Supporting Information

Identification of Non-Nucleoside DNA Synthesis Inhibitors of Vaccinia Virus by High-Throughput Screening

*Mihai Ciustea [a], Janice Elaine Y. Silverman [a], Abigail M. Druck Shudofski [b] and Robert P. Ricciardi [a,c]**

[a] Department of Microbiology, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA

[b] Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

[c] Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA

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Table S1. Screened libraries, number of compounds in each library and number of inhibitors of vaccinia virus DNA synthesis from each library identified in the primary HTS.

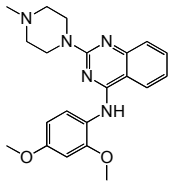
Library Type	Library Name	Compounds in Library	Strong Inhibition >70% Inhibition	Intermediate Inhibition 50%-70% Inhibition	Weak Inhibition 30%-50% Inhibition
Known Bioactives	Biomol ICCB Known Bioactives	480	6 (1.3%)	8 (1.7%)	11 (2.3%)
	NINDS Custom Collection	1,040	15 (1.4%)	8 (0.8%)	10 (1.0%)
Natural Extracts	Starr Foundation Extracts 2	1,000	43 (4.3%)	41 (4.1%)	57 (5.7%)
	ICBG Fungal Extracts 1	851	59 (6.9%)	57 (6.7%)	54 (6.3%)
	ICBG Fungal Extracts 2	460	18 (3.9%)	30 (6.5%)	24 (5.2%)
Synthetic Compounds	1 ChemDiv 3	16,544	21 (0.1%)	64 (0.4%)	89 (0.5%)
	2 MixCommercial 5	268	0 (0.0%)	0 (0.0%)	1 (0.4%)
	3 Maybridge 4	4,576	2 (<0.1%)	9 (0.2%)	28 (0.6%)
	4 ActiMol TimTec 1	8,518	6 (0.1%)	19 (0.2%)	25 (0.3%)
	5 Bionet 2	1,700	2 (0.1%)	2 (0.1%)	14 (0.8%)
	6 Enamine 1	6,004	1 (<0.1%)	9 (0.1%)	19 (0.3%)
	7 I.F. Lab 1	6,543	4 (0.1%)	17 (0.3%)	40 (0.6%)
	8 I.F. Lab 2	292	0 (0.0%)	4 (1.4%)	0 (0.0%)
	9 Maybridge 2	704	1 (0.1%)	1 (0.1%)	5 (0.7%)
	10 MixCommercial 4	331	0 (0.0%)	1 (0.3%)	3 (0.9%)
	11 Peakdale 2	352	0 (0.0%)	1 (0.3%)	0 (0.0%)
Total		49,663	178 (0.4%)	271 (0.5%)	380 (0.8%)

Table S2. Summary of activities for 13 synthetic hit compounds that are trapped by DNA.

compd[a]	DNA Synth IC_{50} (μM)	Cell Prot EC_{50} (μM)	Plaque Red EC_{50} (μM)	Tox 20 h TC_{50} (μM)	Tox 72 h TC_{50} (μM)	Tox 120 h TC_{50} (μM)	Selectivity Index[b]	Trapped by dsDNA
17	65	18	40	192			10.7	Yes
18	25	14	25	133			9.5	Yes
19	42	23	25	100			4.3	Yes
20	25	57	40	>200		12	>3.5	Yes
21	18	57	25	>200		10	>3.5	Yes
22	46	41	25	143			3.5	Yes
23	25	87	100	>200	152	60	>2.3	Yes
24	30	40	25	87			2.2	Yes
25	56	12	6	23			1.9	Yes
26	39	98	100	135			1.4	Yes
27	25	23	10	29			1.3	Yes
28	708	193	100	>200	35		>1	Yes
29	5.6	25	10	20			0.8	Yes
CDV		132	50	>500	>500	300	>3.8	No

[a] Refer to Chart S1 for the structures of compounds **17** to **29**. [b] The Selectivity Index was obtained as the ratio between TC_{50} in the 20 h cytotoxicity assay and EC_{50} in the 20 h cell protection assay.

Chart S1. Structures of the 13 synthetic hit compounds whose activities are shown in Table S2.



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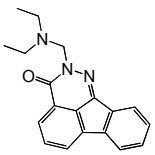

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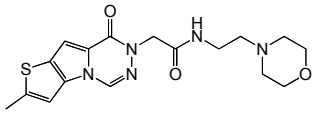

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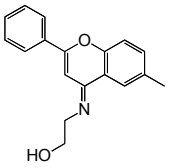

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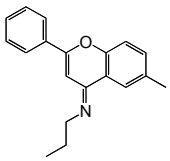

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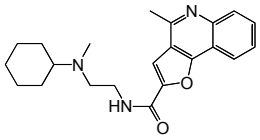

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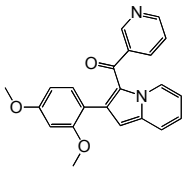

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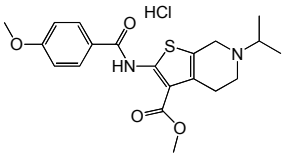

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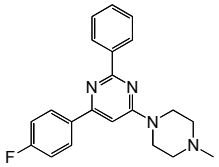

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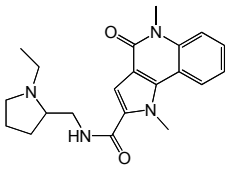

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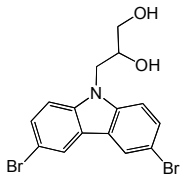

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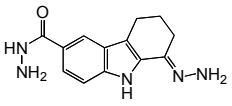

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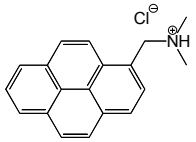
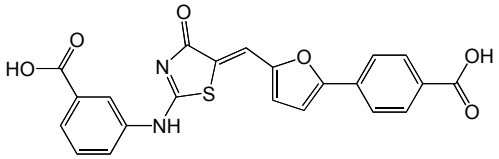
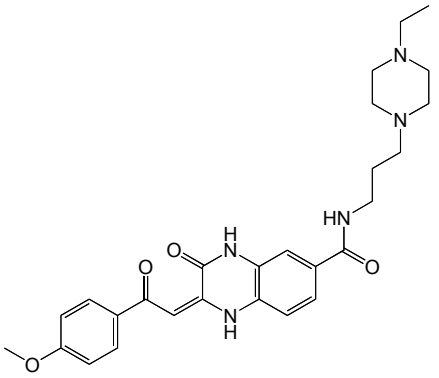
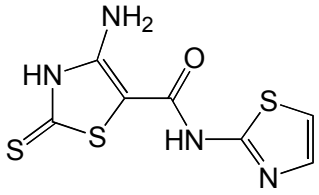
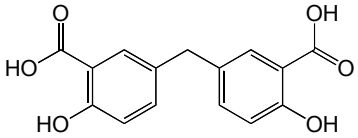
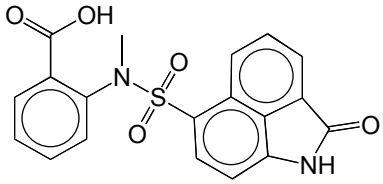
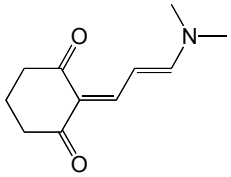
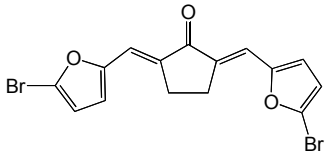
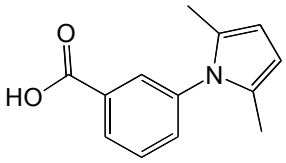
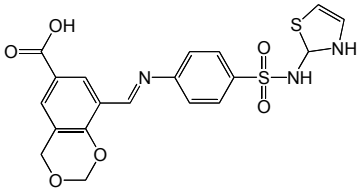
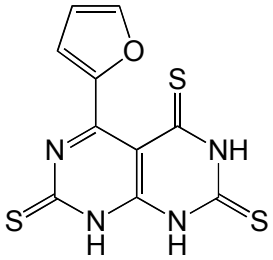
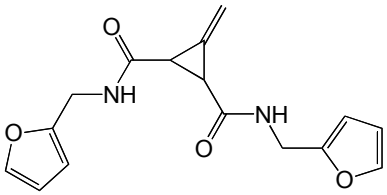
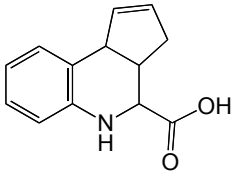
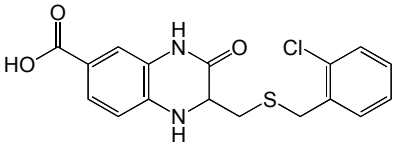
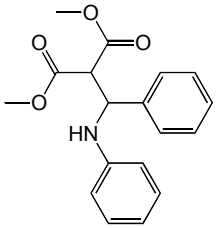
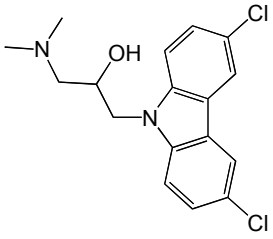


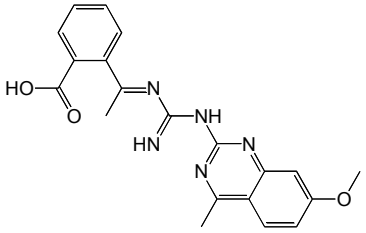
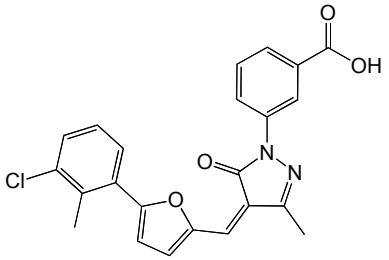
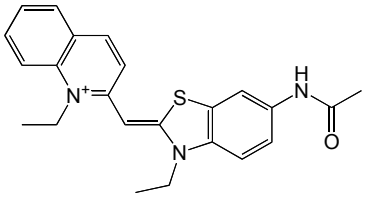
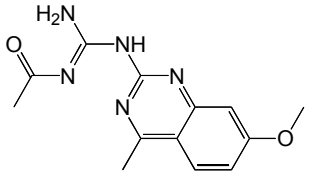
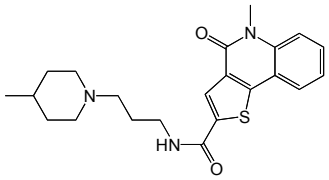
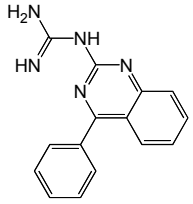
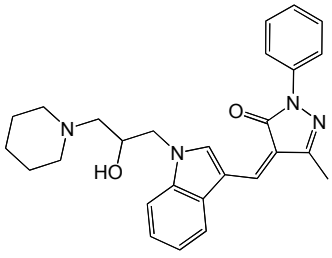
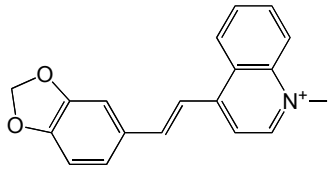
29

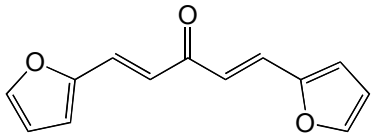
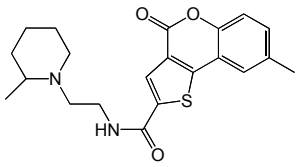
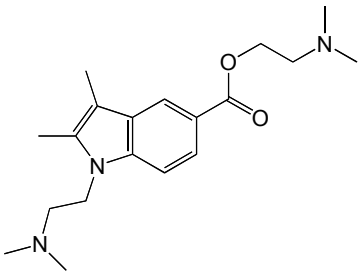
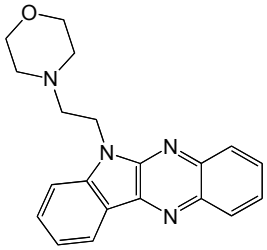
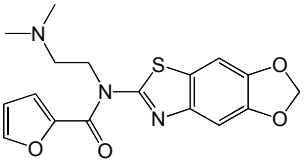
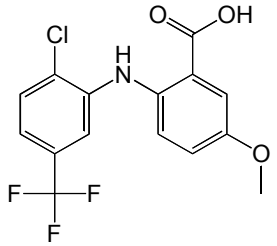
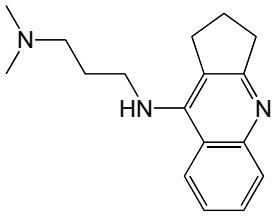
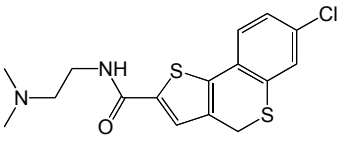
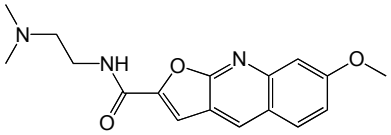
Compounds **17** (706769-72-4), **18** (664972-89-8), **20** (914071-96-8), **21** (913525-45-8), **22** (902627-18-3), **26** (902561-11-9), **28** (68339-98-0) belong to ChemDiv 3 library; compound **27** (173157-92-1) belongs to Maybridge 4 library; compounds **23** (327978-52-9), **29** (94256-44-7) belong to Enamine 1 library; compound **24** (524679-67-2) belongs to I.F. Lab 1 library; compound **19** (902915-02-0) belongs to Maybridge 2 library; compound **25** (881194-03-2) belongs to MixCommercial 4 library.

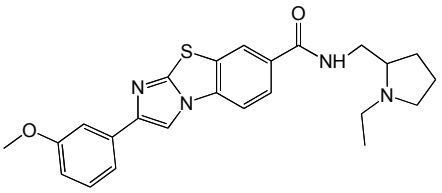
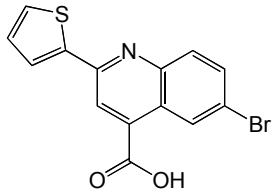
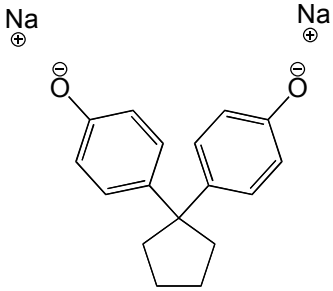
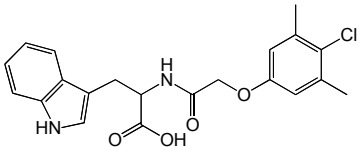
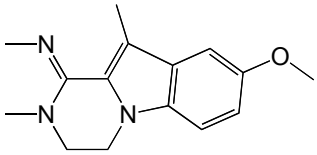
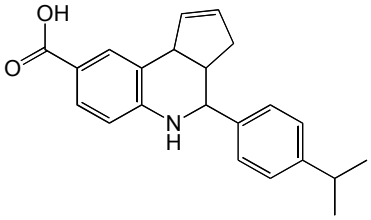
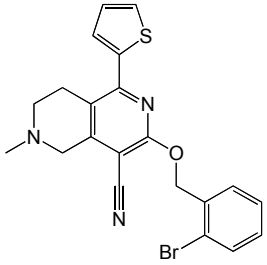
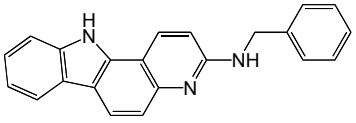
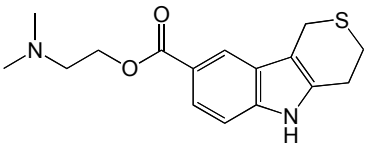
Table S3. Structures and follow-up study results of 45 synthetic compound inhibitors identified in the HTS.

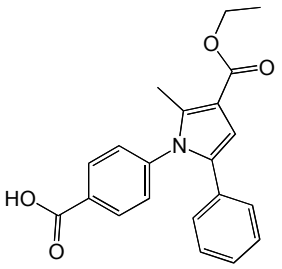
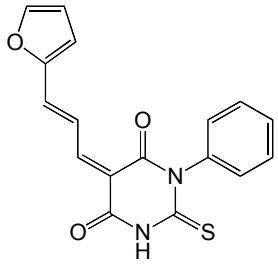
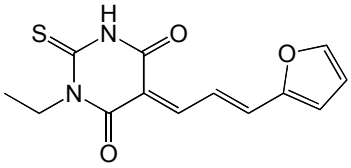
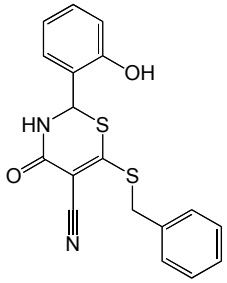
Library[a]	CAS Registry Number[b]	Compound Structure	DNA Synthesis IC_{50} (μM)	Plaque Reduction EC_{50} (μM)	Toxicity 20 h TC_{50} (μM)
1	864840-81-3		30		>200
1	900319-37-1		36		>200
4	329691-77-2		41		>200
4	122-25-8		51		>200
1	823823-44-5		75		>200
1	112338-04-2		86		>200
1	683267-38-1		95		>200

4	26180-28-9		100		>200
4	329061-45-2		104		>200
4	331455-31-3		142		>200
6	303787-08-8		272		>200
4	354815-90-0		>400		>200
7	847362-46-3		450		>200
4	164172-51-4		638		>200
9	253448-96-3		12	10	17

1	332382-71-5		13	50	164
1	317326-06-0		14	50	>200
1	860348-43-2		15	200	>200
4	329213-15-2		16	40	90
1	901748-23-0		19		75
1	92555-63-0		21	50	49
1	899390-65-9		24	25	79
1	113657-49-1		24	75	>200

1	886-77-1		25	75	>200
1	902600-80-0		25	50	147
1	31386-24-0		26	75	>200
1	172038-68-5		28	30	72
1	913526-66-6		29	100	>200
3	883044-26-6		29	100	>200
1	913526-79-1		32	50	172
1	890809-88-8		37	5.0	3.9
1	901014-60-6		46	75	>200

1	891882-35-2		47	50	75
4	33289-49-5		63	100	>200
6	479664-71-6		71	75	35
1	1008981-46-1		73	200	>200
1	515867-15-9		89	75	>200
1	957287-11-5		100	100	>200
4	329057-10-5		112	40	82
1	127040-12-4		114	10	26
1	7489-66-9		115	100	>200

1	361158-00-1		120	100	>200
1	346643-31-0		143	50	>200
1	346643-69-4		160	12	72
1	780810-55-1		250	150	>200

[a] The synthetic compounds belong to the following libraries: ChemDiv 3 (library 1); Maybridge 4 (library 3); ActiMol TimTec (library 4); Enamine 1 (library 6); I.F. Lab 1 (library 7); and Maybridge 2 (library 9). [b] CAS (Chemical Abstracts Service) Registry Numbers are unique identifiers for chemical substances.