Dichloro-phenyl-benzotriazoles: a new selective class of Human Respiratory Syncytial virus entry inhibitors

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TABLE S1. Activity of 5,6-dichloro-1-phenyl-benzotriazole amides(**5a-d** and **7a-h**) against viruses representative of positive-sense, single-stranded RNAs (ssRNA+): i) Retroviridae: HIV-1; ii) Flaviviridae: YFV and BVDV; iii) Picornaviridae: CV-B5 and Sb-1. Viruses representative of negative-sense, single-stranded RNAs (ssRNA-); i) Rhabdoviridae: VSV. Virus representative of double-stranded RNAs (dsRNA): Reoviridae: Reo-1. DNA virus representatives: i) Poxviridae: VV; ii) Herpesviridae: HSV-1. Efavirenz, 2'-C-methyl-guanosine, and Pleconarilwere used as reference inhibitors.Data represent mean values ± SD for three independent determinations. For values where SD is not shown, variation among duplicate samples was less than 15%.Efavirenz (EFV), 2'-C-methyl-guanosine (2MG), and Pleconaril (PCL) were used as reference inhibitors.

Cpd	**MT-4 cells**	**HIV-1_{IIIB}**	**MDBK cells**	**BVDV**	**BHK cells**	**YFV**	**Reo-1**	**Vero-76 cells**	**CV-B5**	**Sb-1, VSV, VV, HSV-1**
	CC_{50}[a]	EC_{50}[b]	CC_{50}[c]	EC_{50}[d]	CC_{50}[e]	EC_{50}[f]	EC_{50}[g]	CC_{50}[h]	EC_{50}[i]	EC_{50}[j]
1a	>100	>100	>100	**75**	>100	>100	>100	>100	85	>100
5a	35	>35	43	>43	53	>53	>53	30	**17**	>30
5b	28	>28	>100	>100	54	>54	>54	30	>30	>30

5c	60	>60	>100	>100	>100	>100	>100	10	**9**	>100
5d	35	>35	14	>14	16	>16	>16	20	>20	>20
7a	>100	>100	>100	>100	44	>44	>44	>100	>100	>100
7b	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7c	33	>33	>100	>100	>100	>100	>100	>100	>100	>100
7d	77	>77	>100	>100	>100	**78**	>100	90	>90	>90
7e	>100	>100	>100	>100	96	>96	>96	>100	>100	>100
7f	>100	>100	>100	>100	84	>84	>84	>100	>100	>100
7g	>100	>100	>100	>100	>100	>100	>100	9	>90	>90
7h	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Ref Cpd										
EFV	40	0.002 ± 0.0002								
2MG			>100	1.1 ± 0.1	>100	1.9 ± 0.1	0.7 ± 0.2			
PCL								>100	0.005 ± 0.001	

[a]Compound concentration (μM) required to reduce the proliferation of mock-infected MT-4 cells by 50%, as determined by the MTT method. [b]Compound concentration (μM) required to achieve 50% protection of MT-4 cells from HIV-1 induced cytopathogenicity, as determined by the MTT method. [c]Compound concentration (μM) required to reduce the viability of mock-infected MDBK cells by 50%, as determined by the MTT method. [d]Compound concentration (μM) required to achieve 50% protection of MDBK cells from BVDV-induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration (μM) required to reduce the viability of mock-infected BHK cells by 50%, as determined by the MTT method. [f]Compound concentration (μM required to achieve 50% protection of BHK cells from YFV-induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration (μM) required to achieve 50% protection of BHK cells from Reo-1-induced cytopathogenicity, as determined by the MTT method. [h]Compound concentration (μM) required to reduce the viability of mock-infected VERO-76 cells by 50%. as determined by the MTT method. [i]Compound concentration (μM) required to reduce the plaque number of CV-B5 by 50% in VERO-76 monolayers. [j]Compound concentration (μM) required to reduce the plaque number of Sb-1, VSV, VV and HSV-1 by 50% in VERO-76 monolayers.

TABLE S2. Activity of 5,6-dichloro-2-phenyl-benzotriazole amides(**6a-h** and **8a-h**), and 5,6-dichloro-2-phenyl-benzotriazole urees**(10a-k)** against viruses representative of positive-sense, single-stranded RNAs (ssRNA+): i) Retroviridae: HIV-1; ii) Flaviviridae: YFV and BVDV; iii) Picornaviridae: CV-B5 and Sb-1. Viruses representative of negative-sense, single-stranded RNAs (ssRNA-); i) Rhabdoviridae: VSV. Virus representative of double-stranded RNAs (dsRNA): Reoviridae: Reo-1. DNA virus representatives: i) Poxviridae: VV; ii) Herpesviridae: HSV-1. For values where SD is not shown, variation among duplicate samples was less than 15%.Efavirenz (EFV), 2'-C-methyl-guanosine (2MG), and Pleconaril (PCL) were used as reference inhibitors.

Cpd	**MT-4 cells**	**HIV-1_{IIIB}**	**MDBK cells**	**BVDV**	**BHK cells**	**YFV**	**Reo-1**	**Vero-76 cells**	**CV-B5**	**Sb-1, VSV, VV, HSV-1**
	CC_{50}[a]	EC_{50}[b]	CC_{50}[c]	EC_{50}[d]	CC_{50}[e]	EC_{50}[f]	EC_{50}[g]	CC_{50}[h]	EC_{50}[i]	EC_{50}[j]
1b	52	>52	≥100	20	>100	>100	>100	>100	>100	>100
6a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
6b	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
6c	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
6d	33	>33	100	>100	72	>72	>100	>100	>100	>100
6e	15	>15	72	>72	26	>26	>26	>100	**61**	>100
6f	24	>24	84	>84	62	>62	>26	>100	**33**	>100
8a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
8b	>100	>100	>100	>100	>100	**73**	>100	>100	>100	>100
8c	>100	>100	>100	>100	>100	>100	>100	>100	**14**	>100
8d	63	>63	>100	**35**	35	>35	>35	80	>80	>80
8e	>100	>100	>100	**4**	68	>68	>68	>100	>100	>100
8f	>100	>100	>100	**60**	>100	>100	>100	80	>100	>100

8g	>100	>100	>100	**28**	>100	>100	>100	>100	>100	>100
8h	>100	>100	>100	**11**	>100	>100	>100	>100	>100	>100
10a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10b	>100	>100	78	>78	40	>40	>40	30	>30	>30
10c	>100	>100	>100	>100	>100	>100	>100	90	>95	>95
10d	>100	>100	>100	>100	71	>71	>71	90	>90	>90
10e	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10f	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10g	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10h	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10i	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10j	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10k	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
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concentration (μM required to achieve 50% protection of BHK cells from YFV-induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration (μM) required to achieve 50% protection of BHK cells from Reo-1-induced cytopathogenicity, as determined by the MTT method. [h]Compound concentration (μM) required to reduce the viability of mock-infected VERO-76 cells by 50%. as determined by the MTT method. [i]Compound concentration (μM) required to reduce the plaque number of CV-B5 by 50% in VERO-76 monolayers. [j]Compound concentration (μM) required to reduce the plaque number of Sb-1, VSV, VV and HSV-1 by 50% in VERO-76 monolayers.

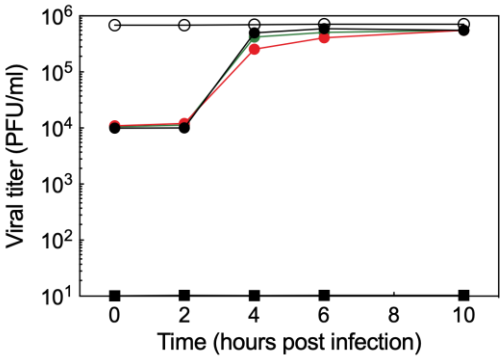

Figure S1. Inhibition of RSV (m.o.i = 1) by addition of 20 μM of compound **10d** (black filled circles), **10b** (green filled circles), and **8d** (red filled circles) at different times. Data for untreated virus (open circles) and for addition of 6-azauridine (filled squares) are also shown for comparison. Data represent mean values from two independent determinations; variation among duplicate samples was less than 15%. Data for **10b** and **8d** were obtained under the same conditions employed for **10d** (see main text, Materials and Methods section).

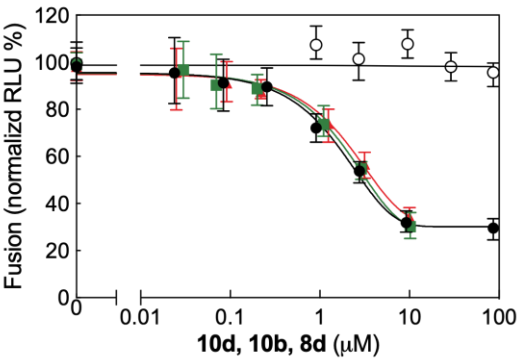

Figure S2. Quantitative dose-response cell-to-cell fusion assay using the DSP-chimeric reporter proteins and the ViviRenrenilla luciferase substrate in the presence of compounds**10d** (black filled symbols), **10b** (green filled symbols), and **8d** (red filled symbols). The MeV (Measles Virus) F and H glycoprotein expression constructs (open symbols) were included for selectivity control. Reported values are normalized for DMSO-treated samples and are expressed as the mean of three experiments ± standard deviation. The EC_{50} valuesfor the three compounds, obtained by 4-parameter variable slope regression fitting, are: 3.2 μM for **10d**, 3.9 μM for **10b**, and 4.5 μM for **8d**, respectively. Data for **10b** and **8d** were obtained under the same conditions employed for **10d** (see main text, Materials and Methods section).