5 |
| Meglumine | 2.4 |
| Ammonium | 2.0 |
| Aluminum | 1.4 |
| Zinc | 1.1 |
| Piperazine | 0.90 |
| Tromethamine | 0.90 |

The salt form of the drug product is also selected for its optimal physicochemical properties, such as crystallinity, morphology, hygroscopicity, stability, and powder properties.

### 7.6.4 Precautions for Using Salt Forms

The solubility of an HCl salt in the stomach can be limited by the "common ion effect." The high concentration of $Cl^-$ (0.1–0.15 M) in the stomach can limit salt form dissolution, according to the $K_{sp}$ (Figure 7.27). If this is the case, other salts such as sulfate or phosphate can be used.

If a compound is a very weak acid ($pK_a > 6$) or a very weak base ($pK_a < 5$) with very low intrinsic solubility, conversion of a small amount of the salt to the free acid or free base can cause precipitation and lead to various issues. For example, for phenytoin, a very weak acid with very low solubility (~20 μg/mL), the IV formulation of the sodium salt can precipitate because of conversion of the salt to the free acid. Precipitation during IV dosing can cause problems.

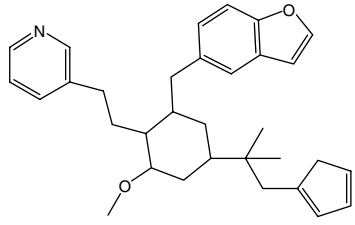
When the salt form particles enter the stomach or intestine, conversion to free acid, free base, or hydrate on the surface of the particle can cause formation of an insoluble film or coating that prevents further dissolution of the salt. In this case, the salt form will not enhance the dissolution rate.

## Problems

(Answers can be found in Appendix I at the end of the book.)

1. A basic amine ($pK_a$ 9) is dissolved in DMSO and tested in phosphate buffer saline (PBS, pH 7.4) for biological activity. An HCl salt of this compound is prepared and tested under the same condition. Will the $IC_{50}$ be the same, lower, or higher?
2. A free acid ($pK_a$ 4) and its sodium salt are tested for solubility. Will they have the same solubility in water? Why? Will they have the same solubility in pH 7.4 potassium phosphate buffer?
3. What approaches can be used to increase solubility? What is the most effective chemical modification to increase solubility? What approaches can be taken to increase dissolution rate?

4. Compound A was dosed in rat as an oral suspension at 100 mg/kg, 200 mg/kg, and 300 mg/kg. $C_{max}$ and AUC of all three doses were the same. What is the potential cause?
5. An acidic compound has intrinsic solubility of 2 μg/mL and p$K_a$ of 4.4. What is the approximate solubility of the compound at pH 7.4?
6. Why does the solubility of subsequent analog compounds in a lead series tend to be lower during lead optimization?
7. List components and characteristics of the aqueous solution matrix that affect solubility.
8. List structural properties that affect solubility.
9. What is the difference between solubility and dissolution rate?
10. Why is thermodynamic solubility not as important as kinetic solubility in early drug discovery?
11. What solubility should the following compounds have for complete human absorption when orally dosed?: (a) dose of 1 mg/kg and high permeability, (b) dose of 10 mg/kg and high permeability, (c) dose of 10 mg/kg and average permeability.
12. Structural modifications to improve solubility often decrease what other property?
13. What usually is the most successful structure modification to improve solubility?
14. Making a salt improves the: (a) intrinsic solubility, (b) dissolution rate.
15. For the following lead structure, what structural modifications could you make to improve solubility?




16. Low solubility can cause which of the following?: (a) low oral bioavailability, (b) low metabolism, (c) low permeability, (d) increased burden on patients, (e) less expensive drug product formulation.
17. Which of the following are true about kinetic solubility measurements?: (a) compound is first dissolved in DMSO then added to aqueous buffer, (b) can be used to develop structure–solubility relationships, (c) is affected by solution pH or components, (d) can be used to recognize solubility limitations and guide structure modifications to improve solubility, (e) better for high throughput analysis than equilibrium solubility.
18. The minimum acceptable solubility to produce in vivo efficacy in humans is predictable using which of the following?: (a) target dose, (b) toxicity, (c) hERG blocking concentration, (d) permeability, (e) intestinal transit time.
19. Which of the following can a salt form change after oral dosing, as compared to the free acid or base?: (a) $T_{max}$, (b) $C_{max}$, (c) AUC, (d) oral bioavailability, (e) efficacy.

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# Permeability

*Overview*

- *Permeability is the velocity of molecule passage through a membrane barrier.*
- *Permeability is a determinant of intestinal absorption and oral bioavailability.*
- *Optimizing passive diffusion is productive because it is the predominant mechanism for absorption of most commercial drugs.*
- *Permeability is increased by removing ionizable groups, increasing* Log P*, and decreasing size and polarity.*

Permeability is the velocity of drug passage through a biological membrane barrier. This is a necessary process for absorption in the intestine, passage through blood–organ barriers, penetration into cells containing the therapeutic target, and elimination by the liver and kidney. Permeability also is important in cell-based biological assays in discovery, where the compound must permeate through the cell membrane to reach an intracellular therapeutic target. Prediction of in vitro permeability can enhance a wide range of drug discovery investigations, help with understanding cell-based bioassays, and assist prediction and interpretation of in vivo pharmacokinetics results.

## 8.1 Permeability Fundamentals

Drug molecules encounter several different membrane barriers in living systems. They include gastrointestinal (GI) epithelial cells, blood capillary wall, hepatocyte membrane, glomerulus, restrictive organ barriers (e.g., blood–brain barrier [BBB]), and the target cell membrane.

Different membranes can have different permeabilities for a compound. These differences are caused by differences in the membrane lipid mixture (passive diffusion), membrane transporter expression (active transport), or tightness of junctions between cells (paracellular).

Different mechanisms of membrane permeation were introduced in Chapter 3. They are passive diffusion, active uptake, endocytosis, efflux, and paracellular (Figure 8.1). Each of these permeation mechanisms is discussed in the following sections.

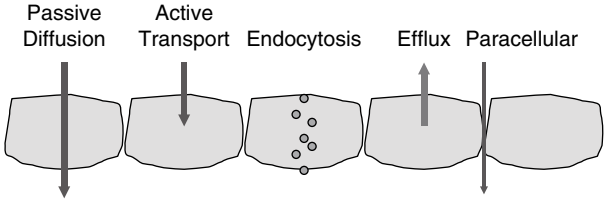



**Figure 8.1** ► Major permeability mechanisms.[12]

### 8.1.1 Passive Diffusion Permeability

The most important permeability mechanism for drug discovery is *passive diffusion*. A compound moves by brownian motion from the aqueous phase through the cellular lipid bilayer membrane to the aqueous phase on the other side. The compound must first pass through the luminal (apical) lipid bilayer membrane, then pass through the cytoplasm and exit the cell through the abluminal (basolateral) membrane. Alternatively, a molecule may pass into the membrane, move laterally through the membrane, and exit elsewhere. Passive diffusion is driven by a concentration gradient, with the net movement of molecules from the area of higher concentration to the area of lower concentration.

During intestinal absorption, drug molecules move from the relatively high concentration of the gastric lumen, through the intestinal membrane to the capillary blood vessels, which have a relatively low drug concentration. It has been estimated that 95% of commercial drugs are predominantly absorbed in the GI tract by passive diffusion.[1,2] Even though the compound may be a transporter substrate, transporters can become saturated in the GI tract at the concentrations produced by oral administration.

It is important to remember that permeability is much higher for more lipophilic molecules than for polar molecules. This is primarily because molecules must pass through the highly nonpolar lipid bilayer membrane. Neutral molecules are much more permeable than their charged forms (anionic or cationic). (Ions may permeate, to some extent, by ion pairing to form a neutral species.)

For this reason, pH and p$K_a$ play important roles in passive diffusion. This is illustrated in Figure 8.2 using passive diffusion permeability data from the parallel artificial membrane

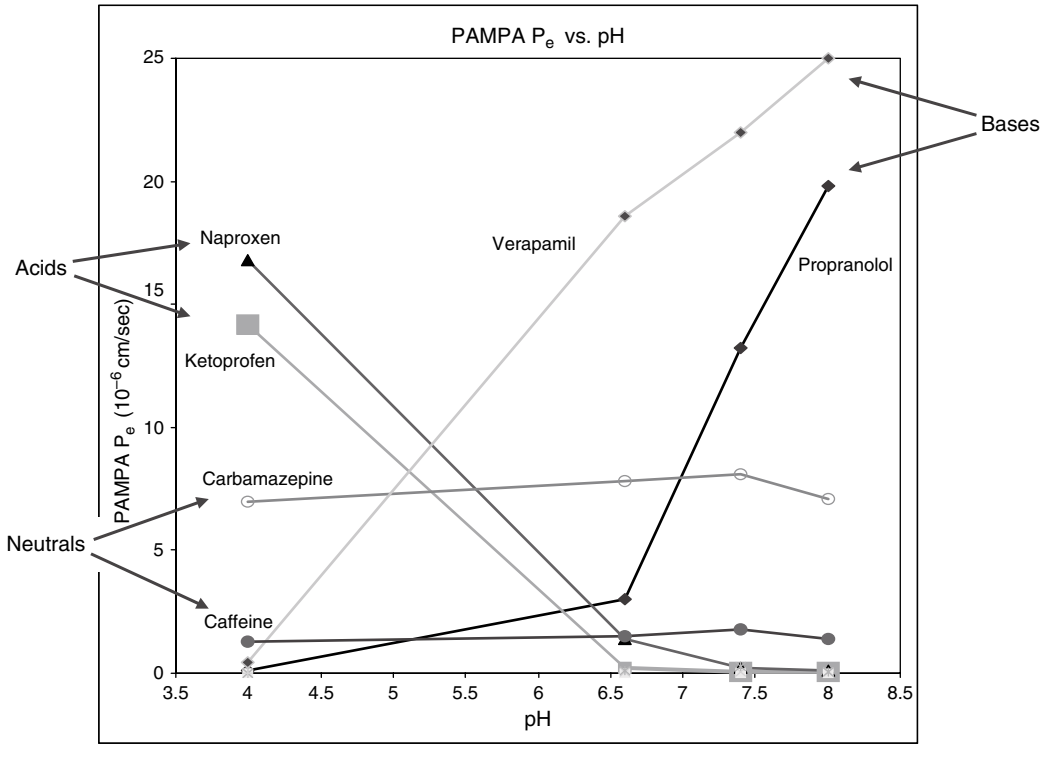



**Figure 8.2** ► Passive diffusion across a membrane is affected by the solution pH and compound p$K_a$. In this PAMPA permeability experiment (see Chapter 26), acidic, basic, and neutral compounds have different permeability at different pH values. (Reprinted with permission from [13].)

permeability assay (PAMPA; see Chapter 26), with the same pH on both sides of the membrane. This diagram illustrates the effect of pH on the permeability of acids, bases, and neutrals at different pH values. The passive diffusion of acids is much higher at low pH, where a large percentage of the acid molecules in solution is neutral but drops with increasing pH as the percentage of neutral molecules drops and the percentage of anions increases. Conversely, the passive diffusion of bases is low at low pH, where a large percentage of the basic molecules in solution is protonated (cations) and increases with increasing pH as the percentage of neutral molecules increases. The ionization behaviors of acids and bases and their effects on permeability are illustrated in Figure 8.3. The passive diffusion permeability of neutral molecules is unaffected by pH.

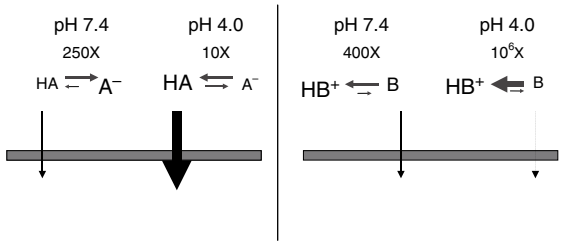



**Figure 8.3** ► Effects of pH on passive diffusion of acids and bases across a lipid membrane. Permeability is highly favored for the neutral form. Thus, passive diffusion is greatest for bases at higher pH values and for acids at lower pH values. In this example, the acid has $pK_a = 5$ and the base has $pK_a = 10$. The fold ratios of the higher population species are shown.

pH and $pK_a$ also affect passive diffusion across a lipid membrane that has a different pH on each side, as occurs in the GI. For an acid (e.g., $pK_a = 5$), passive diffusion is enhanced in the direction of the higher pH because of the ionization equilibrium. On the other hand, for a base (e.g., $pK_a = 10$), passive diffusion is enhanced in the direction of the lower pH. The reason for this behavior is shown in Figure 8.4. The acid anions ($A^-$) are "trapped" on the side of the higher pH. The base cations ($BH^+$) are "trapped" on the side of the lower pH. In the living system, this is not so obvious, because the bloodstream traps the drug molecules and moves them away (often termed *sink effect*). This effect, however, is apparent for in vitro permeability experiments when there is a difference of pH on either side of the membrane. For example, basic compounds may appear to be effluxed in a Caco-2 experiment, but this is really the "secretory" permeability of bases toward the lower pH.

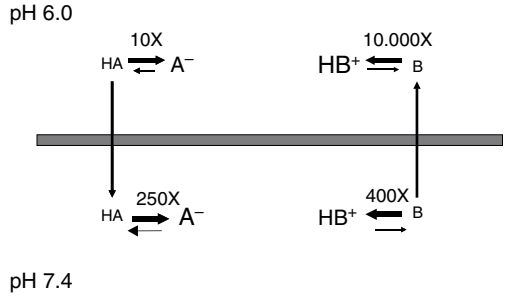



**Figure 8.4** ► pH differences across a lipid membrane, such as in the GI tract, affect passive diffusion. For an acid (e.g., $pK_a = 5$), passive diffusion is enhanced in the direction of the higher pH because of the ionization equilibrium. On the other hand, for a base (e.g., $pK_a = 10$), passive diffusion is enhanced in the direction of the lower pH. The fold ratio of the higher population species is shown.

### 8.1.2 Endocytosis Permeability

Another route of permeability is *endocytosis*. Compounds may be engulfed by the membrane, pass through the cell within the vesicle, and be released on the other side. This has been of only minor interest for small molecule drug discovery.

### 8.1.3 Active Uptake Permeability

Molecules may be permeable by *active uptake transport*, in which a compound binds to a transmembrane protein and moves through the membrane. Active transport requires the expenditure of energy, commonly two ATPs for each molecule transported. Active transport often occurs against the concentration gradient. There must be affinity of the drug for the transporter. Although transporters serve a vital function for the permeability of natural ligands, such as nutrients, they also can be responsible for the permeability of some drug molecules. Transporters are discussed in Chapter 9.

### 8.1.4 Paracellular Permeability

If molecules are small and polar, they might pass by *paracellular* permeability between the epithelial cells through "pores" or channels that are approximately 8 Å in size. Cells in the GI tract or other organs, such as the glomerulus in the kidney, are sometimes termed *leaky* because of the somewhat loose junctions between the cells that allow molecules to slip between. In other tissues, such as the BBB, the junctions are very tight and there is no appreciable paracellular permeability. In the intestine, this route of permeation is observed for less than 5% of drug compounds. The pores represent less than 0.3% of the total membrane surface, so this route of absorption has limited capacity. Generally, paracellular permeability in the GI tract is available primarily to compounds that have a molecular weight less than 180 Da and are polar.

### 8.1.5 Efflux Permeability

Another major mechanism of permeability is *efflux*, the active transport of compounds from inside the cell or membrane back into the lumenal space. P-glycoprotein (Pgp) and breast cancer resistance protein (BCRP) are well-known efflux transporters. The net effect of efflux transport is the reduction of drug concentration within the cell or permeation across the membrane. Pgp is a member of the ABC (ATP binding cassette) family of transporters, which utilize the energy from cleavage of two molecules of ATP to ADP and inorganic phosphate for the transport of each drug molecule. Efflux transport is also found in other membranes, such as the BBB, where it serves a protective function by opposing the exposure of brain tissue to some xenobiotic compounds. In liver hepatocytes, efflux enhances removal of drugs and metabolites from within the hepatocytes to the bile canaliculus for elimination from the body. In the nephron, Pgp is one of the transporters involved in active secretion into the proximal tubule. The expression level of transporters, such as Pgp, varies along the length of the small intestine. It has been reported that compounds can move out of systemic circulation and be secreted into the intestinal lumen by efflux transporters.[3] Pgp is discussed further in Chapter 9.

### 8.1.6 Combined Permeability

The permeability of a compound is the composite of permeability from all of the mechanisms available to it. The term *absorptive transport* is often used to denote compound flux from

the GI lumen toward the bloodstream. As shown in Figure 8.5, absorptive transport is the result of passive diffusion, which is driven by the concentration gradient and pH effects, active transport, which is driven by affinity for the transporter, and paracellular permeability, which is driven by size, polarity, and concentration gradient. Conversely, the term *secretory transport* is often used to denote compound flux in the direction of the GI lumen. Secretory transport is the result of passive diffusion and efflux.

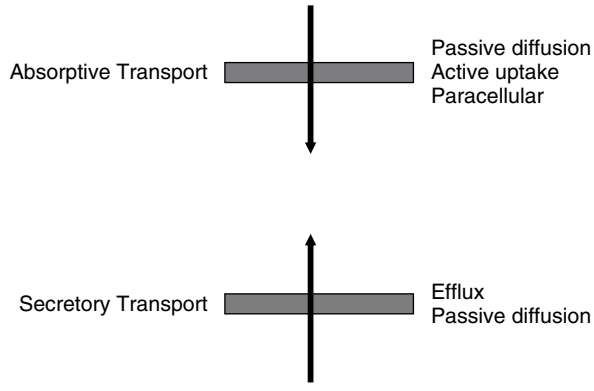



**Figure 8.5** ► Composite permeability for a particular compound is the result of dynamic interaction of local conditions and how they affect the various permeability mechanisms. Conditions include concentration gradient, pH gradient, transporter affinity, molecular size, and polarity.

The flux of a compound is affected by concentration. For example, after oral administration, the concentration of a compound in the GI tract may be so high that (a) transporters are saturated, and (b) the high concentration gradient drives passive diffusion. Thus, passive diffusion becomes the major route of compound absorption. By contrast, at the BBB, the circulating drug concentration is much lower and usually is not near the transporter saturating concentration. Under these conditions, active transport mechanisms, either uptake or efflux, can have a much greater effect on total permeability if the compound is a substrate for a transporter.

The total permeability of a compound differs with the tissue. Factors that affect permeability are the local concentration gradient, pH polarity across membranes, expression of transporters, transporter $K_m$, the side of the cell the transporters are present (i.e., apical or basolateral), affinity of the compound for various transporters, and the size of the pores between cells that form the membrane barrier. There is a dynamic modulation of permeability mechanisms as a result of the conditions.

## 8.2 Permeability Effects

Permeability affects many factors that determine pharmacology in both living systems and in vitro discovery experiments. Two of these factors are bioavailability and cell-based biological activity assays.

### 8.2.1 Effect of Permeability on Bioavailability

Drug absorption in the GI tract after oral administration depends heavily on permeability. Compounds with low permeability typically have low bioavailability. For example, Table 8.1 shows the case of a potent, highly charged acidic compound. The compound has a bioavailability of less than 1%. This is indicated by the PAMPA passive diffusion permeability method (see Chapter 26), with a low permeability value ($0.1 \times 10^{-6}$ cm/s). When a prodrug

**TABLE 8.1 ► Example of Effect of Permeability on Oral Bioavailability of an Acidic Compound**

| | Compound | Prodrug |
|---|---|---|
| PAMPA ($P_e \times 10^{-6}$ cm/s) | 0.1 | 7.0 |
| Oral bioavailability | <1% | 18% |

A compound with good potency ($K_i = 7$nM) had low permeability and bioavailability. Its prodrug had good permeability and bioavailability.
PAMPA, parallel artificial membrane permeability assay.

was made, the compound had much higher PAMPA permeability value ($7 \times 10^{-6}$ cm/s) and a much higher bioavailability of 18%. In this case, passive diffusion is limited for the highly charged acidic compound ($pK_a = 4.5$).

### 8.2.2 Effect of Permeability on Cell-Based Activity Assays

Permeability can limit the activity of compounds in cell-based assays. For intracellular therapeutic targets, the compound must penetrate the cellular membrane to show activity. Therefore, as discovery project teams progress from non–cell-based assays (e.g., enzymes, receptors) to cell-based assays, the activity can be severely reduced for some compounds. If this is due to the unrecognized cell membrane barrier rather than intrinsic activity, the project team might drop the compound from further consideration. A more successful approach is to recognize when permeability may be limiting cell-based activity. Analogs with improved permeability can be synthesized and tested for activity. It would be unfortunate to discard an active lead that could be improved by synthetic modification if the property (here permeability) causing the poor bioactivity can be identified and the structure modified.

An example of the effect of permeability on cell-based assays is shown in Table 8.2. Both good enzyme activity and permeability were required in order to produce good bioactivity in the cell-based assay. In some cases, compounds that are only moderately potent in the enzyme assay can be the most potent of the group of analogs in the cell-based assay if permeability limits intracellular exposure of the compounds that are more active in the enzyme assay (Table 8.3).

**TABLE 8.2 ► Example of Effect of Permeability on Cell-based Assay Bioactivity**

| Compound | In vitro $K_i$ (μM) | PAMPA ($P_e \times 10^{-6}$ cm/s) | Cell-based $IC_{50}$ (μM) |
|---|---|---|---|
| A | 0.007 | 4.9 | 10.5 |
| B | 0.02 | 1.0 | 22.1 |
| C | 0.01 | 0.02 | Inactive |
| D | 0.05 | 0.1 | Inactive |
| E | 3.5 | 14.3 | Inactive |
| F | 17 | 6.6 | Inactive |
| G | 4.3 | 0.01 | Inactive |

White, gray, and dark gray cells categorize data for high-, moderate-, and low-activity ranges, respectively. Cell-based activity requires both good enzyme activity and permeability.
PAMPA, parallel artificial membrane permeability assay.

**TABLE 8.3 ► Example of How Two Compound Series Can Demonstrate Different Activities in Cell-Based Assays and Enzyme Assays**

| | Compound series I | Compound series II |
|---|---|---|
| Enzyme activity assay | High potency | Moderate potency |
| PAMPA permeability | Low | High |
| P-glycoprotein efflux permeability | Yes | No |
| Cell-based activity assay | Inactive | Active |

PAMPA, parallel artificial membrane permeability assay.

## 8.3 Permeability Structure Modification Strategies

The best way to improve permeability is structural modification. Formulations are not effective in fixing permeability. Thus, it is important to assess permeability early and to build permeability improvement into the synthetic plan from the beginning. This could rescue a chemical series that has great potential and improve drug exposure in animal pharmacology and pharmacokinetic studies.

Several strategies for improving permeability are listed in Table 8.4. These strategies are based on a few fundamental concepts: reduce ionizability, increase lipophilicity, reduce polarity, or reduce hydrogen bond donors or acceptors.

**TABLE 8.4 ► Strategies for Improving Permeability by Structural Modification**

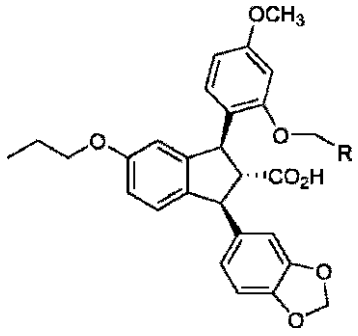
| Structure modification strategy | Section |
|---|---|
| Ionizable group to non-ionizable group | 8.3.1 |
| Add lipophilicity | 8.3.2 |
| Isosteric replacement of polar groups | 8.3.3 |
| Esterify carboxylic acid | 8.3.4 |
| Reduce hydrogen bonding and polarity | 8.3.5 |
| Reduce size | 8.3.6 |
| Add nonpolar side chain | 8.3.7 |
| Prodrug | 8.3.8 |

### 8.3.1 Ionizable Group to Non-ionizable Group

The effect of permeability on absorption after oral administration is shown by the example in Figure 8.6.[4] For the compound where R is $CO_2H$, in vitro Caco-2 permeability is low and *in vivo* oral bioavailability (%F) is low (4%). When R is the less polar and non-ionizable $CH_2OH$ group, in vitro Caco-2 permeability is 30-fold higher and *in vivo* oral bioavailability is much higher (66%).

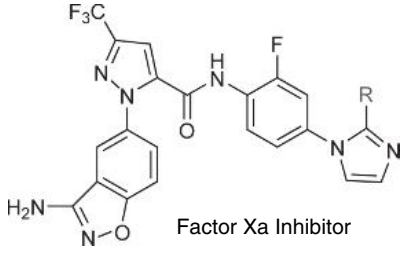
### 8.3.2 Add Lipophilicity

In another example of bioavailability,[5] Figure 8.7 shows that when R is $CH_2NHCH_3$, in vitro Caco-2 permeability is moderate, which is consistent with the moderate 24% in vivo oral bioavailability. When R is the more lipophilic $CH_2N(CH_3)_2$, in vitro permeability is much higher, resulting in a high 84% in vivo oral bioavailability.




| R | ETA, Ki (nM) | Caco-2 (cm/h) | % F (rat) |
|---|---|---|---|
| $CO_2H$ | 0.43 | 0.0075 | 4 |
| $CH_2OH$ | 1.1 | 0.2045 | 66 |

**Figure 8.6** ► Effect of permeability on oral absorption.




Factor Xa Inhibitor

| R | FXa $K_i$ (nM) | Caco-2 $P_{app}$ ($\times 10^{-6}$ cm/s) | CL (L/h/Kg) | $T_{1/2}$ (h) | Vdss (L/Kg) | F (%) |
|---|---|---|---|---|---|---|
| $CH_2NHMe$ | 0.12 | 0.2 | 1.1 | 3.7 | 4.6 | 24 |
| $CH_2NMe_2$ | 0.19 | 5.6 | 1.1 | 3.4 | 5.3 | 84 |

**Figure 8.7** ► Substitution at R of $CH_2N(CH_3)_2$ for $CH_2NHCH_3$ in these factor Xa inhibitors increases Caco-2 permeability and bioavailability. (Reprinted with permission from [14].)

### 8.3.3 Isosteric Replacement of Polar Groups

When a carboxylic acid was replaced with an isosteric tetrazole moiety,[6] Caco-2 permeability increased (Figure 8.8). The tetrazole had the same PTP1B enzymatic activity ($K_i = 2\,\mu M$). The carboxylic acid did not have cellular activity in vitro; however, the tetrazole exhibited positive cellular activity.

### 8.3.4 Esterify Carboxylic Acid

The PTP1B lead in Figure 8.9 was a dicarboxylic acid and was potent and selective in an in vitro enzyme assay.[7] Its activity in a cell-based model was low, which was consistent with the low permeability in the in vitro MDCK cell monolayer permeability assay (see Chapter 26). Synthesis of the diethyl ester prodrug greatly improved permeability and activity in the cell-based assay.

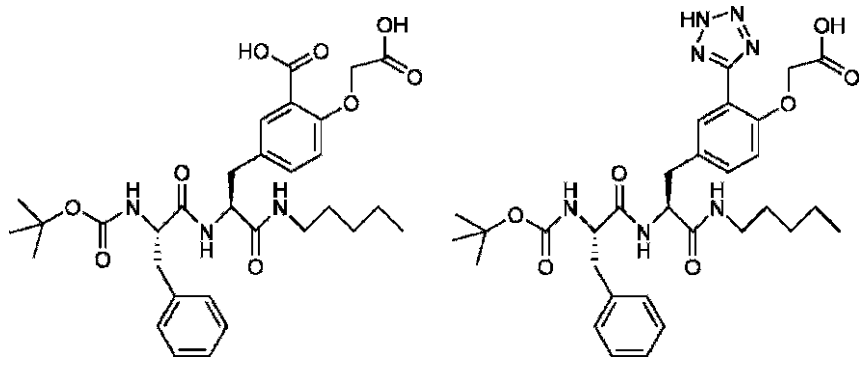
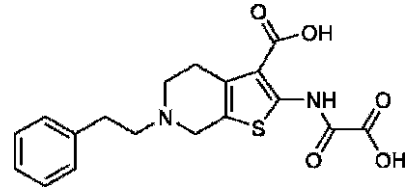



**Figure 8.8** ► Replacement of a carboxylic acid with the bioisosteric tetrazole maintained activity and increased permeability, resulting in cell-based assay activity. (Reprinted with permission from [14].)



| | Diacids | Di-Ethyl Ester Prodrug |
|---|---|---|
| In vitro (PTP1B) | Potent & Selective | |
| Oral Bioavailability (Rat) | 13% | Not Determined |
| Permeability (MDCK) | Low | High |
| 2-DOG Uptake in C2C12 Cell | Inactive | 70% |

**Figure 8.9** ► Effects of permeability on cell-based assay activity for PTP1B lead. (Reprinted with permission from [14].)

### 8.3.5 Reduce Hydrogen Bonding and Polarity

The deleterious effects of hydrogen bonding and polarity on passive diffusion permeability are shown in the series in Figure 8.10. As Cl is modified to F, polarity increases and passive diffusion permeability decreases. As $CH_3$ is modified to $OCH_3$, a hydrogen bond acceptor is added and permeability decreases.

### 8.3.6 Reduce Size

Figures 8.11[8] and 8.12[9] show examples of permeability structure–property relationships. If we examine the cases where one R group is held the same and the other R group is varied, the permeability effects of size and polarity are observed. Increasing size (e.g., methyl, ethyl, butyl, phenyl) reduced Caco-2 permeation or percent of the dose that was absorbed. Increasing polarity (e.g., $CH_3$ to $CF_3$, or $CH_2CH_3$ to $CF_2CF_3$) also reduced permeability. Because all of the compounds have similar activity, the structural analog series allows prioritization of compounds based on their properties that will enhance bioavailability and penetration of permeation barriers on the way to the therapeutic target.

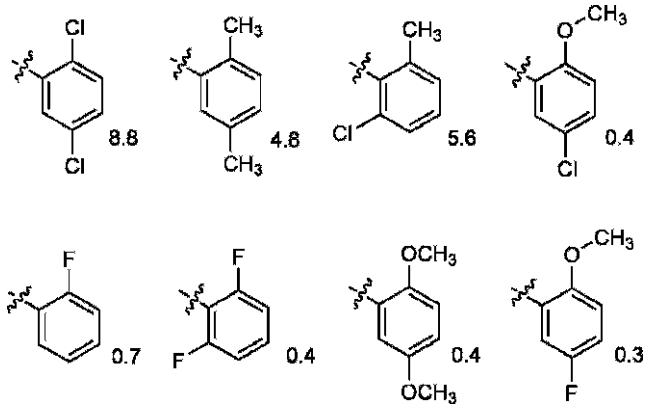
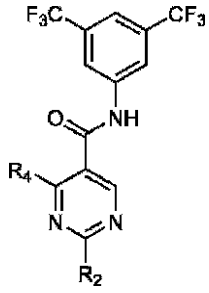
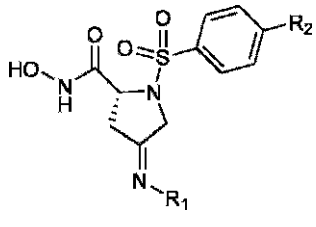



**Figure 8.10** ► Example of the reduction in permeability by passive diffusion as polarity or hydrogen bonding increases. Permeability values are for PAMPA in units of $10^{-6}$ cm/s. (Reprinted with permission from [14].)




| R4 | R2 | Caco-2 Permeability ($\times 10^{-7}$ cm/s, n = 3, mean ± SD) |
|---|---|---|
| $CF_3$ | Cl | 11 ± 4 |
| H | Cl | 61 ± 7 |
| $CH_3$ | Cl | 62 ± 6 |
| $CH_2CH_3$ | Cl | 58 ± 9 |
| $CH_2CH_2CH_3$ | Cl | 31 ± 9 |
| $CF_2CF_3$ | Cl | 9 ± 9 |
| Cl | Cl | 31 ± 6 |
| Ph | Cl | 9 ± 7 |
| $CF_3$ | F | 19 ± 6 |

**Figure 8.11** ► Effects of substitutions on permeability.




| R1 | R2 | % Dose Absorbed (rat ileum) |
|---|---|---|
| OH | OMe | 29–35 |
| OH | O$^n$Bu | 2–5 |
| OMe | O-4-Pyr | 50–68 |
| O$^t$Bu | O-4-Pyr | 10–18 |
| OPh | O-4-Pyr | not detected |
| OMe | OMe | 78–81 |
| OMe | OEt | 23–42 |
| OMe | O$^n$Bu | 28–36 |
| OMe | OPh | 15–18 |

**Figure 8.12** ► Effects of substitutions on permeability.

### 8.3.7 Add Nonpolar Side Chain

Modification of a cyclic peptide to increase permeability is shown in Figure 8.13. By adding the nonpolar side chain, the lipophilicity was increased, resulting in an improvement of permeability.[10]

A series of phenylalanine dipeptides (Figure 8.14) was modified with increasingly lipophilic side chains.[11] This modification resulted in increasing Caco-2 permeability.

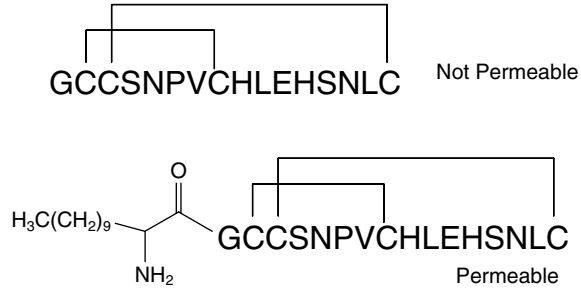



**Figure 8.13** ► Adding the nonpolar side chain to this cyclic peptide increased the lipophilicity and resulted in improved permeability.

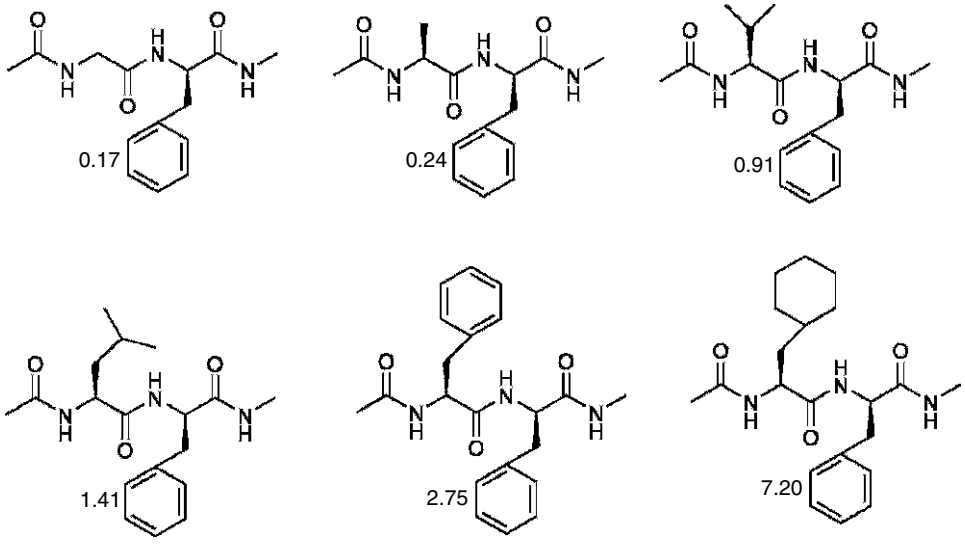



**Figure 8.14** ► For this series of phenylalanine dipeptides, Caco-2 permeability (apical to basolateral, units of $10^{-6}$ cm/s) improved with increasing lipophilicity. (Reprinted with permission from [14].)

### 8.3.8 Prodrug

Prodrugs have been used to increase permeability. Figure 8.15 shows several prodrugs that have been made for permeability purposes.

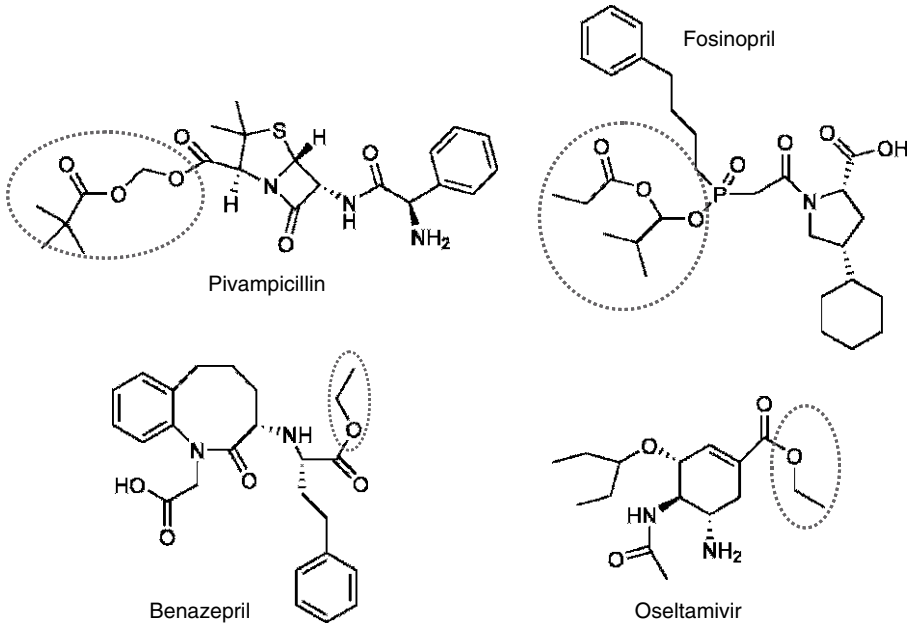
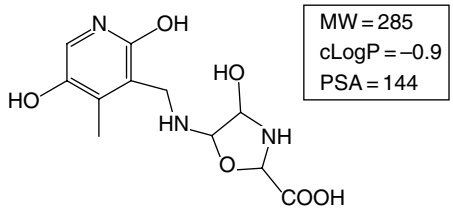



**Figure 8.15** ► Prodrugs with improved passive diffusion permeability. The pro-moiety is circled.

## Problems

(Answers can be found in Appendix I at the end of the book.)

1. What is the predominant permeability mechanism for absorption of most commercial drugs?
2. What are the structural properties of compounds that undergo paracellular permeation?
3. How will passive diffusion permeability change as pH increases from 4.5 to 8 for: (a) basic compound, (b) acidic compound?
4. List important permeability barriers for drug discovery.
5. Which of the following structural modifications likely will improve permeability?: (a) change an amine to a methyl, (b) add a hydroxyl group, (c) remove a propyl group, (d) change a carboxylic acid to an ethyl ester, (e) change a carboxylic acid to a tetrazole.
6. For the following lead compared, what structural modifications could you make that might improve permeability?



7. Permeability is important for which of the following?: (a) absorption in intestine, (b) CYP metabolism, (c) BBB penetration, (d) dissolution in the intestinal lumen, (e) in vitro cell-based assay, (f) to reach intracellular targets in vivo.
8. Following are groups that could be added to a lead compound that is MW 300 and has ClogP 2.0. Rank them from lowest to highest predicted permeability of the product: (a) $–CH_3$, (b) –OH, (c) $–OCH_3$, (d) –COOH.
9. Following are groups that could be added to a lead compound that is MW 450 and has ClogP 4.5. Rank them from lowest to highest predicted permeability of the product: (a) $–C_6H_5$, (b) $–CH_3$, (c) $–C_3H_7$.
10. Following are groups that could be added to a lead compound that is MW 250 and has ClogP 0.0. Rank them from lowest to highest permeability of the product: (a) $–CH_3$, (b) $–C_6H_{11}$, (c) $–C_3H_7$.

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