# Predicting Oral Absorption of Drugs: A Case Study with a Novel Class of Antimicrobial Agents

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***Purpose.*** The purpose of this work was to evaluate an oral absorption prediction model, maximum absorbable dose (MAD), which predicts a theoretical dose of drug that could be absorbed across rat intestine based on consideration of intestinal permeability, solute solubility, intestinal volume, and residence time.

***Methods.*** In the present study, Caco-2 cell permeability, as a surrogate for rat intestinal permeability, and aqueous solubility were measured for 27 oxazolidinones. The oxazolidinones are a novel class of potential antibacterial agents currently under investigation. These values were used to estimate MAD for each of the compounds. Finally, these predicted values were compared to previously measured bioavailability data in the rat in order to estimate oral absorption properties.

***Results.*** A reasonably good correlation between predicted dose absorbed and bioavailability was observed for most of the compounds. In a few cases involving relatively insoluble compounds, absorption was underestimated. For these compounds while aqueous solubility was low, solubility in 5% polysorbate 80 was significantly higher, a solvent possibly more representative of the small intestinal lumen.

***Conclusions.*** These results suggest that MAD may be useful for prioritizing early discovery candidates with respect to oral absorption potential. In the case of compounds with poor aqueous solubility, additional factors may have to be considered such as solubility in the intestinal lumen.

**KEY WORDS:** prediction; *in vitro-in vivo*; oral absorption; Caco-2 cell; solubility; biopharmaceutics.

## INTRODUCTION

Oral bioavailability of a drug is determined by a number of properties, including solubility, intestinal permeability, and presystemic metabolism. Recent reviews have pointed out the overall importance of these properties in contributing to the failure of candidate drugs in clinical trials (1,2). This in turn has led to a number of proposals to measure or estimate these properties early in the drug discovery process to bias the selection of compounds for more serious consideration toward those with a more favorable distribution of these attributes. Among these are the so-called "rule of five" developed by Lipinski *et al.* (3), and implementation of medium to high-throughput *in vitro* methods for measuring solubility, permeability, and metabolic stability (4–6).

Although such properties as permeability, solubility, metabolic stability, and toxicity may be important individually, it is the interrelationship of these properties that determines the *in vivo* performance of a drug. In particular, the role of solubility is dependent upon potency, which will determine dose. That is, although low micromolar solubility may be problematic for a high-dose compound, it may be perfectly acceptable for a low dose drug. To accommodate these situations, a simple model was proposed for relating aqueous solubility and intestinal permeability to absorption where the dose is not known. This model was introduced by Johnson and Swindell and is referred to as maximum absorbable dose (MAD; Ref. 7). The model assumes a highly idealized situation for absorption: the intestine is exposed to a saturated solution of the compound of interest for a time equal to the normal small intestinal transit time (8). Dissolution of the solute is allowed to proceed at a rate equal to the absorption rate, in order to maintain saturation. Under these conditions, a total mass of solute, MAD, is calculated. These values can then be used to rank order the absorption potential of compounds without knowing the expected clinical dose.

In the present study, MAD was evaluated for potential utility in predicting the oral absorption potential for oxazolidinones. The oxazolidinones are novel, synthetic antimicrobial agents that show good activity against a variety of gram-positive and -negative organisms (9,10). To validate the performance of the models, aqueous solubilities and Caco-2 cell permeabilities were used to classify the compounds and calculate MAD for a series of oxazolidinones for which bioavailability in the rat had previously been obtained.

## METHODS

Samples of the oxazolidinones were provided by Pharmacia Research Compound Collection. Structures of the compounds used in this study are shown in Fig. 1.

### General Procedure for Determination of Clearance and Oral Bioavailability of Oxazolidinones in Rats

Solutions of the test compound for intravenous administration were prepared at a concentration of 10 mg/mL in aqueous 25% hydroxypropyl-β-cyclodextrin adjusted to pH 4.9. Formulations for oral administration were either solutions or suspensions prepared to give a concentration of 4 mg/mL in 50 mM acetate buffer containing 10 mg/mL Avicel® (FMC BioPolymer, Philadelphia, PA, USA) and 50 mg/mL polysorbate 80, pH 4.5. On the day of the study, animals were weighed and doses calculated. The dosing syringe was also weighed before and after administration to determine the exact quantity of compound administered. Intravenous doses (10 mg/kg) were administered via a cannula previously placed in the superior vena cava. Oral doses (20 mg/kg) were administered by gastric intubation using an appropriately sized syringe and stainless steel intubation needle. Two animals were used for each dosing regimen. After administration, 0.25 mL of blood was drawn and transferred into appropriately sized

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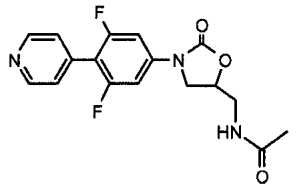
PNU-93936A



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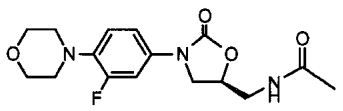

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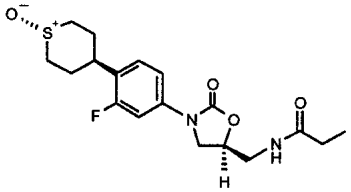

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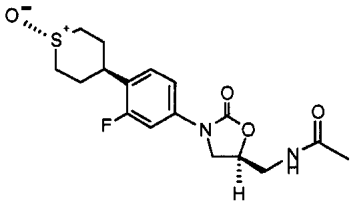

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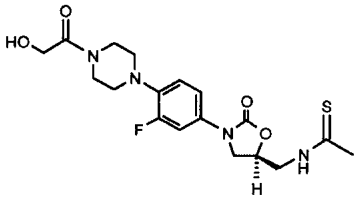

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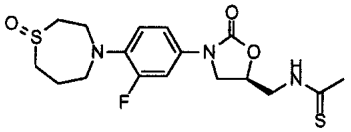

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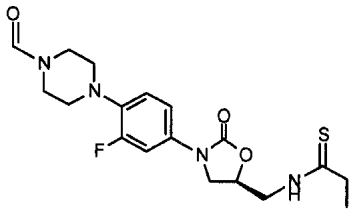

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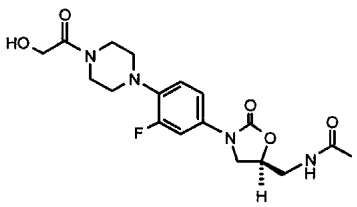

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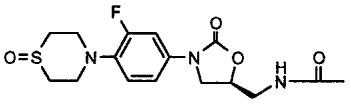

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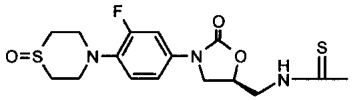

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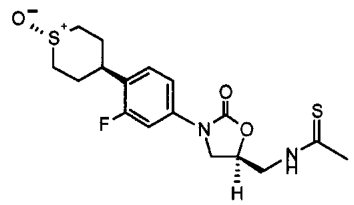

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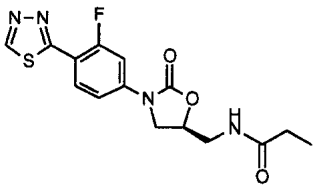

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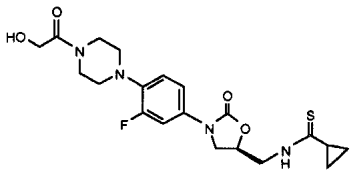

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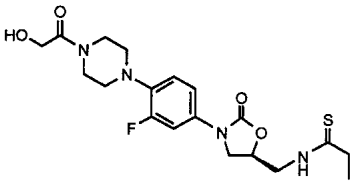

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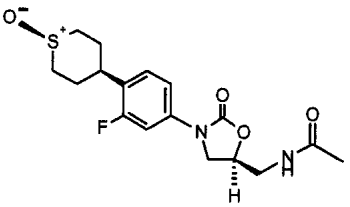

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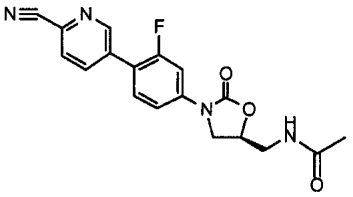

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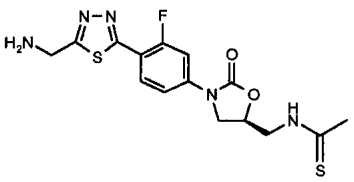

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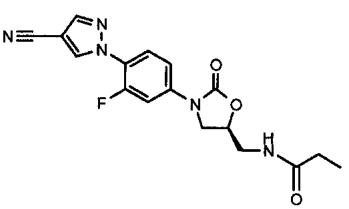

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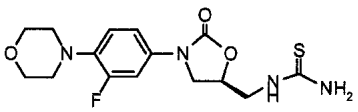

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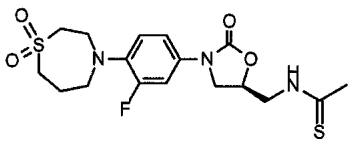

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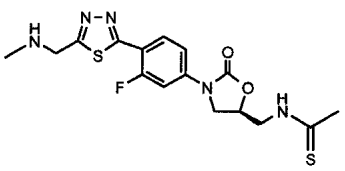

**Fig. 1.** Structures of oxazolidinones used in this study.

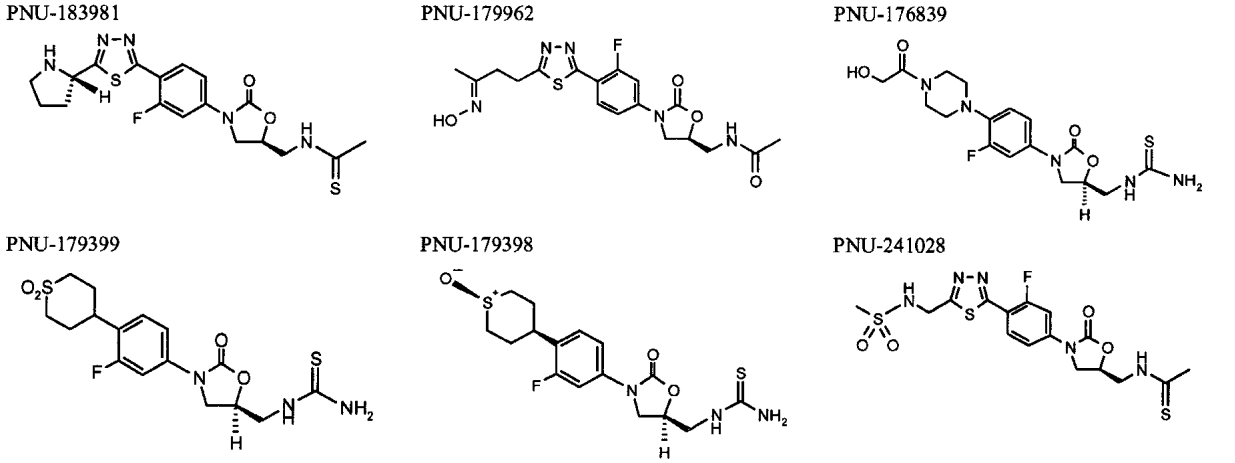


**Fig. 1.** Continued.

EDTA polypropylene centrifuge tubes for collection of plasma. Collection times were 0 (predose), 2 min, 30 min, 1, 2, 4, 6, 8, 12, and 24 h after iv administration; and 0, 30 min, 1, 2, 4, 6, 8, 12, and 24 h for the oral dose. After centrifugation, plasma samples were analyzed by high-performance liquid chromatography (HPLC) optimized for each individual compound.

### Solubility Determinations

Aqueous solubilities were measured in 50 mM phosphate buffer, pH 7, after equilibration with excess drug for 24 h. Typically, sufficient drug was added to 0.5 mL of buffer to achieve a slurry. The slurry was stirred vigorously for 24 h at room temperature, then filtered using 0.22 (μm Millipore Ultrafree-MC centrifuge units (Millipore Corporation, Bedford, MA). Aliquots of each filtrate were diluted appropriately and assayed by reversed-phase HPLC, using a YMC ODS-AM C18 column (4.6 × 150 mm) (Capital HPLC Limited, United Kingdom) at ambient temperature and 1 ml/min flow rate. A linear gradient ranging from 10-72% acetonitrile with 0.1% trifluoroacetic acid over 16 min was used for elution of the oxazolidinone analogs. UV detection was in the range of 240–310 nm, depending on the absorption maximum of the analog.

### Transport Experiments

Caco-2 cells were cultured as previously described (11). Monolayers used in this study were between passages 28 to 37 and were 2 to 3 weeks old. Just before the transport experiment, the monolayers were washed 3 times with phosphate-buffered saline, pH 7.40 that was supplemented with 15 mM HEPES and 1 g/L D-glucose (PBS). All transport studies were conducted in PBS, pH 7.40, in a 37°C incubator. A stock solution (50 mL) of each compound was targeted at 50 μM in PBS (pH 7.40). However, this concentration could not be obtained for all compounds because of low solubilities. After adding a known quantity of drug to 50 mL of PBS, the solutions were sonicated, vortexed, and filtered using 0.2-μm Gelman 25-mm Acrodisc® syringe filters (Pall Co, Ann Arbor, MI). The solutions were assayed after being diluted with buffer to within the range of standards and assayed by HPLC, initially and after several hours. Permeability was measured in the apical to basolateral (absorption) and basolateral to apical (excretion) direction. To maintain sink conditions during the absorption studies, the Transwell® cup (Corning Costar Corporation; Bridgeport, NJ) was transferred to a fresh receiver after each time point. In the case of measuring permeability in the opposite direction, the buffer was removed from the apical chamber and replaced with fresh PBS after each time point. The effective permeability coefficient (Pe) was determined from the appearance data using the following equation:

$$P_e = \frac{V_D}{A M_D(0)} \left( \frac{\Delta M_R(t)}{\Delta t} \right) \quad (1)$$

where $V_D$ = volume of the donor (1.5 mL for Ap→Bl flux and 2.5 mL for Bl→Ap flux), A = surface area of the filter on which the monolayer was grown (4.71 $cm^2$), $M_D(0)$ = mass in the donor at time zero, $M_R(t)$ = mass in the receiver at time $t$, and $t$ = time in seconds. Mass balance was calculated at the end of the experiment from total mass of solute in the basolateral chamber plus solute in the apical chamber, calculated as a percentage of the initial mass of solute in the donor.

### Calculation of MAD

MAD calculates the total mass of drug which could presumably be absorbed if a saturated solution of the solute with solubility $S$ in the small intestinal volume, $SIV$, were absorbed with a rate constant $K_a$ for a time $SITT$ equivalent to the small intestinal transit time:

$$\text{MAD} = S \times K_a \; x \; SIV \times SITT \quad (2)$$

For the calculation of MAD in the rat, the intestinal absorption rate constant was derived from Caco-2 cell permeability values with the assumption that Caco-2 cell permeability values and rat ileum permeability values are approximately equal. This is based on earlier work comparing the permeability behavior of the two models for a number of different model solutes (12). The estimated rat permeability number was converted to an absorption rate constant, Ka, from the relationship:

$$K_a = P_e \times (A/V) \quad (3)$$

where $P_e$ is the measured Caco-2 cell monolayer value in the apical to basolateral direction, $V$ is the volume of the intestinal lumen, and $A$ is the surface area. For our rat intestinal permeability protocol, this $(A/V)$ ratio is 10 $cm^{-1}$ [Day *et al.*,

unpublished observations]. The small intestinal volume used in the MAD calculation was assumed to be 20 mL and the residence time was defined as 270 min. This value is significantly different from the 90 min small intestinal transit time in the rat. In several of the plasma level time profiles after oral dosing we saw evidence for continuing absorption up to 4–5 h. Therefore, for the purposes of comparing predicted to experimental results we assumed that absorption could take place over a 4.5-h time interval.

## RESULTS AND DISCUSSION

Table I summarizes the systemic clearance and oral bioavailability data for oxazolidinones in the rat. In general, the clearances ranged from low to moderate. Thus, to a first approximation, we assume that the oral bioavailability is a reasonable measure of the fraction of the administered dose absorbed (Fa). This assumption was supported by additional studies in which several of the compounds (PNU-100592, PNU-100766, PNU-177553) were administered in radiolabeled form. The clearances of these compounds ranged from 15-25 ml/min/kg. In all cases, bioavailability of radiolabel was similar to bioavailability of the intact compound (data not shown). Further, as shown in Fig. 2, a poor relationship of bioavailability with clearance is observed except for the few compounds with clearances greater than 30 ml/min/kg, or about half hepatic blood flow. However, despite the poor correlation for the more slowly cleared solutes ($r^2 = 0.24$) the trend was marginally significant ($p = 0.02$). Therefore, for the purposes of estimating absorption for these compounds, the bioavailabilities should be considered lower limits of Fa as clearance does seem to play a role, despite the modest values observed.

**Table I.** Bioavailability and Clearance Results from Oral Dosing of Oxazolidinones in the Rat

| Compound | F (%) | Cl (mL/min/kg) |
|---|---|---|
| PNU-93936A | 100 | – |
| PNU-100766 | 100 | 15 |
| PNU-179954 | 55 ± 11 | 15.5 ± 2.5 |
| PNU-177780 | 59 ± 9 | 14 ± 2 |
| PNU-173995 | 64 ± 14 | 20 ± 1 |
| PNU-184435 | 34 ± 5 | 30 ± 4.5 |
| PNU-184249 | 79 ± 32 | 9 ± 2 |
| PNU-100592 | 56 | 25 |
| PNU-101603 | 58 ± 9 | 24 ± 3 |
| PNU-177553 | 55 ± 6 | 15 ± 2 |
| PNU-179759 | 68 ± 3 | 18 ± 0.7 |
| PNU-179450 | 127 ± 7 | 0.6 |
| PNU-184148 | 51 ± 42 | 8 ± 2 |
| PNU-184147 | 33 ± 2 | 9 ± 0.5 |
| PNU-176723 | 55 ± 22 | 21 ± 6 |
| PNU-184421 | 77 ± 4 | 1 ± 0.1 |
| PNU-182347 | 14 ± 4 | 34 ± 2.5 |
| PNU-179397 | 85 ± 10 | 1 ± 0.04 |
| PNU-173335 | 39 ± 12 | 31 ± 5 |
| PNU-184470 | 4 ± 1.3 | 39 ± 5 |
| PNU-182945 | 35 ± 6 | 23 ± 4 |
| PNU-183981 | 52 ± 15 | 5.6 ± 0.2 |
| PNU-179962 | 2 ± 0.2 | 22 ± 3 |
| PNU-176839 | 3 ± 0.4 | 22 ± 3 |
| PNU-179399 | 1 ± 0.3 | 23 ± 5 |
| PNU-179398 | 1.4 ± 0.3 | 13 ± 3 |
| PNU-241028 | 0.2 ± 0.05 | 12 ± 2 |

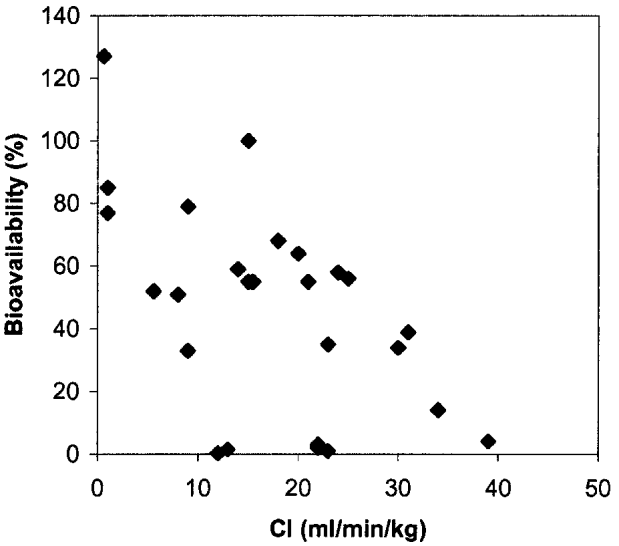


**Fig. 2.** Relationship of oral bioavailability in the rat with systemic clearance for oxazolidinones. In general, a poor correlation is seen except for the more rapidly cleared compounds.

Table II summarizes the Caco-2 cell permeability values. In many cases, secretory permeability is significantly greater than that in the absorptive direction. This is presumed to be due to the presence of p-glycoprotein (P-gp), or other secretory transporters, in these cells (13,14) for which these antibiotic agents may be substrates. In the situation where significant efflux transporter activity is present for a given compound, absorptive permeability can be concentration dependent and, in principle, impact the absorption of the compound. This can present a problem in trying to predict absorption characteristics if permeability *in vivo* changes as a function of concentration in the intestinal lumen. As shown in Fig. 3, the magnitude of secretory permeability to these solutes depends upon the magnitude of the absorptive permeability. For highly permeable solutes, little or no polarization is seen. However, for the more modestly permeable compounds, polarization can be significant, suggesting that the *in vivo* absorption characteristics of these compounds may be concentration dependent, or similarly problematic due to this non-ideal permeability behavior. To better understand this polarization behavior we examined the concentration dependence of PNU-100592 on absorptive and secretory permeability. As shown in Fig. 4, when the donor concentration was increased from 50 to 3000 μM secretory permeability decreased significantly without a significant change in absorptive permeability. At 3000 μM concentration, permeability in the two directions are about the same and equal to that in the absorptive direction at much lower concentrations. Similar results were found when the bi-directional permeability of PNU-176839 was examined in the presence or absence of 5 μM cyclosporin, a potent inhibitor of P-gp in Caco-2 cells (15), suggesting that P-gp is a major contributor to secretion of these compounds. In this case, absorptive permeability was unchanged in the presence of cyclosporin, while secretory permeability decreased from $12.4 \times 10^{-6}$ cm/s (Table II), to $1.9 \times 10^{-6}$ cm/s with cyclosporin. These results suggest that the polarization of oxazolidinones is the result of P-gp mediated

**Table II.** Summary of Aqueous Solubility and Caco-2 Cell Permeability Values for the Oxazolidinones

| Compound | Solubility (mg/mL) | Apical to basolateral permeability ($\times 10^{-6}$, cm/s) | Basolateral to apical permeability ($\times 10^{-6}$, cm/s) | Efflux ratio[a] |
|---|---|---|---|---|
| PNU-93936A | 72 | 51 | 53 | 1.0 |
| PNU-100766 | 3.74 | 21 | 17 | 0.81 |
| PNU-179954 | 5 | 2.1 | 9.5 | 4.5 |
| PNU-177780 | >10 | 1.0 | 2.5 | 2.5 |
| PNU-173995 | 0.27 | 13 | ND | – |
| PNU-184435 | 0.57 | 4.6 | 19 | 4.1 |
| PNU-184249 | 0.056 | 35 | ND | – |
| PNU-100592 | 4.2 | 1.3 | 3.0 | 2.3 |
| PNU-101603 | 5.15 | 0.3 | 0.9 | 3.0 |
| PNU-177553 | 0.6 | 2.5 | 19 | 7.6 |
| PNU-179759 | 0.27 | 4.6 | 21 | 4.6 |
| PNU-179450 | 0.035 | 37 | 36 | 1.0 |
| PNU-184148 | 0.032 | 37 | 29 | 0.78 |
| PNU-184147 | 0.054 | 18 | 30 | 1.7 |
| PNU-176723 | 2.23 | 0.42 | 2.9 | 6.9 |
| PNU-184421 | 0.014 | 42 | 38 | 0.90 |
| PNU-182347 | 0.033 | 15 | ND | – |
| PNU-179397 | 0.01 | 49 | 44 | 0.90 |
| PNU-173335 | 0.05 | 9.25 | 14 | 1.5 |
| PNU-184470 | 0.02 | 23 | 29 | 1.3 |
| PNU-182945 | 0.016 | 17 | ND | – |
| PNU-183981 | 0.007 | 29 | 31 | 1.1 |
| PNU-179962 | 0.02 | 5.6 | 19 | 3.4 |
| PNU-176839 | 0.1 | 0.7 | 12 | 17 |
| PNU-179399 | 0.02 | 1.3 | 16 | 12 |
| PNU-179398 | 0.02 | 0.9 | 6.7 | 7.4 |
| PNU-241028 | 0.004 | 2.4 | 28 | 12 |

[a] The efflux ratio is the ratio of the secretory (Bl → Ap) permeability divided by the absorptive (Ap → Bl) permeability coefficient. A ratio of one is indicative of passive diffusion. Ratios greater than one suggest polarized secretion of the specific solute.

secretion, which has little or no effect on the absorptive permeability, similar to the case with cimetidine and ranitidine (16). Therefore, we assume that the apical to basolateral permeabilities in Table II are reflective of the absorption characteristics of the oxazolidinones in the rat, independent of potential differences in concentration between the Caco-2 cell experiments and rat bioavailability studies.

A number of recent studies have attempted to correlate fraction dosed absorbed, especially in man, with biopharmaceutical characteristics of the drugs. In one such report, Caco-2 cell monolayer permeability values were compared directly to fraction dose absorbed in humans for approximately 20 structurally diverse drugs (17). If we attempt to correlate Caco-2 cell apical to basolateral permeability with bioavailability in the rat for the oxazolidinones, the relationship shown in Fig. 5 is obtained. Clearly, little, if any, correlation is found between these two parameters. Low permeability compounds in the Caco-2 cell model have bioavailabilities ranging from about 0 to 60%. However, the higher permeability solutes do tend to show high bioavailability. However, the majority of the oxazolidinones have permeabilities that fall between $0.5 \times 10^{-6}$ and $20 \times 10^{-6}$ cm/s with associated bioavailabilities showing characteristics of a scatter plot. In this case, permeability alone is insufficient to

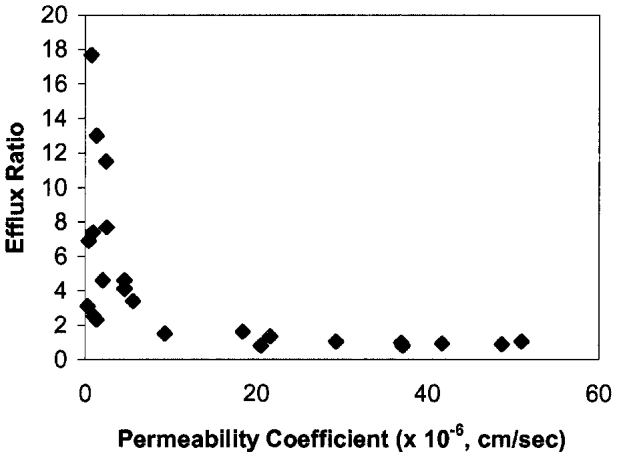


**Fig. 3.** Relationship of efflux ratio with absorptive permeability coefficient in Caco-2 cell monolayers for oxazolidinones. The efflux ratio is the ratio of the secretory (Bl → Ap) permeability divided by the absorptive (Ap → Bl) permeability coefficient. A ratio of one is indicative of passive diffusion. Ratios greater than one suggest polarized secretion of the specific solute.

retrospectively correlate, and therefore predict, absorption in the rat.

The lack of general correlation between permeability and absorption is not unexpected. The simplest model describing the absorptive flux of a drug across the intestinal mucosa is the product of the permeability coefficient of the mucosa to the drug, the surface area available for absorption, and the concentration gradient across the mucosa (18). Since the concentration on the serosal side of the mucosa will, for all practical purposes, be zero, this concentration gradient represents the solubility of the solute in the intestinal lumen, relative to the administered dose. Clearly, for two solutes of

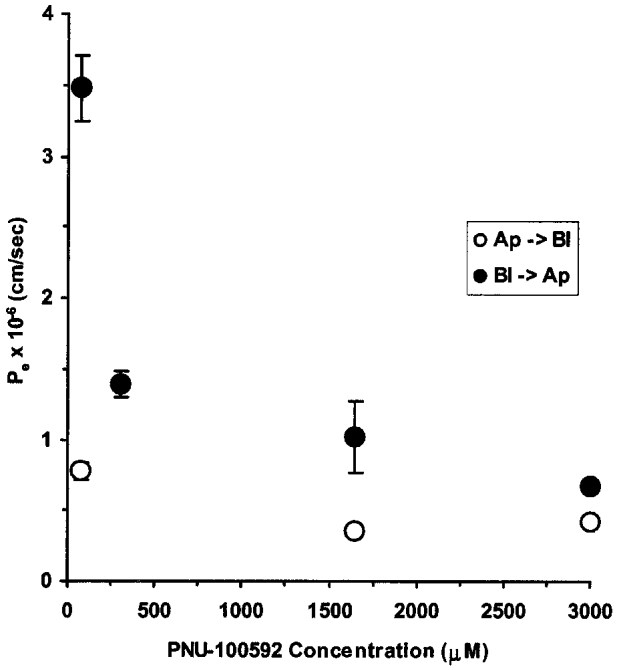


**Fig. 4.** Effect of donor concentration on the Ap → Bl and Bl → Ap permeability of PNU-100592 in Caco-2 cell monolayers. Values shown are mean and standard deviations from three replicates.

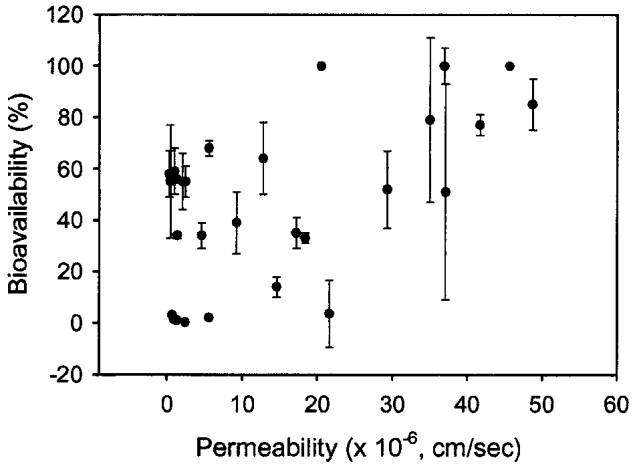


**Fig. 5.** Relationship of oral bioavailability in rats of oxazolidinones with Ap → Bl permeability coefficients in Caco-2 cell monolayers. Caco-2 cell values are mean values from Table II and oral bioavailabilities are mean and range from dosing of two animals.

equal permeability but differing solubility, absorption will be faster and potentially more complete for the more soluble compound, all other things being equal. Conversely, for two equally soluble compounds administered at the same dose, absorption rate will be correlated with permeability. The extent of absorption is a function of residence time in the gastrointestinal lumen relative to the absorptive flux and dose. Given the interdependencies of a number of factors contributing to oral absorption, one might expect a simple correlation, such as that between bioavailability, or fraction absorbed, and permeability, only when a number of other factors have been normalized. However, when this is not the case, we would expect absorption to depend at a minimum on solute permeability, solubility and dose.

In the discovery setting, more often than not the dose is not known but is related to intrinsic potency. A simple model for relating aqueous solubility and intestinal permeability to absorption for experimental drug candidates when the dose is not known was described several years ago and referred to as the maximum absorbable dose (MAD; Ref. 7). Briefly, MAD calculates the total mass of a compound which could be presumably absorbed if a saturated solution of the solute in the small intestine were absorbed with a given absorption rate constant for a time equal to the small intestinal transit time. In this case, dissolution is allowed to take place from an infinite reservoir of solid at a rate equivalent to the absorption rate, in order to maintain saturation. Although originally developed for estimating absorption potential in man, we have modified the model to reflect the characteristics of oxazolidinone absorption in the rat, as discussed in Methods.

Because no previous knowledge of dose is necessary for calculating MAD, the resulting values may be used to rank order the absorption potential for a series of solutes (8). Shown in Table III are the MAD values calculated for the oxazolidinones in this study which are predicted to be well absorbed, based on their solubility and permeability properties. The solutes are arranged in order of decreasing MAD. Also included in the Table is a predicted fraction dose absorbed in the rat, which is the ratio of the MAD to the administered dose used in evaluating the bioavailability of the

**Table III.** Maximum Absorbable Dose (MAD) Prediction for Oxazolidinones: Compounds with Complete Fraction Dose Absorbed

| Compound | MAD (mg) | Predicted Fa | Bioavailability (%) |
|---|---|---|---|
| PNU-93936A | 10637 | 1 | 100 |
| PNU-100766 | 248 | 1 | 100 |
| PNU-179954 | 33 | 1 | 55 ± 11 |
| PNU-177780 | 32.4 | 1 | 59 ± 9 |
| PNU-173995 | 11.2 | 1 | 64 ± 14 |
| PNU-184435 | 8.5 | 1 | 34 ± 5 |
| PNU-184249 | 6.3 | 1 | 79 ± 32 |
| PNU-100592 | 17.7 | 1 | 56 |
| PNU-101603 | 5 | 1 | 58 ± 9 |

oxazolidinones. Because MAD assumes an infinite dose, the actual ratio of MAD to administered dose may exceed one. Given that this is physically impossible, in these situations, the fraction absorbed was assigned a value of 1. For all the compounds in Table III, the Fa is predicted to be 1. In comparing these values to the bioavailabilities obtained for the individual solutes, the prediction was close to that obtained *in vivo* (PNU93936, 100766 and 184249) or absorption was overpredicted. The most significant overprediction was found for PNU184435, which also had one of the highest systemic clearances in the rat (Table I). As discussed earlier, this is a limitation in using bioavailability as a surrogate for fraction absorbed. However, from the perspective of applying MAD prospectively, this is the type of error that would be preferred.

Table IV summarizes the MAD, predicted fraction absorbed and bioavailability comparisons for the oxazolidinones predicted to be poorly absorbed. Again, the comparison of the predicted absorption with that observed is very good for these five solutes. Finally, Table V contains the oxazolidinones predicted to show good to moderate absorption in the rat. For these examples MAD again tends to be close to that observed or overpredicted, except in the case of PNU184421, 179397, and 183981. For these solutes, MAD significantly underpredicted absorption in the rat. It should be noted that these three compounds have the lowest clearance of all the oxazolidinones. In general, low systemic clearance will favor absorption, other things being equal. However, since MAD is focused explicitly on the absorption step before systemic distribution and clearance processes, it is not clear how low clearance could account for an underestimation of fraction absorbed. However, PNU-184421, 179397, and 183981 are high-permeability, low-solubility solutes. In fact, they have the lowest aqueous solubility of the oxazolidinones included in this study. These aqueous solubility values were used in the MAD calculations. However, the intestinal lumen is known to contain bile salts and other agents that may potentially serve to increase the apparent solubility of such compounds (19,20).

**Table IV.** Maximum Absorbable Dose (MAD) Predictions for Oxazolidinones: Compounds with Poor Fraction Dose Absorbed

| Compound | MAD (mg) | Predicted Fa | Bioavailability (%) |
|---|---|---|---|
| PNU-179962 | 0.4 | 0.08 | 2 ± 0.2 |
| PNU-176839 | 0.2 | 0.04 | 3 ± 0.4 |
| PNU-179399 | 0.08 | 0.016 | 1 ± 0.3 |
| PNU-179398 | 0.05 | 0.01 | 1.4 ± 0.3 |
| PNU-241028 | 0.03 | 0.01 | 0.2 ± 0.05 |

**Table V.** Maximum Absorbable Dose (MAD) Predictions for Oxazolidinones: Compounds with Good to Moderate Fraction Dose Absorbed

| Compound | MAD (mg) | Predicted Fa | Bioavailability (%) |
|---|---|---|---|
| PNU-177553 | 4.8 | 0.96 | 55 ± 6 |
| PNU-179759 | 4.8 | 0.96 | 68 ± 3 |
| PNU-179450 | 4.2 | 0.84 | 127 ± 7 |
| PNU-184148 | 3.8 | 0.76 | 51 ± 42 |
| PNU-184147 | 3.2 | 0.64 | 33 ± 2 |
| PNU-176723 | 3.0 | 0.6 | 55 ± 22 |
| PNU-184421 | 1.9 | 0.38 | 77 ± 4 |
| PNU-182347 | 1.6 | 0.32 | 14 ± 4 |
| PNU-179397 | 1.6 | 0.32 | 85 ± 10 |
| PNU-173335 | 1.5 | 0.3 | 39 ± 12 |
| PNU-184470 | 1.4 | 0.28 | 3.6 ± 13 |
| PNU-182945 | 0.9 | 0.18 | 35 ± 6 |
| PNU-183981 | 0.7 | 0.14 | 52 ± 15 |

Further, the formulation vehicle for delivering the oxazolidinone suspension in the bioavailability study was not a purely aqueous medium but contained 5% polysorbate 80 as a wetting agent. When we measured the apparent solubilities of these three solutes in the suspension vehicle, a significant increase in solubility was found relative to a simple pH 7 buffer. For example, PNU-183981 solubility increased 10-fold, to 0.07 mg/mL, in the suspension solution relative to buffer. Similar increases were found for PNU-179397 (0.05 mg/mL compared with 0.01 mg/mL in buffer) and PNU-184421 (0.1 mg/mL vs 0.014 mg/mL). These results suggest that these compounds may show increased solubility in the lumen in the presence of other micellar materials such as bile salts. Therefore, solubility contributing to oral absorption for these compounds, and others showing similar micelle enhanced solubilization, may be considerably greater than that expected from a simple aqueous buffer. In practice then, this result suggests that in the case of these high permeability, very low aqueous solubility solutes, MAD may give a value lower than that which may be achievable *in vivo*.

## CONCLUSION

Caco-2 cell permeability coefficients alone do not predict oral absorption potential for oxazolidinones. Utilization of a maximum absorbable dose (MAD) type algorithm, which takes solubility into consideration, in addition to permeability, is preferred for identification of better behaved oxazolidinones. However, MAD tends to underestimate absorption for high-permeability, low-solubility oxazolidinones. In this situation, solubility in a more physiologically relevant vehicle may improve the predictive capability. Finally, MAD is an effective model for prioritizing oral absorption potential of oxazolidinones in a discovery setting. This model will prove useful in conjunction with other determinants of *in vivo* performance such as metabolic liability, toxicity and intrinsic activity, in identifying more successful development candidates in the drug discovery setting.

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