Application of Oral Bioavailability Surrogates in the Design of Orally Active Inhibitors of Rhinovirus Replication

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Abstract □ Previous studies in rats and humans demonstrated poor oral bioavailability of potent in vitro 2-aminobenzimidazole inhibitors of rhinovirus replication due to significant first-pass elimination and possibly also to poor aqueous solubility. Estimations of aqueous solubility, as well as measurements of caco-2 permeability and NADPH dependent compound loss in rat liver microsomal incubations were employed alongside traditional in vivo experiments in rats to guide subsequent chemistry efforts. Retention of activity upon replacement of the metabolically labile vinyl oxime in the lead molecule with a vinyl carboxamide was a major breakthrough; however, oral bioavailability among the latter compounds was variable. Based on the ability to independently measure solubility, permeability, and metabolic stability of new compounds, variable solubility across the series (ranging from approximately 1 to 10 μg/mL) was identified as the cause of the inconsistent performance. Subsequent efforts to improve solubility led to the discovery of highly soluble (>10 mg/mL) and potent dessulfonyl vinyl carboxamide benzimidazoles. Determination of the metabolic stability of these compounds as a surrogate of the extent of their first-pass elimination supported a prediction of excellent oral bioavailability. In comparison to the sulfonyl-containing vinyl carboxamides, caco-2 permeabilities were reduced 5 to 10-fold; however, these were considered to be in the range of well-absorbed compounds based on comparison to a series of reference compounds of known percentage absorption in humans. Subsequent experiments in the rat verified the oral bioavailability of these *N*-alkyl compounds, with one compound (368177) having an absolute oral bioavailability of 89.4%. The application of solubility and caco-2 permeability as surrogates for oral absorption potential, in conjunction with the use of microsomal incubations as a surrogate for first-pass metabolism, was shown to augment a rational chemistry approach to discover orally bioavailable inhibitors of rhinovirus replication. Future expanded use of these surrogates is planned.

Introduction

The discovery and development of new drugs that are orally bioavailable continues to be a tremendous challenge in the pharmaceutical industry. In the discovery setting, increased reliance on genomics and high throughput screening to identify pharmacologically active lead compounds,[1,2] as well as reliance on combinatorial chemistry to increase molecular diversity and achieve desired activity through structural optimization,[3−6] can result in drug candidates having pharmaceutical properties that are not conducive to oral bioavailability.[7] Realization of this problem has led to a desire for earlier and even parallel consideration in the discovery process of pharmaceutical properties influencing oral bioavailability.[8] In response to the challenge of supplying structure−absorption and structure−metabolism information simultaneous with structure−activity relationships on an increasing supply of new chemical entities, scientists in preclinical drug disposition are increasingly relying on in vitro techniques and more sophisticated analytical methods such as LC-MS and LC-NMR.[9−11] For example, the insightful use of a computational alert approach and turbidimetric solubility measurements to identify compounds with potential for poor oral absorption has recently been employed.[7] With respect to drug transport, caco-2 monolayers have demonstrated potential as a predictive model of human intestinal absorption.[12−15] Coupled with their higher throughput capacity relative to traditional animal experiments, theoretical models of permeability across caco-2 cells show promise to provide additional structure−transport relationship throughput capacity.[16−18] Estimates of drug metabolism using in vitro techniques as a surrogate for first-pass elimination are also being suggested.[19−22]

The objective of the work reported herein was to apply such in vitro approaches to the discovery of orally bioavailable 2-aminobenzimidazoles that inhibit RNA replication in rhinoviruses and enteroviruses.[23] Previous studies demonstrated that, while analogues of this template possessed potent and broad-spectrum inhibition of virus replication in vitro, upon oral administration to both rats and humans these compounds underwent extensive first-pass elimination.[24] An additional objective was to gain experience integrating the results from the various drug absorption and metabolism in vitro techniques, as well as integrating these with in vivo results stemming from traditional bioavailability experiments in animals. This experience could then represent the initial installment of an iterative process to maximize drug discovery and development through parallel potency and bioavailability structural optimization efforts.

Materials and Methods

Materials—2-Aminobenzimidazoles were synthesized and characterized at the Lilly Research Laboratories as previously reported.[25,26]

Culture medium components and reagents for cell culture were obtained from GIBCO Life Technologies, Inc. (Grand Island, NY).

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Fetal bovine serum for caco-2 culture was obtained from Hyclone Corp. (Logan, CT). Sulforhodamine 101, used as a marker for caco-2 monolayer integrity,[27] was from Molecular Probes (Eugene, OR). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific, Inc. (Fair Lawn, NJ) and were used as received.

Inhibition of Rhinovirus Replication—Human rhinovirus 14 strain 1059 and H-1 HeLa cells were received as a gift from R. Rueckert, University of Wisconsin, Madison. Virus stocks were amplified and diluted as previously described.[23] Plaque reduction assays were conducted by inoculating confluent cell monolayers grown in a 60-mm dish using minimum essential medium, 1% nonessential amino acids, and 10% new born calf serum with 0.2 mL of growth medium containing approximately 150 plaque-forming units of RV14. Following a 30 min attachment period at room temperature, 5 mL of medium containing the desired concentration of an inhibitor was added. Virus-infected cultures were incubated for 48 h at 35 °C, fixed with 10% formalin, and stained with 0.5% crystal violet (which stains only noninfected cells). Results from duplicate flasks at each inhibitor concentration were averaged and compared to nondrug control flasks. The inhibition of plaque formation by 50% (IC_{50}) was calculated from the linear region of the inhibition−concentration curve using the standard method of Reed and Muench as previously described.[23]

Physicochemical Properties—*Solubility*—A rapid throughput solubility screen was implemented to determine if there were gross differences in solubility across the SAR. This assay was based on visual observation 24 h after placing different amounts of compound (0.1, 1, 10, and 100 mg) in 1.0 mL of water. Water was selected as the solvent in this assay because of its fundamental importance in drug solubility for purposes of oral absorption[28] and because of limited compound availability and time in the early stages of an SAR to evaluate the effects of pH, ionic strength, and biological surfactants. In a more detailed evaluation of the solubility of compounds 341908 and 354400, determinations were made in 0.05 M phosphate buffers prepared over the pH range 1.7 to 8.0. Preliminary studies with 341908 indicated that bile salts were unlikely to improve solubility. Namely, the solubility of this compound was not improved in the presence of sodium deoxycholate. Type I flint glass amber vials containing a known quantity of either compound (approximately 5 mg) and 5 mL of buffer were tumbled end-over-end at ambient temperature for up to 7 days. Aliquots of approximately 2 mL were removed on days 3 and 7 and passed through a 0.45 μm Teflon filter. The first 1 mL was discarded and the remaining filtrate assayed by HPLC with UV detection. A Zorbax SB-phenyl column (25 cm × 4.6 mm maintained at 35 °C) was used along with a mobile phase consisting of an equal volume mixture of 0.1% (v/v) trifluoroacetic acid in water and acetonitrile. The flow rate was 1 mL/min and detection was at 244 nm.

Determination of pK_a—Ionization constants were measured using the Sirius PCA 101 Potentiometric System. Compounds were dissolved in various proportions of methanol and 0.15 M KCl, and adjusted to pH 6 or lower. For a given titration, an approximately 0.5 mM solution of each compound was titrated from a low to a high pH. The acid used was 0.5 N HCl, and the base, 0.5 N NaOH. The acid and base were standardized to four decimal places using NIST traceable standards. For a given compound, titrations were performed in triplicate for each of three different proportions of methanol and 0.15 M KCl. These titrations were done under an argon atmosphere at a constant temperature of 25 °C. Estimate of pK_a in 100% water was made using the Yasuda−Shedlovsky method of extrapolation.[29,30]

Caco-2 Permeability—Caco-2 cells were obtained from the Memorial Sloan-Kettering Cancer Center and were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air. They were grown in DMEM:F12 (3:1) media supplemented with 5% fetal bovine serum and 50 μg/mL tobramycin. Monolayers at 75−90% confluency were either subcultured on a weekly basis using a 1:10 split ratio, or seeded onto Millicell-PCF polycarbonate inserts (30 mm diameter, 0.4 μm pore size; Millipore Corp., Bedford, MA) at a density of 600 000 cells per filter. The culture medium was replaced with fresh medium every other day. For transport studies, cells from passages 43−56 were used at 21−30 days postseeding. Measurement of transepithelial electrical resistance was made immediately prior to an experiment using a Millicell-ERS system (Millipore Corp.). Cells with a net resistance less than 300 $\Omega \cdot cm^2$ were not used.

Measurements of transepithelial flux of the various inhibitors were made at 37 °C using a side-by-side diffusion chamber described previously.[31] This system has now been coupled to a robotics arm to increase throughput capacity.[32] The transport medium consisted of 25 mM HEPES (pH 7.4) or 25 mM MES (pH 6.0) as buffering agents along with 125 mM NaCl, 5.2 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 10 mM glucose. To initiate an experiment, a stock solution of an inhibitor in DMSO was added to either the apical or basal side of the monolayer. The final concentration of DMSO was 1.4% (v/v). Samples (200 μL) were taken from the contralateral compartment at various times up to 120 min and replaced with an equal volume of fresh transport buffer. Rates of transport under initial rate conditions (<10% transport) were determined by linear regression analysis; these were divided by the initial donor concentration and filter surface area to calculate the corresponding permeability coefficients. Experiments were conducted in duplicate or triplicate and under limited lighting conditions to minimize light catalyzed *Z*-isomer to *E*-isomer transformation. At the end of an experiment, integrity of each monolayer was assessed by evaluating the percentage transport of sulforhodamine 101 in 30 min. Monolayers with greater than 0.2% transport in this time period were not included in the analysis of permeability (there were never fewer than two monolayers used in the estimation of a given permeability coefficient). Analysis of the various inhibitors via HPLC−UV was conducted as previously described for solubility measurements.

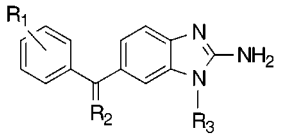
Microsomal-Mediated Metabolism—Using standard techniques,[33] microsomes were prepared from fresh livers obtained from male F344 rats weighing approximately 250 g. Microsomes were diluted to 1 mg protein/mL in 0.1 M sodium phosphate buffer, pH 7.4. Inhibitors were dissolved in either ethanol or acetonitrile to a concentration of 1 mM and spiked into the microsomal suspension such that the final concentration was 10 μM. This concentration was selected based on analytical sensitivity requirements and the desire to keep the concentration in the low micromolar, pharmacologically relevant, range. To start the reaction, either NADPH or, in the case of controls, buffer without NADPH was added. After 30 min, the reaction was stopped with an equal volume of acetonitrile, and the incubation mixture was centrifuged and analyzed for parent compound using HPLC−UV as described previously for measurement of solubility. The integrated peak areas from the HPLC chromatograms were compared for an inhibitor in the presence or absence of NADPH, and the amount of loss of parent compound was calculated.

***In Vivo* Studies**—Pharmacokinetic profiles and absolute oral bioavailability in 24 h fasted male F344 rats weighing approximately 200 to 250 g were determined for select inhibitors in the series. Plasma concentrations of an inhibitor following either an intravenous (iv) dose ($n = 2-3$ rats) or an oral dose ($n = 3$ rats) were quantitated by HPLC−UV analysis as described for solubility measurements. For iv dosing, compounds 341908 and 354400 were prepared in poly(ethylene glycol) PEG-400, ethanol, water mixtures (30%, 30%, 40%; w,v,v), and dosed via the tail vein. Compounds 366094 and 368177 were dissolved in saline and administered similarly. The latter two compounds were also dosed orally as solutions; whereas compounds 341908 and 354400 were dosed orally either as aqueous suspensions (10% acacia/0.5% polysorbate 80) of controlled particle size (5−25 μm), or as solutions (85% PEG-400/0.5% polysorbate 80). Precipitation of formulated solutions of 341908 and 354400 (10 mg/mL) was not observed upon 1:10 dilution with either 0.1 N HCl or 0.1 μ (ionic strength) pH 7.4 buffer. Pharmacokinetic parameters were obtained using model independent methodology. With respect to estimation of $AUC_{0-\infty}$, percent extrapolated areas were never greater than 10%. Percent oral bioavailability was calculated by comparison of dose normalized $AUC_{0-\infty}$ values for iv and oral doses (the equation used is summarized in Table 4B).

Results

In Vitro Potency and Metabolic Stability—Table 1 shows the structures of the compounds evaluated according to structural class. Also included are the potency and metabolic stability of the compounds. Enviroxime, a compound that was tested in Phase II clinical trials in the early 1980s, is included for comparison. Replacing the hydroxyl-

Table 1—Structure, pK_a, Potency, and Metabolic Stability of Vinyl Carboxamide 2-Aminobenzimidazoles



compd no.	R1	R2	R3	structural class	pK_a	potency (mean IC_{50}, μg/mL)[a]	metabolic stability (% loss in 30 min)
enviroxime	all H	oxime	isopropyl sulfonamide	sulfonamide	nd[b]	0.05	39
153186	all H	carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.04	18
341908	3-F	carboxamide	isopropyl sulfonamide	sulfonamide	3.41	0.04	11
354400	2,5-di-F	carboxamide	isopropyl sulfonamide	sulfonamide	3.40	0.08	17
355081	2,5-di-F	*N*-methyl carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.06	28
357132	all H	*N*-methyl carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.07	5
357822	2,3-di-F	*N*-methyl carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.01	22
362546	3-F	*N*-methyl carboxamide	*n*-propyl sulfonamide	sulfonamide	nd	0.04	12
362683	2,3-di-F	carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.01	3
366092	naphthyl	*N*-methyl carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.21	11
366347	2,3,4-tri-F	*N*-methyl carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.01	6
366349	3-F,4-MeO	carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.05	9
366572	3,5-di-F	carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.06	16
366659	2,3,5,6-F	carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.05	4
366799	2-F	carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.03	14
366856	2,3,4-tri-F	carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.02	5
368227	3,4-di-F	carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.05	1
368228	2,4-di-F	carboxamide	isopropyl sulfonamide	sulfonamide	nd	0.05	7
354030	3-F	*N*-methyl carboxamide	*N*,*N*-di-Me sulfonylurea	sulfonylurea	3.41	0.03	19
353462	3-F	carboxamide	*N*,*N*-di-Me sulfonylurea	sulfonylurea	3.41	0.05	13
362898	3-F	*N*-methyl carboxamide	morpholino sulfonylurea	sulfonylurea	3.41	0.05	24
359353	3-F	*N*-methyl carboxamide	pyrrolidino sulfonylurea	sulfonylurea	3.41	0.02	41
368288	3-F	carboxamide	morpholino sulfonylurea	sulfonylurea	3.41	0.08	32
366094	2,3-di-F	*N*-methyl carboxamide	isopropyl	*N*-alkyl	6.69	0.06	7
366853	2,3-di-F	carboxamide	isopropyl	*N*-alkyl	6.70	0.07	4
368177	2-F	*N*-methyl carboxamide	cyclopentyl	*N*-alkyl	6.70	0.03	10

[a] IC_{50}'s are from a plaque reduction assay using Rhinovirus 14. $n = 2$–3. [b] Not determined.

containing oxime in enviroxime at R2, which was extensively conjugated following oral administration of a 10 mg/kg dose in the rat (unpublished results from studies conducted to support enviroxime IND), with a vinylcarboxamide conferred resistance to metabolism by a primary conjugative mechanism. Specifically, incubation of enviroxime and 153186 in rat and human liver microsomes in the presence of UDP-glucuronic acid resulted in a 21% and an 11% loss of enviroxime from rat and human sources, respectively; whereas there was no loss of 153186 from either source. In addition, fluorine substitution at R1, which was a site of aromatic hydroxylation in enviroxime, was expected to provide a further increase in metabolic stability. The percent loss of enviroxime under oxidative conditions was 39% in 30 min. By comparison, the mean percent loss in 30 min of the sulfonamide containing vinylcarboxamides was 11 ± 7.3% (± 1 sd, $n = 17$ compounds). The nonsulfonyl *N*-alkyl compounds were similarly relatively resistant to oxidative metabolism, having a mean percent loss in 30 min of 7 ± 3.0% ($n = 3$ compounds). Importantly, while these structural modifications increased metabolic stability in vitro, they had no effect on antiviral potency relative to enviroxime (Table 1). Mean percentage loss of the sulfonylureas was 26 ± 11.0% ($n = 5$ compounds).

Permeability—Permeability coefficients across caco-2 monolayers relative to several marker compounds of known and varying percentage absorption in humans provided an estimate of the absorption potential of the novel antirhinovirus compounds. These results are shown in Figure 1 according to the three structural classes. The *N*-alkyl compound, 366853, had the lowest permeability ($0.5 \pm 0.05 \times 10^{-5}$ cm/s) while the sulfonamide, 366092, had the highest ($9.3 \pm 0.16 \times 10^{-5}$ cm/s). By comparison to the

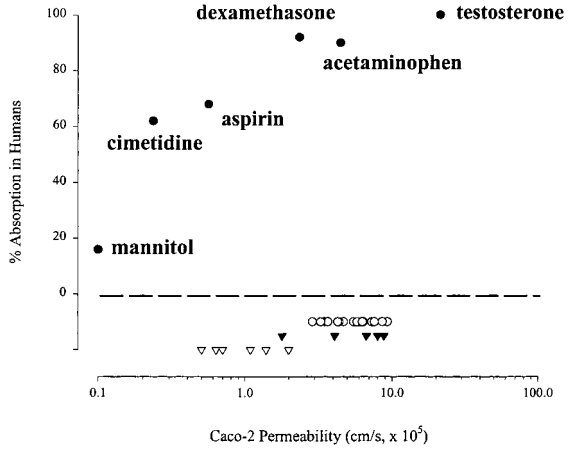


Figure 1—Permeability coefficients of 2-aminobenzimidazoles across caco-2 monolayers in relation to permeability coefficients of reference compounds (●). The three classes of 2-aminobenzimidazoles were sulfonamides, ○; sulfonylureas, ▼; and *N*-alkyls, ▽.

marker compounds, the observed range of permeabilities corresponded to a percentage absorption in humans of approximately 60% to 100%, thus indicating that these compounds should be well absorbed in vivo notwithstanding any potential dissolution or solubility limitations. Since, with a limited set of structurally different compounds, there was no asymmetry with respect to permeability (Table 2), the transport mechanism appeared to be passive. Table 3 indicates the pH dependent permeability of the *N*-alkyl compounds and the lack of pH dependence on the permeability of a sulfonamide. These results were expected due to pK_a differences (Table 1). Indeed, the lower permeability

Table 2—Apical to Basolateral versus Basolateral to Apical Transcellular Permeability of Vinyl Carboxamide 2-Aminobenzimidazoles[a]

compd no.	structural class	donor concentration (μM)	permeability (cm/s $\times 10^5$)[a] apical to basolateral	basolateral to apical
354400	sulfonamide	70	2.9 ± 0.09	2.9 ± 0.15
353462	sulfonylurea	15	6.7 ± 1.20	5.1 ± 0.63
366094	*N*-alkyl	80	1.0 ± 0.06	0.7 ± 0.04

[a] Results are expressed as mean ± one standard deviation; $n = 3$ filters. For the three compounds, $p > 0.05$ based on an unpaired Student's *t*-test, thus indicating no asymmetry with respect to transport direction. pH was 7.4 on both sides.

Table 3—pH-Dependent Permeability of Vinyl Carboxamide 2-Aminobenzimidazoles

compd no.	structural class	permeability (cm/s $\times 10^5$)[a] apical (donor) pH 6.0	apical (donor) pH 7.4
354400	sulfonamide	3.5 ± 0.28	2.9 ± 0.09
366094	*N*-alkyl	0.6 ± 0.01	1.0 ± 0.06[b]
366853	*N*-alkyl	0.5 ± 0.05	0.7 ± 0.03[b]
368177	*N*-alkyl	0.7 ± 0.01	2.0 ± 0.13[b]

[a] Results are expressed as mean ± one standard deviation; $n = 3$ filters. [b] Indicates significantly different from pH 6 result at $p \leq 0.05$ based on an unpaired Student's *t*-test.

of the *N*-alkyl class compared to the sulfonamides and sulfonylureas is attributed to the significant fraction of protonated amine at pH 6.0 for the *N*-alkyl compounds. The fraction present in the ionized form at this pH was estimated to be about 80% for the three *N*-alkyl compounds.

Solubility and Biopharmaceutics of Oral Solution and Suspension Formulations—The equilibrium solubility of enviroxime in water at ambient temperature was approximately 2 μg/mL. The water solubility of compound 341908, one of the earliest compounds in the current SAR, was 1 μg/mL. Based on this finding, there was concern that insufficient solubility in vivo would result in poor bioavailability of the sulfonamide vinyl carboxamide series. These concerns were realized upon administration of compound 341908 to rats. As shown in Figure 2A and summarized in Table 4, systemic exposure from a suspension was substantially lower than from a solution formulation. Interestingly, replacing the fluorine at the 3 position of the phenyl ring at site R1 (compound 341908) with fluorines at positions 2 and 5 (compound 354400), a seemingly minor modification, resulted in a 10-fold enhancement in water solubility (compound 354400 had a solubility of 10 μg/mL in water). As shown in Figure 3, the pH-solubility profiles of these two compounds demonstrate that, over the pH range of 1.75 to 7.9, 354400 solubility in 0.05 M phosphate buffer was consistently 5 to 7 times greater than that of 341908. The cause for this difference may be related more to differences in the interaction of the two compounds with solvent than to crystal lattice energy differences because of the similar melting points of the two compounds, ca. 210 °C. Importantly, as shown in Table 4, the absolute bioavailability of 354400 was similar from both solution and suspension dosage forms and was markedly greater compared to 341908 bioavailability from a suspension. The lower absolute bioavailability of 354400 compared to 341908 bioavailability from solution dosage forms may be due to greater first-pass elimination of 354400. This interpretation is supported by the approximate 6-fold higher systemic clearance of 354400 (Table 4A). While loss in liver microsomes was similar for the two compounds (17% for 354400 versus 11% for 341908, Table 1), as previously indicated, this assay was not scaled to in vivo clearance, nor does it take into account the possibility for conjugative metabolism. The possibility for biliary elimination was also not evaluated. Thus, the cause for the greater clearance of 354400 is not known.

Given the lack of predictability in the solubility properties of the sulfonamides, efforts were made to identify compounds of consistently greater solubility. These efforts led to removal of the sulfonyl group resulting in direct attachment of various alkyl groups to the benzimidazole nitrogen in the R3 position. This modification resulted in conversion of the primary amine to a significantly more basic functional group, as shown by the pK_a values (Table 1). Importantly, this modification had no impact on antiviral potency (Table 1). The water solubility of these so-called *N*-alkyl vinyl carboxamides was consistently greater than 10 mg/mL. As shown in Figure 2C and Table 4, systemic exposure of compound 368177, dosed as neat drug dissolved in water, was excellent, resulting in an absolute bioavailability of 89%.

Discussion

Evaluation of enviroxime disposition in rats demonstrated that glucuronidation of the hydroxyl containing oxime at position R2 was the major metabolic pathway, accounting for 60% to 70% of an oral dose in 24 h and up to 80% in 48 h (unpublished results). Hydroxylation of the phenyl moiety (R1) also significantly contributed to loss of parent. Identification of strategies to obviate these two metabolic pathways while simultaneously preserving or enhancing antiviral potency were the two most important goals of the current structural optimization effort. Discovery of the potent vinyl carboxamide series was a major breakthrough due to the resistance of the acidic primary amide to direct conjugation. Subsequently, attempts to achieve a balance between structural effects on potency and susceptibility to oxidative metabolism became the focus of our optimization work. A 30 min incubation in the presence of rat liver microsomes was used to rank order the compounds with respect to their susceptibility to metabolism, measured as percent loss of parent compound, and was placed after the initial potency screen. No attempt was made to correlate this screen with an in vivo measure of elimination; rather, the intent of the screen was to look for gross effects of structure on susceptibility to metabolism. Although the number of compounds was few, it seemed that the cyclic sulfonylurea compounds 362898, 359353, and 368288 were metabolized to a greater extent than the noncyclic sulfonylureas and sulfonamides. On the basis of loss of parent compound, aromatic fluorine substitution at R1 did not appear to reduce metabolism. However, to conclude that fluorine substitution had no effect is equivocal because metabolites were not identified; also, if fluorine did not have an effect, whether this would translate to the in vivo situation is also not known. Fortunately, fluorine substitution had no effect on antiviral potency.

On the basis of experience with enviroxime, a poor dose to aqueous solubility ratio of the benzimidazoles was considered a potential barrier to achieving adequate and reproducible systemic exposure following oral administration.[34] With a solubility of 2 μg/mL, complete dissolution of a therapeutic dose of enviroxime as low as 10 mg would require 5 L of fluid, which approximates the entire volume consumed and produced by secretions in the human gastrointestinal tract in a 24 h period.[35] The water solubility of compound 341908, one of the earliest vinyl carboxamides made, was 1 μg/mL. Concern that poor solubility of vinyl carboxamide sulfonamides and sulfonylureas would result

Table 4—Summary Single Dose Pharmacokinetics of Vinyl Carboxamide 2-Aminobenzimidazoles in Male F344 Rats

A. Intravenous Bolus Administration

compd no.	structural class	dose (mg/kg)	$AUC_{0-\infty}$[a] (ng·hr/mL)	clearance (L/h/kg)	$T_{1/2}$ (mean ± se) (h)
341908	sulfonamide	3	7703.6	0.37	0.8 ± 0.14
354400	sulfonamide	3	1211.9	2.42	1.8 ± 0.02
366094	*N*-alkyl	5	4042.5	1.18	1.0 ± 0.05
368177	*N*-alkyl	5	6974.7	0.68	1.0 ± 0.09

B. Oral Administration

compd no.	formulation	dose (mg/kg)	$AUC_{0-\infty}$[a] (ng·hr/mL)	C_{max} (mean ± se) (ng/mL)	T_{max} (h)	bioavailability[b] (%)
341908	solution	30	61076.4	10834	0.5	79.3
341908	suspension	30	8971.6	1076	3.0	11.6
354400	solution	6	1175.5	304 ± 51	2.0	48.5
354400	suspension	30	6038.8	1162 ± 361	2.0	49.8
366094	solution	30	8059.3	2128 ± 184	1.0	33.2
368177	solution	30	37404.4	4202 ± 2041	6.0	89.4

[a] Extrapolated areas were less than 10% in all cases. [b] Absolute bioavailability = $(AUC_{0-\infty,po}/AUC_{0-\infty,iv}) \times (dose_{iv}/dose_{po}) \times 100$.

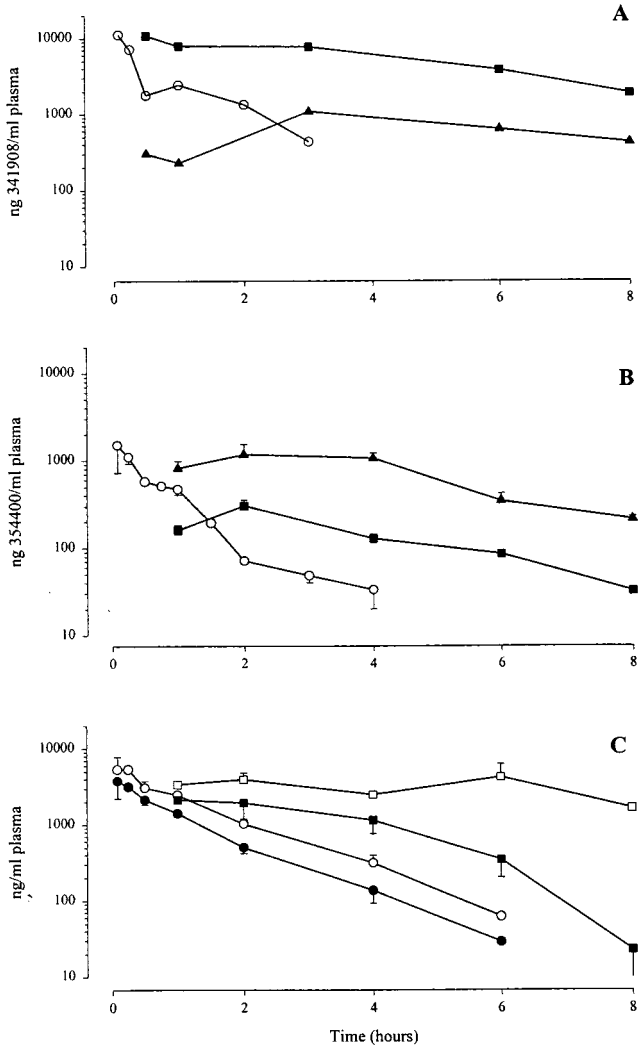


Figure 2—Rat plasma concentration versus time profiles of rhinovirus inhibitors following either intravenous (○ or ●), oral solution (□ or ■), or oral suspension (▲) administration. (A) Compound 341908. (B) Compound 354400. (C) Compounds 366094 (●, ■) and 368177 (○, □). Refer to Table 4 for actual doses administered. For consistency of presentation, 24 h levels following oral administration of compounds 341908, 366094, and 368177 have been omitted; these were <100 ng/mL.

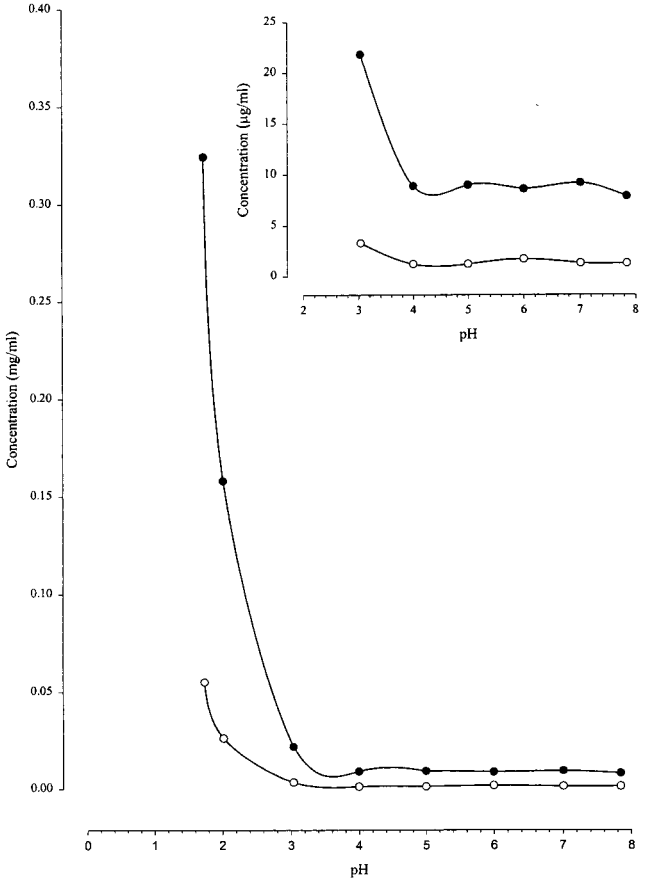


Figure 3—pH versus solubility profiles for compounds 341908 (○) and 354400 (●). Phosphate buffers, 0.05 M, were prepared over the pH range 1.7 to 8.0. Inset graph is of the same results, but expressed in μg/mL over the pH range 3 to 8.

in poor systemic exposure was verified upon oral administration of compound 341908. As shown in Table 4B, absolute bioavailability of this compound when dosed as an aqueous suspension was approximately 10%. In contrast, administration of a solution of 341908 prepared using a mixture of PEG-400, ethanol, and water resulted in a bioavailability of 80%. These results, as well as dose/solubility ratios based on projected human doses and gastrointestinal volumes, indicated that solubility could be problematic in relation to the clinical development of this therapeutic class.

A simplified solubility screen in water was consequently implemented; this was based on visual observation following incubation at room temperature for 24 h. Samples of a given compound were prepared in 10-fold increments of

concentration ranging from 0.1 mg/mL to 100 mg/mL. On the basis of this assay, all compounds tested had a solubility in water less than 0.1 mg/mL (data not shown). Compound 354400 was one of these compounds. HPLC analysis of its pH dependent solubility versus that of compound 341908 indicated a 5- to 7-fold higher solubility throughout the pH range of 1.7 to 7.9 (Figure 3). For the two compounds, an approximate 20-fold increase in solubility was observed at pH 2 relative to pH 4; this is attributed to protonation of the primary amine at the 2 position of the benzimidazole ring, which had a measured pK_a of 3.4 (Table 1). Importantly, the absolute bioavailability of a suspension of 354400 was 49.8%, which was similar to the bioavailability of a solution formulation, 48.5% (Figure 2, Table 4B). On the basis of the physical stability of the solution formulation of 354400 to a 10-fold dilution in either 37 °C 0.1 N HCl, pH 1.2 or pH 7.4 buffer, these results suggested that dissolution of the suspension was sufficient to produce a comparable bioavailability. Furthermore, given the excellent permeabilities of the two compounds (7.3 and 3.5 $\times$ 10^{-5} cm/s, for 341908 and 354400, respectively) and the greater systemic clearance of 354400 (Table 4A), greater solubility of 354400 was the attributed cause of its superior bioavailability from a suspension dosage form. The different effects of the two formulations on the bioavailability of these two compounds suggested that, under the experimental conditions tested, systemic exposure was acutely dependent on solubility and dissolution. Considering the less than 10-fold difference in the solubility of these two compounds, the similar solubilities of the compounds that had thus far been synthesized and the aforementioned unfavorable dose/solubility ratios applicable to human dosing, an effort to identify more soluble compounds was made.

Elimination of the R3 sulfonyl portion of these compounds significantly increased the pK_a of the primary amine at the 2 position of the benzimidazole ring (Table 1). Improvement in aqueous solubility to greater than 10 mg/mL was a consequence of this modification. Importantly, these so-called *N*-alkyl compounds had similar potency and metabolic stability compared to the sulfonamides and sulfonylureas. Given that these compounds had pK_a values in the range of small intestinal pH, it was expected that the *N*-alkyls would have a pH dependent permeability influenced by the ratio of protonated to unprotonated species. As shown in Table 3, *N*-alkyl permeability at pH 7.4 was significantly greater than at pH 6.0. Furthermore, as expected, permeability of compound 354400 was not similarly influenced by pH. It was also expected that the permeability of the *N*-alkyls in general would be less than that of the sulfonylureas and sulfonamides. While this was the case, the permeability of the *N*-alkyls was still in the range of what would be considered moderately well-absorbed compounds (Figure 1). Oral administration of aqueous solutions of compounds 366094 and 368177 to rats resulted in absolute bioavailability estimates of 33.2% and 89.4%, respectively (Table 4B). The approximate 3-fold greater bioavailability of 368177 may be due to either greater absorption, which is consistent with a larger permeability coefficient relative to 366094, or to reduced first-pass elimination, which is supported by the approximate 2-fold lower clearance compared to 366094, or to a combination of these two factors. According to the testing conditions used, both compounds exhibited prolonged absorption behavior, with absorption-rate limited kinetics indicated for compound 368177 (oral terminal half-life was 3.9 h vs 1.0 h following iv administration). The cause of this apparent slow absorption is not known, but may be due to prolonged absorption throughout the rat gastrointestinal tract, possibly including colonic absorption. This possibility is consistent with the known progressive increase in pH along the longitudinal axis of the intestinal tract and the observed increase in permeability of these compounds from pH 6 to 7.4. However, precipitation of drug in the stomach or in the duodenum with subsequent dissolution along the tract cannot be ruled out. The high caco-2 permeability coefficients of these compounds, even at pH 6, are indicative of excellent absorption, but are by no means definitive for novel compounds such as these for which a definitive in vitro/in vivo correlation has not been established.

In conclusion, this work has demonstrated the ability through structural modifications to independently influence potency, solubility, permeability, and metabolism in order to achieve oral bioavailability of potent inhibitors of rhinovirus replication. This positive experience has enabled this laboratory to move forward with a broader implementation of bioavailability surrogates to achieve a more expeditious discovery and development process.

References and Notes

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