Notes

Synthesis and Antiviral Activity of C2 Analogs of Enviroxime: An Exploration of the Role of Critical Functionality

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Enviroxime is a potent antiviral agent with broad spectrum activity in tissue culture against both rhinoviruses and enteroviruses. We have synthesized and studied a series of C2-substituted analogs in order to identify critical functionality and examine its role in antiviral activity. We have found that primary amino substitution is the most active. *Ab initio* calculations indicate that larger groups at C2 may provide a repulsive steric interaction at N3, and in those cases where this undesirable conformation has limited flexibility, the antiviral activity is severely reduced. Further the results show that an amino hydrogen at C2 is strongly hydrogen bonded to the N1 sulfonyl oxygen, which in the case of Enviroxime may act to enhance the activity by holding the second hydrogen in a desirable orientation for interaction at an antiviral site.

Introduction

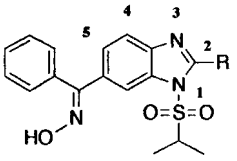
Enviroxime (**1**) and related benzimidazoles have been shown to have a number of properties that are highly desirable in a drug candidate for the treatment of the common cold. Compounds from this structural class have demonstrated potent broad spectrum antiviral activity when tested against a range of both rhinoviruses and enteroviruses.[1−3] To date all attempts to develop drug resistant mutants as measured by a significant increase in the IC_{50} have failed.[4] Further, studies have shown that Enviroxime is a selective antiviral which exhibits its activity by inhibiting the synthesis of plus strand RNA via a mechanism which involves rhinovirus protein 3A.[4,5]

The requirements of a drug to treat the common cold are demanding, and Enviroxime failed in clinical studies because of poor oral bioavailability and emetic side effects.[6,7] However, because Enviroxime does have a number of desirable properties,[1−5] we wanted to determine which substitutions were critical to antiviral activity and which portions of the molecule could be modified without demonstrating adverse results. In addition we wanted to give special consideration to understanding how a critical substitution might interact at an active site. To this end we have investigated the effects of modifying the C2 position. We have also performed an *ab initio* conformational analysis of several C2-substituted analogs to understand the role of this group on conformation and its relation to activity.

Chemistry

The ketones used for preparation of the *trans* oximes **2**−**7** (see Chart 1) were synthesized beginning with compound **14** (see Scheme 1). Ketones **8**−**10** were prepared from **14** by reaction with the appropriate orthoesters (triethyl orthoformate, triethyl orthoacetate, and tetramethyl carbonate). Ketones **11**−**13** were prepared by reacting **14** with methyl isothiocyanate and *N*,*N*-dimethylthiocarbamoyl chloride. Both reagents react with **14** to give thiol intermediate derivatives **15**. These thiol intermediates were isolated and then reacted with sodium hypochlorite to give ketones **12** and **13**, or if instead heating of the thiol intermediates was continued, they both proceed to give thione **16** which then was converted to ketone **11** by reaction with methyl iodide.

Chart 1. Structure of Enviroxime (**1**) and Related C2-Substituted Benzimidazoles



1 R = NH_2
2 R = H
3 R = CH_3
4 R = OCH_3
5 R = SCH_3
6 R = $NHCH_3$
7 R = $N(CH_3)_2$

Reaction of the ketones **8**−**13** with hydroxylamine hydrochloride gave the required oximes as a mixture of *cis* and *trans* isomers. The desired *trans* isomer was then isolated by reverse phase preparative HPLC or by selective crystallization.

Biological Evaluation and *ab Initio* Calculations

The *trans* oximes **2**−**7** were tested side-by-side with Enviroxime (**1**) for their tissue culture antiviral activity against a representative selection of viruses from the picornavirus family (see Table 1). Enviroxime (**1**) with a primary amino function at the C2 position gave superior antiviral activity across the range of picornaviruses tested. Compounds **2**−**5**, with hydrogen, methyl,

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Scheme 1

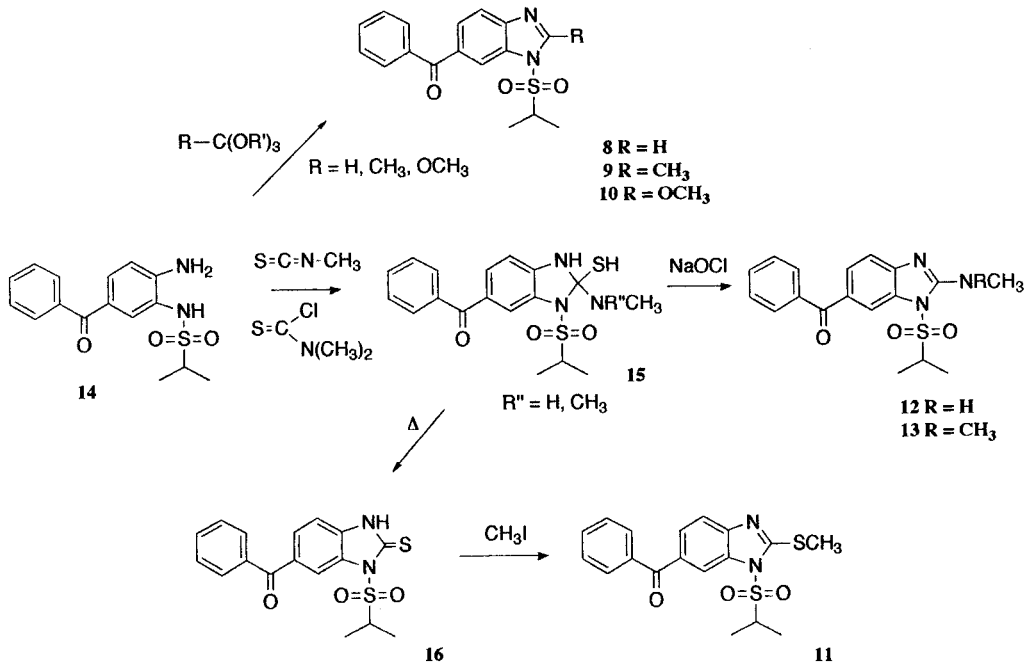


Table 1. Antiviral Activity of C2 Analogs (see Chart 1 for structures)

		IC$_{50}$ (μg/mL)[a]				
no.	C2	RV-2[b]	RV-14	RV-16	PV-1[c]	Cox A21[d]
1	NH_2	0.07	0.04	0.04	0.06	0.05
2	H	0.19	0.53	0.16	0.57	0.12
3	CH_3	0.24	0.34	0.18	0.23	0.05
4	OCH_3	0.54	0.48	0.18	0.57	0.17
5	SCH_3	0.69	0.76	0.18	0.59	0.18
6	$NHCH_3$	54.8	24.4	1.49	6.16	7.11
7	$N(CH_3)_2$	30.3	49.0	0.64	4.55	2.19

[a] CPE/XTT assay using HeLa cells. [b] Rhinovirus. [c] Poliovirus (Mahoney type 1). [d] Coxsackie virus.

O-methyl, and *S*-methyl substitution, respectively, also exhibited substantial although somewhat reduced activity.

The antiviral activity of **1** as compared with **2**−**5** suggests an important, but not absolutely essential, role for hydrogen bond donation at the C2 position. We were not surprised therefore to find that the *N*,*N*-dimethyl derivative **7** showed greatly reduced antiviral activity. Whereas compounds **4** and **5** lack the ability to provide a hydrogen bond, they are monosubstituted and can therefore rotate to avoid forcing a methyl group toward the presumed hydrogen bond acceptor which may be encountered in binding at the active site. In contrast, compound **7**, with *N*,*N*-dimethyl substitution, apparently cannot rotate sufficiently to avoid a significant steric interaction, and hence the observed large loss of activity.

On the basis of these assumptions, we were at first surprised to find that the monosubstituted *N*-methyl analog **6** was also much less active than **1**−**5** when tested against the same viruses. We had initially assumed that compound **6** would show substantial antiviral activity because it possesses monosubstitution similar to **4** and **5**, but like Enviroxime (**1**) it also has the potential to form an important hydrogen bond at the active site. On reflection however, it seemed reasonable that **6** might instead form an internal hydrogen bond with a sulfonyl oxygen, thus locking the *N*-methyl substituent into an undesirable conformation.

In order to test this hypothesis, we carried out *ab initio* calculations[8] of Enviroxime and the C2-substituted analogs. The 3-21G basis set[9] calculations of the L-shaped isomer[2] of Enviroxime indicate the presence of internal hydrogen bonding, namely, an amine hydrogen is 1.956 Å from the sulfonyl oxygen as illustrated in Figure 1. The presence of internal hydrogen bonding is also indicated by analysis of overlap populations. The overlap of the amine hydrogen and the sulfonyl oxygen in **1a** is 0.082, indicating the atoms strongly attract each other. Because of this internal hydrogen bond to the sulfonyl oxygen, the remaining amine hydrogen is held in a fixed position which is nearly *syn* to N3. By comparison, *ab initio* conformational analysis of the *N*-methyl analog **6** resulted in structures **6a**,**b**. These two structures differ in energy by 6.9 kcal/mol, favoring **6a**. Again, in **6a** the amine hydrogen is strongly hydrogen bonded to the sulfonyl oxygen as illustrated by the 1.953 Å distance, and there is a 0.084 overlap population, but in this case the methyl group is held *syn* to N3. In rotamer **6b** the absence of an internal hydrogen bond gives way to a strong steric interaction between the *N*-methylamino group and sulfonyl group which produces a change in conformation of the isopropylsulfonyl group. In addition, the *N*-methyl group of **6b** is orthogonal to N3, as indicated by an N3−C2−N−C torsion angle of −99.5°. Moreover, we could not locate an energy minima for compound **6** in which the amine hydrogen is *syn* (~0°) to position N3. Although starting from a *syn* structure, the geometry optimizes to conformer **6b** in which the N3−C2−N−H torsion angle is nearly *syn* at 30.1°. The structure of **6b** is similar to the only conformation located for the *N*,*N*-dimethyl analog shown by **7a**. The minimum energy structure of **7a** indicates an orthogonal orientation for one of the methyl groups with an N3−C2−N−C torsion angle of −101.9°, while the other methyl has an N3−C2−N−C torsion angle which is nearly *syn* at 29.6°.

A conformational analysis of the *O*-methyl and *S*-

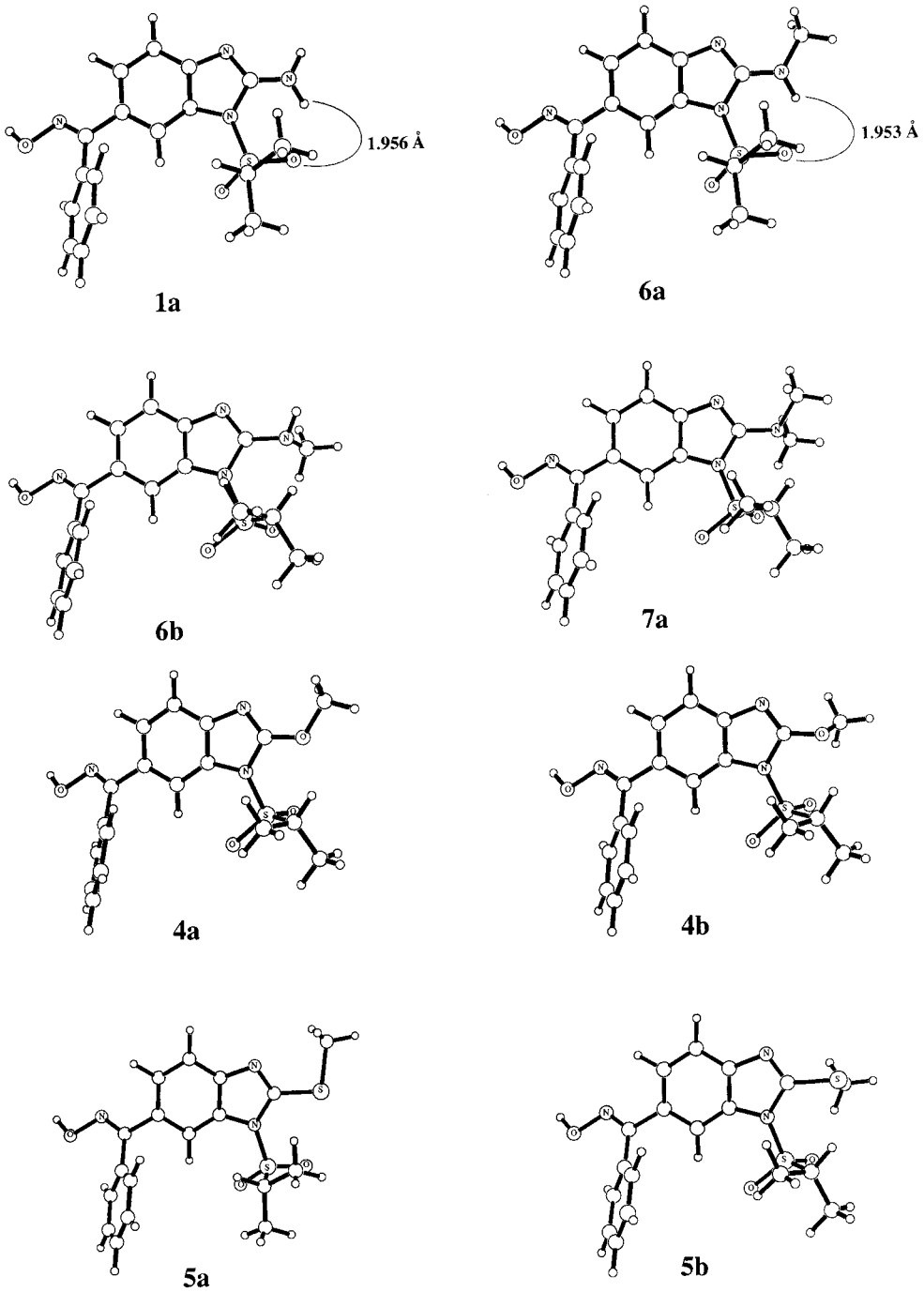
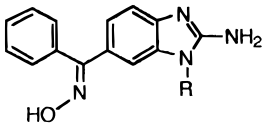


Figure 1. Several conformers of the C2 analogs of Enviroxime calculated at the 3-21G *ab initio* basis level. The absolute energies (au) at the 3-21G level are −1485.836 42 for **1a**, −1524.650 22 for **6a**, −1524.633 91 for **6b**, −1563.452 65 for **7a**, −1544.359 53 for **4a**, −1544.357 61 for **4b**, −1865.460 83 for **5a**, and −1865.464 95 for **5b**.

methyl analogs was also performed. The conformer given by **4a** is 1.2 kcal/mol lower in energy than that of **4b**. In structure **4a** the methyl group is nearly in the plane of the benzimidazole ring; the N3−C2−O−C torsion angle is −9.9°. In conformer **4b** the corresponding torsion angle is −70.2°, indicating an orthogonal orientation. As seen with the C2 analog **7a**, there is a change in conformation of the isopropylsulfonyl group in both **4a**,**b**, but here it is caused by repulsive interactions between the sulfonyl oxygen and the *O*-methyl oxygen. There were also two conformations, **5a**,**b**, that were located for the *S*-methyl analog. In this case conformer **5a** is favored over conformer **5b** by 2.6 kcal/mol. The latter structure is disfavored because of the steric repulsion of the *S*-methyl group and the sulfonyl group. By comparison, the structure located for **5a** differs from that of **4a** because the C2−S bond is longer than the C2−O bond and therefore the isopropylsulfonyl group is not forced to change conformation. In **5b** the N3−C2−S−C torsion angle is −94.7°, whereas in structure **5a** the corresponding angle is 4.7°.

Overall, the conformational analysis of the C2 analogs indicates that the preferred conformations of the less active analogs **4**−**7** exhibit a geometry whereby the bulk of the C2 substituent sterically blocks potential interaction of the benzimidazole N3 position with a presumed receptor binding site. Moreover, in **6a** the C2 conformation of the *N*-methyl group is locked in a *syn* position with little flexibility of the substitution. Likewise, in **7a** the bulky *N*,*N*-dimethyl group has little flexibility

Table 2. Antiviral Activity of N1 Analogs



no.	R	IC_{50} (μg/mL)[a]
1	$S(O)_2CH(CH_3)_2$	0.02
17	$CH(CH_3)_2$	0.09

[a] Poliovirus (Mahoney type 1) plaque reduction assay using BSC1 cells.

for conformational rotation of the C2 substituent. By contrast, the more active analogs **4** and **5** are somewhat more flexible and can more easily obtain conformations (**4b** and **5b**) whereby the bulky C2 substituent can deviate from an undesirable *syn* conformation.

Our calculations provide strong evidence for internal hydrogen bonding and support the hypothesis that compound **6** is inactive because the internal hydrogen bond locks the *N*-methyl group into an undesirable position for binding at the active site. The results also suggest that we should consider a positive role for internal hydrogen bonding in Enviroxime (**1**), where the second hydrogen might be held in an advantageous position for binding at the active site. In fact there is some evidence that suggests this may be true. It has been reported that substitution at N1 is required for antiviral activity,[1] but N1 sulfonyl substitution seems to have special significance. For example, alkyl substitution at N1 results in compounds that are less active than their sulfonyl analogs (see Table 2).[10] Compound **17** cannot form an internal hydrogen bond, and this may be a reason for its somewhat reduced antiviral activity.

Conclusion

We have synthesized a series of C2 analogs of Enviroxime. A comparison of their antiviral activities demonstrates that a primary amino function is the most desirable substitution. *Ab initio* calculations indicate that a primary or secondary amino function at C2 should exhibit a strong internal hydrogen bond with the sulfonyl group at N1. *Ab initio* calculations also show that larger groups at C2 provide a repulsive steric interaction at N3, and in those cases where the conformations having this undesirable N3 interaction are less flexible (due to geminal substitution or internal hydrogen bonding, compounds **6** and **7**), the loss in antiviral activity is severe. Overall our results suggest that N3 may play a critical role in the interaction of the benzimidazoles at an antiviral site and that, in Enviroxime with a primary amino function at C2, the internal hydrogen bond may enhance the antiviral activity by fixing the second hydrogen in a desirable *syn* orientation. Further, this key feature of the most active compound—an amine hydrogen held in a *syn* configuration with N3—is consistent with the requirements needed for binding with a protein amide function (such as a glutamine side chain) at a presumed antiviral site.

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, and FDMS spectra were recorded on a VG Analytical VG 70 SE spectrometer. ^{1}H NMR spectra, FDMS spectra, and microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories.

6-Benzoyl-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (8). The ketone 4-amino-3-[(methylethyl)sulfonamido]-benzophenone] (**14**)[11] (27 g) was dissolved in triethyl orthoformate (300 mL), and *N*,*N*-diisopropylethylamine (12 mL) was added. The reaction mixture was then refluxed under nitrogen for 5 h. At this time the solvent was removed by rotary evaporation and the residue redissolved in ethyl acetate (600 mL). The organic phase was then washed with 1 N HCl (300 mL) and dried over magnesium sulfate, and the solvent was removed by rotary evaporation. The resulting residue was purified by preparative HPLC (silica gel, gradient eluent of 30−60% ethyl acetate/hexane) to give the desired product (24 g or 86% yield): ^{1}H NMR (DMSO-d_6) δ 8.84 (s, 1H), 8.16 (d, J = 1 Hz, 1H), 7.98 (d, J = 9 Hz, 1H), 7.81 (dd, J = 9, 2 Hz, 1H), 7.78−7.72 (m, 2H), 7.69 (d, J = 9 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 7.57 (d, J = 8 Hz, 1H), 4.06 (septet, J = 7 Hz, 1H), 1.28 (d, J = 7 Hz, 6H); FDMS (MeOH) m/z 328 (M^+). Anal. ($C_{17}H_{16}N_2O_3S_1$) C, H, N.

***anti*-6-[(Hydroxyimino)phenylmethyl]-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (2).** Ketone **8** (2 g) was dissolved in dry methanol (30 mL), and dry pyridine (15 mL) was added. This was followed by the addition of hydroxylamine hydrochloride (2 g), and the reaction mixture was stirred under nitrogen overnight. At this time TLC (silica gel plate, with eluent 60% ethyl acetate and 40% hexane) showed complete conversion to a slower running product. The reaction mixture was poured into ethyl acetate (400 mL), the organic phase was washed two times with 1 N HCl (500 mL) and dried over magnesium sulfate, and the solvent was removed by rotary evaporation. The residue was then separated into the *cis* and *trans* isomers by reverse phase HPLC with a gradient of 49−52% acetonitrile/water: ^{1}H NMR (DMSO-d_6) δ 11.50 (s, 1H), 8.65 (s, 1H), 7.92 (d, J = 2 Hz, 1H), 7.80 (d, J = 8 Hz, 1H), 7.54−7.46 (m, 3H), 7.41 (dd, J = 8, 2 Hz, 1H), 7.37−7.29 (m, 2H), 3.97 (septet, J = 6 Hz, 1H), 1.27 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 343 (M^+). Anal. ($C_{17}H_{17}N_3O_3S_1$) C, H, N.

***syn*-6-[(Hydroxyimino)phenylmethyl]-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole:** ^{1}H NMR (DMSO-d_6) δ 11.48 (s, 1H), 8.69 (s, 1H), 7.93 (d, J = 8 Hz, 1H), 7.78 (d, J = 2 Hz, 1H), 7.44−7.35 (m, 5H), 7.33 (dd, J = 8, 2 Hz, 1H), 4.00 (septet, J = 6 Hz, 1H), 1.29 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 343 (M^+). Anal. ($C_{17}H_{17}N_3O_3S_1$) C, H, N.

6-Benzoyl-2-methyl-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (9). The ketone was prepared following the procedure for **8** above using triethyl orthoacetate as solvent. Starting with 14 g of compound **14** the yield of desired product was 12 g or 80%: ^{1}H NMR (DMSO-d_6) δ 8.15 (d, J = 1 Hz, 1H), 7.82, (d, J = 9 Hz, 1H), 7.78−7.71 (m, 3H), 7.68 (d, J = 8 Hz, 1H), 7.58 (d, J = 8 Hz, 1H), 7.56 (d, J = 8 Hz, 1H), 4.03 (septet, J = 7 Hz, 1H), 2.78 (s, 3H), 1.28 (d, J = 7 Hz, 6H); FDMS (MeOH) m/z 342 (M^+). Anal. ($C_{18}H_{18}N_2O_3S_1$) C, H, N, S.

***anti*-6-[(Hydroxyimino)phenylmethyl]-2-methyl-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (3).** The oximes were prepared following the procedure for **2** above: ^{1}H NMR (DMSO-d_6) δ 11.43 (s, 1H), 7.95 (d, J = 2 Hz, 1H), 7.63 (d, J = 8 Hz, 1H) 7.52−7.44 (m, 3H), 7.36−7.29 (m, 3H), 3.94 (septet, J = 6 Hz, 1H), 2.74 (s, 3H), 1.24 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 357 (M^+). Anal. ($C_{18}H_{19}N_3O_3S_1$) C, H, N.

***syn*-6-[(Hydroxyimino)phenylmethyl]-2-methyl-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole:** ^{1}H NMR (DMSO-d_6) δ 11.44 (s, 1H), 7.76−7.73 (m, 2H), 7.43−7.35 (m, 5H), 7.27 (d, J = 2 Hz, 1H), 3.95 (septet, J = 6 Hz, 1H), 2.67 (s, 3H), 1.24 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 357 (M^+). Anal. ($C_{18}H_{19}N_3O_3S_1$) C, H, N.

6-Benzoyl-2-methoxy-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (10). The ketone was prepared following the procedure for **8** above using tetramethyl carbonate as solvent.

The reaction gave two major products. The faster running product (TLC) was identified as the 2,2-dimethoxy derivative; the slower is the desired product. Starting with 8 g of ketone **14** the yield was 3.5 g or 39%: ^{1}H NMR (DMSO-d_6) δ 8.14 (s, 1H), 7.76−7.55 (m, 5H), 7.59 (d, J = 8 Hz, 1H), 7.56 (d, J = 8 Hz, 1H), 4.00 (septet, J = 6 Hz, 1H), 3.34 (s, 3H), 1.34 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 358 (M^+). Anal. ($C_{18}H_{18}N_2O_4S_1$) C, H, N.

***anti*-6-[(Hydroxyimino)phenylmethyl]-2-methoxy-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (4).** The oximes were prepared following the procedure for **2** above except that a quantity of *cis* (*syn*) isomer was selectively crystallized from methanol prior to the preparative HPLC purification step: ^{1}H NMR (DMSO-d_6) δ 11.29 (s, 1H), 7.90 (s, 1H), 7.52−7.34 (m, 3H), 7.31−7.25 (m, 2H), 7.21 (d, J = 9 Hz, 1H), 7.05 (d, J = 9 Hz, 1H), 3.96 (septet, J = 7 Hz, 1H), 3.31 (s, 3H), 1.30 (d, J = 7 Hz, 6H); FDMS (MeOH) m/z 373 (M^+). Anal. ($C_{18}H_{19}N_3O_4S_1$) C, H, N.

***syn*-6-[(Hydroxyimino)phenylmethyl]-2-methoxy-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole:** ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H), 7.58 (s, 1H), 7.40−7.32 (m, 6H), 7.18 (dd, J = 9, 1 Hz, 1H), 3.97 (septet, J = 7 Hz, 1H), 3.37 (s, 3H), 1.30 (d, J = 7 Hz, 6H); FDMS (MeOH) m/z 373 (M^+). Anal. ($C_{18}H_{19}N_3O_4S_1$) C, H, N.

6-Benzoyl-2-(methylthio)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (11). Ketone **14** (19 g, 60 mmol) was dissolved in dry THF (300 mL), and the solution under nitrogen was cooled to 0 °C in an ice bath. Sodium hydride (1 equiv) was added and the reaction mixture stirred for 20 min to allow anion formation. *N*,*N*-dimethythiocarbamoyl chloride was then added (7.4 g, 60 mmol), the ice bath was removed, and the reaction mixture was allowed to stir at room temperature for 3 h. At this point the reaction mixture was heated to reflux for 2 days. After cooling the reaction mixture was added to ethyl acetate (1.5 L) which was washed with brine (700 mL). The organic phase was then dried (magnesium sulfate) and the solvent removed by rotary evaporation. The residue was then purified by preparative HPLC (silica gel, gradient eluent of 30−70% ethyl acetate/hexane) to give thione **16** (6.5 g or 30% yield).

Thione **16** (6.5, 18 mmol) was dissolved in DMF (150 mL), and potassium carbonate (2.5 g, 18 mmol) was added. This was followed by the addition of methyl iodide (5 mL, excess), and the reaction mixture was allowed to stir overnight at room temperature. At this time TLC showed conversion of starting material to a faster running product (60% ethyl acetate/hexane), and the reaction mixture was poured into ethyl acetate (400 mL). The organic phase was washed, first with 1 N HCl (500 mL) and then three times with brine (500 mL), and dried (magnesium sulfate), and the solvent was removed by rotary evaporation to give a residue which crystallized from ether to give the desired product (5.77 g, 85%): ^{1}H NMR (DMSO-d_6) δ 8.12 (s, 1H), 7.83−7.71 (m, 4H), 7.68 (d, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 7.57 (d, J = 8 Hz, 1H), 4.03 (septet, J = 6 Hz, 1H), 2.72 (s, 3H), 1.32 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 374 (M^+). Anal. ($C_{18}H_{18}N_2O_3S_2$) C, H, N.

***anti*-6-[(Hydroxyimino)phenylmethyl]-2-(methylthio)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (5).** The oximes were prepared following the procedure for **2** above: ^{1}H NMR (DMSO-d_6) δ 11.41 (s, 1H), 7.92 (s, 1H), 7.61 (d, J = 9 Hz, 1H), 7.52−7.43 (m, 4H), 7.31 (dd, J = 9, 3 Hz, 1H), 7.27 (dd, J = 9, 2 Hz, 1H), 3.92 (septet, J = 6 Hz, 1H), 2.72 (s, 3H), 1.27 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 389 (M^+). Anal. ($C_{18}H_{19}N_3O_3S_2$) C, H, N.

***syn*-6-[(Hydroxyimino)phenylmethyl]-2-(methylthio)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole:** ^{1}H NMR (DMSO-d_6) δ 11.45 (s, 1H), 7.74 (d, J = 8 Hz, 1H), 7.69 (s, 1H), 7.46−7.34 (m, 5H), 7.27 (dd, J = 8, 2 Hz, 1H), 3.94 (septet, J = 6 Hz, 1H), 2.73 (s, 3H), 1.28 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 389 (M^+). Anal. ($C_{18}H_{19}N_3O_2S_2$) C, H, N.

6-Benzoyl-2-(methylamino)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (12). Ketone **14** (15.9 g, 50 mmol) was dissolved in dry THF (250 mL), placed under a nitrogen atmosphere, and cooled to 0 °C in an ice bath. Sodium hydride (1 equiv) was added, and after stirring for 15 min this was followed by the addition of methyl isothiocyanate (3.7g, 50 mmol). The ice bath was then removed, and after stirring at room temperature for 30 min TLC indicated that only slight reaction had occurred; therefore the reaction mixture was heated to reflux for 2 h. At this point substantial reaction had occurred, and after cooling the reaction mixture was poured into ethyl acetate (400 mL). The mixture was washed with brine (300 mL), the organic phase separated and dried (magnesium sulfate), and the solvent removed by rotary evaporation. The residue was purified by preparative HPLC (silica gel, gradient eluent 30−70% ethyl acetate/hexane) giving a major product (6.2 g) which was identified by FDMS as intermediate **15** [m/z 391 (M^+)].

Compound **15** (3.5 g) was dissolved in ethyl acetate (70 mL), and a solution of 4−5% NaOCl (70 mL) was added. After stirring for 3 min at room temperature the reaction mixture was poured into ethyl acetate (300 mL), which was then washed with brine (300 mL). The organic phase was then dried (magnesium sulfate), and the solvent was removed by rotary evaporation to give a residue which was crystallized from ether to give the desired product (2.45 g): ^{1}H NMR (DMSO-d_6) δ 7.94 (d, J = 1 Hz, 1H), 7.73−7.66 (m, 2H), 7.66−7.59 (m, 2H), 7.56 (d, J = 8 Hz, 1H), 7.53 (d, J = 8 Hz, 1H), 7.39 (d, J = 9 Hz, 1H), 7.14 (q, J = 5 Hz, 1H), 3.90 (septet, J = 7 Hz, 1H), 3.00 (d, J = 5 Hz, 3H), 1.27 (d, J = 7 Hz, 6H); FDMS (MeOH) m/z 357 (M^+). Anal. ($C_{18}H_{19}N_3O_3S_1$) C, H, N, S.

***anti*-6-[(Hydroxyimino)phenylmethyl]-2-(methylamino)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (6).** The oximes were prepared following the procedure described for **2** above: ^{1}H NMR (DMSO-d_6) δ 11.15 (s, 1H), 7.76 (d, J = 2 Hz, 1H), 7.49−7.39 (m, 3H), 7.30−7.25 (m, 2H), 7.21 (d, J = 9 Hz, 1H), 7.02 (dd, J = 9, 2 Hz, 1H), 6.85 (q, J = 5 Hz, 1H), 3.80 (septet, J = 7 Hz, 1H), 2.95 (d, J = 5 Hz, 3H), 1.22 (d, J = 7 Hz, 6H); FDMS (MeOH) m/z 372 (M^+). Anal. ($C_{18}7H_{20}N_4O_3S_1$) C, H, N.

***syn*-6-[(Hydroxyimino)phenylmethyl]-2-(methylamino)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole:** ^{1}H NMR (DMSO-d_6) δ 11.31 (s, 1H), 7.52 (d, J = 2 Hz, 1H), 7.40−7.33 (m, 5H), 7.33 (d, J = 9 Hz, 1H), 7.10 (dd, J = 9, 2 Hz, 1H), 6.85 (q, J = 5 Hz, 1H), 3.80 (septet, J = 7 Hz, 1H), 2.98 (d, J = 5 Hz, 3H), 1.23 (d, J = 7 Hz, 6H); FDMS (MeOH) m/z 372 (M^+). Anal. ($C_{18}H_{20}N_4O_3S_1$) C, H, N.

6-Benzoyl-2-(dimethylamino)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (13). The procedure is the same as for compound **12** above except that dimethylthiocarbamoyl chloride is used ring for closure. The yield, following purification by preparative HPLC, from starting ketone **14** (6.3 g, 20 mmol) was 2 g or 27%: ^{1}H NMR (DMSO-d_6) δ 8.07 (s, 1H), 7.78−7.71 (m, 3H), 7.67 (d, J = 8 Hz, 1H), 7.63−7.54 (m, 3H), 3.71 (septet, J = 6 Hz, 1H), 3.08 (s, 6H), 1.05 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 371 (M^+). Anal. ($C_{19}H_{21}N_3O_3S_1$) C, H, N.

***anti*-6-[(Hydroxyimino)phenylmethyl]-2-(dimethylamino)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole (7).** The oximes were prepared following the procedure described for **2** above: ^{1}H NMR (DMSO-d_6) δ 11.30 (s, 1H), 7.88 (s, 1H), 7.54−7.37 (m, 4H), 7.36−7.27 (m, 2H), 7.23 (d, J = 8 Hz, 1H), 3.62 (septet, J = 6 Hz, 1H), 3.01 (s, 6H), 1.02 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 386 (M^+). Anal. ($C_{19}H_{22}N_4O_3S_1$) C, H, N.

***syn*-6-[(Hydroxyimino)phenylmethyl]-2-(dimethylamino)-1-[(1-methylethyl)sulfonyl]-1*H*-benzimidazole:** ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H), 7.68 (d, J = 2 Hz, 1H), 7.44−7.35 (m, 5H), 7.23 (dd, J = 8, 2 Hz, 1H), 3.64 (septet, J = 6 Hz, 1H), 3.30 (s, 6H), 1.05 (d, J = 6 Hz, 6H); FDMS (MeOH) m/z 386 (M^+). Anal. ($C_{19}H_{22}N_4O_3S_1$) C, H, N.

CPE/XTT Assay. The CPE/XTT assay was carried out following the method described in ref 12.

Computational Method. We undertook a theoretical investigation of the conformations of Enviroxime in order to assess the conformational preference between the crystal structure forms.[2] Although all molecules and conformations are not describe here, we performed 3-21G *ab initio* calculations on a number of benzimidazole analogs to study the energetic preference between the L-shaped and V-shaped

isomers. In general, the L-shaped isomers are lower in energy than the V-shaped isomers. In all of these compounds we investigated we placed each molecule in an L-shaped conformer with the phenyl ring above the plane of the benzimidazole ring and the oxime group below the plane of the benzimidazole ring. Moreover, our calculations sought to understand the effects of substitution at the C2 position. The minimum energy conformations for the ground state structures were obtained with Pople's GAUSSIAN 94 program[8] via the RHF/3-21G basis level.[9] All structures were fully optimized with analytical gradient methods on a CRAY J90 computer.

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References

(1) 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.

(2) 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]-1*H*-benzimidazol-2-amine. Inhibitors of Rhinovirus Multiplication. *J. Med. Chem*. **1980**, *23*, 368−372.

(3) 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.

(4) 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.

(5) 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.

(6) Enviroxime (IND 17152). IND Protocol No. 1 and Amendment to IND Protocol No. 1, Phase 1, Final Report, Summary; unpublished results.

(7) 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**, *1/8234*, 1342−1344.

(8) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, revision C.3; Gaussian, Inc.: Pittsburgh, PA, 1995.

(9) 3-21G: Binkley, J. S.; Pople, J. A.; Hehre, W. J. Self-Consistent Molecular Orbital Methods. 21. Small Split-Valence Basis Sets for First-Row Elements. *J. Am. Chem. Soc.* **1980**, *102*, 939−947.

(10) Spitzer, W. A. Antiviral Benzimidazoles. U.S. Patent 4,492,708, 1985.

(11) Ryan, C. W.; Slomaski, B. A. Synthesis of Alkylidene Intermediates. U.S. Patent 4,501,921, 1985.

(12) Heinz, B. A.; Tang, J.; Labus, J. M.; Chadwell, F. W.; Kaldor, S. W.; Hammond, M. A Simple In Vitro Translation Assay to Analyze Inhibitors of Rhinovirus Proteases. *Antimicrob. Agents Chemother.* **1996**, *40/1*, 267−270.

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