Journal of Pharmaceutical Sciences

FEBRUARY 1975
VOLUME 64 NUMBER 2

*REVIEW ARTICLE*

# Rationale for Design of Biologically Reversible Drug Derivatives: Prodrugs

**A. A. SINKULA ˣ and S. H. YALKOWSKY**

**Keyphrases** □ Drug derivatives, biologically reversible—rationale for design of prodrugs, physicochemical and biological considerations, applications, review □ Prodrugs—rationale for design, physicochemical and biological considerations, applications, modifications affecting absorption, site direction, depot, taste and odor, and irritation, review □ Reversible drug derivatives—rationale for design for prodrugs, physicochemical and biological considerations, applications, review □ Chemical modification—prodrugs, physicochemical and biological considerations, applications, review □ Latentiated drugs—design of prodrugs, review

CONTENTS

*General Considerations* ..... 183
Physicochemical ..... 183
Absorption ..... 183
Solubility ..... 185
Biological ..... 186
Enzymes ..... 186
Esterases ..... 188
Alkaline Phosphatase ..... 189
Acid Phosphatase ..... 189
Sulfatases ..... 191
*Applications* ..... 191
Absorption ..... 191
Site Direction ..... 192
Depot ..... 198
Organoleptic Properties ..... 200
Taste ..... 200
Mechanism of Taste Receptor Stimulation ..... 200
Odor ..... 202
Taste Studies ..... 202
Irritation ..... 203
Intramuscular or Intravenous Injection ..... 203
GI Disturbances ..... 204
*References* ..... 205

Nearly all therapeutic agents possess various physicochemical and biological properties, some desirable and others undesirable. In general, the pharmaceutical world is concerned with minimizing the number and magnitude of undesirable properties of a drug while retaining the desirable therapeutic activity.

Improvement of drug efficacy can be accomplished by biological, physical, or chemical means. The biological approach entails varying the route of administration. Examples include the injectable route to optimize onset of action, maximize bioavailability (enhanced blood levels), and eliminate gastric irritation and acid-catalyzed drug degradation. Versatility is severely limited when utilizing the biological approach, because alternative routes of administration are frequently unavailable and are always less convenient than oral administration.

A greater degree of flexibility of drug modification is offered by the physical approach, commonly referred to as dosage form design. The elements and philosophy of this approach were discussed by Schroeter (1) and others (2–8). The highest degree of flexibility in altering drug efficacy, however, is offered by the chemical approach.

Drug derivatization has been long recognized as an important means of producing better pharmaceuti-

cals. Bayer, as far back as 1899, synthesized the drug aspirin in an attempt to improve the therapeutic activity of salicylic acid. Since that time, literally thousands of drug derivatives have been synthesized and tested. These drug derivatives can be broadly classified into two categories: irreversible or reversible. Irreversible derivatives or analogs are usually synthesized for the purpose of finding a similar, new, biologically active entity possessing increased potency, a broader spectrum of activity, or some other desirable property not possessed by the parent compound. A reversible drug derivative utilizes a chemical moiety of proven biological activity (the parent molecule) and seeks to deliver it to the site of action while overcoming some inherent drawback to the use of the parent compound.

In the case of the analog, precautions must be taken as to what functional group can be modifed since indiscriminate modification may destroy all bioactivity. The reversible derivative can be modified at any functionality without undue concern for its involvement at the receptor level since it is reversible by definition. These facts eliminate the need to determine the bioactive center(s) in the molecule and offer the chemist a greater number of chemical sites at which to modify.

In general, three approaches are followed in the search for new drug agents: *(a)* the general screening approach in which chemical substances from any source are tested for their effect against a predetermined disease or disease state, *(b)* the chemical modification of existing drug substances whose biological effects are known, and *(c)* mimicking nature by biochemical design, where a compound is made to exert an action in a manner similar to a known biochemical substance (9). Any lead compounds obtained from these three approaches are usually further modified chemically to gain the biologically most potent representatives of the series.

This review will focus on approach *(b)*, the chemical modification of existing drug substances whose biological effects are known. It will be limited solely to biologically reversible derivatives, *i.e.*, those compounds that, upon introduction to the appropriate biological system, revert back to the parent molecule by virtue of enzymatic and/or chemical lability. To provide an interpretive review of the area, references from the journal and patent literature will be selected to illustrate the various principles discussed. The discussion will of necessity not be comprehensive but will nonetheless cover the significant aspects of the discipline.

Reversible derivatives (10–14) have also been termed prodrugs (15–22) and latentiated drugs (23–25) and have been designed to eliminate a variety of undesirable properties such as bitterness, odor, gastric upset, and poor absorption. Many comprehensive reviews of both reversible and irreversible drug derivatives (23–32) have appeared recently.

Harper (25) must be credited with coining the term "drug latentiation," and he defined it as "the chemical modification of a biologically active compound to form a new compound, which upon *in vivo* enzymatic attack will liberate the parent compound." Kupchan *et al.* (33) extended this definition operationally by including nonenzymatic processes as well for regeneration of the parent compound. By inference, latentiation implies a time lag element or time component involved in regenerating the bioactive parent molecule *in vivo*. Since most latentiated drug substances *per se* are biologically inactive, this concept is important for those drugs that are metabolized or excreted too rapidly to provide adequate clinical efficacy.

Albert (34, 35), in discussing the selective toxicity of drug molecules, elucidated the proagent or prodrug concept. The term prodrug is general in that it includes latentiated drug derivatives as well as substances that are converted after administration to the actual substance that combines with receptors. These actual substances may be active metabolites of the parent molecule. Harper (24), on the other hand, discussed the concept of structural formulation and defined it as "the modification of a biologically active compound at a point not essential for binding to an active site in the biological receptor, so that although the desired biological effect is retained, the resulting changes in physicochemical properties cause alteration in the absorption, distribution or metabolism of the drug; the parent compound, however, is not liberated in the body." Structural formulation differs from irreversible derivative formation in that the former retains bioactivity *in vivo* whereas the latter may or may not do so. The distinction is subtle but may have profound ramifications in the rational approach to the synthesis of bioactive agents.

Any number of inherent disadvantages may preclude the use of the parent drug molecule in clinical practice. Among those properties considered disadvantageous in a drug molecule are bitterness or tartness, offensive odor, gastric or intestinal upset and irritation, pain on injection, lack of absorption, slow or rapid metabolism, and lack of stability in the bulk state, the dosage form, or *in vivo* (*i.e.*, gastric instability).

In many cases, undesirable properties in a drug molecule cannot be overcome by conventional pharmaceutical formulation or route of administration changes, so the method of choice becomes reversible derivative formation. In the intelligent design of reversible drug derivatives, it is necessary to consider two questions:

1. What structural modification(s) of the parent molecule are necessary to reduce or eliminate the particular undesirable effect?
2. What conditions are available *in vivo* (enzymes, pH, *etc.*) to regenerate the parent molecule from the derivative?

The first question requires an extensive knowledge of structure–activity relationships as they apply to elimination of these undesirable properties. The second question is dependent on a rather sophisticated knowledge of biology. Complete answers to these two questions are obviously not yet available to the medicinal chemist. A limited body of knowledge is available, however, and this knowledge, if used judiciously, can form a basis for the rational design of revers-

ible drug derivatives. The remainder of this review will consider specific undesirable drug properties and possible means of eliminating these properties by using reversible drug derivatives. Examples of drugs whose properties have been successfully modified are provided in the tables.

## GENERAL CONSIDERATIONS

**Physicochemical**—*Absorption*—Probably the most fruitful area of reversible derivatization is the improvement of passive drug absorption through epithelial tissue. Many studies involving *in vivo, in situ,* and *in vitro* systems have been conducted to elucidate the role of chemical structure in drug absorption. The similarities and differences among some more widely accepted theories also will be discussed here.

Nearly all of the empiricisms and theories in current use agree that the addition of a hydrophobic group to a compound usually increases its absorption. It is also agreed that this increase in absorption is a direct consequence of the increase in the biological lipid–water partition coefficient resulting from the added hydrophobic moiety. Although there is a lack of agreement as to what, if any, *in vitro* partitioning system best mimics the biological situation, octanol–water apparently is the most useful for correlative purposes.

From both the physicochemical and the biological point of view, octanol–water is the most extensively studied system. Leo *et al.* (36) tabulated and critically evaluated thousands of octanol–water partition coefficients and developed a system of rules for estimating values for compounds that have not yet been studied.

It is important to realize that the theoretical and empirical relationships to be discussed are equally applicable to reversible derivatives as well as nonreversible derivatives (analogs), because all of these relationships rely upon partitioning and because the presence of a reversible linkage does not normally alter the relative partition coefficients of the members of a series. For example, in the octanol–water system, the ratio of the partition coefficients of ethyl and butyl benzoates is the same as the ratio for *p*-ethyl- and *p*-butylbenzoic acids, even though the esters are reversible while the acids are not. Another consequence of the dependence of absorbability on partitioning is the fact that the unionized form of a dissociable molecule is absorbed more efficiently than its ionic species. The quantitative relationships between pH, pK, and partition coefficient are well known (37, 38) and will not be discussed here.

To get a clear picture of the role of hydrophobicity in drug transport, it is necessary to cover a wide range of partition coefficients. The most convenient and economical way of accomplishing this task is to construct a homologous series. The partition coefficient of the *n*th number of any homologous series, $PC_n$, in any solvent system can be described by:

$$\log(PC_n) = \log(PC_0) + \pi^s n \qquad \text{(Eq. 1)}$$

**Table I**[a]—Values of $\pi_{CH_2}$ for Some Common Solvents and for Red Blood Cell Ghosts

| Solvent | $\pi_{CH_2}$ |
|---|---|
| Ether | 0.573 |
| Ether | 0.612 |
| Octanol | 0.500 |
| Chloroform | 0.609 |
| Olive oil | 0.525 |
| Castor oil | 0.545 |
| Red blood cell ghosts | 0.526 |

[a] Adapted, with permission, from Ref. 46.

where $PC_0$ is a constant dependent on both the series and the solvent system, and $\pi^s$ is a constant dependent only on the solvent system. According to the notation of Leo *et al.* (36), $\pi$ can be an incremental constant for any substituent; but when dealing primarily with homologous series, $\pi$ will designate $\pi_{CH_2}$ unless otherwise specified.

The values of $\pi$ ($\pi_{CH_2}$) for several organic solvents and red blood cell ghosts against water are listed in Table I. From the octanol–water $\pi$ value of 0.5, it can be seen that only seven consecutive homologs are needed to cover a 1000-fold range of partition coefficients in half-log increments. Spanning this broad range of values minimizes the effects of random error and normal biological variation. Homologous series are particularly well suited for studying transport because the alkyl group usually does not interfere with the interaction of the active portion of the molecule and the receptor site. Therefore, it is frequently possible to measure the relative biological activities of homologs and then equate these values to relative transport rates.

The earliest structure–activity or structure–transport workers all recognized the parallelism between the logarithms of the biological response *(BR)* and the lipid–water partition coefficient *(PC)*. Equations of the form:

$$\log(BR) = a + b\log(PC) \qquad \text{(Eq. 2)}$$

were proposed (39–44). For the simple case of a homologous series, Eqs. 1 and 2 are combined giving:

$$\log(BR) = b\pi^s + b\log(PC_0) + a \qquad \text{(Eq. 3)}$$

which describes the so-called linear structure–activity relationship.

The value of $a$ in Eq. 2 or 3 is a measure of the degree of similarity of the *in vitro* and the true *in vivo* partitioning systems. If $a$ is equal to unity, the systems are equivalent in their relative affinities for a methylene group. (It is possible for $\pi_{CH_2}$ to be the same for a pair of systems and, at the same time, for other substituent constants such as $\pi_{OH}$ to be quite different. For specific examples, see Ref. 36.) When considering a homologous series, it is not necessary to use any reference *in vitro* partitioning system. Flynn and Yalkowsky (45) showed that $b\pi^s$ is equal to $\pi^B$, the $\pi$ value in the biological system. Thus, Eq. 3 becomes:

$$\log(BR) = A + \pi^B n \qquad \text{(Eq. 4)}$$

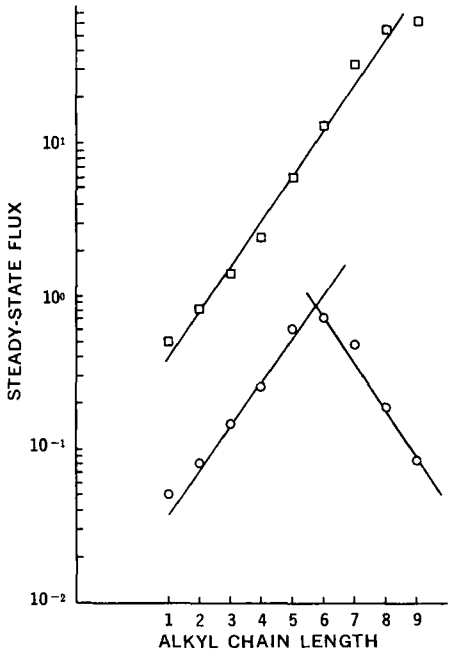


**Figure 1**—*Data of Scheuplein and Blank (48) for permeation of normal aliphatic alcohols across human stratum corneum* in vitro. *Key: □, concentration-normalized flux; and ○, flux from 0.10* M *solution (last four points represent saturated solutions). (Adapted, with permission, from Ref. 46.)*

where $A$ is equal to the constant portion of Eq. 3 and is, therefore, dependent upon both the biological system and the homologous series.

Yalkowsky and Flynn (46) recently evaluated a large number of linear chain-length–activity relationships which are dependent upon passive transport. They observed, as expected, that $\pi^B$ for a particular biological system is essentially independent of the series under study. They found that the value of $\pi^B$ for most simple organisms (bacteria, fungus, erythrocytes, *etc.*) is $0.46 \pm 0.03$; *i.e.*, on the average, there is a 2.9-fold increase in activity for each additional methylene group. It was also observed that the value of $\pi^B$ for epithelial and GI tissue of higher animals is around 0.25, which corresponds to an incremental constant for each methylene group of about 1.8 for the change in activity (or transport) with chain length. This low value is in agreement with the fact that these tissues contain a significant fraction of polar components.

The linear increase in the logarithm of the transport rate with chain length is expected from basic permeability theory. The flux, $F$, or transport rate of a substrate across a rate-determining lipid phase or membrane separating two aqueous phases at a concentration differential of $\Delta C$ is:

$$F = \frac{PC}{R}\Delta C \qquad \text{(Eq. 5)}$$

where $PC$ is the lipid–water partition coefficient of the substrate, and $R$ is the resistance of the membrane to its diffusion. Collander (47), using chara cells, was among the first to show the relationship between permeability and partitioning in a biological system. More recently, excellent correlation was shown (48) between the permeability of nine aliphatic alcohols across excised human stratum corneum and their stratum corneum–water partition coefficients. From these data (Fig. 1), it can be seen that there is a great increase in permeability between methanol and nonanol.

The exponential increase in transport rate and thus activity with extention of the alkyl group cannot go on indefinitely. If experiments are carried to high enough chain lengths, there is a leveling off or plateauing of the curve and ultimately a decrease in activity with increasing hydrophobicity. While there is reasonably good agreement in the literature about the ascending portion of the structure–activity curve, there is a great deal of controversy about the reason for and even the shape of the apical or descending portions of the curve.

Hansch (49) described the overall shape of the structure–activity curve by a parabolic equation of the form:

$$\log(BR) = a + b\log(PC) + c\log^2(PC) \qquad \text{(Eq. 6)}$$

He showed that Eq. 6 gives better statistical fit to many sets of data than the linear Eq. 2. The improved correlation is undoubtedly at least partially due to the greater degree of flexibility produced by the additional variable $c$; but since $c$ is always negative, it cannot be regarded as simply another adjustable parameter. One difficulty that arises with the parabolic equation is that the values of the coefficients, $a$ and $b$ in Eqs. 2 and 6, have no relationship to one another and parabolic equations having the same ascending slopes can appear quite different.

Since Hansch's parabolic relationship is based upon a countercurrent distribution-type model (49), the curve is nowhere linear and is symmetrical about an optimum value of log $(PC)$. The nonlinearity of the parabola makes it difficult to reconcile with the linear case described by Eq. 2. Furthermore, the symmetry is not consistent with the many reasons given by Hansch and Clayton (50) to explain the decrease in activity with chain length. Nevertheless, in spite of the theoretical shortcomings, the Hansch parabolic relationship can be extremely useful in the empirical analysis of structure–activity data and in the prediction of optimum partition coefficients for biological activity. From a practical standpoint, it is frequently more useful than the more theoretically valid relationships that will be discussed.

Wagner and Sedman (51) recently analyzed much of Hansch's data and found that a statistically better fit is obtained with an equation of the form:

$$(BR) = \frac{1}{a + (b/PC)} \qquad \text{(Eq. 7)}$$

Others also showed good fit of biological activity data and transport data to equations similar to Eq. 7 (46, 52, 53). One of the earliest uses of this equation was by Zwolinski *et al.* (54) who studied the permeability of various plant cells to the members of several homologous series. They also showed that double-recip-

rocal plots [$(BR)^{-1}$ *versus* $(PC)^{-1}$] are linear and can be used to evaluate the constants $a$ and $b$ conveniently. Another important feature of Eq. 7 is that it is applicable to both linear and nonlinear data.

The mathematical form of Eq. 7 can be obtained directly from Eq. 5 by the incorporation of an additional resistance in series with that of the membrane. Equation 5 then becomes:

$$BR \propto F = \frac{\Delta C}{(R_m/PC) + R_{aq}} \qquad \text{(Eq. 8)}$$

This added resistance, $R_{aq}$, results from the unstirred aqueous layers adjacent to the membrane which must be traversed by any solute passing through the membrane. For a more complete description of the derivation of Eq. 8 and the importance of unstirred layers (or diffusion layers as they are often called), the reader is referred to Refs. 45 and 46. For an alternative treatment based on extraction theory leading to another equation of the same form as Eq. 7, see Ref. 51.

For the study of a homologous series, it is convenient to combine Eq. 1 with the logarithmic form of Eq. 8 (or 7) to get:

$$\log(BR) = \log(\Delta C) - \log(PC_0) - \pi n - \log(R_m + R_{aq}PC_0 10^{\pi n}) \qquad \text{(Eq. 9)}$$

which is shown schematically in Fig. 2. It can be seen that, according to Eq. 9, there is a linear increase in log $BR$ with chain length but that at some point [when $R_{aq} \geq (R_m/PC)$] the curve levels off and approaches a limiting value. These equations can satisfactorily describe most structure–activity data, but they do not explain the descending portion of the curve.

Because of the wide variety of reasons for a decline in activity with increasing chain length, no single equation can explain all available data. Hansch and Clayton (50) and Yalkowsky and Flynn (46) listed about a dozen possible reasons for this decline. These reasons can be broadly classified into those that are dependent upon a biological parameter (*e.g.*, enzyme specificity, conformational distortion of the active site, metabolism, and poisoning of enzymes) and those that are related to some physical property (*e.g.*, solubility, complex formation, micelle formation, partitioning into inert phases, and binding to inert surfaces). The former are the most difficult to correlate by simple theories but can be handled quite satisfactorily by equations such as Eq. 6. The latter all have one important feature in common; they can generally be described mathematically by:

$$\log(P_n) = \log(P_0) + \alpha n \qquad \text{(Eq. 10)}$$

where $P_n$ is the value of any property of the $n$th homolog of the series, $P_0$ is a reference value, and $\alpha$ is a well-defined constant[1].

[1] Occasionally, it is necessary to use a higher order polynomial of $n$ to describe $P_n$. This would alter the subsequent mathematical treatment slightly but not the general conclusions (see Ref. 52).

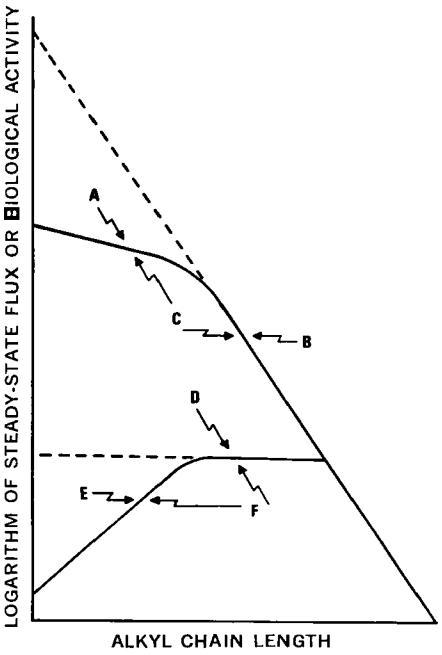


**Figure 2**—*Hypothetical chain-length activity relationships. (Adapted, with permission, from Ref. 52.)*

Yalkowsky *et al.* (46) theoretically characterized the effects of behavior on the transport rate by using solubility as an example. Based on literature data for nearly 20 homologous series in water, they found[1]:

$$\log(S_n) = \log(S_0) - \delta n \qquad \text{(Eq. 11)}$$

where $S_n$ is the solubility of the $n$th member of the series, and $S_0$ is a constant.

The effect of solubility on transport rate is to limit the attainable concentration differential so that the maximum value of Eq. 8 becomes:

$$F = \frac{S}{(R_m/PC) + R_{aq}} = \frac{(S_0 10^{-\delta n}/R_m)}{PC_0 10^{\pi n} + R_{aq}} \qquad \text{(Eq. 12)}$$

which, in logarithmic form, is:

$$\log(F) = \log(S_0 PC_0) + (\pi - \delta)n - \log(R_m + R_{aq}PC_0 10^{\pi n}) \qquad \text{(Eq. 13)}$$

These equations can now describe a "parabolic" structure–activity curve on the basis of transport-limited activity and basic physical–chemical relationships. Figure 2 shows the expected dependence of transport across a biological barrier (and activity dependent thereupon) for the members of a homologous series predicted by Eqs. 9 and 13. The scales are arbitrary but show that the break occurs at the same chain length for the equimolar and saturated cases. Figure 3 shows an experimental verification of Eqs. 9 and 13. These data were obtained from turnover time experiments with goldfish (52). The agreement between experimental and theoretical data, while not necessarily proving the theory, gives a positive indication of its utility.

*Solubility*—The primary role of solubility in determining drug absorption is obvious since only the

DOCKET ALARM

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

**LAW FIRMS**
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

**FINANCIAL INSTITUTIONS**
Litigation and bankruptcy checks for companies and debtors.

**E-DISCOVERY AND LEGAL VENDORS**
Sync your system to PACER to automate legal marketing.