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Fundamental Structure–Activity Relationships Associated with a New Structural Class of Respiratory Syncytial Virus Inhibitor

Kuo-Long Yu,^a Yi Zhang,^a Rita L. Civiello,^a Kathleen F. Kadow,^b
Christopher Cianci,^b Mark Krystal^b and Nicholas A. Meanwell^{a,*}

^a*Department of Chemistry, The Bristol-Myers Squibb Pharmaceutical Research Institute,
5, Research Parkway, Wallingford, CT 06492, USA*

^b*Department of Virology, The Bristol-Myers Squibb Pharmaceutical Research Institute,
5, Research Parkway, Wallingford, CT 06492, USA*

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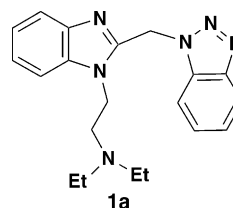
Abstract—Structure–activity relationships surrounding the dialkylamino side chain of a series of benzotriazole-derived inhibitors of respiratory syncytial virus fusion based on the screening lead **1a** were examined. The results indicate that the topology of the side chain is important but the terminus element offers considerable latitude to modulate physical properties.

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Outbreaks of respiratory syncytial virus (RSV) occur annually in the United States, generally emerging in the Fall and lasting until the Spring, although infections have also been detected in the summer months.^{1–4} Almost all children encounter RSV before the age of 2 years and reinfection is a common occurrence. The disease is particularly dangerous for those infants with underlying cardiopulmonary problems^{1–3} and in a recent study RSV was identified as the most common viral cause of death in children under 5 years of age.⁵ Moreover, the morbidity and mortality associated with RSV infections of the elderly may be significantly underestimated since it is frequently misdiagnosed as influenza.⁵ In bone marrow transplant units, RSV is the leading cause of death of this immunocompromised population, with a fatality rate of 80%.^{6,7} Prevention of RSV infection relies upon a series of injections of the humanized monoclonal antibody palivizumab (Respi-gam®),⁸ whilst therapeutic options are restricted to a single agent, the teratogen ribavirin, which was originally licensed specifically for this indication.⁹ As a consequence, the identification of potent and selective inhibitors of RSV has recently attracted considerable attention and several classes of inhibitor have been described.^{1,10} A number of these agents have, interestingly, been shown to interfere

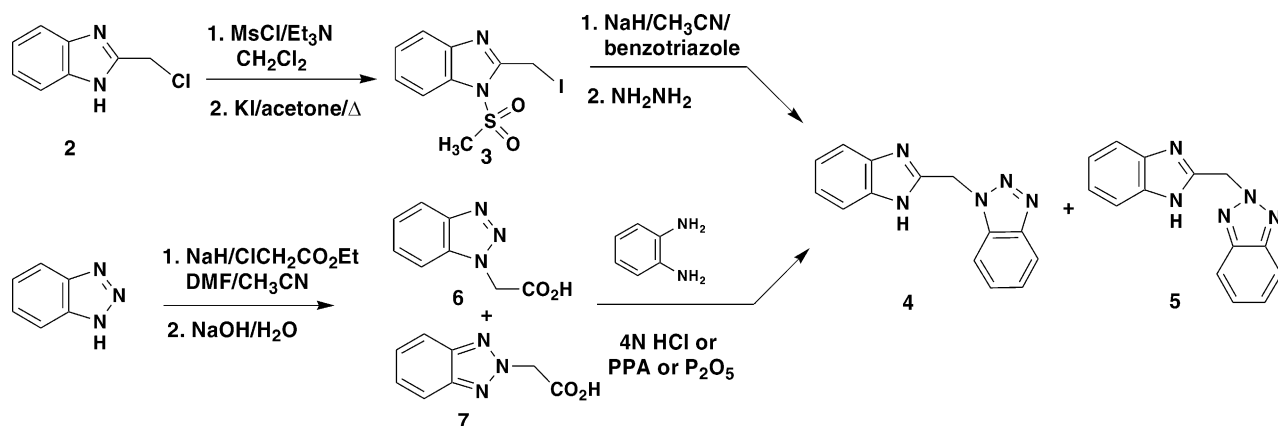
with the fusion of virus and host cell membranes, mechanistic insight based on an analysis of biochemical pharmacology and the identification of mutations associated with the selection of resistant virus.

We have recently described the discovery and antiviral activity associated with the benzotriazole derivative **1a** as the first representative of a new structural class of RSV inhibitor.¹¹ This compound, which appears to act by preventing the fusion of virus and host membranes, possesses drug-like qualities¹² and provided a compelling opportunity for the discovery of orally effective RSV inhibitors.¹³ In this communication, we delineate the first steps in that process by describing fundamental aspects associated with the relationship between the structure and antiviral activity of **1a**.



Scheme 1 depicts two complementary approaches to the synthesis of the key heterocyclic precursors **4** and **5**.¹⁴ The nitrogen atom of 2-chloromethyl benzimidazole **2** was protected by mesylation and the chlorine exchanged under Finkelstein reaction conditions to provide the

*Corresponding author. Tel.: +1-203-677-6679; fax: +1-203-677-7702; e-mail: nicholas.meanwell@bms.com

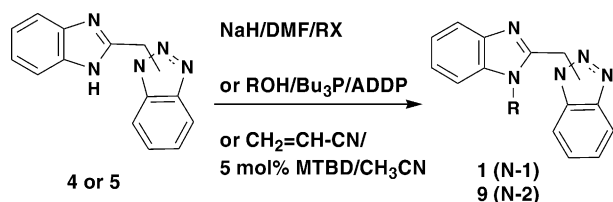


Scheme 1.

iodide **3**. Alkylation of the sodium salt of benzotriazole with **3** followed by exposure of the crude reaction product to an excess of hydrazine at 65 °C afforded a mixture of the N-1 and N-2 derivatives **4** and **5**, respectively. Chromatographic resolution was readily accomplished and the minor, more mobile isomer identified as the N-2-substituted compound **5** based upon the ¹H NMR spectrum, the simplicity of which reflected the symmetry inherent to this topology.^{14,15} Minor isomer **5** was isolated in 5% yield, an order of magnitude less than the 48% yield recorded for the major isomer **4**. Alternatively, alkylation of benzotriazole with ethyl chloroacetate in a mixture of DMF and CH₃CN followed by hydrolysis afforded a mixture of the N-1- and N-2-substituted carboxylic acids **6** and **7**, respectively. Condensation with phenylenediamine in the presence of 4N HCl, polyphosphoric acid or P₂O₅ in toluene at reflux afforded **4** and **5**.^{14,16,17}

The benzimidazole moiety of **4** and **5** was readily derivatized using the three fundamental protocols depicted in Scheme 2.¹⁸ Alkylation using halides or sulfonates was readily accomplished using NaH as the base in DMF. Alcohols participated as coupling partners when activated by the more powerful conditions associated with the use of Bu₃P and 1,1'-(azodicarbonyl)dipiperidine (ADDP), a Mitsunobu protocol.¹⁹ A Michael reaction using acrylonitrile, methyl vinyl ketone, acrylamide or *t*-butyl acrylate as the acceptor afforded the adducts **1u**, **1v**, **1ad** and **1af**, respectively. Ester **1aa** was hydrolyzed to the corresponding acid using NaOH in aqueous EtOH whilst exposure of the *t*-Bu ester **1ad** to 50% trifluoroacetic acid in CH₂Cl₂ provided acid **1ae**.

Ketones **1v** and **1ai** and ester **1aa** were reduced to the corresponding alcohols in straightforward fashion by



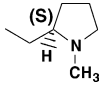
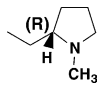
Scheme 2.

treating with NaBH₄ and LiAlH₄, respectively. The dimethylamine **1b** was quaternized with methyl iodide to afford **1c**. Heating **4** with ethylene carbonate at 120 °C provided access to alcohol **1t**, which was mesylated, exposed to NaN₃ in DMF and the resultant azide reduced by catalytic hydrogenation to afford the primary amine **1k**. The oxidation state of sulfide **1x** was adjusted by treatment with sodium perborate in acetic acid to afford sulfoxide **1y** or magnesium monoperoxyphthalate hexahydrate in DMF to provide sulfone **1z**.¹⁸

The compounds that constitute this preliminary survey of benzotriazole-derived RSV inhibitors were evaluated as inhibitors of the cytopathic effect induced by the Long (A) strain of RSV in the HEp-2 human lung carcinoma cell line.¹¹ The antiviral activity is reported as an EC₅₀, which represents the concentration of compound required to provide 50% protection, and is compared with the concentration of compound that causes cytotoxicity to uninfected HEp-2 cells. The results are compiled in Tables 1 and 2.

A basic understanding of the effect on antiviral activity of varying the side chain element in the context of amine-containing compounds was developed by evaluating the series **1a–m**. The structure–activity correlate exhibited by the homologous series **1b**, **1a** and **1d** indicates a clear preference for less bulky terminal substitution, particularly evident in the relatively poor activity associated with the diisopropyl derivative **1d**. This trend is reflected in the series of analogues **1e–g**, in which the terminal amine moiety is incorporated into a saturated ring, with pyrrolidine **1e** the most potent. Examination of the alternative cyclization topology represented by the enantiomeric pair **1h** and **1i** revealed some dependence on chirality, with a preference for the (*R*)-configuration. The quaternary ammonium derivative **1c** is a potent antiviral agent, a result compatible with the proposal that the biochemical target is extracellular.¹¹ Whilst the activity of the mono-methyl derivative **1j** is similar to the disubstituted homologue **1b**, reducing the size of the lipophilic umbrella around the amine moiety results in a markedly inferior compound, **1k**. The effect of side chain length was examined in the context of **1l** and **1m**, both exhibiting reduced activity.

Table 1. Structure, RSV inhibitory activity and cytotoxicity associated with a series of N-1-substituted benzotriazole derivatives

Compd	R	Antiviral activity EC ₅₀ (μM) ^a	Cytotoxicity CC ₅₀ (μM) ^b
4	H	31.1 (<i>n</i> = 1)	164 (<i>n</i> = 1)
1a	CH ₂ CH ₂ NEt ₂	0.47 (0.14/0.8)	216 (144/287)
1b	CH ₂ CH ₂ NMe ₂	0.14 (0.03/0.25)	234 (<i>n</i> = 1)
1c	CH ₂ CH ₂ NMe ₃ ⁺ I [−]	0.87 (<i>n</i> = 1)	42.5 (42/43)
1d	CH ₂ CH ₂ NiPr ₂	6.7 (4.5/8.9)	42.3 (40.0/44.5)
1e	CH ₂ CH ₂ N(cCH ₂) ₄	0.6 (0.48/0.72)	80 (71.6/88.3)
1f	CH ₂ CH ₂ N(cCH ₂) ₅	1.1 (0.53/1.62)	75 (57.7/92.4)
1g	CH ₂ CH ₂ N(cCH ₂) ₆	2.8 (2.2/3.4)	67.1 (55.9/78.3)
1h		7.36 (6.25/8.47)	24.4 (10.3/38.4)
1i		1.0 (0.26/1.73)	181 (53/210)
1j	CH ₂ CH ₂ NHMe	0.21 (0.16/0.25)	74.5 (62/87)
1k	CH ₂ CH ₂ NH ₂	3.3 (3.2/3.4)	
1l	CH ₂ CH ₂ CH ₂ NEt ₂	NA	
1m	CH ₂ CH ₂ CH ₂ NMe ₂	1.1 (<i>n</i> = 1)	197 (<i>n</i> = 1)
1n	CHMe ₂	15.5 (3.4/27.5)	129 (86/172)
1o	CH ₂ CHMe ₂	NA	15.5 (5.2/25.8)
1p	CH ₂ CH ₂ CHMe ₂	0.1 (0.078/0.125)	39.9 (17.2/62.7)
1q	CH ₂ CH=CMe ₂	3.3 (0.28/6.3)	102 (94/110)
1r	(CH ₂) ₃ CHMe ₂	0.19 (0.16/0.22)	24 (8.4/39.3)
1s	CH ₂ CH ₂ CH ₂ CH ₃	0.066	12 (10.7/13.11)
1t	CH ₂ CH ₂ OH	7.7 (5.1/10.2)	205 (34.1/375.4)
1u	CH ₂ CH ₂ CN	2.69 (2.61/2.77)	49.3 (5.3/93.3)
1v	CH ₂ CH ₂ C(O)CH ₃	0.52 (0.21/0.82)	59 (57/61)
1w	CH ₂ CH ₂ CH(OH)CH ₃	0.12 (0.09/0.15)	> 309
1x	CH ₂ CH ₂ SCH ₃	0.3 (0.22/0.37)	166 (155/177)
1y	CH ₂ CH ₂ SOCH ₃	0.43 (0.27/0.59)	56.4 (55.4/57.3)
1z	CH ₂ CH ₂ SO ₂ CH ₃	0.25	337 (175/499)
1aa	CH ₂ CO ₂ CH ₃	NA	374 (125/623)
1ab	CH ₂ CO ₂ H	NA	303
1ac	CH ₂ CO ₂ NEt ₂	NA	250
1ad	CH ₂ CH ₂ CO ₂ tBu	4.31	9.98 (2.19/17.76)
1ae	CH ₂ CH ₂ CO ₂ H	18.1 (5.54/2.57/46.1)	184 (31/211/311)
1af	CH ₂ CH ₂ CONH ₂	2.1 (1.78/2.41)	4.28 (2.5/6.06)
1ag	CH ₂ Ph	10.5 (<i>n</i> = 1)	9 (<i>n</i> = 1)
1ah	CH ₂ CH ₂ Ph	0.77 (0.5/1.03)	2.6 (2.5/2.7)
1ai	CH ₂ C(O)Ph	2.2 (0.29/0.5/5.8)	18 (4.5/19.3/31.7)
1aj	CH ₂ CH(OH)Ph	0.59 (0.24/0.94)	37.6 (27.6/47.5)

^aValues are the means of two experiments performed on consecutive weeks with the data from individual experiments shown in parentheses. NA, not active.

Table 2. Structure, RSV inhibitory activity and cytotoxicity for a series of N-2-substituted benzotriazole derivatives

Compd	R	Antiviral activity EC ₅₀ (μM) ^a	Cytotoxicity CC ₅₀ (μM) ^a
9a	CH ₂ CH ₂ NEt ₂	0.22 (0.21/0.23)	83.7 (83.5/83.9)
9b	CH ₂ CH ₂ NMe ₂	0.18 (0.17/0.19)	212 (200/223)
9d	CH ₂ CH ₂ NiPr ₂	0.26 (0.20/0.32)	42 (21/63)
9e	CH ₂ CH ₂ N(cCH ₂) ₄	0.42 (0.24/0.60)	127 (100/154)
9f	CH ₂ CH ₂ N(cCH ₂) ₅	2.6 (0.7/4.3)	64 (21/107)
9g	CH ₂ CH ₂ N(cCH ₂) ₆	1.1 (0.4/0.8)	23 (19/27)
9k	CH ₂ CH ₂ CH ₂ NMe ₂	3.6 (<i>n</i> = 1)	182 (<i>n</i> = 1)
9l	CH(CH ₃) ₂	51.5 (<i>n</i> = 1)	58 (<i>n</i> = 1)
9n	CH ₂ CH(CH ₃) ₂	47.5 (29.7/65.3)	106 (82/130)
9p	CH ₂ CH ₂ CH(CH ₃) ₂	0.44 (0.43/0.45)	13.5 (6.8/20.2)
9ab	CH ₂ CO ₂ NEt ₂	NA	

^aValues are the means of two experiments performed on consecutive weeks with the data from individual experiments shown in parentheses. NA, not active.

The survey of SAR was extended to include a series of non-basic side chains (**1t–1aj**), an exercise that revealed that a range of lipophilic and polar but non-charged functionality is compatible with potent antiviral effect. These data indicate a preference for more lipophilic elements optimally disposed two atoms away from to the heterocyclic core. This is clearly apparent in the series of alkyl-substituted derivatives **1n–s** where branching proximal to the core affords poorly active RSV inhibitors, **1n** and **1o**. Terminal branching that more closely mimics the topology discovered with the lead **1a** is more effective, although the simple *n*-butyl-substituted analogue **1s** emerged as the most potent RSV inhibitor in this study.

The N-2 substituted benzotriazole derivatives were examined less extensively in deference to templates that offered increased structural versatility and inherently greater potency.^{20–22} As a consequence, the SAR for this symmetrical topology is less well developed. However, where comparisons can be made, RSV inhibitory properties resemble the N-1 isomers. The only notable exception is for the pair of diisopropylaminoethyl derivatives **1d** and **9d** where the N-2 isomer is clearly superior. The origin of this difference is not immediately obvious from the limited SAR study conducted but may reflect reduced non-bonded interactions between the side chain and benzotriazole moiety of the less compact **9d** in the bound state.

Several of the RSV inhibitors prepared as part of this study demonstrated significant cytotoxicity towards the host cell, resulting in a poor therapeutic index (TI). Indeed this measure of drug safety ranged from an index essentially non-existent with **9l** and **1ag** to >2575 with the alcohol **1w**. Particularly prominent amongst the more cytotoxic compounds are those in the short series represented by **1ag–1aj**. These compounds all incorporate a benzene ring in the side chain and the simple prototypes **1ag** and **1ah** are the most cytotoxic compounds evaluated. However, the introduction of polar functionality improves this problematic aspect of the profile of these RSV inhibitors and the alcohol **1aj** exhibits an improved TI. A similar correlate can be observed between the alkane **1s** and alcohol **1w**, with the latter compound only 2-fold less potent than the former but offering a much improved TI. Interestingly, cytotoxicity has been equated with lipophilicity within a series of HIV protease inhibitors.²³

In conclusion, basic structure–activity relationships associated with benzotriazole-based RSV fusion inhibitors have been examined, providing, an addition to this newly emerging class of antiviral agents.^{24–29} These studies have established the essential nature of a side-chain moiety appended to the benzimidazole nucleus for expression of potent antiviral activity. Activity is sensitive to the topology of the side-chain element, with branching proximal to the heterocyclic nucleus deleterious. However, there is a clear tolerance for a range of polar functionality that provides considerable latitude to modulate both pharmaceutical and pharmacokinetic properties, of considerable importance in the quest for

orally effective inhibitors of RSV. Progress towards these objectives will be the topic of future publications that describe the further evolution of this chemotype.

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