Antirhino/Enteroviral Vinylacetylene Benzimidazoles: A Study of Their Activity and Oral Plasma Levels in Mice

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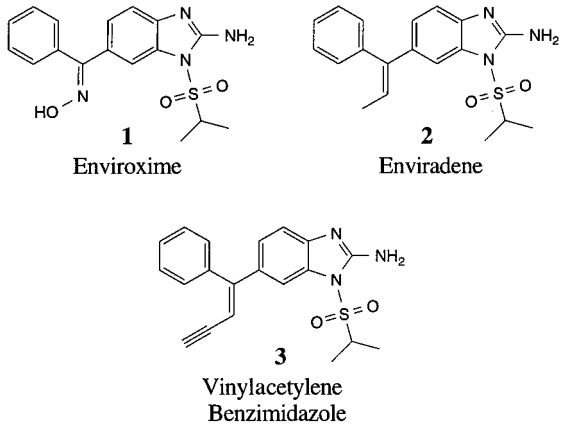
In an effort to find an orally bioavailable antiviral for the treatment of rhino/enteroviral infections, a series of vinylacetylene benzimidazoles (**11a**−**o**, **12**, and **18a**) was made. Initial studies of this class of antivirals showed that fluorine substitution on the left-hand phenyl ring in combination with the vinylacetylene moiety gave the requisite mix of physical properties to achieve good in vitro antiviral activity as well as respectable oral bioavailability in rhesus monkeys. To ascertain the generality of this finding and to broaden the scope of the structure−activity relationship (SAR), the present study concentrated on fluoro substitution of this class of molecules. The initial antiviral activity for each analogue was measured using human rhinovirus 14 (HRV-14). This served as an indicator of general antiviral activity for SAR purposes. Subsequently, the spectrum of antirhino/enteroviral activity of the more interesting analogues was evaluated through testing against a panel of seven additional rhino/enteroviruses. Broad-spectrum activity was present and consistent for all analogues tested, and it tracked closely with the antiviral activity observed against HRV-14. A simple screening protocol for oral bioavailability was established whereby compounds were administered orally to mice and plasma levels were measured. This procedure facilitated the evaluation of numerous analogues in a rapid manner. The C_{max} was used as a measure of oral bioavailability to allow relative ranking of compounds. In general, fluorine substitution directly on the left-hand aromatic ring does give good oral blood levels. However, fluorine incorporation at other positions in the molecule was not as effective at maintaining either the activity or the oral plasma levels. The constructive combination of activity and oral plasma levels was maximized in three derivatives: **11a**,**e**,**g**.

Introduction

The benzimidazoles[1−4] are a unique, potent, and broad-spectrum class of antirhino/enteroviral agents discovered over 25 years ago through routine screening of Lilly's research file compounds against rhino- and polioviruses.[5−9] In the early 1980s, two candidates from this series, Enviroxime and Enviradene (Chart 1), were evaluated in the clinic. Enviroxime (**1**) possesses significant antiviral activity against human rhinovirus 14 (HRV-14) (IC_{50} = 0.05 μg/mL) as well as a broad spectrum of activity against both rhino- and enteroviruses.[10−13] However, when Enviroxime was dosed orally in humans, it showed very low blood levels and caused an emetic response similar to that observed earlier in toxicology studies in dogs. An intranasal route of delivery was then explored, but confounding results obtained in these studies led to the decision to return to the discovery process to look for a compound that would exhibit the desired oral bioavailability but lack the emetic side effect.[14,15] Enviradene (**2**) seemed to meet these criteria in animal models. It possesses improved pharmacokinetics in dogs and caused no emesis.[16] Additionally, when Enviradene was dosed orally to humans, there was no emesis. Unfortunately, the peak plasma levels did not surpass the antiviral IC_{50} value, and development was discontinued before challenge studies were initiated.[17] In light of the nature of the failure of these compounds in the clinic, it should be noted that the key test of efficacy (i.e., administration of an orally bioavailable agent for the treatment of rhino/enteroviral infections) was never accomplished. Notwithstanding these prior disappointments, this series has the potential to provide a compound active against the causative agent and not merely the symptoms of the common cold.

Chart 1



Although the exact mechanism of action of these compounds remains unclear, it is known that they inhibit replication and, specifically, that they selectively inhibit (+)-strand RNA synthesis.[18] Efforts to identify the true target of the benzimidazoles through experiments designed to produce resistant mutants have failed. However, "drug tolerant" viruses that exhibit no increase in their IC_{50} value when tested in standard plaque reduction assays can be isolated. These viruses contain mutations in the RNA genome that code for amino acid changes in the viral protein 3A.[18] This

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protein has no known catalytic function, but it is intimately involved in the replication complex. This potentially implies that these compounds interfere in some way with the replication complex.[19] In the absence of knowledge of the actual molecular target, tissue culture assays served as a guide for the structure–activity relationship (SAR).

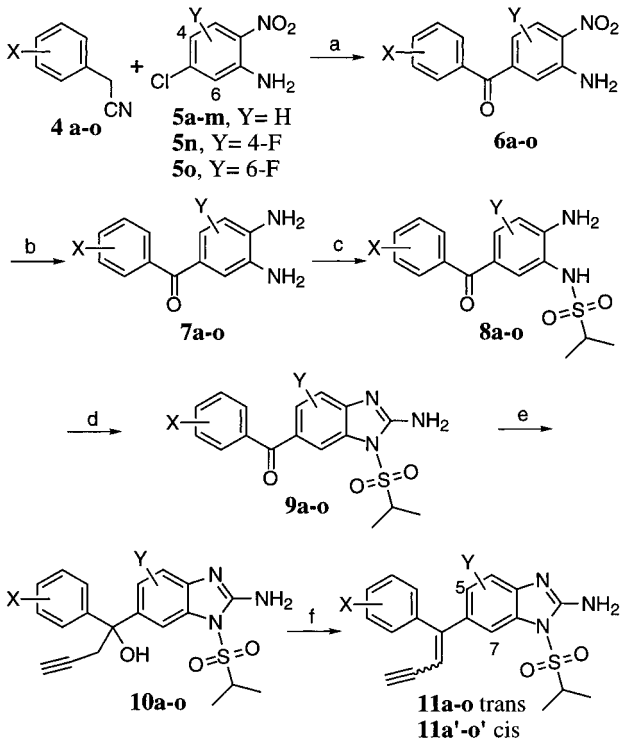
Recognition of the unique nature of these compounds and the fact that the key test of efficacy remained unanswered led to the initiation of an intense study of the benzimidazoles to find an analogue capable of overcoming the lack of oral plasma levels seen with the previous two clinical candidates. These efforts have, therefore, focused on optimization of the oral bioavailability of these compounds along with the antiviral activity since these two parameters are directly related and largely determine the deliverable activity for an orally administered compound. Our initial communication in this area detailed the information on the first members of the vinylacetylene family,[20] and now we wish to report on the expansion of the SAR, synthetic improvements in this series, and oral plasma concentrations in mice.

Chemistry

The intriguing combination of activity and oral bioavailability seen in rhesus monkeys for the *p*-fluoro acetylene derivative **11c** provided the impetus for further studies of this class of molecules. The original route used to synthesize this compound was cumbersome and inefficient and did not allow for rapid synthesis of additional analogues in sufficient quantities to be useful; therefore, a new synthetic route was required.

The first of the changes to the synthetic scheme came in the initial step. Previously, joining of the left-hand phenyl with the benzimidazole portion had been accomplished with Friedel–Crafts chemistry. This limited both the type and pattern of substitution available on the left-hand side of the molecule. In the new route (Scheme 1), it was found that 5-chloro-2-nitroanilines **5** served very nicely as activated acceptors in nucleophilic substitution reactions with the anion of variously substituted phenylacetonitriles **4**.[21] Additionally, the nitrile intermediate (not shown) could be oxidatively decyanated in situ to provide the desired 3-amino-4-nitrobenzophenone **6**. This modification in the reaction sequence offers a number of advantages. First, the procedure is simple, and it affords the desired intermediate **6** in high yield (79%). Second, the reaction is quite general, accommodating a variety of substitutions in both the phenylacetonitrile (**4**) and the nitroaniline (**5**) portions of the benzophenone without significant modification of the reaction conditions. Furthermore, a wide variety of substituted phenylacetonitriles are commercially available, and additional substitution patterns can be accessed readily through synthesis. The oxidation step was initially performed by bubbling oxygen through the reaction mixture; however, it was subsequently found that 30% aqueous hydrogen peroxide was easier to manipulate and resulted in better yields. Finally, it was found that 2 equiv of base was necessary to drive the first step (nucleophilic aromatic substitution) of the reaction to completion due to the increased acidity of the α hydrogen of the nitrile

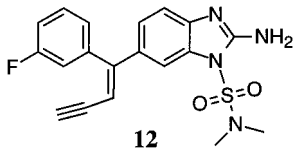
Scheme 1[a]



a: X= 3-F, Y= H; b: X= 2-F, Y= H; c: X= 4-F, Y= H; d: X= 2,5-diF, Y= H; e: X= 3,5-diF, Y= H; f: X= 3,4-diF, Y=H; g: X= 3-F,4-OMe, Y= H; h: X= 4-OMe, Y= H; i: X= 4-Me, Y= H; j: X= 4-NMe_2, Y= H; k: X= 3-CF_3, Y=H; l: X= 4-OCF_3, Y=H; m: X= 3-Cl, Y= H; n: X= H, Y= 5-F; o: X= H, Y= 7-F

[a] (a) i. KO^tBu (2.0 equiv), 0 °C, DMF, ii. H_2O_2 (3.25 equiv), 0 °C; (b) H_2, Ra-Ni, THF; (c) $^iPrSO_2Cl$ (1.4 equiv), pyridine (5.0 equiv), CH_2Cl_2; (d) CNBr (1.0 equiv), NaOH (1.0 equiv), ^iPrOH; (e) $HCCCH_2Br$ (3.5 equiv), Mg (4.2 equiv), $HgCl_2$ (0.088 equiv), THF:Et_2O, 1:1; (f) i. TBDMSOTf (1.1 equiv), 2,6-lutidine (1.2 equiv), CH_2Cl_2, ii. Et_3N (4.5 equiv), DMAP (2.5 equiv), MsCl (3.8 equiv).

Chart 2

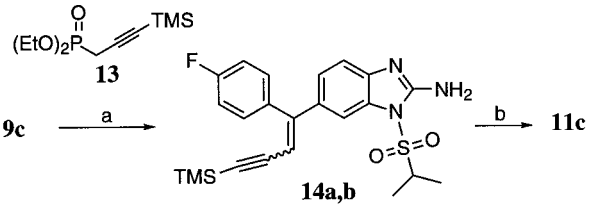


intermediate. By adding the hydrogen peroxide to the reaction mixture following completion of the first step, the overall reaction to give ketone **6** can be conveniently accomplished in a simple one-pot procedure.

The next three steps were carried out unchanged from the former route. Thus, reduction of the nitro group of **6** gave diamine **7** (94% yield), selective sulfonylation of the more nucleophilic aniline nitrogen of **7** furnished the desired sulfonylated product **8** in 88% yield, and reaction of compound **8** with cyanogen bromide provided the desired 2-aminobenzimidazole **9** (54% yield), a versatile intermediate for analogue preparation. Substitution of the isopropylsulfonyl group with other groups was also desired and was easily attained. For example, by use of dimethylsulfamoyl chloride in place of the 2-propanesulfonyl chloride in the synthesis, **12** was readily produced (Chart 2).

The next task was installation of the vinylacetylene moiety. In the original scheme, this had been achieved through a multistep sequence involving addition of propargylmagnesium bromide to ketone **9**, protection of

Scheme 2[a]



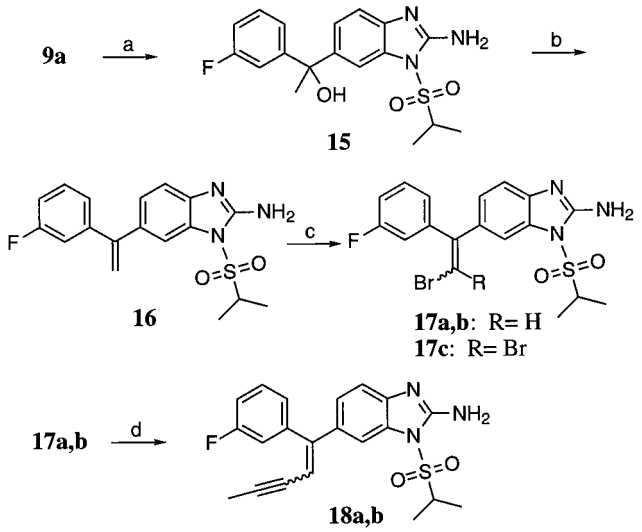
[a] (a) i. LiHMDS (6.0 equiv), **13** (5.0 equiv), −78 °C, THF, ii. separate isomers; (b) **14a**, CsF, CH_3CN:CH_2Cl_2, 5:1.

the alkyne of the resultant propargyl alcohol **10** with $Co_2(CO)_8$, elimination of the tertiary alcohol under acidic conditions, and finally deprotection of the alkyne to reveal the vinylacetylene (**11**). This sequence was employed due to problems with the vinylacetylene's stability when the unprotected propargyl alcohol (**10**) was subjected to the acidic elimination reaction conditions. This route for conversion of the ketone to the vinylacetylene was unattractive for several reasons, and this reaction sequence was ultimately the major impediment to analogue preparation in the overall synthetic scheme. The yield for this particular transformation was only ∼10%, and the purification was complicated by the cobalt byproducts liberated in the last step.

Two alternatives were investigated for the installation of the acetylene moiety. The first utilized the same propargyl Grignard addition as in the initial synthesis to generate **10**, but the elimination was executed under basic conditions. In situ protection of the 2-amino of benzimidazole **10** as the silylamine followed by reaction of the hindered tertiary alcohol with mesyl chloride, Et_3N, and DMAP produced a mixture of the cis and trans olefin isomers from which the desired trans vinylacetylene **11** could be isolated in moderate yields (19%). The use of the in situ protection of the silylamine was not necessary for the reaction to proceed, but it did eliminate the 10−20% of the undesired 2-aminosulfonylated material observed in its absence. This set of conditions offered several advantages to the cobalt protection route. The yield of isolated trans material was doubled to 19%, and the separation was now easier. As well, the three-step sequence previously required for the transformation of the propargyl alcohol to the vinylacetylene had been reduced to one step. A second approach was examined which involved use of Horner−Wadsworth−Emmons (H−W−E) reagent **13**[22] to produce vinylacetylene **14** in one step from ketone **9c** (Scheme 2). Subsequent deprotection of the silylacetylene furnished the desired final compound **11c**. This reaction was first performed using 3 equiv of the H−W−E reagent, but this led to incomplete consumption of starting material. The use of 5 equiv of reagent did drive the reaction to completion; however, the excess reagents required complicated the separation, and a low yield (3.5%) of the desired trans isomer **14a** was obtained. Furthermore, the deprotection of the silylacetylene to give the final product **11c** was not a high-yielding process (45%). Although these reactions were not optimized, it seemed that the first of the two routes examined was superior, and it was routinely used.

Examination of the olefin geometry produced under the mesyl chloride elimination reaction conditions yields an observation worthy of further mention. The ratio of trans to cis obtained under these conditions is dominated by the electronic nature of the left-hand phenyl

Scheme 3[a]

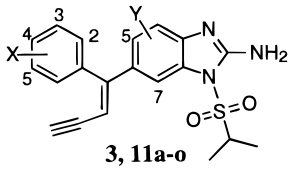


[a] (a) CH_3MgBr (5.0 equiv), 0 °C, THF:Et_2O; (b) *p*-TSA (2.25 equiv), $CHCl_3$; (c) NBS (1.5 equiv), THF, reflux; (d) $(Ph_3)_2PdCl_2$ (0.033 equiv), $^iPr_2NH$ (10.0 equiv), CuI (0.10 equiv), CH_3CCH, THF.

unit.[23] For example, *p*-methoxy- and *p*-dimethylamino-substituted rings give predominantly the undesired cis isomer (trans:cis = ∼1:3), while *p*-fluoro substitution gives mostly the trans isomer (trans:cis = 2:1). One possible explanation of these ratios is afforded upon analysis of the transition state of the elimination. Although the reaction conditions certainly favor an E2-like process, it is known that α-aryl substitution can shift the mechanism toward the E1 end of the mechanistic continuum. In this case, the developing positive charge on the carbon attached to the two aromatic systems could be conjugatively stabilized through the π-framework of one of the two aromatic systems more than by the other due to the steric limitations of the system. If the left-hand phenyl ring has electron-donating substituents, it can better serve to stabilize the partial positive charge than can the pendent benzimidazole moiety. As such, due to the eclipsing effect, the elimination will favor the isomer which places the acetylene group opposite to the left-hand phenyl. When the left-hand phenyl contains electron-withdrawing substituents, the benzimidazole is now better suited to stabilize the transient positive charge. As a result, the elimination places the acetylene away from the benzimidazole portion of the molecule favoring the trans product.

In addition to our examination of substituent effects on the aromatic rings, the consequence of substitution of the acetylene was also briefly studied. Since the routine synthetic scheme was not amenable to the preparation of this type of compound (e.g., methyl-substituted acetylene derivative **18a**), the route shown in Scheme 3 was developed. This process delivered **18a** in four steps from ketone **9a**. Reaction of **9a** with methylmagnesium bromide followed by elimination of water with *p*-TSA easily afforded olefin **16**. The bromination of **16** was not selective and gave a mixture of trans and cis bromides **17a**,**b** as a 1:1 mixture as well as the dibromide product **17c**.[24] The monobromo derivatives were crystallized out of the reaction mixture and used without further purification in a palladium-catalyzed coupling reaction[25] with propyne to yield the

Table 1. Antiviral Activity and Cellular Toxicity against HRV-14



3, 11a-o

compd	X	Y	IC_{50} (μg/mL)[a,b]	TC_{50} (μg/mL)[c]
3	H	H	0.094(3)	11
11a	3-F	H	0.060(6)	5.3
11b	2-F	H	0.092(5)	66
11c	4-F	H	0.13(4)	5.8
11d	2,5-diF	H	0.11(3)	63
11e	3,5-diF	H	0.17(2)	32
11f	3,4-diF	H	0.19(3)	19
11g	3-F, 4-OMe	H	0.061(3)	5.5
11h	4-OMe	H	0.059(1)	5.2
11i	4-Me	H	0.075(1)	5.7
11j	4-NMe_2	H	0.117(3)	5.3
11k	3-CF_3	H	0.40(3)	>100
11*l*	4-OCF_3	H	0.55(3)	18
11m	3-Cl	H	0.21(3)	20
11n	H	5-F	1.5(1) [d]	13
11o	H	7-F	2.8(1)	57

[a] HRV-14 plaque reduction assay except where noted. [b] The superscript number is the number of replicates. [c] HRV-14 CPE/XTT assay. [d] PV-1 plaque reduction assay, tested as a 1:1 mixture of cis and trans isomers.

desired methyl-substituted analogues **18a**,**b**. Subsequent separation of this isomeric mixture furnished pure isomer **18a** for antiviral evaluation.

Results and Discussion

Antiviral Potency Evaluation. HRV-14 was picked as the routine and initial virus for testing due to the amount of information known about this serotype. To establish the antiviral activity of the compounds versus a general cellular toxicity, compounds were first tested in a cytopathic effect assay. As can be seen in Table 1, the therapeutic index for these compounds (ratio of TC_{50} over IC_{50}) is generally quite high (~100−700), and no associations between cellular toxicity and antiviral activity are apparent. Analysis of the antiviral IC_{50} values from the plaque reduction assay (Table 1) does yield several general trends. Although the mono- and disubstituted fluoro analogues that were examined do exhibit similar IC_{50} values, the *m*-fluoro compound **11a** does appear to be slightly more active than the other substitution patterns examined. There is a suggestion of the influence of electronic effects on the activity as evidenced by comparison of derivatives with electron-donating substituents at the para position (**11g**−**i**) versus electron-withdrawing substituents at this site (**11c,l**) with electron-donating groups being favored. Steric interactions at the meta position appear to be important since replacement of the fluorine with a chlorine (**11a** vs **11m**) led to a considerable loss in activity. This was further substantiated by trifluoromethyl analogue **11k**. In an effort to examine substitution of the benzimidazole core, derivatives **11n**,**o** which placed a fluorine in the 5 and 7 positions, respectively, were prepared. These compounds suffer a significant drop in activity. This result was not altogether unexpected due to the work reported by Kelley et al. who demonstrated that replacement of C-4 of the benzimidazole with a nitrogen (to make an imidazopyridine) resulted in complete abrogation of activity.[26] This indicates that the electronics of the benzimidazole core are very important for activity. Apparently, only certain, if any, substitutions on the benzimidazole core will be tolerated.

Table 2. Antiviral Activity and Cellular Toxicity against HRV-14

compd	IC_{50} (μg/mL)[a,b]	TC_{50} (μg/mL)[b,c]
9a	2.1(1)	15.9
12	0.061(2)	>100
18a	0.26(2)	3.1

[a] HRV-14 plaque reduction assay. [b] The superscript number is the number of replicates. [c] HRV-14 CPE/XTT assay.

In an effort to begin to probe the effect of changes at the N-1 position, dimethylsulfamoyl derivative **12** was evaluated (Table 2). This change delivered essentially an equipotent analogue of its isopropylsulfonyl counterpart **11a** but offered a compound with substantially different chemical, and potentially different pharmaceutical, properties: a result that could be of paramount importance in further studies. One example of the differences imparted by this isosteric replacement is the ability of **12** to readily form salts.

Although it was known that the sp^2 substituent should be trans and sterically small, the effect of substitution of the terminal acetylene hydrogen for a methyl group was unknown. Synthesis and testing of analogue **18a** confirmed the stringent spatial requirement in this region of the molecule and show that the addition of a methyl group reduces activity substantially (Table 2). Furthermore, ketone **9a** was also tested to confirm that the trans functionality is important and that an sp^2 center is not sufficient for good activity. Although this ketone certainly exhibits an antiviral effect, the approximate 50-fold drop in activity underscores the importance of the trans substituent.

Broad-Spectrum Testing. To ensure that the typical broad-spectrum nature of the benzimidazole class was present and consistent among the vinylacetylene subclass, a panel of seven additional viruses was chosen as representative of the large number of human rhinoviruses (at least 110 serotypes) and human enteroviruses (at least 68 serotypes). The constituents of this panel include two viruses from the minor rhinovirus receptor subgroup, human rhinovirus 1A (HRV-1A) and human rhinovirus 2 (HRV-2); one additional virus from the major rhinovirus receptor subgroup,[27] human rhinovirus 16 (HRV-16); a prototypical enterovirus, poliovirus 1 (PV-1); and three non-polio enteroviruses, coxsackievirus A21 (CA21), coxsackievirus A21 mouse muscle-adapted (CA21M), and coxsackievirus B3 (CB3). By carefully selecting these viruses to span the major classes of rhino/enteroviruses, it was felt that any divergence of the SAR would become apparent. As can be seen in Table 3, the broad-spectrum activity was found to be intact in all of the analogues tested. Indeed, it is also gratifying to note that the magnitude as well as the absolute number for the antiviral activity against HRV-14 is predictive of the activity across the spectrum of viruses tested (compare IC_{50} data in Table 1 to averaged, av, data in Table 3).

Oral Plasma Level Screening. On the basis of the limited number of analogues made in the previous study,[20] the combination of fluorine and acetylene

Table 3. Broad-Spectrum Antiviral Activity Evaluation[a]

compd	HRV-1A	HRV-2	HRV-16	PV-1	CA21	CA21M	CB3	av
3	0.033	0.065	0.039	0.037	0.037	0.089	0.10	0.057
11a	0.027	0.069	0.057	0.037	0.085	0.046	0.032	0.050
11b	0.035	0.050	0.070	0.045[b]	0.079	0.10	0.050	0.061
11c	0.078	0.091	0.098	0.067	0.10	0.062	0.095	0.085
11d	0.096	0.079	0.099	0.032	0.069	0.10	0.10	0.083
11e	0.12	0.18	0.26	0.12	0.19	0.23	0.14	0.18
11f	0.12	0.23	0.55	0.082[b]	0.247	0.22	0.10	0.22
11g	0.049	0.054	0.060	0.016	0.032	0.029	0.035	0.039
11h	0.030	0.041	0.029	0.040	0.047	0.051	0.055	0.042
11j	0.048	0.060	0.080	0.059[b]	0.14	0.13	0.060	0.082
11*l*	0.26	0.35	0.22	0.27	0.22	0.50	0.70	0.36
11m	0.13	0.12	0.19	0.15	0.23	0.18	0.17	0.17
12	0.053	0.050	0.040	0.047[b]	0.10	0.090	0.070	0.064
18a	0.14	0.28	0.090	0.12[b]	0.33	0.36	0.19	0.22

[a] Values are IC_{50}'s in μg/mL, are single determinations, and were run with Enviroxime as a standard. [b] Enviroxime was unusually high in this assay (0.48 μg/mL), so values were corrected back to 0.05 μg/mL.

appeared promising in terms of attaining adequate oral plasma levels. This led to a focus on fluoro-containing derivatives to try and understand the scope of the initial trend that was observed. With the clinical experience of Enviroxime and Enviradene and the followup studies done with the vinylacetylenes both in rodents and in monkeys, a diverse set of data existed for consideration of how best to approach the issue of optimizing oral plasma levels. Since a relatively large number of compounds needed to be evaluated, it was not practical to use monkeys as a screening animal. Evaluation of the metabolism data generated earlier for **11c** indicated that the mouse would be a good small-animal model for testing of oral absorption of the vinylacetylenes.[20] Therefore, a screening protocol was developed in which mice were dosed by oral gavage with the test compound as a 1:5 mixture with poly(vinylpyrrolidone) (PVP-30). Since this was being run as a screening tool, C_{max}'s were chosen as the criteria for comparison of compounds. As such, blood was drawn more frequently early in the experiment (0.25, 0.5, 1, 2, and 4 h) to ensure that the C_{max} was represented. In the rare cases where the C_{max} was not found in the 4-h time frame, the experiment was repeated with extended time points. Plasma samples were then analyzed by HPLC to determine the C_{max}. These data along with the antiviral activity were then employed to rank compounds for further pharmacokinetic and toxicological evaluation.

On the basis of the results from the oral dosing, the compounds could be roughly subdivided into three main groups (Table 4). Those derivatives in the lowest group (C_{max}'s ≤ 5 μg/mL) were considered to be poor candidates for oral delivery no matter what antiviral activity they possessed. This eliminated 7 of the 16 compounds evaluated in the present study. For comparative purposes Enviroxime (**1**) and Enviradene (**2**) were both analyzed in this model. These two compounds which suffered from poor oral bioavailability in man would have been eliminated from consideration based on the results obtained serving to help validate this model. The next group was one with intermediate plasma levels (C_{max}'s $= 9-12$ μg/mL). Six compounds (**11a**−**d**,**g** and **12**) fell into this class. The next level was the highest (C_{max}'s ~ 18 μg/mL), and only three compounds (**11e**,**m**,**n**) achieved this level in the plasma. It is of interest to note that **11c**,**n** were both previously dosed in monkeys and found to have 23% and 46% oral bioavailability, respectively. These data add further support to the applicability of this crude screening model.

Table 4. Oral Plasma Concentrations in Mice

compd	C_{max} (μg/mL)[a]	compd	C_{max} (μg/mL)[a]
1	0.89	**11i**	0.61
2	5.1	**11j**	0.21
11a	12	**11k**	4.9[b]
11b	9.2	**11*l***	2.0
11c	9.2	**11m**	18
11d	9.3	**11n**	16[c]
11e	18[b]	**11o**	0.52
11f	2.9	**12**	8.4
11g	9.2[d]	**18a**	0.64

[a] Compounds were dosed at 100 mg/kg. Blood from three mice was pooled. The C_{max} was within a 4-h time frame unless noted. [b] The C_{max} was at 6 h. [c] Dosed as a 1:1 mixture of cis:trans isomers which were indistinguishable by HPLC. [d] C_{max} estimated at 9.2 μg/mL for a 100 mg/kg dose of pure **11g** (actually dosed at 100 mg/kg as a 1:1 mixture of cis:trans isomers which were distinguishable by HPLC: C_{max}(**11g**) = 4.6 μg/mL, C_{max}(**11g′**) = 5.5 μg/mL).

With the blood level data in hand, the antiviral activity was next taken into consideration. Deliverable activity was the criterion by which compounds were ranked, and the oral plasma level and antiviral activity of the compounds were weighted equally. Using these criteria, analysis of the compounds which produced the highest C_{max}'s shows that one compound rises above the other two: the 3,5-difluoro analogue **11e** is plainly superior to **11m** or **11n**. Evaluation of the middle group of compounds was approached in a similar manner, although the differences in this set are definitely more subtle. The 3-fluoro compound **11a** seemed to stand out given its higher C_{max} value and its slightly better activity. Likewise, the 3-fluoro-4-methoxy derivative **11g** was also considered to be of interest due to its equivalent blood level to the others in this group but its apparently superior activity. The remaining compounds in this group (**11b**−**d** and **12**) were all viewed as being slightly below this first tier of three compounds picked for advancement into more detailed studies.

Conclusion

The discovery and development of a drug to treat the common cold requires a compound which meets a very stringent set of conditions. First, a successful compound must possess activity against a subset of a virus family that minimally encompasses one genus (rhinovirus) with at least 110 serotypes as well as a second genus (enterovirus) which adds at least 68 additional serotypes as far as human pathogenicity is concerned. If this requirement can be met with sufficient potency across this vast number of viruses, the target chosen must be one such that resistance does not rapidly become a problem. Once these criteria are fulfilled, the challenges turn toward development issues. For a drug to be of use for this indication, it will have to be available to the patient quickly and easily, and it will almost certainly have to be delivered either orally or intranasally. The other and most important development issue is safety. A compound for a self-limiting illness with the potential for multiple courses of therapy per year and certain use in pediatrics demands a compound with little or no side effects. Last, the compound will have to be efficacious in humans, if not providing relief for those who are already infected at least being useful as a prophylactic.

We have shown that the vinylacetylene benzimidazoles certainly possess the potency and broad spectrum of activity necessitated as a first step toward a treatment of the common cold. By incorporating concomitant evaluation of oral plasma levels along with antiviral activity, we were able to optimize both of these parameters to identify not only the most active analogues but also those that have the highest potential for oral bioavailability. The generality of the link between fluorine substitution of the vinylacetylenes and oral plasma levels was found to be present but not absolute. Although it is true that each of the derivatives picked for further evaluation do incorporate a fluorine, a compound which showed one of the highest plasma levels was a chlorine-containing molecule. Perhaps then the discernible trend is that there is an association between halogen-containing left-hand phenyl units and oral plasma levels within this series.

Having aptly demonstrated both antiviral activity and oral plasma levels, this class has cleared the first two hurdles outlined above. Additionally, one of the members of this group (**11c**) has already been shown to have good oral bioavailability in monkey (23%) and in rat (51%) as well as activity in the coxsackievirus A21 mouse model. Further evaluation of the lead compounds identified in this study including more extensive ADME and toxicological studies is clearly warranted, and progress toward these goals will be reported in due course.

Experimental Section

Reactions were carried out with continuous stirring under a positive pressure of nitrogen except where noted. Reagents and solvents were purchased and used without further purification. TLC was performed with 0.25-mm silica gel 60 plates with a 254-nm fluorescent indicator from E. Merck. Plates were developed in a covered chamber and visualized by ultraviolet light or by treatment with 5% phosphomolybdic acid in ethanol followed by heating. Flash chromatography was carried out with silica gel 60, 230−400 mesh (0.040−0.063-mm particle size), purchased from EM Science. Reverse-phase chromatography was carried out with a Waters PrepLC System 500A instrument using PrepPAK 500 cartridges for preparative liquid chromatography. Analytical data for compounds **3** and **11c**,**h** were reported previously.[20] Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR spectra are reported as chemical shifts in parts-per-million (ppm) downfield from a tetramethylsilane internal standard (0 ppm). ^{1}H NMR spectra were recorded in the solvent indicated on a GE QE-300 spectrometer at 300.15 MHz. IR spectra were recorded on a Nicolet 510P FT-IR spectrometer, UV spectra were recorded on a Shimadzu UV-2101 PC spectrometer, and field desorption (FD) mass spectra were recorded on either a VG ZAB-3F or VG 70-SE instrument. IR spectra, UV spectra, FDMS spectra, elemental analyses, and some ^{1}H NMR spectra were provided by the Physical Chemistry Department at Lilly Research Laboratories.

General Procedure for Vinylacetylenes 11a−o: A. 3-Amino-4-nitro-3′-fluorobenzophenone (6a). 3-Fluorophenylacetonitrile (**4a**) (13.5 g, 100 mmol) and 5-chloro-2-nitroaniline (**5a**) (17.25 g, 100 mmol) were placed in a 500-mL round-bottom flask and dissolved in anhydrous DMF (200 mL). The reaction was cooled to 0 °C with an ice bath, and potassium *tert*-butoxide (22.44 g, 200 mmol) was added in one portion causing the reaction mixture to turn an intense dark blue. The ice bath was then removed and the reaction mixture allowed to stir at room temperature for approximately 4 h at which time the initial reaction was complete by TLC (20% ethyl acetate/hexane—fast running yellow spot is converted to a slower running yellow-orange spot). At this point the reaction mixture was again cooled to 0 °C with an ice bath, and hydrogen peroxide (30 mL of a 30% solution, 326 mmol) was added. The ice bath was left in place, and the reaction mixture was stirred overnight, slowly allowing the reaction mixture to come to room temperature. At this time the color had changed from an intense blue to dark brown, and the reaction mixture was poured into approximately 1 L of 1 N HCl giving a bright orange precipitate. The HCl suspension was stirred vigorously for about 30 min, and then the orange solid was collected by filtration and dried in a vacuum oven at 30 °C for 12 h furnishing **6a** (20.5 g, 78.8 mmol, 79% yield) as an orange solid: ^{1}H NMR (DMSO-d_6) δ 6.86 (dd, J = 8.9, 1.6 Hz, 1H), 7.37 (d, J = 1.5 Hz, 1H), 7.43−7.72 (m, 6H), 8.08 (d, J = 8.9 Hz, 1H); IR ($CHCl_3$) 3401, 1671, 1623, 1588, 1501, 1331, 1252 cm^{-1}; UV λ_{max} 234 (ϵ = 20 905), 281 (ϵ = 11 673), 395 (ϵ = 4091), 423 nm (ϵ = 5473); FDMS (MeOH) m/z 260 (M^+). Anal. ($C_{13}H_9FN_2O_3$) C, H, N.

B. 3,4-Diamino-3′-fluorobenzophenone (7a). Compound **6a** (20.5 g, 78.8 mmol) was placed in a Parr-type hydrogenation vessel along with THF (500 mL). To this was added Raney nickel (2.05 g, 10 wt %) which was purchased from the Davidson Specialty Chemical Co. and then washed with ethanol and stored under ethanol. The reaction mixture was shaken for 12 h under hydrogen pressure (60 psi, 4 atm). After this time, the reaction mixture was filtered to remove the catalyst. The solvent was then removed in vacuo to give **7a** (17.1 g, 74.1 mmol, 94% yield), the desired diamino compound, as a yellow solid which can be used in the next step without further purification: ^{1}H NMR (DMSO-d_6) δ 4.76 (s, 2H), 5.58 (s, 2H), 6.91 (d, J = 8 Hz, 1H), 7.07 (s, 1H), 7.32−7.47 (m, 3H), 7.49−7.61 (m, 1H); IR ($CHCl_3$) 3438, 3377, 3010, 1648, 1618, 1585, 1516, 1446, 1311, 1271, 1249 cm^{-1}; FDMS (MeOH) m/z 230 (M^+). Anal. ($C_{13}H_{11}FN_2O_1$) C, H, N.

C. 4-Amino-3-(1-methylethanesulfonamido)-3′-fluorobenzophenone (8a). Compound **7a** (17.1 g, 74.1 mmol) was dissolved in methylene chloride (150 mL) and pyridine (30 mL, 371 mmol). 2-Propanesulfonyl chloride (12 mL, 105 mmol) was added, and the reaction mixture was stirred at room temperature for 5 h. At this point, the reaction mixture was poured into a separatory funnel, 1 N HCl (500 mL) was added, and the mixture was extracted with ethyl acetate (500 mL). The organic phase was then dried ($MgSO_4$) and the solvent removed in vacuo. The crude product was then purified by HPLC (Waters Prep 500, gradient eluent 30−60% ethyl acetate/hexane) to give the desired product **8a** (21.9 g, 65 mmol, 88% yield) as a yellow gum which can be used in the next step without further purification: ^{1}H NMR (DMSO-d_6) δ 1.26 (d, J = 7.4 Hz, 6H), 3.24 (septet, J = 7.1 Hz, 1H), 6.17 (s, 2H), 6.81 (d, J = 8.9 Hz, 1H), 7.38−7.73 (m, 6H), 8.90 (s, 1H); IR ($CHCl_3$) 3405, 2995, 1649, 1619, 1585, 1441, 1314, 1291, 1142 cm^{-1}; UV λ_{max} 246 (ϵ = 13 961), 336 nm (ϵ = 15 859); FDMS (MeOH) m/z 336 (M^+). Anal. ($C_{16}H_{17}FN_2O_3S$) C, H, N, S.

D. 6-(3-Fluorobenzoyl)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazol-2-amine (9a). Compound **8a** (21.9 g, 65 mmol) was dissolved in 2-propanol (120 mL), and 2 N NaOH (32.5 mL, 65 mmol) was added. The reaction mixture was cooled to 0 °C with an ice bath, and cyanogen bromide (13 mL, 5 N solution in acetonitrile, 65 mmol) was added. After the mixture stirred for approximately 1 h at room temperature, a precipitate began to form. Stirring was continued overnight, and in the morning the precipitate was collected by filtration and washed with ether to give **9a** (12.7 g, 35.2 mmol, 54% yield) as an off-white solid: ^{1}H NMR (DMSO-d_6) δ 1.30 (d, J = 6.8 Hz, 6H), 3.95 (septet, J = 6.8 Hz, 1H), 7.35 (d, J = 8.3 Hz, 1H), 7.46−7.65 (m, 7H), 7.96 (d, J = 0.9 Hz, 1H); IR ($CHCl_3$) 3397, 3016, 1639, 1603, 1586, 1541, 1443, 1283, 1271 cm^{-1}; UV λ_{max} 246 (ϵ = 15 589), 320 nm (ϵ = 17 567); FDMS (MeOH) m/z 361 (M^+). Anal. ($C_{17}H_{16}FN_3O_3S$) C, H, N, S.

E. DL-α-(3-Fluorophenyl)-α-[1-[(1-methylethyl)sulfonyl]-2-amino-1*H*-benzimidazol-6-yl]-α-propargylmethanol (10a). To a dry 500-mL three-necked round-bottom flask equipped with an addition funnel, reflux condenser, and nitrogen inlet were added magnesium turnings (3.6 g, 148 mmol) and mercuric chloride (0.84 g, 3.10 mmol). Anhydrous Et_2O (150 mL) was added to the flask, and the whole assembly

was placed in a water-filled sonicator at room temperature. Next, the propargyl bromide (13.7 mL, 123 mmol, 80% in toluene) was added to the addition funnel. This was diluted with Et_2O (100 mL) and then slowly dripped into the flask. An ice bath was also prepared and kept on hand to control the reaction. Once initiated, the reaction started refluxing, and the addition of propargyl bromide was metered to maintain a controlled reaction. Sonication was begun at this point to facilitate complete reaction. This gave a clear, greenish-colored solution with only a small amount of Mg metal in the bottom of the flask. At this point, ketone **9a** (12.7 g, 35.2 mmol) was placed in a 1000-mL round-bottom flask, and THF (200 mL) was added (**9a** was not totally in solution). The Grignard reagent was then cannulated into the flask containing **9a**. After 2 h, the reaction was quenched by the addition of ice and 1 N HCl (200 mL). The mixture was then extracted with ethyl acetate (2 × 100 mL). The organic layers were combined and washed with saturated aqueous NH_4Cl (1 × 80 mL) and brine (1 × 80 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to give product **10a** (13.6 g, 33.8 mmol, 96% yield) as an orange solid which was taken on to the next step without further purification: ^{1}H NMR (DMSO-d_6) δ 1.23 (dd, J = 8.4, 6.9 Hz, 6H), 2.69 (t, J = 2.3 Hz, 1H), 3.15 (m, 2H), 3.80 (septet, J = 6.7 Hz, 1H), 6.06 (s, 1H), 6.91 (s, 2H), 6.94−7.37 (m, 5H), 7.68 (s, 1H); IR ($CHCl_3$) 3506, 3398, 3307, 1639, 1588, 1551, 1441, 1359, 1152, 1043 cm^{-1}; UV λ_{max} 257 (ϵ = 15 350), 213 nm (ϵ = 36 480); FDMS (MeOH) m/z 401 (M^+). Anal. ($C_{20}H_{20}FN_3O_3S$) C, H, N.

F. (*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(3-fluorophenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11a). To a solution of **10a** (13.6 g, 33.8 mmol) in methylene chloride (500 mL) were added 2,6-lutidine (4.71 mL, 40.6 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (8.53 mL, 37.2 mmol). After this mixture stirred for approximately 1 h, 4-(dimethylamino)pyridine (10.3 g, 84.5 mmol), triethylamine (21.1 mL, 152 mmol), and methanesulfonyl chloride (9.95 mL, 128 mmol) were added. The reaction mixture was allowed to stir for 2 h. After this time, the reaction mixture was diluted with methylene chloride (300 mL) and extracted with 1 N HCl (1 × 100 mL), saturated aqueous $NaHCO_3$ (1 × 100 mL), and brine (1 × 100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a reddish solid. This solid was dissolved in acetonitrile (100 mL), and water (∼50 mL) was added until a brownish solid started to come out of solution. The mixture was then cooled to 4 °C overnight. This typically resulted in the formation of a tan precipitate (mostly cis material) that was subsequently removed by filtration. The filtrate was ∼3:1 trans:cis. The isomers were separated using reverse-phase chromatography (gradient eluent of 45−50% acetonitrile in water). Two grams of material (filtrate) was loaded per run in acetonitrile (∼14 mL) to give almost baseline separation of the trans and cis isomers. This gave **11a**[28] (2.46 g, 6.42 mmol, 19% yield) with only traces of the cis material. In cases where the isomeric ratio was only ∼90:10, the ratio could be furthered improved by slurrying the compound after reverse-phase chromatography with acetonitrile (∼5 mL/g). Filtration of this slurry then yielded a solid with an improved ratio of trans:cis: ^{1}H NMR ($CDCl_3$) δ 1.39 (d, J = 6.8 Hz, 6H), 3.05 (d, J = 2.4 Hz, 1H), 3.61 (septet, J =6.8 Hz, 1H), 6.02 (d, J = 2.4 Hz, 1H), 6.05 (s, 2H), 7.04−7.10 (m, 2H), 7.14−7.39 (m, 4H), 7.55 (s, 1H); IR ($CHCl_3$) 3506, 3398, 3306, 2999, 1639, 1547, 1442, 1381 cm^{-1}; UV λ_{max} 317 (ϵ = 21 897), 263 (ϵ = 15 248), 212 nm (ϵ = 31 161); mp 150−155 °C dec; FDMS (MeOH) m/z 383 (M^+). Anal. ($C_{20}H_{18}FN_3O_2S$) C, H, N.

(*Z*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(2-fluorophenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11b): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR ($CDCl_3$) δ 1.38 (d, J = 6.9 Hz, 6H), 3.00 (d, J = 2.4 Hz, 1H), 3.60 (septet, J = 6.8 Hz, 1H), 6.18 (s, 2H), 6.21 (d, J = 2.5 Hz, 1H), 7.07−7.27 (m, 4H), 7.35−7.42 (m, 2H), 7.59 (d, J = 1.4 Hz, 1H); FDMS (MeOH) m/z 383 (M^+). Anal. ($C_{20}H_{18}FN_3O_2S$) C, H, N.

(*Z*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(2,5-difluorophenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11d): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR ($CDCl_3$) δ 1.38 (d, J = 6.8 Hz, 6H), 3.05 (d, J = 2.5 Hz, 1H), 3.61 (septet, J = 6.8 Hz, 1H), 5.93 (s, 2H), 6.21 (d, J = 2.5 Hz, 1H), 7.04−7.15 (m, 4H), 7.28 (s, 1H), 7.58 (s, 1H); IR ($CHCl_3$) 3506, 3398, 3307, 2986, 1639, 1493, 1362 cm^{-1}; UV λ_{max} 319.5 (ϵ = 24 117), 242.5 (ϵ = 15 234), 211 nm (ϵ = 27 698); FDMS (MeOH) m/z 401 (M^+). Anal. ($C_{20}H_{17}F_2N_3O_2S$) C, H, N, S.

(*Z*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(3,5-difluorophenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11e): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR ($CDCl_3$) δ 1.40 (d, J = 6.8 Hz, 6H), 3.10 (d, J = 2.5 Hz, 1H), 3.61 (septet, J = 6.8 Hz, 1H), 6.03 (d, J = 2.6 Hz, 1H), 6.04 (s, 2H), 6.82 (dt, J = 8.8, 2.1 Hz, 1H), 7.00 (d, J = 6.4 Hz, 2H), 7.08 (d, J = 8.2 Hz, 1H), 7.28 (d, J = 8.3 Hz, 1H), 7.53 (d, J = 0.9 Hz, 1H); IR ($CHCl_3$) 3306, 1694, 1640, 1620, 1547, 1482, 1361, 1155, 1121, 1046 cm^{-1}; UV λ_{max} 318 (ϵ = 15 655), 263 (ϵ = 17 393), 213 nm (ϵ = 32 410); FDMS (MeOH) m/z 401 (M^+). Anal. ($C_{20}H_{17}F_2N_3O_2S$) C, H, N.

(*Z*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(3,4-difluorophenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11f): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR (DMSO-d_6) δ 1.24 (d, J = 6.6 Hz, 6H), 3.90 (septet, J = 6.6 Hz, 1H), 4.10 (d, J = 2.6 Hz, 1H), 6.22 (d, J = 2.6 Hz, 1H), 7.07−7.12 (m, 3H), 7.18−7.23 (m, 2H), 7.37 (s, 1H), 7.41−7.58 (m, 2H); IR ($CHCl_3$) 3398, 3306, 2981, 1639, 1517, 1274, 1043 cm^{-1}; UV λ_{max} 319 (ϵ = 22 343); 261 (ϵ = 15 525), 213 nm (ϵ = 31 019); FDMS (MeOH) m/z 401 (M^+). Anal. ($C_{20}H_{17}F_2N_3O_2S$) C, H, N.

(*Z*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(3-fluoro-4-methoxyphenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine 11g: prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR ($CDCl_3$) δ 1.39 (d, J = 6.7 Hz, 6H), 3.06 (s, 1H), 3.62 (septet, J = 6.8 Hz, 1H), 3.93 (s, 3H), 5.91 (d, J = 0.9 Hz, 1H), 6.45 (s, 2H), 6.95 (t, J = 8.4 Hz, 1H), 7.09 (d, J = 8.2 Hz, 1H), 7.24 (m, 3H), 7.54 (s, 1H); IR ($CHCl_3$) 3398, 3306, 2960, 2815, 1638, 1271 cm^{-1}; UV λ_{max} 317 (ϵ = 22 342), 273 (ϵ = 17 661), 213 nm (ϵ = 33 257); mp 160−165 °C dec; FDMS (MeOH) m/z 413 (M^+). Anal. ($C_{21}H_{20}FN_3O_3S$) C, H, N.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(4-methylphenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11i): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 6.8 Hz, 6H), 2.44 (s, 3H), 3.06 (d, J = 3.0 Hz, 1H), 3.67 (septet, J = 6.8 Hz, 1H), 6.00 (d, J = 2.2 Hz, 1H), 7.12 (d, J = 8.1 Hz, 1H), 7.23 (d, J = 7.9 Hz, 2H), 7.29 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 7.9 Hz, 2H), 7.64 (s, 1H); IR ($CHCl_3$) 2970, 1638, 1609, 1547, 1310, 1268, 1156, 1044 cm^{-1}; UV λ_{max} 315 (ϵ = 20 514), 267 (ϵ = 17 276), 212 nm (ϵ = 32 191); FDMS (MeOH) m/z 379 (M^+). Anal. ($C_{21}H_{21}N_3O_2S$) N; C: calcd, 66.47; found, 65.79.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-[4-(dimethylamino)phenyl]-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11j): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR ($CDCl_3$) δ 1.45 (d, J = 6.8 Hz, 6H), 3.05 (s, 6H), 3.09 (d, J = 2.4 Hz, 1H), 3.68 (septet, J = 6.8 Hz, 1H), 5.82 (d, J = 2.5 Hz, 1H), 6.23 (s, 2H), 6.73 (d, J = 8.8 Hz, 2H), 7.17 (m, 1H), 7.30 (m, 1H), 7.46 (d, J = 8.8 Hz, 2H), 7.66 (s, 1H); IR ($CHCl_3$) 3507, 3398, 3306, 1638, 1608, 1584, 1547, 1523, 1439, 1359, 1267, 1155, 1044, 822 cm^{-1}; UV λ_{max} 304 (ϵ = 21 040), 211 nm (ϵ = 26 310); FDMS (MeOH) m/z 408 (M^+). Anal. ($C_{22}H_{24}N_4O_2S$) C, H, N.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-[3-(trifluoromethyl)phenyl]-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11k): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR ($CDCl_3$) δ 1.39 (d, J = 6.8 Hz, 6H), 3.04 (d, J = 2.5 Hz, 1H), 3.59 (septet, J = 6.8 Hz, 1H), 5.74 (s, 2H), 6.07 (d, J = 2.2 Hz, 1H), 7.09−7.14 (m, 1H), 7.23−7.33 (m, 1H), 7.50−7.56 (m, 2H), 7.63 (m, 1H), 7.71 (s, 1H), 7.73 (m, 1H); IR ($CHCl_3$) 3398, 3306, 2960, 1639, 1547, 1360, 1328, 1170, 1132, 1045 cm^{-1}; UV λ_{max} 319 (ϵ = 19 672), 265 (ϵ = 14 485), 214 nm (ϵ = 29 852); FDMS (MeOH) m/z 433 (M^+). Anal. ($C_{21}H_{18}F_3N_3O_2S$) C, H, N, F, S.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-[4-(trifluoromethoxy)phenyl]-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11l): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 6.8 Hz, 6H), 3.09 (d, J = 2.1 Hz, 1H), 3.66 (septet, J = 6.8 Hz, 1H), 6.02 (s, 2H), 6.05 (d, J = 2.7 Hz, 1H), 7.11 (m, 1H), 7.25−7.35 (m, 3H), 7.53−7.63 (m, 3H); IR ($CHCl_3$) 3398, 3306, 1638, 1547, 1387, 1360, 1262, 1227, 1174, 1044 cm^{-1}; UV λ_{max} 319 (ϵ = 21 800), 265 (ϵ = 16 340), 213 nm (ϵ = 30 850); FDMS (MeOH) *m/z* 449 (M^+). Anal. ($C_{21}H_{18}F_3N_3O_3S$) C, H, N.

(*Z*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(3-chlorophenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11m): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a**; ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 6.8 Hz, 6H), 3.10 (d, J = 2.1 Hz, 1H), 3.66 (septet, J = 6.8 Hz, 1H), 6.07 (d, J = 2.6 Hz, 1H), 6.15 (s, 2H), 7.13 (m, 1H), 7.30−7.47 (m, 5H), 7.57 (s, 1H); IR ($CHCl_3$) 3506, 3398, 3306, 2983, 1639, 1547, 1361, 1269, 1155, 1044, 823 cm^{-1}; UV λ_{max} 318 (ϵ = 21 950), 265 (ϵ = 16 040), 215 nm (ϵ = 37 200); FDMS (MeOH) *m/z* 399 (M^+). Anal. ($C_{20}H_{18}ClN_3O_2S$) C, H, N.

(*E*)-5-Fluoro-1-[(1-methylethyl)sulfonyl]-6-(1-phenyl-1-buten-3-ynyl)-1*H*-benzimidazol-2-amine (11n) and (*Z*)-5-Fluoro-1-[(1-methylethyl)sulfonyl]-6-(1-phenyl-1-buten-3-ynyl)-1*H*-benzimidazol-2-amine (11n′): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a** except these isomers could not be separated and as such were tested as a 1:1 mixture; isomer 1 ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 6.4 Hz, 6H), 2.99 (s, 1H), 3.62 (m, 1H), 6.20 (s, 1H), 6.79 (s, 2H), 7.00−7.50 (m, 6H), 7.75 (s, 1H); isomer 2 ^{1}H NMR ($CDCl_3$) δ 1.46 (d, J = 6.5 Hz, 6H), 3.16 (s, 1H), 3.62 (m, 1H), 5.95 (s, 1H), 6.70 (s, 2H), 7.00−7.80 (m, 7H); FDMS (MeOH) *m/z* 383 (M^+). Anal. ($C_{20}H_{18}FN_3O_2S$) C, H, N, F, S.

(*E*)-7-Fluoro-1-[(1-methylethyl)sulfonyl]-6-(1-phenyl-1-buten-3-ynyl)-1*H*-benzimidazol-2-amine (11o): prepared and purified substantially in accordance with procedures (A−F) listed above for **11a** except that these isomers could only be separated by crystallization; trans isomer was crystallized from acetonitrile:water, ∼4:1, at 4 °C, and structure of this isomer was determined by heteronuclear NOE experiments between the fluorine and the olefinic hydrogen; ^{1}H NMR ($CDCl_3$) δ 1.42 (d, J = 6.8 Hz, 6H), 3.11 (d, J = 2.6 Hz, 1H), 3.65 (septet, J = 6.8 Hz, 1H), 6.01 (d, J = 2.6 Hz, 1H), 6.27 (s, 2H), 6.86 (dd, J = 6.8, 8.2 Hz, 1H), 7.30−7.39 (m, 4H), 7.50−7.53 (m, 2H); IR ($CHCl_3$) 3506, 3398, 3306, 1646, 1384, 1087 cm^{-1}; UV λ_{max} 287 (ϵ = 16 790), 240 (ϵ = 37 382), 204 nm (ϵ = 25 933); FDMS (MeOH) *m/z* 383 (M^+). Anal. ($C_{20}H_{18}FN_3O_2S$) H, N; C: calcd, 62.65; found, 62.22.

(*Z*)-1-[(Dimethylamino)sulfonyl]-6-[1-(4-fluorophenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (12). This was prepared and purified substantially in accordance with procedures (A−F) listed above for **11a** except that dimethylsulfamoyl chloride was used in place of 2-propanesulfonyl chloride in procedure C. Subsequent compounds were capable of forming salts. Therefore, isolation of materials had to be somewhat altered based on typical procedures for salt-forming compounds (i.e., after acid washes the water layer was collected, adjusted to a basic pH, and then extracted with an organic): ^{1}H NMR ($CDCl_3$) δ 2.94 (s, 6H), 3.09 (d, J = 2.3 Hz, 1H), 6.07 (d, J = 2.5 Hz, 1H), 6.21 (brs, 2H), 7.07−7.46 (m, 6H), 7.50 (s, 1H); IR ($CHCl_3$) 3398, 3306, 3019, 2976, 1636, 1610, 1544, 1476, 1442, 1391, 1275, 1170, 1052, 969, 884, 823 cm^{-1}; UV λ_{max} 318 (ϵ = 22 150), 259 (ϵ = 14 930), 222 nm (ϵ = 27 680); FDMS (MeOH) *m/z* 384 (M^+). Anal. ($C_{19}H_{17}FN_4O_2S$) C, H, N, F, S.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(4-fluorophenyl)-4-(trimethylsilyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (14a) and (*Z*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(4-fluorophenyl)-4-(trimethylsilyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (14b). To a −78 °C solution of diethoxy 3-(trimethylsilyl)prop-2-ynylphosphonate (**13**) (6.21 g, 25 mmol) in anhydrous THF (5 mL) was added lithium bis(trimethylsilyl)amide (30 mL, 1 M solution in THF, 30 mmol). The resultant mixture was stirred for approximately 30 min and was then added to a −78 °C solution of **9c** (1.81 g, 5 mmol, general preparation A−D) in THF (20 mL). After approximately 15 min, the reaction mixture was warmed to room temperature, reacted overnight, and then partitioned between ethyl acetate (500 mL) and saturated aqueous ammonium chloride (60 mL). The resultant layers were separated, and the organic layer was washed with brine (1 × 50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to provide 6.16 g of an oil. This oil was purified using flash chromatography (silica gel, gradient eluent of 2−5% methanol in methylene chloride). The fractions containing the desired compound were combined and dried in vacuo to provide **14a** (400 mg, 0.879 mmol, 3.5% yield) and **14b** (207 mg, 0.455 mmol, 1.8% yield) as impure solids. Data for **14a**: ^{1}H NMR ($CDCl_3$) δ 0.12 (s, 9H), 1.37 (d, J = 6.8 Hz, 6H), 3.58 (septet, J = 6.8 Hz, 1H), 5.96 (s, 1H), 6.66 (brs, 2H), 7.00 (d, J = 8.6 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 7.47 (d, J = 10.6 Hz, 2H), 7.48 (dd, J = 23.0, 8.7 Hz, 2H), 7.53 (d, J = 1.2 Hz, 1H). Data for **14b**: ^{1}H NMR ($CDCl_3$) δ 0.14 (s, 9H), 1.35 (d, J = 7.0 Hz, 6H), 3.58 (septet, J = 7.0 Hz, 1H), 5.93 (s, 1H), 6.14 (brs, 2H), 6.96 (d, J = 8.6 Hz, 1H), 6.99 (d, J = 8.6 Hz, 1H), 7.22 (dd, J = 8.7, 5.6 Hz, 2H), 7.35 (dd, J = 22.7, 8.7 Hz, 2H), 7.64 (s, 1H).

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(4-fluorophenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (11c). To a solution of **14a** (280 mg, 0.614 mmol) in methylene chloride and acetonitrile (1:5) was added cesium fluoride (93.3 mg, 0.614 mmol). After approximately 2 h at room temperature, the reaction mixture was partitioned between brine (30 mL) and methylene chloride (30 mL). The resultant layers were separated, and the organic layer was dried (Na_2SO_4), filtered, and then concentrated in vacuo to provide an oil. This oil was purified using reverse-phase column chromatography (eluent of 0−5% acetonitrile in water) followed by reverse-phase HPLC (eluent of 60% acetonitrile in water) to provide **11c** (106 mg, 0.277 mmol, 45% yield; data previously reported).[20]

DL-α-(3-Fluorophenyl)-α-methyl-α-[1-[(1-methylethyl)-sulfonyl]-2-amino-1*H*-benzimidazol-6-yl]methanol (15). To a 0 °C solution of **9a** (25.4 g, 70 mmol) in THF (600 mL) was slowly added methylmagnesium bromide (117 mL, 350 mmol, 3 M solution in diethyl ether). The reaction was monitored to ensure that the temperature stayed below room temperature. After approximately 1 h, the reaction was quenched by the slow addition of a saturated ammonium chloride solution. The resulting layers were separated, and the desired compound was extracted from the aqueous layer with an additional ethyl acetate (500 mL) wash. The resultant organic portions were combined, washed with brine (1 × 100 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to provide **15** (26.4 g, 70 mmol, 100% yield). The compound was taken to the next step without further purification.

1-[(1-Methylethyl)sulfonyl]-6-[1-(3-fluorophenyl)ethylene]-1*H*-benzimidazol-2-amine (16). To a solution of **15** (26.4 g, 70 mmol) in chloroform (300 mL) was added *p*-toluenesulfonic acid (27 g, 157 mmol). The resultant reaction mixture was refluxed for approximately 2 h. The reaction was cooled to room temperature and washed sequentially with water (1 × 50 mL), saturated sodium bicarbonate (1 × 50 mL), and brine (1 × 50 mL). It was then dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a brown foam. This foam was triturated in diethyl ether and then filtered to provide **16** (22.7 g, 63 mmol, 90% yield) as a tan solid. This compound can be used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.45 (d, J = 6.8 Hz, 6H), 3.68 (septet, J = 6.8 Hz, 1H), 5.52 (s, 2H), 6.35 (s, 2H), 7.04−7.10 (m, 2H), 7.17−7.25 (m, 2H), 7.29−7.38 (m, 2H), 7.64 (s, 1H); IR ($CHCl_3$) 3420, 1639, 1610, 1579, 1550, 1438, 1359, 1155, 1043 cm^{-1}; UV λ_{max} 280 (ϵ = 15 482), 256 nm (ϵ = 14 929); FDMS (MeOH) *m/z* 359 (M^+). Anal. ($C_{18}H_{18}FN_3O_2S$) C, H, N.

(*E*/*Z*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(3-fluorophenyl)-2-bromoethylene]-1*H*-benzimidazol-2-amine (17a,b) and 1-[(1-Methylethyl)sulfonyl]-6-[1-(3-fluorophenyl)-2,2-dibromoethylene]-1*H*-benzimidazol-2-amine (17c). To a solution of **16** (22.6 g, 63 mmol) in THF (500 mL) was added *N*-bromosuccinimide (16.7 g, 93.8 mmol). The resultant reaction mixture was refluxed for approximately 3 h. The reaction mixture was then cooled to room temperature and stirred overnight. The reaction mixture was concentrated in vacuo

to provide a residue which was dissolved in ethyl acetate (600 mL), washed with water (1 × 60 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a red foam. This foam was dissolved in diethyl ether and then dried in vacuo to provide 32 g of a red solid. The desired monobromination products **17a**,**b** were separated from the dibromide **17c** by precipitation from acetonitrile (~100 mL) at 4 °C to provide **17a**,**b** (5.66 g, 12.9 mmol, 20% yield) as a solid. This mixture of compounds was used without further purification. Isomer **17a** was assigned as trans through NMR experiments which showed an NOE between the olefinic hydrogen and the C-7 and C-5 hydrogens of the benzimidazole core. Data for **17a**: ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 6.7 Hz, 6H), 3.66 (septet, J = 6.7 Hz, 1H), 6.19 (s, 2H), 6.81 (s, 1H), 7.05−7.17 (m, 4H), 7.28−7.31 (m, 1H), 7.41−7.46 (m, 1H), 7.55 (s, 1H). Data for **17b**: ^{1}H NMR ($CDCl_3$) δ 1.46 (d, J = 6.5 Hz, 6H), 3.68 (septet, J = 6.5 Hz, 1H), 6.19 (s, 2H), 6.85 (s, 1H), 6.97 (m, 1H), 7.05−7.17 (m, 3H), 7.28−7.31 (m, 1H), 7.41−7.46 (m, 1H), 7.69 (s, 1H). Data for **17a**,**b**: IR ($CHCl_3$) 3430, 1638, 1610, 1548, 1438, 1359, 1155, 1043 cm^{-1}; UV λ_{max} 289 (ϵ = 17 127), 262 nm (ϵ = 16 964); FDMS (MeOH) m/z 439 (M^+). Anal. ($C_{18}H_{17}BrFN_3O_2S$) C, H, N. Data for **17c**: ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 6.8 Hz, 6H), 3.66 (septet, J = 6.8 Hz, 1H), 6.37 (s, 2H), 7.02−7.17 (m, 4H), 7.30−7.42 (m, 2H), 7.67 (s, 1H); IR ($CHCl_3$) 3430, 1638, 1610, 1549, 1439, 1360, 1155, 1044 cm^{-1}; UV λ_{max} 282 (ϵ = 17 107), 257 nm (ϵ = 17 821); FDMS (MeOH) m/z 517 (M^+). Anal. ($C_{18}H_{16}Br_2FN_3O_2S$) H, N; C: calcd, 41.80; found, 43.79.

***(Z)*-1-[(1-Methylethyl)sulfonyl]-6-[1-(3-fluorophenyl)-1-penten-3-ynyl]-1*H*-benzimidazol-2-amine (18a).** To a solution of **17a,b** (4.00 g, 9.1 mmol) in THF (25 mL) was added bis(triphenylphosphine)palladium(II) chloride (210 mg, 0.3 mmol) followed by diisopropylamine (12.7 mL, 91 mmol). After the resultant mixture stirred for approximately 10 min, copper(I) iodide (170 mg, 0.91 mmol) was added. The resultant mixture was stirred for another 10 min, and then propyne(g) was bubbled through the mixture for approximately 1.75 h. The reaction mixture was diluted with diethyl ether (300 mL), washed sequentially with a saturated ammonium chloride solution (1 × 40 mL), a 1 N HCl solution (1 × 40 mL), and a saturated sodium bicarbonate solution (1 × 40 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a residue. This residue was purified using flash chromatography (silica gel, gradient eluent of 60−80% ethyl acetate in hexanes) to give **18a**,**b** (2.0 g, 5.0 mmol, 55% yield) as a solid. The trans isomer **18a** was then separated from the cis isomer using reverse-phase chromatography: ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 6.8 Hz, 6H), 1.99 (s, 3H), 3.66 (septet, J = 6.8 Hz, 1H), 6.04 (d, J = 2.0 Hz, 1H), 6.26 (s, 2H), 7.08−7.13 (m, 2H), 7.27−7.41 (m, 4H), 7.58 (s, 1H); IR ($CHCl_3$) 3398, 2984, 1639, 1639, 1610, 1548, 1441, 1360, 1269, 1174, 1155, 1043 cm^{-1}; UV λ_{max} 315 (ϵ = 22 318), 267 (ϵ = 16 135), 212 nm (ϵ = 32 383); FDMS (MeOH) m/z 397 (M^+). Anal. ($C_{21}H_{20}FN_3O_2S$) C, H, N.

Cytopathic Effect Assay. The cytopathic effect (CPE) assay was performed as described previously[29] with a modification to the method of the quantification of the cytopathic effect of virus on the cells. Instead of using crystal violet staining, XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) was used as substrate to quantify the surviving host cells.[30]

General Plaque Reduction Assay. Susceptible HeLa cells were grown in 6-well tissue culture cluster plates at 37 °C in minimum essential medium (MEM) with 5% fetal bovine serum, 10 mM $MgSO_4$, penicillin (100 U/mL), and streptomycin (100 μg/mL). When confluent monolayers were formed, growth medium was removed and 0.2 mL/well of an appropriate dilution of virus was added. After adsorption for 1−2 h at room temperature, the infected cell sheet was overlaid with equal parts of 1.5% sterile agarose solution and a 2-fold concentration of MEM (2% fetal bovine serum, 10 mM $MgSO_4$, penicillin (100 U/mL), and streptomycin (100 μg/mL)) containing varying concentrations of the compounds to be tested.

The compounds were dissolved in DMSO at a concentration of 20 mg/mL, and an aliquot was diluted to the desired concentration in DMSO before addition to the agar medium mixture. Plates were incubated at 34 °C for rhinoviruses and at 37 °C for the enteroviruses until the DMSO control wells demonstrated plaques of optimal size. At this time, a solution containing 10% formalin and 2% sodium acetate was added to each well to inactivate the virus and fix the cell sheet to the plastic surface. The fixed cell sheets were stained with 0.5% crystal violet, and the plaques were counted. Results from duplicate wells at each concentration were averaged and compared with DMSO control wells. The inhibition of plaque formation by 50% (IC_{50}) was calculated from the linear region of the inhibition concentration curve using the method of Reed and Muench.[31]

Protocol for Mouse Oral Plasma Level Screening Studies. One equivalent of the compound to be studied was dissolved in absolute ethanol along with 5 equiv of PVP-30 (Aldrich; poly(vinylpyrrolidone), av MW= 40 000). The solvent was removed by rotary evaporation to give a glassy foam. The foam was removed from the flask and ground to a fine powder using a mortar and pestle. The compound was suspended in 2% Emulphor (98% water). The resulting suspension was then subjected to extensive sonication to give a fine suspension. The mice (ICR Swiss white, female mice weighing 15−20 g) were then dosed (usually 3/group) by both gavage and ip at 100 mg/kg (dose volume of 0.25 mL).

At selected time intervals (usually 15, 30, 60, 120, and 240 min), the mice were lightly anesthetized over dry ice and bled from the orbital sinus to obtain blood (0.050 mL/mouse). The blood from each group was pooled, and an equal volume of acetonitrile (0.150 mL) was added. After vortexing (10−20 s), the liquid fraction was separated from the coagulated blood cell debris by microcentrifugation in the cold for approximately 5 min. The liquid supernatant was then collected and analyzed by analytical HPLC utilizing C18 reverse-phase columns with UV detection.

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