Supporting Information.

Non-rigid Diarylmethyl Analogs of Baloxavir as CAP-Dependent Endonuclease Inhibitors of Influenza Viruses

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Table S1. 5a and 5d protection of MDCK cells from death caused by influenza A and B virus [a]

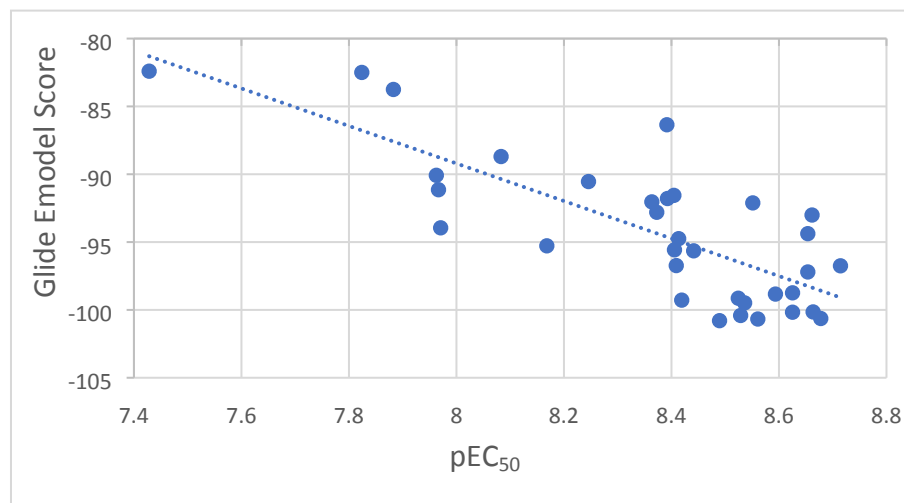| Compound	$EC_{50} \pm SEM$, nM[b]			
	A/Denmark/524/2009 (H1N1)pdm09	A/Fukui/20/2004 (H3N2)	B/Brisbane/60/2008 (Victoria Lineage)	B/Florida/4/2006 (Yamagata lineage)
5a	5.1 ± 3.9	3.6 ± 1.7	9.6 ± 1.0	5.1 ± 3.9
5d	12.5 ± 4.9	10.0 ± 2.8	10.5 ± 1.3	28.3 ± 6.7
3	1.1 ± 0.5	0.4 ± 0.2	5.0 ± 0.6	1.2 ± 0.5

[a] The cells were infected with the corresponding virus at a MOI = 0.001

[b] The EC_{50}, 50% effective concentration, was assessed 72 or 96 hours post infection. 3 or 4 independent dose-response curves were averaged and mean ± SEM values presented.

Table S2. PK parameters of 3, 5a, 5b, 5d, and 5e in CD-1 female mice upon IV administration.

Parameter	Units	5a	5b	5d	5e	3
PK in mice, IV (2 mg/kg)						
		Compound				
kel	1/h	0.62	0.57	0.50	0.5	0.24
T1/2	h	1.11	1.21	1.39	1.3	2.83
Tmax	h	0.083	0.083	0.083	0.1	0.25
Cmax	ng/ml	654	511	600	977.7	198
C0	ng/ml	815	522	642	1073.8	156
AUClast	h*ng/ml	844	1073	927	2213.9	354
AUCinf	h*ng/ml	931	1085	937	2241.0	412
Vz	ml/kg	3446	3231	4279	1655.0	16747
Cl	ml/h/kg	2147	1844	2134	892.4	4101
MRTlast	h	2.02	1.24	1.59	1.9	2.12

Validation of Glide Emodel *vs* pEC$_{50}$ for Known Influenza Virus CEN inhibitors.

A virtual library containing BXA (**3**) and its potent structural analogues was used to validate the docking protocol. 2D structures of these compounds and their CEN inhibitory activity (EC$_{50}$) were manually mined from the patent application [1]. Collected structures were docked into the model of IFV cap-dependent endonuclease and were ranked by Glide Emodel score (Figure). Maestro, release 2015-2, was used for the molecular modelling. The X-ray structure of CEN and BXA complex was downloaded from the Protein Data Bank (PDB code: 6FS6). The target structure was pre-processed using Protein Preparation Wizard tool. Pre-processing included addition of missing hydrogens, bonds assignment, protonation of BXA and generation of charge states for the cofactors (Epik, at pH of 7 ± 2), removal of water molecules, which form less than three hydrogen bonds, and H-bonds optimization (PROPKA, at pH 7). Next, a grid with BXA structure at the centre, was generated using Receptor Grid Generation and was applied for further docking simulations. 2D structures of the ligands were preliminary pre-processed with LigPrep tool. The stereo configurations were manually inspected, and the required isomers were selected. Docking simulations were performed exploiting Glide SP with the post-docking minimization. Compounds were ranked by the values of Glide Emodel scoring function and manually inspected.

Reference:

1. Makoto, K.; Tomita, K.; Akiyama, T.; Okano, A.; Miyagawa, M. Substituted polycyclic pyridone derivative and prodrug thereof. U.S. Patent Appl. US 2018/0118760, May 3, 2018.

HPLC-MS traces

Compound 5a

TIC of +Q1: from Sample 1 (VEM-MOD-1078E3-20MIN) of VEM-MOD-1078E3-20MIN.wiff (Turbo Spray) — Max. 1,1e8 cps.

+Q1: 12,925 to 13,243 min from Sample 1 (VEM-MOD-1078E3-20MIN) of VEM-MOD-1078E3-20MIN.wiff (... — Max. 2,3e6 cps.

A/D Converter 254nm from Sample 1 (VEM-MOD-1078E3-20MIN) of VEM-MOD-1078E3-20MIN.wiff — Max. 78,4 %.

A/D Converter 254nm from Sample 1 (VEM-MOD-1078E3-20MIN) of VEM-MOD-1078E3-20MIN.wiff

Data List | Peak List

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
1	3,8228	1,1540	0,1899	0,1848	0,2359	0,2433	Valley
2	12,9567	606,5749	99,8101	78,1603	99,7641	1,0267	Base to Base

Compound **5b**

TIC of +Q1: from Sample 1 (1788-MOD-1097D-20MIN) of 1788-MOD-1097D-20MIN.wiff (Turbo Spray)   Max. 4

+Q1: 12,321 to 12,525 min from Sample 1 (1788-MOD-1097D-20MIN) of 1788-MOD-1097D-20MIN.wiff (Turbo Spray)   Max. 1

A/D Converter 254 from Sample 1 (1788-MOD-1097D-20MIN) of 1788-MOD-1097D-20MIN.wiff   Max

A/D Converter 254 from Sample 1 (1788-MOD-1097D-20MIN) of 1788-MOD-1097D-20MIN.wiff

Data List Peak List

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
2	3,5352	1,9963	0,2197	0,2064	0,2656	0,3067	Valley
3	3,8152	2,2036	0,2425	0,1884	0,2424	0,3133	Valley
4	12,3250	890,3217	97,9892	75,3140	96,9115	1,1033	Base to Base
5	17,5371	9,0294	0,9938	0,9180	1,1813	0,4733	Valley

Compound **5c**

TIC of +Q1: from Sample 1 (1400-NA-6853-H1-20MIN) of 1400-NA-6853-H1-20MIN.wiff (Turbo Spray)   Max. 5,9e7 cps.

+Q1: 12,715 to 12,949 min from Sample 1 (1400-NA-6853-H1-20MIN) of 1400-NA-6853-H1-20MIN.wiff (T...   Max. 1,7e6 cps.

A/D Converter 254nm from Sample 1 (1400-NA-6853-H1-20MIN) of 1400-NA-6853-H1-20MIN.wiff   Max. 69,8 %.

A/D Converter 254nm from Sample 1 (1400-NA-6853-H1-20MIN) of 1400-NA-6853-H1-20MIN.wiff

Data List Peak List

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
1	7,0919	12,1328	2,4503	1,4808	2,0803	0,5433	Valley
2	12,7159	482,0125	97,3454	69,5501	97,7057	1,0433	Base to Base
3	16,1521	1,0115	0,2043	0,1524	0,2141	0,2767	Valley

Compound **5d**

TIC of +Q1: from Sample 1 (BIC-MOD-1082E-20MIN) of 1788-MOD-1082E-20MIN.wiff (Turbo Spray)   Max. 1,1e8 cps.

+Q1: 15,567 to 15,917 min from Sample 1 (BIC-MOD-1082E-20MIN) of 1788-MOD-1082E-20MIN.wiff (Tu...   Max. 2,5e6 cps.

A/D Converter 254nm from Sample 1 (BIC-MOD-1082E-20MIN) of 1788-MOD-1082E-20MIN.wiff   Max. 60,9 %.

A/D Converter 254nm from Sample 1 (BIC-MOD-1082E-20MIN) of 1788-MOD-1082E-20MIN.wiff

Data List | Peak List

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
1	4,3670	1,6021	0,3270	0,2016	0,3325	0,2633	Valley
2	14,6055	1,2071	0,2464	0,1008	0,1662	0,3833	Valley
3	15,6266	487,1415	99,4266	60,3429	99,5013	1,0233	Base to Base

Compound **5e**

TIC of +Q1: from Sample 1 (BIC-MOD-1082D-20MIN) of 1788-MOD-1082D-20MIN.wiff (Turbo Spray)   Max. 1,7e8 cps.

+Q1: 15,631 to 16,013 min from Sample 1 (BIC-MOD-1082D-20MIN) of 1788-MOD-1082D-20MIN.wiff (Tu...   Max. 3,2e6 cps.

A/D Converter 254nm from Sample 1 (BIC-MOD-1082D-20MIN) of 1788-MOD-1082D-20MIN.wiff   Max. 85,1 %.

A/D Converter 254nm from Sample 1 (BIC-MOD-1082D-20MIN) of 1788-MOD-1082D-20MIN.wiff

Data List | Peak List

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
1	4,3483	1,3612	0,1888	0,2051	0,2402	0,1933	Valley
2	14,6043	1,1153	0,1547	0,1026	0,1201	0,3233	Valley
3	15,6636	717,2783	99,4873	84,8966	99,4349	1,0633	Base to Base
4	18,5356	1,2201	0,1692	0,1749	0,2048	0,3000	Base to Base

Compound 5f

TIC of +Q1: from Sample 1 (1788-MOD-1097B-20MIN) of 1788-MOD-1097B-20MIN.wiff (Turbo Spray)   Max. 3,5e7 cps.

+Q1: 12,025 to 12,284 min from Sample 1 (1788-MOD-1097B-20MIN) of 1788-MOD-1097B-20MIN.wiff (Turbo Spray)   Max. 9,5e5 cps.

A/D Converter 254 from Sample 1 (1788-MOD-1097B-20MIN) of 1788-MOD-1097B-20MIN.wiff   Max. 66,9 %.

A/D Converter 254 from Sample 1 (1788-MOD-1097B-20MIN) of 1788-MOD-1097B-20MIN.wiff

Data List Peak List

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
4	7,9102	5,8107	0,5370	0,3975	0,3204	0,4100	Valley
5	8,1652	1,5196	0,1404	0,1556	0,1254	0,2367	Valley
6	11,9930	303,0806	28,0090	55,9271	45,0725	0,1800	Valley
7	12,0919	763,6738	70,5744	66,2856	53,4206	1,0700	Valley

Compound 5g

TIC of +Q1: from Sample 1 (1788-MOD-1097C1-20MIN) of 1788-MOD-1097C1-20MIN.wiff (Turbo Spray)   Max. 4,2e7 cps.

+Q1: 12,155 to 12,395 min from Sample 1 (1788-MOD-1097C1-20MIN) of 1788-MOD-1097C1-20MIN.wiff (Turbo Spray)   Max. 9,7e5 cps.

A/D Converter 254 from Sample 1 (1788-MOD-1097C1-20MIN) of 1788-MOD-1097C1-20MIN.wiff   Max. 69,4 %.

A/D Converter 254 from Sample 1 (1788-MOD-1097C1-20MIN) of 1788-MOD-1097C1-20MIN.wiff

Data List Peak List

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
2	3,5604	2,0587	0,2398	0,2075	0,2923	0,3467	Base to Base
3	3,8326	3,1522	0,3672	0,1943	0,2736	0,4700	Valley
4	12,1820	844,4625	98,3660	68,8320	96,9568	1,2200	Base to Base
5	13,8842	2,5603	0,2982	0,2811	0,3959	0,3233	Base to Base

Compound 6a

A/D Converter 254nm from Sample 1 (1788-MOD-1087F6-20MIN) of 1788-MOD-1087F6-20MIN.wiff

Data List | Peak List

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
1	3,5864	1,9352	0,4535	0,2502	0,4121	0,3300	Valley
2	12,0019	4,4266	1,0372	0,6665	1,0980	0,2933	Valley
3	13,6105	2,0840	0,4883	0,2810	0,4630	0,3267	Valley
4	14,5275	418,3214	98,0210	59,5103	98,0270	0,9733	Base to Base

Compound 6b

A/D Converter 254nm from Sample 1 (1400-NA-6904-K2-20MIN) of 1400-NA-6904-K2-20MIN.wiff

Data List | Peak List

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
1	3,3072	1,6391	0,3393	0,1900	0,2702	0,3167	Valley
2	14,6094	1,1070	0,2291	0,1725	0,2454	0,1567	Valley
3	14,8938	473,0060	97,9059	68,9165	98,0433	0,6100	Valley
4	15,8669	2,0960	0,4338	0,2103	0,2992	0,3467	Valley
5	16,2294	5,2748	1,0918	0,8027	1,1419	0,3167	Valley

Compound **6d**

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
2	11,3868	1,4086	0,3356	0,1702	0,2667	0,5667	Base to Base
3	12,8038	2,3569	0,5616	0,3721	0,5830	0,2933	Base to Base
4	13,5535	412,2736	98,2305	62,8533	98,4768	0,6600	Valley
5	14,9894	1,4760	0,3517	0,2338	0,3663	0,2533	Base to Base

Compound **6**

	Time (min)	Area (counts)	% Area	Height (%)	% Height	Width (min)	Baseline Type
1	12,6039	3,4423	0,7630	0,5523	0,8073	0,3133	Base to Base
2	14,5098	2,3182	0,5138	0,3729	0,5450	0,2400	Base to Base
3	15,1809	442,9926	98,1903	67,1005	98,0738	0,5900	Valley
4	16,5764	1,4355	0,3182	0,2424	0,3542	0,2233	Base to Base
5	18,5768	0,9684	0,2147	0,1503	0,2197	0,2500	Base to Base