Synthesis, Antiviral Activity, and Biological Properties of Vinylacetylene Analogs of Enviroxime

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A series of vinylacetylene analogs of Enviroxime (**1**) was synthesized. The new compounds are potent inhibitors of poliovirus in tissue culture. Cross-sensitivity with Enviroxime-derived mutants shows that the new compounds have the same mechanism of action as Enviroxime, which involves the viral 3A protein. In studies with Rhesus monkeys, the *p*-fluoro derivative **12** was found to be unique in providing oral bioavailability. Metabolism studies using hepatic microsomes suggest that this procedure would be a useful *in vitro* method for selecting the appropriate animal model for testing oral absorption. Compound **12** was found to be efficacious by oral administration in treating a Coxsackie A21 infection in CD-1 mice.

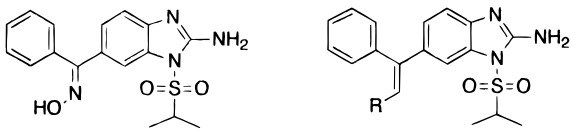
Introduction

Rhinoviruses are the major cause of the "common cold" and may be responsible for 50% of all acute respiratory infections in man.[1] Whereas "colds" are generally mild and of a relatively short duration, the sheer number of infections has a huge economic impact in terms of absenteeism from school and work.[1−3] In addition "colds" cause an enormous number of hours of general human misery.

The search for a drug to effectively treat the "common cold" caused by rhinovirus infection faces a number of significant challenges. First, "colds" can be caused by more than 100 different strains of rhinoviruses,[4,5] and therefore a potential drug must have broad-spectrum antiviral activity. Because the genome of RNA viruses is replicated with notoriously poor fidelity,[6] an antiviral target should be chosen which will minimize the propensity for the development of drug resistance. Also, because rhinovirus infections are typically mild and self-limiting, it is required that an appropriate medication be conveniently administered (most probably by the oral route) and very safe.

In recent years significant progress has been made in developing a treatment for the "common cold". Most notably these efforts have emanated from a class of compounds that exhibit their antiviral effect through viral capsid binding ("canyon compounds").[7] It has been observed, however, that some of these compounds have suffered from several deficiencies. In spite of the fact that they are often very potent antivirals against selected rhinovirus strains, they have frequently shown a limited spectrum of potency when tested against a wider variety of different rhinoviruses.[8] In addition, studies with certain capsid-binding compounds in both tissue culture and human volunteers have shown that drug resistant mutants are readily selected.[9,10] Finally, many of the clinical studies with capsid-binding compounds have required intranasal administration because the candidate drug lacked sufficient bioavailability when given orally.[7]

In considering a strategy for the development of an anti-rhinovirus drug which has both broad-spectrum activity and limited potential for the development of resistance, we felt that it was important to avoid targets associated with the viral protein coat, where one might expect problems in these areas to be most strongly manifested. Instead, we felt that it would be prudent to direct our efforts toward a highly conserved protein target essential to the virus' replicative machinery.



1

2, R = CH_3
3, R = CH_2OH
4, R = CD_3
5, R = $CH{=}CH_2$
6, R = $C{\equiv}CH$

Figure 1.

In the early 1980s, two closely related benzimidazoles, Enviroxime (**1**) and Enviradene (**2**), were evaluated in clinical studies. Enviroxime, which was found to have emetic side effects and poor oral bioavailability in man,[11,12] was evaluated in a series of rhinovirus challenge studies. When the compound was delivered by intranasal administration, it was determined to have limited product potential as a treatment for the "common cold",[12−15] and further studies were discontinued. In subsequent studies, Enviradene, which had demonstrated superior oral bioavailability in rat and dog and did not cause emesis in test animals,[16] was unexpectedly found to give very low oral blood levels in man, and once again further clinical studies were terminated.[17]

In spite of the clinical failures of Enviroxime and Enviradene, we were drawn to these benzimidazoles by the fact that both compounds exhibited potent broad-spectrum anti-rhinovirus and anti-enterovirus activity;[18−20] both compounds were highly selective, with tissue culture activity restricted to the aforementioned members of the picornavirus family, and further, preliminary studies indicated a mechanism of action directed toward the inhibition of viral RNA synthesis.[21]

Intrigued by the potential of these compounds, workers initiated further virology studies to investigate their mechanism of action. From these studies it was determined that Enviroxime selectively inhibits plus strand

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Table 1. Enviroxime (**1**) Sensitive Mutants of Human Rhinovirus 14

3A amino acid substitution	resistance	
	IC_{50} (μg/mL)[a]	survivors (%)[b]
Glu 30 → Asp	0.04	0.6
→ Val	0.04	1.1
→ Gln	0.04	1.5
Ile 42 → Val	0.04	2.1
Met 54 → Ile	0.04	5.5
wild-type	0.04	0.03

[a] Standard plaque reduction assay. [b] Extended cycle replication assay, conducted in 1 μg/mL Enviroxime (**1**). Numbers are relative to no-drug control.

Table 2. Comparison of Enviradene Analogs: *In Vitro* Antiviral Activity and Plasma Levels in Rhesus Monkeys following Oral Dosing

compd[a]	IC_{50} (μg/mL)[b]	peak plasma conctn (ng/mL)[c]
2	0.06	14−140[d]
4	0.10	10−12[e]
5	0.09	none detected[e]
6	0.02	70−300[d]

[a] The structures of the compounds are shown in Figure 1. [b] Poliovirus (type 1, Mahoney) plaque reduction assay. The compounds were not toxic at 1 μg/mL or lower. [c] All compounds were dosed orally at 8 mg/kg formulated 5/1 with PVP-30, except compound **5** which was formulated with 50% cellulose and polysorbate 80. [d] Range for four monkeys. [e] Range for three monkeys.

viral RNA synthesis. In addition, all efforts to develop drug resistant mutants, as measured by a significant increase in the IC_{50}, failed. However, drug sensitive mutants could be selected (see Table 1), and in every case these mutants mapped to rhinovirus protein 3A, clearly indicating that 3A is somehow involved in the antiviral activity of Enviroxime.[22] Protein 3A is thought to serve as a scaffold for the virus replication complex[23] and is highly conserved among both rhinoviruses and enteroviruses.[24] For these reasons, further drug development efforts with the benzimidazoles appeared to be a desirable course of action.

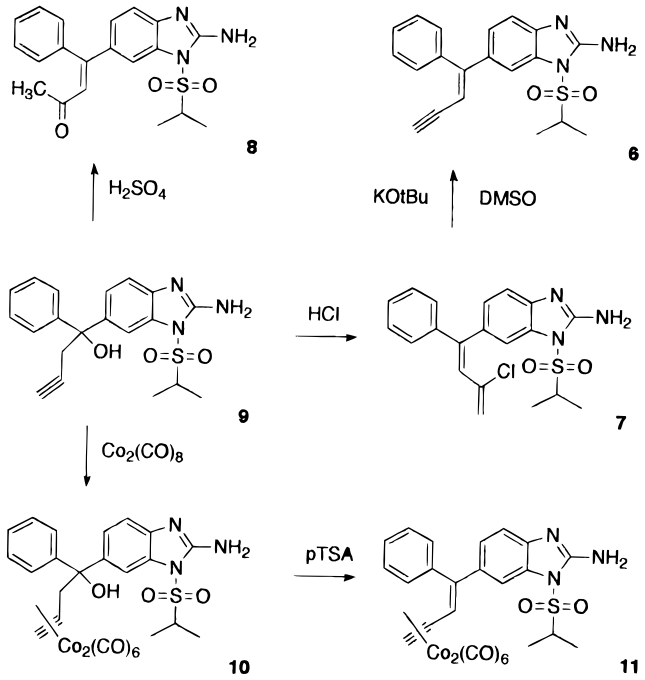
During the clinical evaluation of Enviradene, a radiolabeled drug study had indicated that the very low blood levels in man were due to rapid metabolism.[17] It was subsequently found that Enviradene also gave poor blood levels on oral administration to monkeys.[25] Because the rapid metabolism was in part caused by allylic oxidation of the vinyl methyl group to a hydroxymethyl metabolite (**3**),[17] we began our studies by modifying this functional group, using the monkey as our animal model for oral bioavailability.

When the methyl group was replaced by trideuteriomethyl (**4**), or vinyl (**5**), the oral administration of these modified analogs also gave very poor blood levels in monkeys. However, when methyl was replaced by acetylene (**6**) significant blood levels in monkey were observed (Table 2).[26] We therefore initiated the synthesis of a series of 4′-substituted derivatives of **6** to determine if compounds with further improved antiviral activity and/or oral bioavailability could be obtained.

Chemistry

Compound **4** was prepared following the synthetic procedure for Enviradene.[27] Compound **5** was prepared in a similar manner by reacting the vinyl Grignard reagent with 2-amino-6-benzoyl-1-(isopropylsulfonyl)-

Scheme 1



benzimidazole[19] to give the substituted hydroxy derivative, which was then eliminated under acidic conditions to give a mixture of *cis* and *trans* olefinic isomers.[28] The desired *trans* isomers (the *trans* isomers have considerably more antiviral active than the *cis* isomers)[29] were then purified by preparative reverse phase HPLC. Likewise the propargyl Grignard was reacted to give the hydroxy intermediate **9** in good yield (Scheme 1), but efforts to eliminate this alcohol under acidic conditions failed to give the vinylacetylene **6.** Instead, when HCl was used as the acid, the major product was a *cis*/*trans* mixture of the vinyl chloride **7**. The product apparently arises from the addition of the chloride counterion to a protonated intermediate. Efforts to avoid this problem by using an acid with a weaker counterion (H_2SO_4) resulted in addition of H_2O to give a *cis*/*trans* mixture of methyl ketone **8**. Conversion of the vinyl chloride **7** to acetylene **6** was successfully carried out using potassium *tert*-butoxide in DMSO,[30] but the reaction proceeded in low yield and the route was abandon.

The problem was resolved by first reacting hydroxyacetylene intermediate **9** with dicobalt octacarbonyl to give the hydroxycobalt complex **10**.[31] With the acetylene moiety complexed, the hydroxy group was successfully eliminated under acidic conditions without incident, and the acetylene was then freed from the cobalt complex with ferric nitrate[31] to give **6** as a *cis*/*trans* mixture. During the synthesis of the various 4′-substituted derivatives, when an unfavorable *cis* to *trans* isomer ratio occurred, the mixture was photolized in methanol (Pyrex filter) to restore the isomer ratio to approximately 1/1, prior to purification by reverse phase HPLC.

The basic route for the various 4′-ketones required for starting materials for the synthesis of the acetylene derivatives **12**−**17** is shown in Scheme 2. The appropriate Friedel−Crafts reaction with 3,4-dinitrobenzoyl chloride was followed by catalytic hydrogenation to give the 3,4-diaminobenzophenone; then the more nucleophilic 3-amino group was selectively sulfonylated. At

Scheme 2

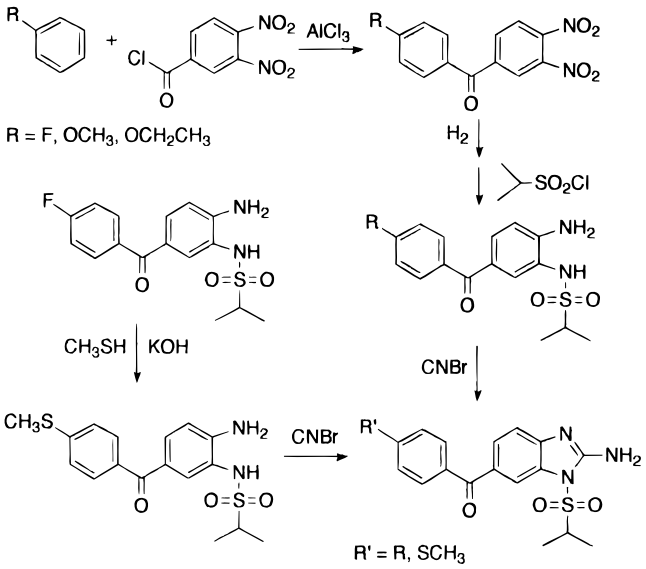
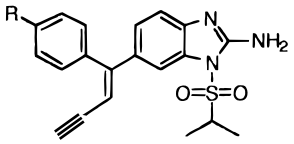


Table 3. Comparison of 4′-Substituted Acetylenic Analogs: *In Vitro* Antiviral Activity and Oral Bioavailability in Rhesus Monkeys



compd	4′ group	IC_{50} (μg/mL)[a]	oral bioavailability (%)[b]
13	OCH_3	0.01	5
6	H	0.02	9
12	F	0.04	23
15	SCH_3	0.05	5
17	$S(O)_2CH_3$	0.05	0
16	$S(O)CH_3$	0.07	3
14	OCH_2CH_3	0.07	4

[a] Polioviurus (type 1, Mahoney) plaque reduction assay. The compounds were not toxic at 1 μg/mL or lower. [b] 16 mg/kg oral dose formulated 5/1 with PVP-30.

this point the 4′-fluorobenzophenone was reacted with methanethiol under basic conditions to give the 4′-methanethiol derivative. The synthesis of the benzimidazole ketones was then completed by reaction with cyanogen bromide in the presence of base. We found it preferable to isolate the *trans*-4′-methanethiol vinylacetylene derivative **15** before oxidation to the methanesulfinyl and methanesulfonyl compounds **16** and **17**.

Biological Evaluation

The *trans*-vinylacetylenes **6** and **12**−**17** were first evaluated for their antiviral activity using poliovirus as a representative for the rhinovirus and enterovirus members of the picornavirus family (Table 3). All seven compounds show good antiviral activity in tissue culture with compound **13** being the most active. It would be tempting to suggest that the improvement in antiviral activity seen with compound **13** is the result of the electron-donating potential of the *p*-methoxy group, especially when coupled with the drop in antiviral activity observed in compound **12**, which has electron-withdrawing *p*-fluoro substitution. However, an important role for electronic effects is not strongly supported by the lack of differentiation in the antiviral activities

Table 4. Enviroxime (**1**) Sensitive Mutants of Human Rhinovirus 14 Selected Using Compound **13**

3A amino acid substitution	resistance (% survivors)[a] to	
	13	**1**
Asp 28 → Tyr	4.7	2.0
Ile 32 → Val[b]	2.3	0.7
Glu 34 → Val	5.0	1.5
Ile 42 → Val[b,c]	5.6	1.7
Glu 51 → Val	3.8	1.3
Met 54 → Ile[c]	3.5	1.9
Gly 72 → Ser[b]	6.8	4.9
Ile 77 → Val	2.2	0.7
wild-type	0.05	0.07

[a] Extended cycle replication assay, conducted in 1 μg/mL compoud. Numbers are relative to no-drug control. [b] Also selected using compound **12**. [c] Also selected using Enviroxime (**1**).

Table 5. Comparison of Hepatic Microsomal Metabolism

	metabolized (%)				
compd[a]	mouse	rat	dog	monkey	human
1	24	39	37	80	98
2	36	27	40	88	73
12	13	29	31	37	12

[a] The structures of the compounds are shown in Figure 1 and Table 3.

of *p*-methyl sulfide **15**, *p*-methyl sulfoxide **16**, and *p*-methyl sulfone **17**. On the other hand, the 7-fold loss in activity in going from *p*-methoxy (**13**) to *p*-ethoxy (**14**) indicates that there is little tolerance for increasing size or lipophilicity at the *para* position.

Rhinovirus mutants were selected for sensitivity to compound **13** following the same procedure used to select for Enviroxime sensitivity shown in Table 1. Eight sensitive mutants were isolated and characterized (see Table 4). All eight isolates selected with compound **13** show mutations in 3A, and all eight show cross-sensitivity with Enviroxime. In fact, two of the mutants, Ile 42 → Val and Met 54 → Ile, have the same mutation as isolates selected using Enviroxime. Further, when rhinovirus mutants were selected with compound **12**, three of these also had the same mutation observed with isolates derived with compound **13**. These results suggest that the antiviral mechanism of action for the vinylacetylene derivatives involves the 3A protein and is similar to the mechanism of action of Enviroxime.

When the compounds were tested for oral bioavailability in Rhesus monkeys (see Table 3), only the *p*-fluoro compound **12** gave good blood levels. Although it was not the major goal of the study, it was noted that none of the acetylene derivatives caused emesis in the monkey when dosed either orally or by iv administration. The oral bioavailability of the *p*-fluoro compound **12** in the monkey prompted further studies; in the Fischer rat, **12** was found to be 51% orally bioavailable when dosed at 20 mg/kg.

With these results in hand, we wondered retrospectively what role metabolism might play in choosing an appropriate animal model for correctly predicting the oral bioavailability of the benzimidazoles in man. To this end, Enviroxime (**1**), Enviradene (**2**), and the *p*-fluoroacetylene **12** were compared in an *in vitro* metabolism study using hepatic microsomes from several potential animal models and from man (see Table 5). In previously reported studies, oral administration of Enviroxime (**1**) gave moderate oral blood levels in rat

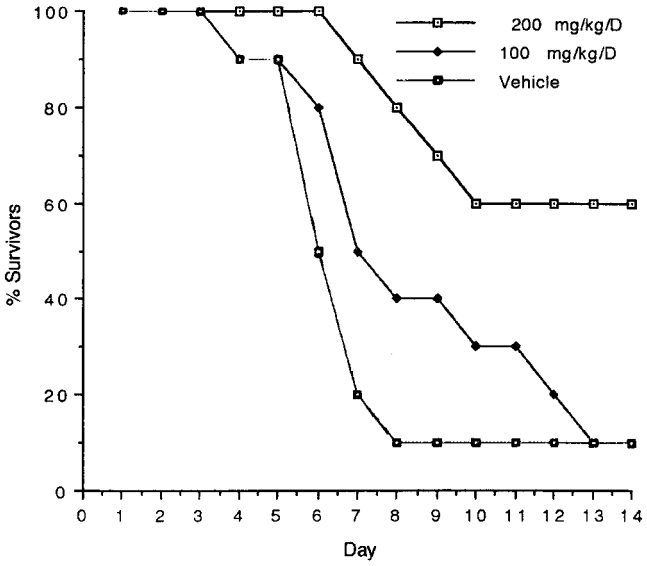


Figure 2. Effects of compound **12** on Coxsackie A21 infection in CD-1 mice. The IC_{50} (plaque reduction assay) for compound **12** against mouse-adapted Coxsackie A21 is 0.06 μg/mL. The survival index (SI) for each mouse was calculated as described in the Experimental Section. The mean SI (±SD) for vehicle, 100 mg/kg/d, and 200 mg/kg/d groups are 2.12 (±0.474), 2.77 (±2.742; p = not significant), and 5.40 (±0.998; p = 0.001), respectively.

and dog but very poor oral blood levels in man.[11] Oral administration of Enviradene (**2**) gave good blood levels in rat and dog but poor blood levels in monkey and man.[16,17,25] These results would have been predicted by the *in vitro* metabolism study shown in Table 5. The study further suggests that for both compounds **1** and **2**, the monkey would have been the most appropriate animal model for man. In contrast, the results for compound **12** show that the rat should be predictive for the monkey (as observed) and further suggest that rat or mouse would be an appropriate choice as a test animal for predicting oral bioavailability in man.

Finally, compound **12** was tested for antiviral efficacy in the Coxsackie A21 mouse model (see Figure 2). While it is not a true model in the sense that it does not cause a respiratory infection in the mouse, it does provide an opportunity to test compound **12** in a live animal infection, against a representative virus from the picornavirus family. When compound **12** was dosed by oral gavage twice a day at 25 mg/kg, there was no difference from vehicle control (result not shown). However at both 50 and 100 mg/kg twice a day by oral gavage, compound **12** afforded protection (statistically significant at 100 mg/kg) in a dose-dependent manner.

Conclusion

We have synthesized a series of vinylacetylene benzimidazoles and have found that these analogs of Enviroxime (**1**) and Enviradene (**2**) are potent antivirals against poliovirus in tissue culture and that they share the same mechanism of action as the earlier reported benzimidazoles. It is therefore reasonable to speculate that these and other structurally related acetylene derivatives will have broad-spectrum antiviral activity against both rhinoviruses and enteroviruses and that they will also have a limited potential for the development of resistance.

We have shown that the *p*-fluoro substitution of compound **12** is critical in providing improved oral bioavailability in the monkey. It is not clear at this time precisely what role fluorine substitution plays. It may be that the electron-withdrawing character of fluorine deactivates the aromatic ring toward metabolic oxidation. Or perhaps the somewhat lipophilic character of fluorine coupled with its ability to hydrogen bond provides a unique mixture of physical properties which assist in the absorption process. In any case, further evaluation of fluorine-substituted analogs and their ability to provide improved oral biavailability seems warranted.

It has been demonstrated that for this series of benzimidazoles, *in vitro* metabolism studies with hepatic microsomes can play an important role in selecting an appropriate animal model for oral bioavailability studies. Further, our results suggest that compound **12** would have good oral bioavailability in man. In addition, it was established that compound **12** is efficacious when dosed by oral gavage in the Coxsackie A21 mouse model.

Taken together, the results reported here indicate that a more intensive study of the antiviral activity and associated properties of fluoro-substituted vinylacetylenic benzimidazoles should be carried out.

Experimental Section

Reactions were followed by TLC with Merck F254 silica gel plates. Reverse phase chromatography was carried out with a Waters PrepLC System 500A instrument using PrepPAK 500 cartridges for preparative liquid chromatography. ^{1}H NMR spectra were recorded on a Bruker QE-300 or a Bruker AC-250 spectrometer; IR spectra were recorded on a Nicolet 5109 FT-IR spectrometer; UV spectra were recorded on a Shimadzu UV-2101 PC spectrometer; FDMS spectra were recorded on a VG Analytical VG 70 SE spectrometer. ^{1}H NMR spectra, IR spectra, UV spectra, FDMS spectra, and microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-(1-phenyl-1-propenyl-*3,3,3-d*$_3$)-1*H*-benzimidazol-2-amine (4): prepared following the procedure described for Enviradene (**2**);[27] ^{1}H NMR (DMSO-d_6) δ 7.41−7.29 (m, 4H), 7.20−7.09 (m, 3H), 6.98−6.91 (m, 3H), 6.11 (s, 1H), 3.84 (septet, J = 7 Hz, 1H), 11.23 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3694, 3398, 1638, 1551, 1385, 1357, 1175, 1154, 1044 cm^{-1}; UV λ_{max} 276 nm (ϵ 17 580); FDMS (MeOH) m/z 358 (M^+). Anal. ($C_{19}H_{18}N_3O_2S_1D_3$) C, H, N, S.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-(1-phenyl-1,3-butadienyl)-1*H*-benzimidazol-2-amine (5). Following the general procedure described in ref 28, 6-benzoyl-1-[(1-methylethyl)-sulfonyl]-1*H*-benzimidazol-2-amine[19] (6.8 g, 20 mmol) was dissolved in dry THF (250 mL) and added to a 500 mL three-necked flask equipped with an addition funnel and nitrogen inlet. The solution was cooled to 0 °C in an ice bath, and a 1 M solution of allylmagnesium bromide in diethyl ether (100 mL) was added dropwise. Following the addition, the ice bath was removed, and the reaction mixture was stirred at room temperature for 2 h. At this time TLC (silica gel with eluent 75% chloroform, 20% ethyl acetate, 5% acetic acid) showed complete reaction of the ketone, and the reaction was quenched by the careful addition of 1 N HCL (200 mL); ethyl acetate (1 L) was added, the organic phase separated and dried over magnesium sulfate, and the solvent removed by rotary evaporation. The residue was then redissolved in chloroform (300 mL), *p*-toluenesulfonic acid (7 g) was added, and the reaction mixture was refluxed under nitrogen for 4 h. The chloroform was then removed by rotary evaporation and replaced by ethyl acetate (1 L). The solution was then washed three times with 500 mL portions of brine, the organic phase separated and dried over magnesium sulfate, and the solvent removed under vacuum to give 3.5 g (47.5% yield) of a residue which is a 1/1 mixture of the *E* and *Z* (*cis* and *trans*) isomers. The desired *trans* isomer was separated by reverse phase HPLC with a

gradient of 35−40% THF/water: ^{1}H NMR (DMSO-d_6) δ 7.48−7.34 (m, 4H), 7.22−7.14 (m, 3H), 7.10−6.98 (m, 3H), 6.74 (d, J = 11 Hz, 1H), 6.33−6.20 (m, 1H), 5.49 (dd, J = 17, 2 Hz, 1H), 5.13 (dd, J = 10, 2 Hz, 1H), 3.83 (septet, J = 7 Hz, 1H), 1.23 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3506, 3398, 1637, 1605, 1548, 1360, 1155 cm^{-1}; UV λ_{max} 309 nm (ϵ 22 036); FDMS (MeOH) m/z 367 (M^+). Anal. ($C_{20}H_{21}N_3O_2S_1$) C, H, N, S.

General Procedure for Vinylacetylenes 6 and 12−15 from the Corresponding Ketones. DL-2-Amino-A-propargyl-1-[(1-methylethyl)sulfonyl]-A-phenyl-1*H*-benzimidazole-6-methanol (9). Magnesium turnings (30 g, 1234 mmol) were added to a 2 L three-necked flask equipped with an addition funnel, reflux condenser, and nitrogen inlet. Anhydrous THF (200 mL) was added followed by mercuric chloride (500 mg) and approximately 3 mL of propargyl bromide (80% in toluene) to initiate the reaction. As the reaction mixture began to reflux, a mixture of 6-benzoyl-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazol-2-amine[19] (30 g, 87.5 mmol) and propargyl bromide (115 mL, for a total of 1060 mmol) in anhydrous THF (1200 mL) was slowly added at a rate to keep the reaction mixture gently refluxing. After the addition was complete, approximately 2.5 h, the reaction mixture was stirred for an additional 90 min. When the ketone was completely reacted as indicated by TLC (silica gel with eluent 66% chloroform, 26% ethyl acetate, 8% acetic acid), the reaction was quenched by the addition of ice and 1 N HCl (1.3 L). The mixture was then extracted with ethyl acetate (1 L), the organic phase was dried over magnesium sulfate, and the solvent was removed by rotary evaporation. The residue was purified by silica gel flash chromatography with a gradient of methanol in dichloromethane (0−5%) to give 27 g of product or 80% yield: ^{1}H NMR (DMSO-d_6) δ 7.69 (s, 1H), 7.43 (d, J = 9 Hz, 2H), 7.32−7.09 (m, 5H), 6.89 (s, 2H), 5.88 (s, 1H), 3.77 (septet, J = 7 Hz, 1H), 3.12 (d, J = 3 Hz, 2H), 2.66 (t, J = 3 Hz, 1H), 1.21 (d, J = 7 Hz, 3H), 1.24 (d, J = 7 Hz, 3H); IR ($CHCl_3$) 3308, 1639, 1551, 1438, 1387, 1359, 1156, 1045 cm^{-1}; UV λ_{max} 213 nm (ϵ 40 025), 257 (ϵ 16 250); FDMS (MeOH) m/z 383 (M^+). Anal. ($C_{20}H_{21}N_3O_3S_1$) C, H, N, S.

***(E)*-1-[(1-Methylethyl)sulfonyl]-6-(1-phenyl-1-buten-3-ynyl)-1*H*-benzimidazol-2-amine (6).** Compound **9** (11.4 g, 30 mmol) was dissolved in anhydrous THF (100 mL), and dicolbalt octacarbonyl (10.2 g, 30 mmol) was added resulting in the generation of a gas. When the gas evolution ceased and TLC (silica gel plate with eluent 66% chloroform, 26% ethyl acetate, 8% acetic acid) showed complete conversion of starting material, the solvent was removed by rotary evaporation. The residue was then redissolved in chloroform (100 mL), *p*-toluenesulfonic (12 g) was added, and the reaction mixture was stirred under nitrogen for 18 h. The reaction mixture was then poured into ethyl acetate (1.5 L) and the organic phase washed with 500 mL portions of water until the aqueous layer had a pH greater than 5.0. The solvent was then removed from the organic phase by rotary evaporation, and the residue was redissolved in ethanol (220 mL). Ferric nitrate (50 g) was then slowly added to the reaction mixture as it was stirred under nitrogen. Removal of the cobalt complex was followed by TLC using the same system as before. When the reaction was substantially complete, THF (100 mL) was added followed by ethyl acetate (1.5 L). The organic phase was washed with water and dried over magnesium sulfate, and the solvent was removed by rotary evaporation. The residue was then purified by column chromatography (silica gel, eluent 8% acetic acid in methylene chloride) to give 6.5 g of product as a 4/1 (*Z*/*E*, *cis*/*trans*) mixture of isomers.

This mixture was dissolved in methanol (200 mL) and subjected to UV irradiation using a 450 W Hanovia lamp with a Pyrex filter. The reaction was monitored by NMR or analytical HPLC (55% acetonitrile/water). When the isomer ratio was restored to approximately 1/1, the irradiation was halted and the solvent removed by rotary evaporation. The desired *E* (*trans*) isomer was then isolated by reverse phase HPLC with a gradient of 38−42% acetonitrile/water. Approximately 300 mg of *E* isomer was purified from about 1.5 g of the mixture: ^{1}H NMR (DMSO-d_6) δ 7.48−7.34 (m, 6H), 7.19 (d, J = 9 Hz, 1H), 7.17 (dd, J = 9, 2 Hz, 1H), 7.07 (s, 2H), 6.16 (d, J = 3 Hz, 1H), 3.97 (d, J = 3 Hz, 1H), 3.89 (septet, J = 7 Hz, 1H), 1.24 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3505, 3397, 3306, 1638, 1608, 1548, 1475, 1442, 1387, 1357, 1269, 1154, 1044 cm^{-1}; UV λ_{max} 262 nm (ϵ 15 188), 315 nm (ϵ 22 084); FDMS (MeOH) m/z 365 (M^+). Anal. ($C_{20}H_{19}N_3O_2S_1$) C, H, N, S.

***(Z)*-1-[(1-Methylethyl)sulfonyl]-6-(3-chloro-1-phenyl-1-butadienyl)-1*H*-benzimidazol-2-amine (7).** The propargyl alcohol **9** (50 g), as prepared above, was dissolved in ethyl acetate (1 L) and placed in a 4 L beaker along with 3 N HCl (2 L). The beaker was covered with a porcelain dish to reduce evaporation, and the mixture was stirred vigorously. Progress of the reaction was followed by TLC (silica gel plate with eluent 66% chloroform, 26% ethyl acetate, 8% acetic acid). When the starting material had reacted (approximately 6 days), ethyl acetate (500 mL) was added, and solid sodium bicarbonate was cautiously added until the aqueous layer was basic. The organic phase was then separated and washed with 5% sodium bicarbonate solution until the aqueous wash remained basic. The organic layer was then dried over magnesium sulfate and concentrated under vacuum until crystallization began. The crystallization was aided by the careful addition of small amounts of hexane, after which 10.5 g of nearly pure *Z* (*cis*) isomer was collected by filtration. The product was further purified by reverse phase HPLC with a gradient of 38−42% acetonitrile/water: ^{1}H NMR (DMSO-d_6) δ 7.38−7.32 (m, 4H), 7.30−7.23 (m, 3H), 7.04 (s, 2H), 6.93 (dd, J = 8, 2 Hz, 1H), 6.23 (s, 1H), 5.33 (d, J = 1 Hz, 1H), 5.22 (s, 1H), 3.81 (septet, J = 7 Hz, 1H), 1.25 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3505, 3398, 1637, 1608, 1550, 1438, 1387, 1359, 1155, 1044 cm^{-1}; UV λ_{max} 261 nm (ϵ 24 793); FDMS (MeOH) m/z 401 (M^+). Anal. ($C_{20}H_{20}N_3O_2S_1Cl_1$) C, H, N, S, Cl.

***(E)*-1-[(1-Methylethyl)sulfonyl]-6-(3-chloro-1-phenyl-1-butadienyl)-1*H*-benzimidazol-2-amine.** Alternatively the *trans* isomer was isolated directly in low yield from the reaction mixture described for **7** above, by reverse phase HPLC with a gradient of 38−40% THF/water: ^{1}H NMR (DMSO-d_6) δ 7.43−7.32 (m, 4H), 7.22−7.15 (m, 3H), 7.12 (s, 2H), 7.05 (dd, J = 8, 2 Hz, 1H), 6.57 (s, 1H), 5.30 (d, J = 2 Hz, 1H), 5.13 (s, 1H), 3.86 (septuplet, J = 7 Hz, 1H), 1.22 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3505, 3397, 1636, 1606, 1548, 1439, 1386, 1175, 1155, 1043 cm^{-1}; UV λ_{max} 312 nm (ϵ 21 715); FDMS (MeOH) m/z 401 (M^+). Anal. ($C_{20}H_{20}N_3O_2S_1Cl_1$) C, H, N, S.

***(E)*-1-[(1-Methylethyl)sulfonyl]-6-(1-phenyl-1-buten-3-ynyl)-1*H*-benzimidazol-2-amine (6) Prepared from 7.** Compound **7** (3.5 g, 8.7 mmol) was dissolved in dry DMSO (15 mL) and cooled to 0 °C with an ice bath. Potassium *tert*-butoxide (4.9 g, 43.5 mmol) was added, and after a few minutes the ice bath was removed. The reaction mixture was then stirred at room temperature for 2 h. At this time, water (40 mL) was slowly added. The resulting precipitate was filtered and washed with water giving 2.3 g (~74%) of crude product which was approximately 1/2, *cis*/*trans*, by NMR. The desired *trans* isomer was separated by reverse phase HPLC as described for **6** above.

***(E)*-1-[(1-Methylethyl)sulfonyl]-6-(3-oxo-1-phenyl-1-butenyl)-1*H*-benzimidazol-2-amine (8).** Compound **9** (2.5 g) was dissolved in 3-methyl-2-butanone (100 mL), and the solution was poured into a beaker followed by the addition of 6 N H_2SO_4 (100 mL). The reaction mixture was then stirred at room temperature overnight, and then 2 N NaOH (300 mL) was added. The mixture was extracted two times with 200 mL portions of ethyl acetate. The combined ethyl acetate portions were then dried over magnesium sulfate, and the solvent was removed by rotary evaporation to give a brown gum. Separation of the desired *trans* isomer was carried out by reverse phase HPLC with a gradient of 38−40% acetonitrile/H_2O: ^{1}H NMR (DMSO-d_6) δ 7.47−7.37 (m, 4H), 7.23−7.08 (m, 6H), 6.62 (s, 1H), 3.86 (septet, J = 7 Hz, 1H), 1.87 (s, 3H), 1.22 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3505, 3397, 3011, 1638, 1604, 1545, 1443, 1360, 1261, 1155 cm^{-1}; UV λ_{max} 256 nm (ϵ 12 113), 336 (ϵ 13 926); FDMS (MeOH) m/z 383 (M^+). Anal. ($C_{20}H_{21}N_3O_3S_1$) C, H, N, S.

General Procedure for Compounds 12−14. A. 3,4-Dinitro-4′-fluorobenzophenone. 3,4-Dinitrobenzoic acid (20 g, 94.2 mmol) was suspended in fluorobenzene (200 mL) in a 1 L three-necked flask equipped with stirring bar, reflux

condenser, and drying tube; PCl_5 (27.8 g, 133.5 mmol) was added. After stirring for 10 min at room temperature, the reaction mixture was heated to 45 °C with an oil bath for 45 min. The reaction mixture was then cooled to below 10 °C with an ice bath before careful addition of anhydrous $AlCl_3$ (37.6 g, 282 mmol). After the addition, the reaction mixture was then heated at 50 °C for 2 h. The reaction mixture was then cooled in an ice bath, and concentrated HCl (35 mL) was added dropwise, keeping the temperature below 20 °C. After the mixture stirred for 1 h in the ice bath, water (400 mL) was carefully added. The reaction mixture was then extracted with ethyl acetate (200 mL) and the ethyl acetate layer washed four times with an equal volume of brine. The organic phase was then separated and dried over magnesium sulfate, and the solvent was removed by rotary evaporation. The resulting residue was then slurried with hexane and filtered to give 25.5 g of the desired product: ^{1}H NMR (DMSO-d_6) δ 8.46 (d, J = 2 Hz, 1H), 8.37 (d, J = 7 Hz, 1H), 8.23 (dd, J = 7, 2 Hz, 1H), 7.93 (dd, J = 8, 5 Hz, 2H), 7.43 (dd, J = 8, 8 Hz, 2H); IR ($CHCl_3$) 1671, 1600, 1551, 1278, 1245, 845 cm^{-1}; UV λ_{max} 228 nm (ϵ 19 928); FDMS (MeOH) m/z 290 (M^+). Anal. ($C_{13}H_7N_2O_5F_1$) C, H, N, F.

B. 3,4-Diamino-4′-fluorobenzophenone. 3,4-Dinitro-4′-fluorobenzophenone (51 g, 176 mmol) was dissolved in THF (940 mL), and Raney nickel catalyst (10 g) was added. The reaction mixture was then stirred at room temperature for 7 h under 60 psi of hydrogen. The reaction mixture was then filtered and the solvent removed by rotary evaporation to give 37.5 g or 92% yield of the desired product as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 7.65 (dd, J = 7, 5 Hz, 2H), 7.30 (dd, J = 7, 7 Hz, 2H), 7.04 (d, J = 2 Hz, 1H), 6.90 (dd, J = 7, 2 Hz, 1H), 6.53 (d, J = 7 Hz, 1H), 5.49 (bs, 2H), 4.73 (bs, 2H); IR ($CHCl_3$) 3436, 3374, 1644, 1619, 1599, 1585, 1515, 1506, 1309, 1155, 850 cm^{-1}; UV λ_{max} 251 nm (ϵ 13 721), 360 (ϵ 10 161); FDMS (MeOH) m/z 230 (M^+). Anal. ($C_{13}H_{11}N_2O_1F_1$) C, H, N, F.

C. 4-Amino-3-(1-methylethanesulfonamido)-4′-fluorobenzophenone. 3,4-Diamino-4′-fluorobenzophenone (18.14 g, 79 mmol) was dissolved in dry methylene chloride (160 mL), and dry pyridine (32 mL) was added. This was followed by the addition of isopropylsulfonyl chloride (13.25 mL, 118 mmol), and the reaction mixture was then stirred under nitrogen at room temperature for 5 h. At this time the methylene chloride was removed by rotary evaporation, ethyl acetate (300 mL) was added, and the mixture was washed with 1 N HCl (400 mL). The organic phase was separated and dried over magnesium sulfate, and the solvent was removed in vacuo to provide a red gummy residue. The residue was purified using preparative HPLC (gradient eluent of 30−60% ethyl acetate/hexane). The fractions containing the desired product were combined, and the solvent was removed by rotary evaporation to give 17.11 g (65%) of product as a yellow gum: ^{1}H NMR (DMSO-d_6) δ 8.89 (s, 1H), 7.73 (dd, J = 7, 5 Hz, 2H), 7.65 (d, J = 2 Hz, 1H), 7.46 (dd, J = 7, 2 Hz, 1H), 7.36 (dd, J = 7, 7 Hz, 2H), 6.82 (d, J = 7 Hz, 1H), 6.12 (bs, 2H), 3.24 (septet, J = 6 Hz, 1H), 1.27 (d, J = 6 Hz, 6H); IR ($CHCl_3$) 3453, 3367, 1626, 1598, 1589, 1333, 1317, 1307, 1231, 1158, 768 cm^{-1}; UV λ_{max} 248 nm (ϵ 14 828), 332 (ϵ 15 737); FDMS (MeOH) m/z 336 (M^+). Anal. ($C_{16}H_{17}N_2O_3S_1F_1$) C, H, N, S, F.

D. 6-(4-Fluorobenzoyl)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazol-2-amine. 4-Amino-3-(2-propanesulfonamido)-4′-fluorobenzophenone (17.11 g, 51 mmol) was added to dry 2-propanol (100 mL) and dissolved by the addition 2 N NaOH (25 mL). The solution was cooled in an ice bath, and a 5 N solution of cyanogen bromide in acetonitrile (10 mL) was added. After the mixture stirred overnight at room temperature, a precipitate had formed which was collected by filtration and resuspended in 2-propanol (250 mL). The suspension was then refluxed until solution occurred. On cooling, the desired product crystallized out giving 10.0 g or 55% yield: ^{1}H NMR (DMSO-d_6) δ 7.95 (d, J = 2 Hz, 1H), 7.83−7.79 (m, 2H), 7.64 (dd, J = 8, 2 Hz, 1H), 7.44−7.34 (m, 5H), 3.96 (septet, J = 7 Hz, 1H), 1.32 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3506, 3457, 3396, 2990, 1640, 1600, 1542, 1444, 1361, 1280, 1156 cm^{-1}; UV λ_{max} 247 nm (ϵ 17 115), 318 (ϵ 19 608); FDMS (MeOH) m/z 361 (M^+). Anal. ($C_{17}H_{16}N_3O_3S_1F_1$) C, H, N.

E. (*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(4-fluorophenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (12). Compound **12** was prepared from the ketone of step D following the procedure for compound **6** above: ^{1}H NMR ($CDCl_3$) δ 7.56 (d, J = 3 Hz, 1H), 7.46 (m, 2H), 7.28 (d, J = 9 Hz, 1H), 7.07 (m, 3H), 6.46 (bs, 2H), 5.98 (d, J = 4 Hz, 1H), 3.64 (septet, J = 7 Hz, 1H), 3.06 (d, J = 4 Hz, 1H), 1.38 (d, J = 7 Hz, 1H); IR ($CHCl_3$) 3504, 3397, 3306, 1638, 1602, 1547, 1509, 1361, 1268, 1158 cm^{-1}; UV λ_{max} 212 nm (ϵ 29 163), 259 (ϵ 13 100), 312 (ϵ 11 252); FDMS (MeOH) m/z 383 (M^+). Anal. ($C_{20}H_{18}N_3O_2S_1F_1$) C, H, N, S, F.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-(4-methoxyphenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (13): prepared following the procedure for compound **12**; ^{1}H NMR (DMSO-d_6) δ 7.38 (m, 3H), 7.33 (d, J = 9 Hz, 2H), 7.22 (d, J = 8 Hz, 1H), 7.10 (dd, J = 8, 2 Hz, 1H), 6.97 (d, J = 9 Hz, 2H), 6.01 (d, J = 2 Hz, 1H), 3.99 (d, J = 2 Hz, 1H), 3.91 (septet, J = 7 Hz, 1H), 3.83 (s, 3H), 1.26 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3434, 3397, 3305, 1692, 1637, 1607, 1512, 1193, 1178, 1154 cm^{-1}; UV λ_{max} 291 nm (ϵ 15 681), 315 (ϵ 16 749); FDMS (MeOH) m/z 395 (M^+). Anal. ($C_{21}H_{21}N_3O_3S_1$) C, H, N, S.

(*E*)-1-[(1-Methylethyl)sufonyl]-6-[1-(4-ethoxyphenyl)-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (14): prepared following the procedure for compound **12**; ^{1}H NMR ($CDCl_3$) δ 7.59 (d, J = 3 Hz, 1H), 7.41 (d, J = 9 Hz, 2H), 7.31 (d, J = 10 Hz, 1H), 7.15 (dd, J = 10, 3 Hz, 1H), 6.87 (d, J = 9 Hz, 2H), 6.30 (bs, 2H), 5.88 (d, J = 4 Hz, 1H), 4.07 (q, J = 8 Hz, 2H), 3.64 (septet, J = 7 Hz, 1H), 3.03 (d, J = 4 Hz, 1H), 1.46 (t, J = 8 Hz, 3H), 1.42 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3505, 3397, 3306, 1638, 1607, 1511, 1359, 1248, 1176, 1045 cm^{-1}; UV λ_{max} 288 nm (ϵ 18 184), 315 (ϵ 18 530); FDMS (MeOH) m/z 409 (M^+). Anal. ($C_{22}H_{23}N_3O_3S_1$) C, H, N, S.

A. 4-Amino-3-(1-methylethanesulfonamido)-4′-(methylthio)benzophenone. DMF (450 mL) and H_2O (100 mL) were added to a 2 L three-necked round bottom flask equipped with addition funnel, thermometer, and nitrogen inlet. The solvent was cooled to −5 °C in an ice/acetone bath, and methanethiol (525 g, 10.9 mol) and NaOH (144 g, 3.6 mol) were added. The mixture was stirred until all of the NaOH was dissolved, and then 4-amino-3-(1-methylethanesulfonamido)-4′-fluorobenzophenone (150 g, 0.45 mol), dissolved in DMF (150 mL), was slowly added keeping the temperature below 0 °C. The reaction mixture was then allowed to stir overnight allowing the reaction mixture to warm to room temperature. At this point ethyl acetate (2.5 L) was added, the organic layer washed three times with 1 N HCl (1.5 L) and dried over magnesium sulfate, and the solvent removed by rotary evaporation to give 147 g or 90% yield of the desired product: ^{1}H NMR (DMSO-d_6) δ 8.85 (s, 1H), 7.62 (d, J = 2 Hz, 1H), 7.59 (d, J = 7 Hz, 2H), 7.43 (dd, J = 7, 2 Hz, 1H), 7.35 (d, J = 7 Hz, 2H), 6.78 (d, J = 7 Hz, 1H), 6.03 (s, 2H), 3.20 (septet, J = 6 Hz, 1H), 2.53 (s, 3H), 1.25 (d, J = 6 Hz, 6H); IR ($CHCl_3$) 3502, 3406, 3369, 1618, 1591, 1325, 1314, 1289, 1143 cm^{-1}; UV λ_{max} 244 nm (ϵ 12 807), 335 (ϵ 24 088); FDMS (MeOH) m/z 364 (M^+). Anal. ($C_{17}H_{20}N_2O_3S_2$) C, H, N, S.

B. (*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-[4-(methylthio)-phenyl]-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (15): prepared from 4-amino-3-(2-propanesulfonamido)-4′-(methylthio)benzophenone (A above) following the procedure for compound **12** beginning with step D: ^{1}H NMR (DMSO-d_6) δ 7.36 (d, J = 2 Hz, 1H), 7.20 (d, J = 9 Hz, 2H), 7.27 (d, J = 9 Hz, 2H), 7.18 (d, J = 8 Hz, 1H), 7.05 (m, 3H), 6.05 (d, J = 2 Hz, 1H), 3.98 (d, J = 2 Hz, 1H), 3.87 (septet, J = 7 Hz, 1H), 2.50 (s, 3H), 1.23 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3397, 3306, 1638, 1549, 1440, 1358, 1269, 1155, 1044, 824 cm^{-1}; UV λ_{max} 298 nm (ϵ 26 835); FDMS (MeOH) m/z 411 (M^+). Anal. ($C_{21}H_{21}N_3O_2S_2$) C, H, N, S.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-[4-(methylsulfinyl)-phenyl]-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (16). Compound **15** (1.2 g or 2.9 mmol) was dissolved in methylene chloride (150 mL), and 85% *m*-chloroperoxybenzoic acid (600 mg, 2.9 mmol) was added. The reaction mixture was stirred overnight at room temperature at which time the reaction was complete as indicated by TLC. The reaction mixture was then washed once with an equal volume of saturated sodium bicarbonate solution and twice with brine. The organic phase

was then separated and dried over magnesium sulfate, and the solvent was removed in vacuo to give 1.0 g (80% yield) of the desired sulfoxide: ^{1}H NMR ($CDCl_3$) δ 7.67 (m, 4H), 7.28 (d, J = 9 Hz, 1H), 7.05 (d, J = 9 Hz, 1H), 6.07 (d, J = 4 Hz, 1H), 5.93 (s, 2H), 3.62 (septet, J = 7 Hz, 1H), 3.04 (d, J = 4 Hz, 1H), 2.80 (s, 3H), 1.39 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3398, 3306, 3005, 1638, 1547, 1360, 1268, 1225, 1044 cm^{-1}; UV λ_{max} 214 nm (ϵ 31 300), 276 (ϵ 19 800), 318 (ϵ 17 600); FDMS (MeOH) m/z 427 (M^+). Anal. ($C_{21}H_{21}N_3O_3S_2$) C, H, N, S.

(*E*)-1-[(1-Methylethyl)sulfonyl]-6-[1-[4-(methylsulfonyl)-phenyl]-1-buten-3-ynyl]-1*H*-benzimidazol-2-amine (17). The sulfone was prepared as described for the sulfoxide above, except that 2.5 equiv of *m*-chloroperoxybenzoic acid was employed and 1.4 g of **15** giving 1.4 g of sulfone, or 93% yield: ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 8 Hz, 2H), 7.68 (d, J = 8 Hz, 2H), 7.57 (d, J = 4 Hz, 1H), 7.29 (d, J = 10 Hz, 1H), 7.01 (dd, J = 10, 4 Hz, 1H), 6.11 (d, J = 4 Hz, 1H), 6.02 (s, 2H), 3.63 (septet, J = 7 Hz, 1H), 3.13 (s, 3H), 3.05 (d, J = 4 Hz, 1H), 1.41 (d, J = 7 Hz, 6H); IR ($CHCl_3$) 3400, 3105, 2955, 1639, 1319, 1269, 1155, 1139, 1044 cm^{-1}; UV λ_{max} 216 nm (ϵ 28 400), 278 (ϵ 17 100), 318 (ϵ 14 900); FDMS (MeOH) m/z 443 (M^+). Anal. ($C_{21}H_{21}N_3O_4S_2$) C, H, N, S.

Polio Plaque Reduction Assay. Susceptible BSC-1 cells were grown in 25 cm^2 tissue culture flasks treated at 37 °C in medium 199 with 5% fetal bovine serum, 10 mM Mg_2SO_4, penicillin (100 units/mL), and streptomycin (100 mg/mL). When confluent monolayers were formed, growth medium was removed and 0.3 mL/flask 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 medium 199 (2% fetal bovine serum, 10 mM Mg_2SO_4, 100 mg/mL penicillin, and 100 mg/mL streptomycin) 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. Flasks were incubated at 37 °C until the DMSO control flasks demonstrated plaques of optimal size. At thus time a solution containing 10% formalin and 2% sodium acetate was added to each flask 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 flasks at each concentration were averaged and compared with DMSO control flasks. 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.[32]

Drug Resistant Mutants. Drug resistant mutants of rhinovirus 14 were isolated, sequenced, and assayed for resistance using procedures previously described.[22]

Oral Bioavailability in Rhesus Monkeys. Pharmacokinetic studies were conducted with a series of benzimidazole derivatives to evaluate the oral bioavailability of these derivatives in monkeys. In these studies two female and two male monkeys were administered compounds **6** and **12**−**17** first orally (16 mg/kg) and then intravenously (1 mg/kg) after a 2 week washout period. Plasma samples from these monkeys were collected at various time intervals from 1 min to 8 h after dosing and then stored at −70 °C until analysis.

Compounds were isolated from the plasma by liquid−liquid extraction with benzene (compounds **6**, **13**, and **15**−**17**) or cyclohexane (compounds **12** and **14**). Reverse phase chromatography was used in the separation and quantitation of all samples using a 4 cm × 4.6 mm, 5 μm Zorbax ODS (Dupont Chemical, Wilmington, DE) column with a RP18 Brownlee guard column. An isocratic method utilizing 0.1 M ammonium acetate/acetonitrile (Burdick and Jackson, HPLC grade), 50/50 (v/v) mobile phase, and a flow rate of 1.0 mL/min was employed for compounds **12**, **14**, and **16**. The mobile phase was modified for the analysis of compounds **15** and **17** to 0.02 M phosphate buffer/acetonitrile, 50/50 (v/v). Further alterations of the mobile phase were required for elution of compounds **6** and **13** with the mobile phase consisting of 0.05 M sodium acetate/methanol (Burdick and Jackson, HPLC grade), 25/75 (v/v). Column elutions were monitored by UV detection at 314 nm (compound **12**), 315 nm (compound **14**), 319 nm (compounds **6**, **13**, **15**, and **17**), and 320 nm (compound **16**). Data were captured using an HP1000 computer system with Lilly software. The limit of detection of the assays for all compounds was 10 ng/mL.

Area under the curve (AUC) values were calculated using the trapezoidal rule.[33] Bioavailabilty (%) was calculated using the following equation: $AUC_{po/dose}/AUC_{iv/dose} \times 100$.

Oral Bioavailability in the Fischer Rat. Pharmacokinetic studies were also conducted with compound **12** in Fischer 344 (F344) rats. In these studies, male F344 rats were administered either an oral or intravenous dose of compound **12** at 20 mg/kg. For the orally dosed rats, plasma samples were collected simultaneously from the portal vein and the systemic vena cava at various time points from 15 min to 24 h. For the iv dosed rats, plasma samples were collected from a cardiac puncture at various time points from 5 min to 8 h. All plasma samples were stored at −20 °C until analysis.

Compound **12** was extracted from the plasma using a solid phase extraction method. Bond Elut CN-N (100 mg/1 cc) SPE columns were prepared by washing with methanol and then 20% acetonitrile, the plasma was loaded onto the column, the column was washed with 20% acetonitrile, compound **12** was eluted with 100% acetonitrile, the elution was dried, and then the sample was reconstituted with the HPLC mobile phase.

Reverse phase chromatography was used in the separation and quantitation of all samples. The isocratic method utilized a 4.6 × 150 mm, 5 μm Alltech Econosphere C8 (Alltech Associates, Inc., Deerfield, IL) column, 0.025 M sodium acetate with EDTA (5 mg/L)/methanol (Mallinckrodt HPLC grade), 32/68 (v/v), mobile phase, and 1.0 mL/min flow rate. Column elutions were monitored by UV detection at 260 nm. The limit of detection was approximately 30 ng/mL. Data were captured using the ACCESS*CHROM system from Perkin-Elmer Nelson. AUC values were calculated using MIKAPC, a noncompartmental method of pharmacokinetic analysis written at the Lilly Laboratory for Clinical Research.

***In Vitro* Metabolism Studies Using Hepatic Microsomes.** The *in vitro* procedure to determine oxidative and conjugative metabolism used solutions of compound **1**, **2**, or **12** incubated in a shaking water bath at 370 °C with F344 rat, beagle dog, cynomolgus monkey, or pooled human microsomes and coenzymes in a buffer. The resulting amount of compound **1**, **2**, or **12** was determined using the F344 rat HPLC bioassay described in the previous section.

The incubate for oxidative metabolism was a solution of 666 μL of 66 mM Tris buffer (pH 7.4), 283 μL of animal microsomes (adjusted to 3.53 mg of microsomal protein/mL), and 40 μL of 50 mM NADPH solution which was preincubated for 3 min before 40 μL of compound **1**, **2**, or **12** (approximately 3−4 mg/mL in acetone) was added. This mixture was incubated for 3 h before the reaction was stopped by addition of 1000 μL of chilled acetonitrile. The protein was pelleted by centrifugation. The resulting supernatant was then assayed. The oxidation blank used the same procedure as above but substituted 40 μL of acetone for the compound solution. The oxidation control was a mixture of Tris, microsomes, NADPH, and acetonitrile vortexed together before addition of 40 μL of compound **1**, **2**, or **12**. The control solution was not incubated but immediately centrifuged to pellet the protein.

The incubate for conjugative (glucuronidation) metabolism was a solution of 444 μL of 150 mM Tris-Brij buffer (pH 7.4), 32 μL of 400 μM $MgCl_2$ solution, 160 μL of 8 mM UDPGA solution, and 4.1 μL of compound **1**, **2**, or **12** (approximately 3−4 mg/mL in acetone) which was mixed together before 160 μL of animal microsomes (3.53 mg of microsomal protein/mL) was added. This mixture was incubated for 1 h before the reaction was stopped by addition of 800 μL of chilled acetonitrile. The protein was pelleted by centrifugation. The resulting supernatant was then assayed. The conjugative blank used was the same procedure as above but substituted acetone for the compound **1**, **2**, or **12** solution. The conjugative control was a mixture of Tris, $MgCl_2$, UDPGA, microsomes, compound **1**, **2**, or **12** solution, and acetonitrile vortexed together. The control solution was not incubated but immediately centrifuged to pellet the protein.

Coxsackie A21 Mouse Infection Model. The Coxsackie A21 virus used in the infection model was mouse adapted. The CD-1 white Swiss mice (Charles Rivers, Wilmington, MA) needed to be recently weaned and weighed 8−10 g. A dose of 10 LD_{50} of Coxsackie A21 (~4×10^4 PFU) in 0.25 mL vol was administered to the mice by the intraperitoneal route. Compound **12** was formulated 1/5 with PVP-30 and dissolved in 2% emulphor solution to give the proper dose concentrations. Mice (10/dose level, 20 for control) were treated at the proper dose by oral gavage with 0.25 mL of the compound solution. Treatment began with dosing 2 h prior to infection and 4 h after infection on day 1 and then twice daily for an additional 4 days. The mortality of mice was recorded daily through day 15 postinfection. A composite measure of effectiveness of the drug, the survival index (SI), which incorporates both time of death and number of survivors into a single parameter, was used to aid in the interpretation of the *in vivo* animal data.[34] The SI of animals dying on day N was calculated according to the following formula: SI for day N = (N − 1)[number of control animals dying on day (N − 1)]/(total number of control animals) + SI for day (N − 1). The p values were determined by comparing treated groups with the vehicle control by Student's t-test.

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References

(1) Couch, R. B. The Common Cold: Control? *J. Infect. Dis.* **1984**, *150* (2), 167−173.
(2) Sperber, S. J.; Hayden, F. G. Chemotherapy of Rhinovirus Colds. *Antimicrob. Agents Chemother.* **1988**, *32* (4), 409−419.
(3) Monto, A. S.; Bryan, E. R., Ohmit, S. Rhinovirus Infections in Tecumseh Michigan: Frequency of Illiness and Number of Serotypes. *J. Infect. Dis.* **1987**, *156* (1), 43−49.
(4) Hamparian, V. V.; et al. A Collaborative Report: Rhinoviruses-Extension of the Numbering System from 89 to 100. *Virology* **1987**, *159*, 191−192.
(5) Uncapher, C. R.; DeWitt, C. M.; Colonno, R. J. The Major and Minor Group Receptor Families Contain All but One Human Rhinovirus Serotype. *Virology* **1991**, *180*, 814−817.
(6) Holland, J.; Spindler, K.; Horodyski, F.; Grabau, E.; Nichol, S.; VandePol, S. Rapid Evolution of RNA Genomes. *Science* **1982**, *215*, 1577−1585.
(7) McKinlay, M. A.; Pevear, D. C.; Rossmann, M. G. Treatment of the Picornavirus Common Cold by Inhibitors of Viral Uncoating and Attachment. *Annu. Rev. Microbiol.* **1992**, *46*, 635−654.
(8) Andries, K.; Dewindt, B.; Snoeks, J.; Willebrords, R.; Stokbroekx, R.; Lewi, P. J. A Comparative Test of Fifteen Compounds Against All Known Human Rhinovirus Serotypes as a Basis for a More Rational Screening Program. *Antiviral Res.* **1991**, *16*, 213−225.
(9) Heinz, B. A.; Rueckert, R. R.; Shepherd, D. A.; Dutko, F. J.; McKinlay, M. A.; Fancher, M.; Rossmann, M. G.; Badger, J.; Smith, T. J. Genetic and Molecular Analyses of Spontaneous Mutants of Human Rhinovirus 14 That Are Resistant to an Antiviral Compound. *J. Virol.* **1989**, *63* (6), 2476−2485.
(10) Dearden, C.; Al-Nakib, W.; Andries, K.; Woestenborghs, R.; Tyrrell, D. A. J. Drug Resistant Rhinoviruses from the Nose of Experimentally Treated Volunteers. *Arch. Virol.* **1989**, *109*, 71−81.
(11) Enviroxime (IND 17152). IND Protocol No. 1 and Amedment to IND Protocol No. 1, Phase 1, Final Report, Summary; unpublished results.
(12) Phillpotts, R. J.; DeLong, D. C.; Wallace, J.; Jones, R. W.; Reed, S. E.; Tyrrell, D. A. J. The Activity of Enviroxime Against Rhinovirus Infection in Man. *Lancet* **1981**, *1*, 1342−1344.
(13) Hayden, F. G.; Gawaltney, J. M., Jr. Prophylatic Activity of Intranasal Enviroxime Against Experimentally Induced Rhinovirus Type 39 Infection. *Antimicrob. Agents Chemother.* **1982**, *21* (6), 892−897.
(14) Phillpotts, R. J.; Wallace, J.; Tyrrell, D. A. J.; Tagart, V. B. Therapeutic Activity of Enviroxime Against Rhinovirus Infection in Volunteers. *Antimicrob. Agents Chemother.* **1983**, *23* (5), 671−675.
(15) Dewolfe Miller, F.; Monto, A. S.; DeLong, D. C.; Exelby, A.; Bryan, E. R.; Srivastava, S. Controlled Trial of Enviroxime Against Natural Rhinovirus Infections in a Community. *Antimicrob. Agents Chemother.* **1985**, *27* (1), 102−106.
(16) Enviradene (IND 20204). Virology Section, Summary, p 58; unpublished results.
(17) Enviradene (IND 20204). IND Protocol No. 3, pp 1457−1461; unpublished results.
(18) DeLong, D. C.; Nelson, J. D.; Wu, C. Y. E.; Warren, B. R.; Wikel, J.; Chamberlin, J.; Montgomery, D.; Paget, C. J. Virus Inhibition Studies with AR-336 I. Tissue Culture Activity. *Abstracts of the Annual Meeting of the American Society for Microbiology*; American Society for Microbiology: Washington, DC, 1978; p 234.
(19) Wikel, J. H.; Paget, C. J.; DeLong, D. C.; Nelson, J. D.; Wu, C. Y. E.; Paschal, J. W.; Dinner, A.; Templeton, R. J.; Chaney, M. O.; Jones, N. D.; Chamberlin, J.W. Synthesis of Syn and Anti Isomers of 6-[(Hydroxyimino)phenyl]-1-[(1-methyl)sulfonyl]-1H-benzimidazol-2-amine. Inhibitors of Rhinovirus Multiplication. *J. Med. Chem.* **1980**, *23*, 368−372.
(20) DeLong, D. C.; Reed, S. E. Inhibition of Rhinovirus Replication in Organ Culture by a Potential Antiviral Drug. *J. Infect. Dis.* **1980**, *141* (1), 87−91.
(21) Wu, C. Y. E.; Nelson, J. D.; Warren, B. R.; DeLong, D. C. Virus Inhibition Studies with AR-336 II. Mode of Action Studies. *Abstracts of the Annual Meeting of the American Society for Microbiology*; American Society for Microbiology: Washington, DC, 1978; p 234.
(22) Heinz, B. A.; Vance, L. M. The Antiviral Compound Enviroxime Targets the 3A Coding Region of Rhinovirus and Poliovirus. *J. Virol.* **1995**, *69* (7), 4189−4197.
(23) Heinz, B. A.; Vance, L. M. Sequence Determinants 3A-Mediated Resistance to Enviroxime in Rhinoviruses and Enteroviruses. *J. Virol.* **1996**, *70* (7), 4854−4857.
(24) Palmenberg, A. C. Unpublished results.
(25) Enviradene (IND 20204). Twelve-Month Report, pp 1450−1456; unpublished results.
(26) Bopp, R. J.; Quay, J. F. Unpublished results.
(27) Ryan, C. W.; Slomski, B. A. Synthesis of Alkylidene Intermediates. U.S. Patent 4,501,921, 1985.
(28) Paget, C. J.; Chanberlin, J. W.; Wikel, J. H. Carbonyl-Substituted 1-Sulfonylbenzimidazoles. U.S. Patent 4,118,742, 1978.
(29) DeLong, D. C.; Nelson, J. D.; Wu, C. Y. E.; Warren, B.; Wikel, J.; Templeton, R. J.; Dinner, A. Eighteenth Interscience Conference on Antimicrobial Agents and Chemotherapy, 1978; Abstract 34.
(30) Parham, W. E.; Sperley, R. J. Reactions of Enol Ethers with Carbenes. VIII, Rearrangement of the Dichlorocyclopropane Derived from 1-Ethoxycyclododecene. *J. Org. Chem.* **1967**, *32*, 926−931.
(31) Nicholas, K. M.; Pettit, R. An Alkyne Protecting Group. *Tetrahedron Lett.* **1971**, 3475−3478.
(32) Reed, L. J.; Muench, H. A Simple Method of Estimating Fifty Per Cent Endpoints. *J. Am. Hyg.* **1938,** *27*, 493−497.
(33) Gibaldi, M.; Perrier, D. *Pharmacokinetics*, 2nd ed.; Marcel Dekker: New York,1982; p 445.
(34) Redman, C. E.; Streightoff, F.; Jones, M.; Bonice, W. S.; DeLong, D. C. *In Vivo* Antiviral Chemotherapy. I. Experimental Designs and Statistical Evaluations. *Antimicrob. Agents Chemother.* **1966**, 497−502.

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