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Bioorganic & Medicinal Chemistry Letters 16 (2006) 1115–1122

Bioorganic & Medicinal Chemistry Letters

Respiratory syncytial virus fusion inhibitors. Part 3: Water-soluble benzimidazol-2-one derivatives with antiviral activity in vivo[☆]

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Received 11 November 2005; revised 24 November 2005; accepted 28 November 2005
Available online 20 December 2005

Dedicated to the memory of John E. Lawson, Ph.D.

Abstract—The introduction of acidic and basic functionality into the side chains of respiratory syncytial virus (RSV) fusion inhibitors was examined in an effort to identify compounds suitable for evaluation in vivo in the cotton rat model of RSV infection following administration as a small particle aerosol. The acidic compounds **2r**, **2u**, **2v**, **2w**, **2z**, and **2aj** demonstrated potent antiviral activity in cell culture and exhibited efficacy in the cotton rat comparable to ribavirin. In a BALB/c mouse model, the oxadiazolone **2aj** reduced virus titers following subcutaneous dosing, whilst the ester **2az** and amide **2aab** exhibited efficacy following oral administration. These results established the potential of this class of RSV fusion inhibitors to interfere with infection in vivo following topical or systemic administration.

Annual outbreaks of respiratory syncytial virus (RSV) infections occur most prominently during the winter season in the United States, although the virus has also been detected during the summer months.[3–8] Exposure to RSV is essentially universal for those under 2 years of age and re-infection is common, a consequence of an incomplete immune response of limited durability. RSV can also occur as a co-infection with other respiratory pathogens, resulting in exacerbated symptoms.[9,10] Those most vulnerable to serious complications from RSV infection are infants with underlying cardiopulmonary problems.[8] RSV infections are also problematic in the elderly and immunocompromised, populations where morbidity and mortality are underestimated since it is frequently misdiagnosed as influenza.[8,11–13] Of particular note, outbreaks of RSV in bone marrow transplant units are associated with high levels of mortality.[14,15] The treatment of RSV infection is currently limited to administration of the nucleoside analogue ribavirin, a teratogen with an enigmatic mode of action that is frequently administered as an aerosol.[16] Prophylaxis with the humanized monoclonal antibody palivizumab (Synagis®), delivered by a series of injections during the disease season, has shown benefit in the prevention of RSV infections.[17,18] However, the high cost of palivizumab[19–22] restricts its widespread use and there remains a need for potent, selective, and efficacious inhibitors of RSV suitable

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Keywords: Water-soluble respiratory syncytial virus inhibitors; Antiviral; Benzimidazol-2-one derivatives.

[☆] For Parts 1 and 2 of this series, see Refs. 1 and 2.

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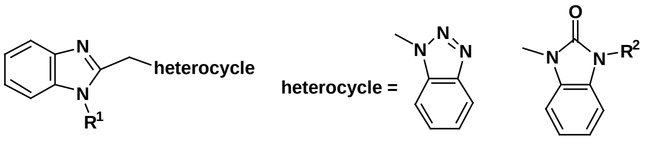
doi:10.1016/j.bmcl.2005.11.109



1a 1b

for clinical use.[3–6,23–26] Against this background, there has been considerable recent interest in discovering and developing inhibitors of RSV, a process that has generally relied upon screening campaigns using a virus replication assay. Interestingly, whilst this strategy is essentially devoid of mechanistic bias, the majority of RSV inhibitors that have been characterized appear to interfere with the fusion of virus and host cell membranes.[27–38]

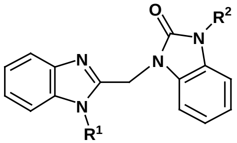
The discovery and preliminary structure–activity relationships associated with a new structural class of RSV fusion inhibitors[1,2] have been described, and this provided the foundation for the discovery of BMS-433771, an orally active antiviral agent.[39–41] Whilst a series of benzotriazole derivatives **1a** were the focus of initial studies,[1] evolution of this chemotype resulted in the development of a series of more potent RSV inhibitors based on the benzimidazol-2-one template generically represented by **1b**.[2] However, reproducible antiviral activity in vivo in rodent models of RSV infection following oral or subcutaneous administration with representative compounds from either series **1a** or **1b** could not be obtained. As a consequence, we sought to establish RSV inhibitory activity in vivo using topical delivery of compounds via small particle aerosol.[42] In order to pursue this strategy, RSV inhibitors with a high level of aqueous solubility suitable for formulation as aerosols were required. In this communication, the synthesis and SAR associated with a series of RSV inhibitors that incorporate polar functionality conferring aqueous solubility are described. Representative compounds from this study demonstrated antiviral activity in the cotton rat model of RSV infection, establishing proof of concept in vivo for this chemotype, which has been shown to function through destabilization of the 6-helix bundle assembly that is a critical step in the function of the fusion protein.[41]

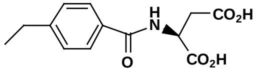
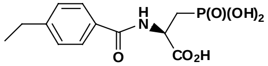
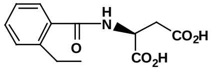
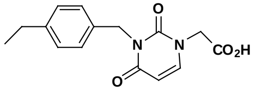
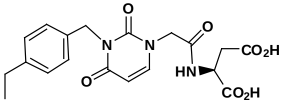
The introduction of side chains containing both acidic and basic moieties to the benzimidazole and the benzimidazol-2-one heterocycles of the core template **2** were examined as part of this study. The scope of the survey is compiled in Tables 1 (acidic compounds) and 2 (basic amine-containing derivatives). The three general synthetic strategies that had been optimized in earlier studies were utilized to assemble target compounds and are summarized in Scheme 1.[2,43–45] Side-chain elements were generally introduced to substrates **3** and **6** using alkylation methodology and subsequent functional group manipulation was straightforward, with simple alkyl esters converted to carboxylic acids under alkaline conditions and *tert*-butyl esters (for the preparation of **2z**) unmasked under acidic conditions. The sulfonic acids **2l** and **2m** were accessed in a direct fashion using the appropriate commercially available sultones as electrophiles. Carboxylic acids **2n** and **2p** were derivatized to the dicarboxylates **2v** and **2x**, respectively, by coupling with the dimethyl ester of L-aspartic acid using PyBroP in DMF in the presence of (i-Pr)$_2$NEt, followed by ester hydrolysis. The iminodiacetic acid derivative **2at** was obtained from **2ar** in a similar fashion. The acylsulfonamides **2f** and **2g** originated from acid **2a** using protocols described in the literature,[46,47] while the tetrazoles **2h–k** and **2aa–2ac** were prepared from the corresponding nitriles[2] by heating with NaN$_3$ and NH$_4$Cl in DMF at 110 °C.[48] For compounds **2ag** and **2ah**, benzylation of the benzimidazol-2-one nitrogen was conducted on the trityl-protected form of tetrazole **2ae**, a compound procured by exposure of **2ae** to trityl chloride in the presence of Et$_3$N in DMF at 70 °C.[43] Removal of the trityl moiety subsequent to alkylation was accomplished by exposure to HCl in MeOH or one equivalent of NaOH in MeOH. BBr$_3$-mediated demethylation of **2ah** afforded **2ai**. The oxadiazolone **2aj** was prepared from the corresponding amidoxime, derived by exposing the appropriate nitrile to hydroxylamine, after treatment with phosgene in toluene at 120 °C for 8 h.[49] Phosphonic acids **2r**, **2t**, and **2ap** were liberated from their esters using TMSBr in CH$_2$Cl$_2$.[50]

The compounds presented in Tables 1 and 2 were evaluated as inhibitors of the RSV-induced cytopathic effect (CPE) of the Long (a subtype A) strain of virus replicating in HEp-2 human lung epithelial carcinoma cells.[39] The antiviral activity, defined as the EC$_{50}$, is the concentration of test compound preserving 50% of infected cells, whilst the CC$_{50}$ is the concentration of drug that manifests cytotoxicity toward 50% of uninfected HEp-2 cells. The therapeutic index reported in the tables is the ratio of CC$_{50}$ to EC$_{50}$. Test compounds were typically evaluated in two consecutive experiments with additional experiments conducted in the absence of reasonable concordance. Where the data reported are the average of 2 or 3 experiments, the individual results provide a measure of assay variability.

The results presented in Tables 1 and 2 extend and refine the structure–activity correlates developed earlier for this class of RSV fusion inhibitors and demonstrate quite clearly that polar, water-solubilizing elements deployed at the terminus of either the benzimidazole or benzimidazol-2-one side chains are compatible with potent antiviral activity.[1,2] The initial survey was conducted with the *iso*-amyl side chain appended to the benzimidazole moiety since this configuration had emerged as optimal from earlier work.[1,2] For the series of simple alkyl carboxylates defined by **2a–e**, superior potency is observed with longer alkyl chains that project the polar functionality away from the benzimidazol-2-one nucleus, a trend largely reproduced with the homologous series of tetrazoles **2h–k**, an established carboxylic acid isostere.[51] Another carboxylate isostere, the acylsulfonamide moiety,[46] was examined only in the context of the acetic acid derivative **2a**. However, both **2f** and **2g** are weaker antiviral agents than the progenitor. These observations are consistent with the proposed mode of binding of this class of inhibitors to the trimeric amino

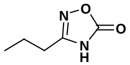
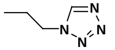
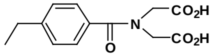
Table 1. Structure RSV inhibitory activity, and cytotoxicity associated with a series of carboxylic acid-substituted benzimidazol-2-one derivatives



Compound	R^1	R^2	Antiviral activity EC_{50}[a] (μM)	Cytotoxicity CC_{50} (μM)	Therapeutic index[b]
2a	$CH_2CH_2CH(CH_3)_2$	CH_2CO_2H	0.163 ± 0.064 (*n* = 3)	194.3 ± 48.0 (*n* = 3)	
2b	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CO_2H$	0.015 (0.013/0.016)	102.1 (99.6/104.6)	6806
2c	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CO_2H$	0.075 (0.047/0.104)	138.2 (172.8/103.5)	1843
2d	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CH_2CO_2H$	0.008 (0.003/0.013)	16.5 (18.9/14.1)	2062
2e	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CH_2CH_2CO_2H$	0.015 (0.015/0.015)	16.5 (20.5/12.5)	1100
2f	$CH_2CH_2CH(CH_3)_2$	$CH_2CONHSO_2CH_3$	0.613 (1.110/0.116)	151.7 (116.4/187.0)	247
2g	$CH_2CH_2CH(CH_3)_2$	$CH_2CONHSO_2Ph$	1.214 (0.979/1.449)	14.1 (9.8/18.5)	12
2h	$CH_2CH_2CH(CH_3)_2$	CH_2CN_4H	0.500 (0.428/0.572)	>210	>420
2i	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CN_4H$	0.235 (0.240/0.229)	37.1 (21.2/15.9)	158
2j	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CH_2CN_4H$	0.015 (0.015/0.014)	14.9 (20.8/9.0)	993
2k	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CH_2CH_2CN_4H$	0.013 (0.013/0.013)	90.0 (18.6/161.5)	6923
2l	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH_2SO_3H$	0.038 (0.042/0.034)	114.5/>209	3013
2m	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH_2CH_2SO_3H$	0.052 (0.003/0.101)	2.9 (2.5/3.3)	56
2n	$CH_2CH_2CH(CH_3)_2$	CH_2-4-C_6H_4-CO_2H	0.011 ± 0.0006 (*n* = 3)	27.3 ± 10.5 (*n* = 3)	2482
2o	$CH_2CH_2CH(CH_3)_2$	CH_2-3-C_6H_4-CO_2H	0.010 (0.012/0.007)	10.7 (16.0/5.4)	1070
2p	$CH_2CH_2CH(CH_3)_2$	CH_2-2-C_6H_4-CO_2H	0.011 (0.010/0.012)	15.7 (16.5/14.9)	1427
2q	$CH_2CH_2CH(CH_3)_2$	CH_2-3,5-C_6H_3-$(CO_2H)_2$	0.656 ± 0.923 (*n* = 3)	>168 (*n* = 3)	>256
2r	$CH_2CH_2CH(CH_3)_2$	CH_2-4-C_6H_4-$P(O)(OH)_2$	0.027±0.036 (*n* = 3)	81/>175/>175	3000
2s	$CH_2CH_2CH(CH_3)_2$	CH_2-4-C_6H_4-P(O)(OH)(OEt)	0.048 ± 0.027 (*n* = 3)	>180/>180/161	3354
2t	$CH_2CH_2CH(CH_3)_2$	CH_2-4-C_6H_4-$CH_2P(O)(OH)_2$	0.051 (0.050/0.051)	2.8 (3.0/2.7)	55
2u	$CH_2CH_2CH(CH_3)_2$	CH_2-2-C_6H_4-SO_3H	0.007 ± 0.004 (*n* = 5)	>19 (19/>189/>189/28.5)	>2714
2v	$CH_2CH_2CH(CH_3)_2$		0.005 ± 0.003(*n* = 4)	>144 (*n* = 4)	>28800
2w	$CH_2CH_2CH(CH_3)_2$		0.034 ± 0.025 (*n* = 3)	>114 (*n* = 3)	>3353
2x	$CH_2CH_2CH(CH_3)_2$		0.006 (0.006/0.005)	154/>159	⩾25667
2y	$CH_2CH_2CH(CH_3)_2$		0.003 (0.003/0.002)	>159	>53000
2z	$CH_2CH_2CH(CH_3)_2$		0.008 ± 0.003(*n* = 4)	>106 (*n* = 4)	>13250
2aa	CH_2CN_4H	*iso*-Propenyl	8.437 (7.668/9.205)	181/>227	21

(*continued on next page*)

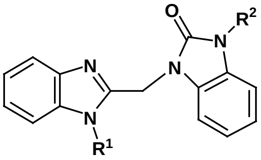
Table 1 (*continued*)

Compound	R^1	R^2	Antiviral activity EC_{50}[a] (μM)	Cytotoxicity CC_{50} (μM)	Therapeutic index[b]
2ab	$CH_2CH_2CN_4H$	*iso*-Propenyl	0.008 ± 0.003 (*n* = 3)	>249/>249/116.4	14550
2ac	$CH_2CH_2CH_2CN_4H$	*iso*-Propenyl	0.014 ± 0.009 (*n* = 3)	>0.33 (1.6/0.33/>205.9)	>23
2ad	$CH_2CH_2CN_4H$	*iso*-Propyl	0.015 (0.014/0.016)	177.1/>226.0	11806
2ae		H	1.007 (0.661/1.352)	228/>228	226
2af		CH_3	0.593 (0.495/0.691)	>201.8	>340
2ag		CH_2Ph	0.114 (0.091/0.136)	129.3/>192.3	1134
2ah		CH_2-4-C_6H_4-OCH_3	0.333 (0.218/0.448)	41.1 (38.9/43.3)	123
2ai		CH_2-4-C_6H_4-OH	0.063 (0.043/0.083)	58.7 (94.0/23.5)	932
2aj		*iso*-Propyl	0.021 ± 0.014 (*n* = 6)	75.45 ± 75.75	3545
2ak		H	1.196 (1.365/1.026)	92/>251	77
2al		CH_2CO_2H	0.155 (0.126/0.184)	20.6 (8.3/32.9)	133
2am		CH_2-4-C_6H_4-CO_2H	0.014 ± 0.011 (*n* = 6)	75.53 ± 82.68 (*n* = 6)	5395
2an		CH_2-3-C_6H_4-CO_2H	0.007 ± 0.006 (*n* = 3)	>193.6/>193.6/45	6428
2ao		CH_2-2-C_6H_4-SO_3H	0.013 ± 0.012 (*n* = 5)	129.6 ± 106.8	9969
2ap		CH_2-4-C_6H_4-$P(O)(OH)_2$	0.368 ± 0.31 (*n* = 3)	>174.1 (*n* = 3)	>473
2aq	$CH_2CH_2CH_2OH$	CH_2CO_2H	1.93 (2.47/1.38)	229 (229/229)	119
2ar	$CH_2CH_2CH_2OH$	CH_2-4-C_6H_4-CO_2H	0.049 (0.076/0.022)	209.7 (202.9/216.4)	4280
2as	$CH_2CH_2CH_2CH_2OH$	CH_2-4-C_6H_4-CO_2H	0.004 (0.005/0.003)	>196 (*n* = 2)	>49000
2at	$CH_2CH_2CH_2CH_2OH$		0.057 (0.070/0.044)	112.0 (148.8/75.2)	1965

[a] Values are means of two or more experiments performed on consecutive weeks with the data from individual experiments shown in parentheses.

[b] Therapeutic index = CC_{50}/EC_{50}.

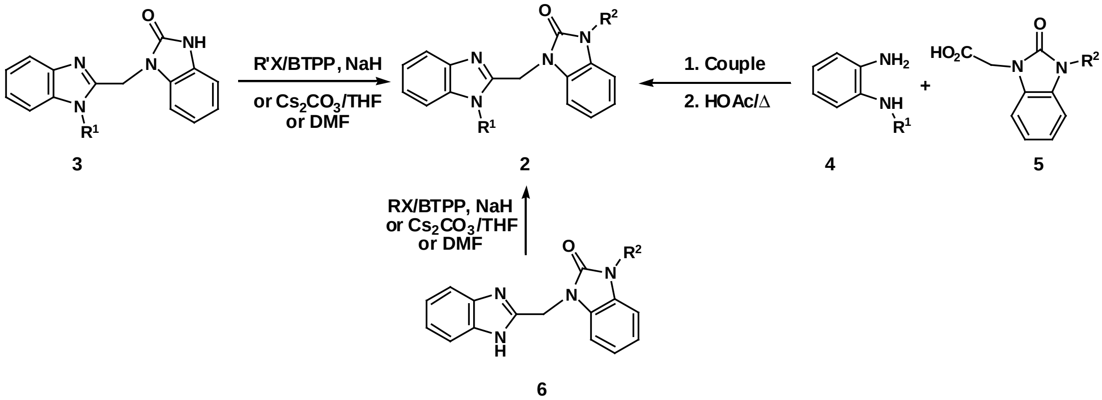
Table 2. Structure, RSV inhibitory activity, and cytotoxicity associated with a series of amine-substituted benzimidazol-2-one derivatives



Compound	R^1	R^2	Antiviral activity EC_{50}[a] (μM)	Cytotoxicity CC_{50} (μM)	Therapeutic index[b]
2au	$CH_2CH_2N(CH_3)_2$	H	0.062 (0.049/0.075)	292.2 (286.3/298.1)	4712
2av	$CH_2CH_2N(CH_3)_2$	*iso*-Propenyl	0.011 (0.014/0.007)	9.9 (10.2/9.7)	900
2aw	$CH_2CH_2N(CH_3)_2$	*iso*-Propyl	0.014 (0.010/0.018)	9.5 (14.3/4.6)	678
2ax	$CH_2CH_2CH_2N(CH_3)_2$	H	1.665 (1.066/2.263)	89.1 (152.6/25.6)	53
2ay	$CH_2CH_2CH_2N(CH_3)_2$	*iso*-Propenyl	0.075 (0.093/0.056)	19.9 (20.3/19.5)	265
2az	$CH_2CH_2N(CH_3)_2$	CH_2-4-C_6H_4-CO_2CH_3	0.023 ± 0.01 (*n* = 6)	28.0 ± 16 (*n* = 6)	1217
2aaa	$CH_2CH_2N(CH_3)_2$	CH_2-4-C_6H_4-CO_2H	0.196 ± 0.26 (*n* = 5)	>150 (*n* = 5)	>765
2aab	$CH_2CH_2N(CH_3)_2$	CH_2-4-C_6H_4-$CO{\cdot}N(CH_3)_2$	0.184 ± 0.127 (*n* = 3)	68.7 ± 52.0 (*n* = 3)	369
2aac	$CH_2CH_2N(CH_3)_2$	CH_2-2-C_6H_4-CO_2CH_3	0.019 (0.023/0.014)	11.5 (11.1/11.9)	605
2aad	$CH_2CH_2N(CH_3)_2$	CH_2-2-C_6H_4-CO_2H	0.127 (0.168/0.087)	3.2 (0.7/5.6)	25
2aae	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2N(CH_3)_2$	0.400 (0.376/0.425)	10.0 (15.5/4.5)	25
2aaf	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH_2N(CH_3)_2$	0.292 (0.206/0.378)	5.3 (2.9/7.6)	18

[a] Values are means of two or more experiments performed on consecutive weeks with the data from individual experiments shown in parentheses.

[b] Therapeutic index = CC_{50}/EC_{50}.



Scheme 1.

terminal heptad repeats of the RSV fusion protein in which the benzimidazol-2-one substituent is considered to project away from the protein toward solvent.[41] The introduction of a benzene spacer element into the alkyl chain allowed an appreciation of topological preferences with the result that, perhaps somewhat surprisingly, the 3 isomeric benzoic acid derivatives **2n**–**p** were not differentiated by RSV. There is a tolerance for a broad range of acidic moieties within this structural context with the phosphonates **2r**–**t** and sulfonate **2u** providing RSV inhibitors with potency comparable to that of alkyl analogues, to the extent that comparisons can be made. However, while the introduction of a second carboxylate to the aromatic ring of **2o** led to markedly weaker antiviral activity (**2q**), the introduction of additional spacer elements between the aryl ring and a diacidic moiety fully restored potency (**2v**, **2w**, **2x**, and **2z**).

The introduction of carboxylic acid isosteres to the terminus of the benzimidazole side chain revealed a pronounced preference for a 2- or 3- atom separation (**2aa**, **2ab**, and **2ac**), with simple, branched benzimidazol-2-one substituents being optimal (**2ad**–**aj**). The combination of non-acidic but polar benzimidazole side-chain moieties with acidic elements projected from the benzimidazol-2-one heterocycle (**2ak**–**at)** produced structure–activity patterns similar to that seen with the *iso*-amyl series, although phosphonate **2ap** is notably less potent than **2s**.

The basic amine-containing side chain found in analogues **2au**–**aad**, characteristic of the original series of benzotriazole-based RSV inhibitors,[1] conferred excellent antiviral properties, provided that the benzimidazol-2-one nitrogen atom was also substituted (Table 2). Combining a basic amine element with a remote carboxylic acid moiety provided a zwitterionic species in which the *para*-substituted derivative **2aaa** is comparable to the *ortho* isomer **2aad**. However, altering the topological disposition of functionality by combining an *iso*-amyl benzimidazole substituent with a basic amine projecting from the benzimidazol-2-one heterocycle provided the generally poorer RSV inhibitors **2aae** and **2aaf**.

Based on a combination of high in vitro potency and aqueous solubility exceeding 10 mg/mL, several compounds were selected for evaluation in the cotton rat model of RSV infection[42,52] with drug delivered as a small particle aerosol.[35,53–56] In this model, in which viral infection occurs asymptomatically, titers peak on day 4 post-inoculation and ribavirin shows efficacy after intraperitoneal administration at a dose of 90 mg/Kg BID. A typical result for ribavirin, which was used as a positive control in the initial experiments, is presented in entry 1 of Table 3. In order to maximize drug exposure of test compounds in the initial experiments, which were designed to establish proof of concept for this chemotype, the 4 test animals were continuously exposed to drug-containing aerosol for 21 h per day for 4 consecutive days beginning 1 h after intranasal inoculation with the Long strain of RSV. For each 21 h experiment, the test compounds were dissolved in water and delivered by aerosol at a rate of 12.5 L/min to animals maintained in plastic cages covered with plastic tops. The concentration of the inhibitor examined in individual experiments is reported in Table 3 and approximately 600 mL of solution was consumed over a 21 h period. All of the animals were sacrificed on day 4, approximately 16 h after completing the final exposure period to drug, since this is the time at which RSV titers are maximal in untreated or placebo-treated animals. The lungs of each animal were removed, transpleurally lavaged, and evaluated for virus levels by inoculating samples of each lung lavage fluid into wells containing HEp-2 cells. Efficacy was defined as the reduction in $\log_{10}$ median tissue culture infectious dose ($TCID_{50}$) of RSV per gram of lung compared to the levels of virus in the control animals. The limit of detection in this assay was 1.6 $\log_{10}$ and this figure rather than zero was used to calculate the average virus titer for those animals in which virus was determined to be undetectable. Because of this limit of detection, the efficacy of drug is potentially underestimated where virus titers were undetectable and note is made in Table 3 of the number of animals in each experiment in which this circumstance prevailed. Of the compounds evaluated, **2r**, **2u**, **2v**, **2w**, **2z**, and **2aj** exhibited efficacy comparable to that of ribavirin, whilst **2am** and **2aaa** were inactive at the concentrations tested, and **2k** and **2ab** were toxic, in each case killing 3 of 4 animals. From this cohort, both **2r** and **2z** demonstrated robust reductions in virus titers and were examined at lower concentrations in the aerosol. At the lower concentration of 0.2 mg/mL, aspartate derivative **2z** produced a reduction in virus titers similar to the 1 mg/mL aerosol concentration (Table 3, entries 7 and 8). However,

Table 3. In vivo efficacy of RSV inhibitors

Entry	Compound	Dose of drug (mg/mL = concentration on aerosol)	$\log_{10}$ $TCID_{50}$/g lung	
			Treated animals	Control
1	Ribavirin	90 mpk BID ip cotton rat	2.5 ± 0.3 (*n* = 4)	4.1 ± 0.4 (*n* = 4)
2	**2k**	2.0 mg/mL cotton rat	Toxic (3 of 4 died)	4.1 ± 0.4 (*n* = 4)
3	**2r**	2.0 mg/mL cotton rat	2.1 ± 0.58 (*n* = 4)[a]	4.1 ± 0.4 (*n* = 4)
4		0.2 mg/mL cotton rat	3.5 ± 0.6 (*n* = 4)[a]	4.1 ± 0.6 (*n* = 4)
5		0.05 mg/mL cotton rat	4.0 ± 0.5 (*n* = 4)	4.1 ± 0.6 (*n* = 4)
6		0.01 mg/mL cotton rat	4.4 ± 0.5 (*n* = 4)	4.1 ± 0.6 (*n* = 4)
7	**2u**	1.6 mg/mL cotton rat	2.25 ± 0.75 (*n* = 4)[a]	4.1 ± 0.4 (*n* = 4)
8	**2v**	2.0 mg/mL cotton rat	2.6 ± 0.71 (*n* = 4)[b]	4.1 ± 0.4 (*n* = 4)
9	**2w**	0.5 mg/mL cotton rat	2.43 ± 0.97 (*n* = 4)[a]	3.9 ± 0.3 (*n* = 4)
10	**2z**	1.0 mg/mL cotton rat	1.83 ± 0.45 (18 h) (*n* = 4)[c]	3.0 ± 0.7 (*n* = 4)
11		0.2 mg/mL cotton rat	3.22 ± 0.78 (*n* = 6)	4.59 ± 0.44 (*n* = 6)
12	**2ab**	1.33 mg/mL cotton rat	Toxic (3 of 4 died)	4.1 ± 0.4 (*n* = 4)
13	**2aj**	0.5 mg/mL cotton rat	2.1 ± 0.71 (*n* = 4)[a]	4.1 ± 0.4 (*n* = 4)
14		120 mpk BID sc mouse	2.55 ± 0.24 (*n* = 10)	3.39 ± 0.17 (*n* = 9)
15		120 mpk BID sc mouse	2.38 ± 0.48 (*n* = 10)	3.31 ± 0.35 (*n* = 9)
16	**2az**	50 mpk BID sc mouse	2.59 ± 0.1 (*n* = 10)	3.69 ± 0.31 (*n* = 10)
17		50 mpk BID sc mouse	2.94 ± 0.85 (*n* = 10)	4.25 ± 0.35 (*n* = 9)
18		50 mpk BID po mouse	3.47 ± 0.42 (*n* = 10)	4.36 ± 0.38 (*n* = 10)
19	**2am**	0.67 mg/mL cotton rat	3.60 ± 0.3 (*n* = 4)	3.80 ± 0.3 (*n* = 4)
20	**2aaa**	0.5 mg/mL cotton rat	4.30 ± 0.3 (*n* = 4)	3.90 ± 0.3 (*n* = 4)
21		50 mpk BID po mouse	2.72 ± 0.19 (*n* = 7)	3.22 ± 0.23 (*n* = 8)
22	**2aab**	50 mpk BID po mouse	3.09 ± 0.52 (*n* = 10)	4.41 ± 0.33 (*n* = 10)
23		15 mpk BID po mouse	3.88 ± 0.29 (*n* = 7)	4.70 ± 0.24 (*n* = 7)
24	Ribavirin	90 mpk BID sc mouse	1.86 ± 0.39 (*n* = 9)	2.98 ± 0.18 (*n* = 9)

ip, Intraperitoneal administration; sc, subcutaneous administration; po, oral administration. BID indicates that the noted dose was administered twice per 24 h period.

[a] Virus titers were undetectable in 2 cotton rats.

[b] Virus titers were undetectable in 1 cotton rat.

[c] Virus titers were undetectable in 3 cotton rats.

the phosphonic acid **2r** was inactive at concentrations of 0.2, 0.05, and 0.01 mg/mL in the aerosol (Table 3, entries 4–6). The lack of activity under these circumstances presumably reflects inadequate exposure to drug at the site of virus replication and may account for the inactivity associated with **2am** and **2aaa**, both of which were tested at relatively low aerosol concentrations.

Having established in vivo efficacy following topical drug delivery, several compounds were selected for evaluation in a BALB/c mouse model of RSV infection following either subcutaneous (sc) or oral (po) administration.[40] Ribavirin demonstrated efficacy in this model following sc doses of 90 mpk BID with the first dose administered 1 h post-infection (Table 3, entry 24). Subcutaneous administration of the acidic oxadiazolone **2aj** produced reproducible antiviral activity at a dose of 120 mpk BID (Table 3, entries 14 and 15), establishing the potential for this class of RSV inhibitors to interfere with infection in vivo, following delivery to lung tissue by systemic exposure. The basic dimethylamine **2az** also demonstrated reproducible efficacy after daily exposure to 50 mpk BID SC (Table 3, entries 16 and 17) and, more importantly, following oral administration at the same dose, representing a significant advance in the search for orally active inhibitors of RSV based on this mechanistic chemotype (Table 3, entry 18). In vivo, the ester moiety of **2az** was found to be readily metabolized to the acid **2aaa**, a compound that is an 8-fold weaker RSV inhibitor in vitro.[55] Nevertheless, **2aaa** appeared to show some efficacy following po administration, although the reduction in viral titers is not statistically significant (Table 3, entry 21). In an effort to identify a metabolically more stable functionality, the amide **2aab** was examined in vivo, providing a promising compound that demonstrated efficacy after doses of both 50 and 15 mpk BID po. However, amide **2aab** is extensively metabolized in both mouse and human liver microsomes, undergoing N-demethylation of both the amine and amide moieties and hydroxylation of both heterocyclic rings,[57] eliminating this compound from further consideration but providing a clear focus for further structural optimization, studies that will be described in due course.

In summary, a series of potent and water-soluble inhibitors of RSV fusion have been identified that demonstrate activity in models of virus infection following topical exposure to drug via small particle aerosol administration. Structural optimization has led to the identification of compounds with antiviral activity following oral administration, providing a basis for further structural optimization in the quest for candidates suitable for clinical development.

Acknowledgments

We thank Drs. J. J. Kim Wright and Richard J. Colonno for their support and encouragement throughout this work.

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