

# Discovery of SARS-CoV-2 nsp14 and nsp16 methyltransferase inhibitors by high-throughput virtual screening

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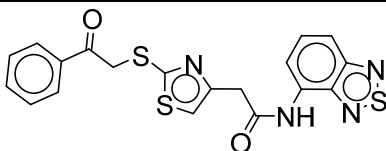
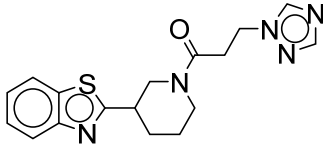
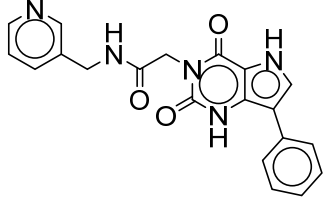
<sup>†</sup>Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga LV-1006, Latvia

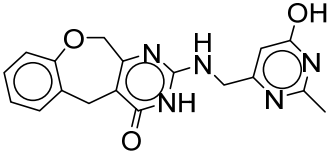
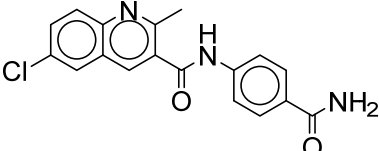
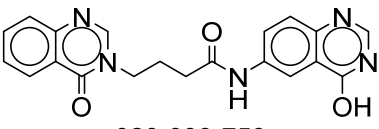
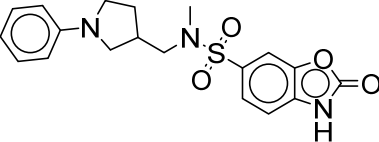
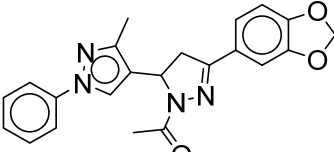
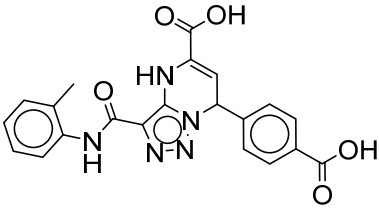
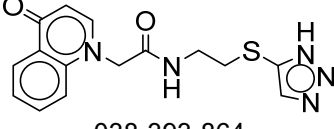
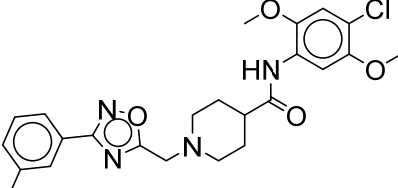
<sup>‡</sup>University of Latvia, Jelgavas 1, Riga LV-1004, Latvia

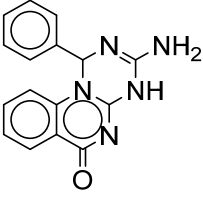
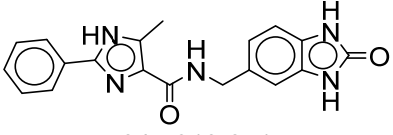
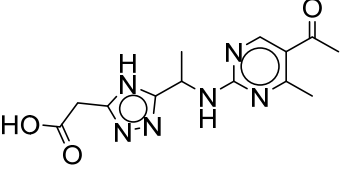
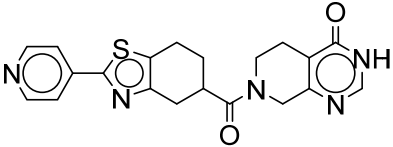
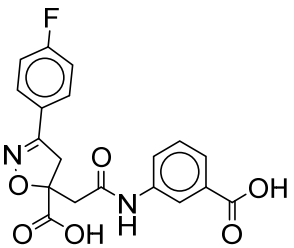
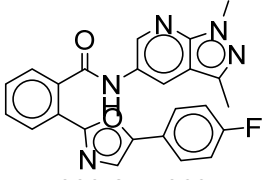
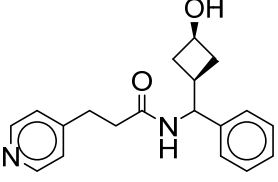
<sup>§</sup>Latvian Biomedical Research and Study Centre, Ratsupites 1 k1, Riga LV-1067, Latvia

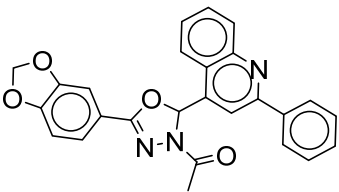
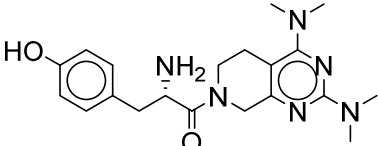
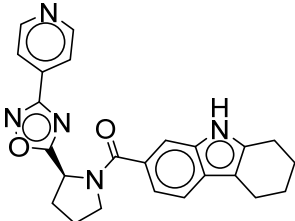
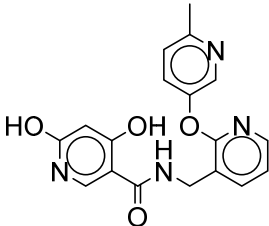
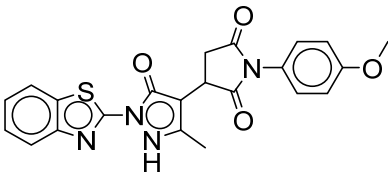
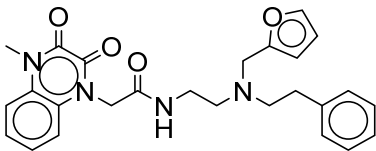
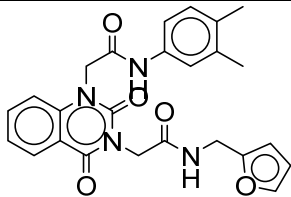
## Enzymatic assay data

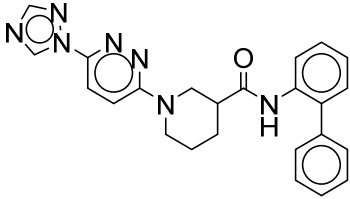
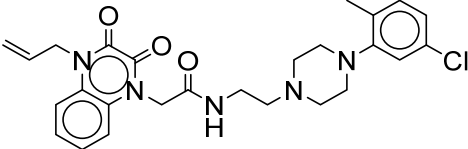

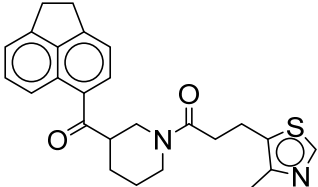
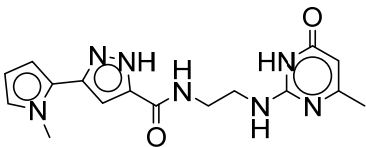
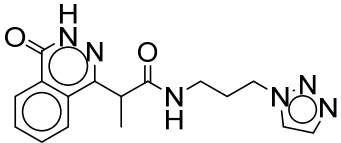
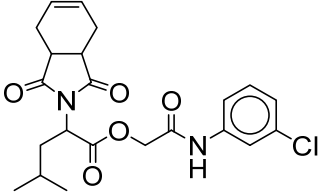
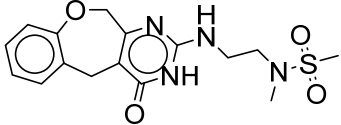
Table S1. The inhibition potency, ligand efficiency and target MTase of commercially obtained HTVS hits

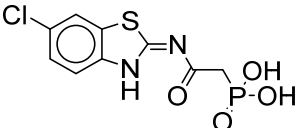
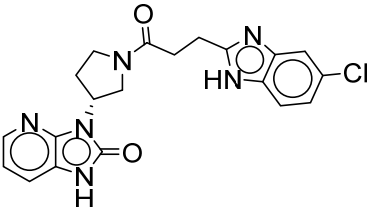
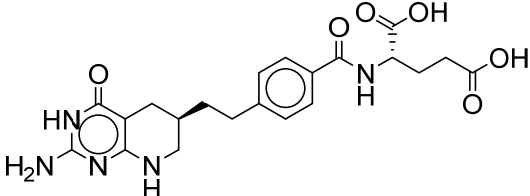
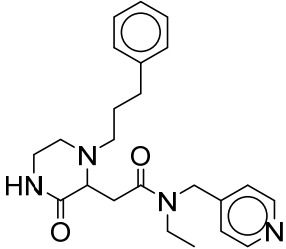
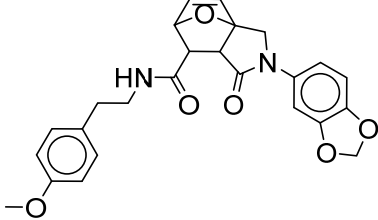
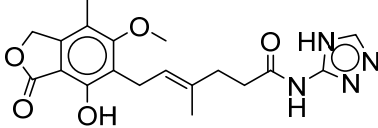
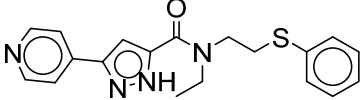
No	Compound (MolPort ID) (ZINC ID)	Target MTase	Percent inhibition at 100 $\mu$ M, % (IC <sub>50</sub> , $\mu$ M) (Ligand Efficiency (LE), kcal/mol)		
			nsp14	nsp16	Human
1	 009-724-498 ZINC000038661771	nsp14	58 $\pm$ 7 IC <sub>50</sub> =118 $\pm$ 4 LE=0.19	69 $\pm$ 8 IC <sub>50</sub> =51 $\pm$ 2 LE=0.21	63 $\pm$ 5 IC <sub>50</sub> =58 $\pm$ 3 LE=0.21
2	 009-161-166 ZINC000023398144	nsp14	57 $\pm$ 8 IC <sub>50</sub> =77 $\pm$ 3 LE=0.23	39 $\pm$ 7 IC <sub>50</sub> =197 $\pm$ 9 LE=0.21	21 $\pm$ 2 IC <sub>50</sub> =315 $\pm$ 5 LE=0.20
3	 007-818-785 ZINC000033037945	nsp14	56 $\pm$ 5 IC <sub>50</sub> =106 $\pm$ 5 LE=0.19	-8 $\pm$ 6 inactive	53 $\pm$ 8 IC <sub>50</sub> =125 $\pm$ 6 LE=0.19

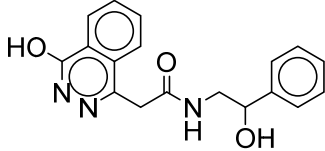
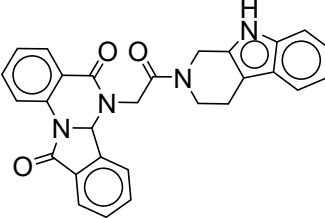
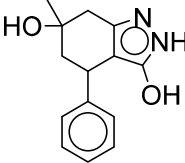
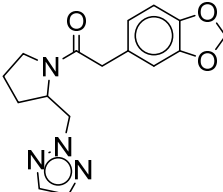
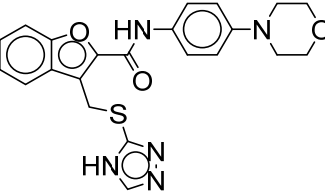
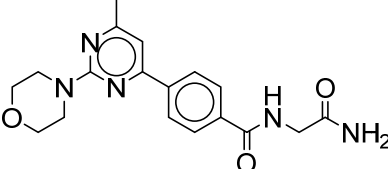
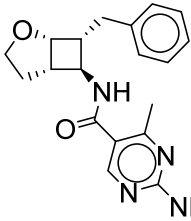
4	 <p>028-735-286* ZINC000095519272</p>	nsp16	49 ±17 IC <sub>50</sub> =~200 LE=0.19	57 ±13 IC <sub>50</sub> =95 ±4 LE=0.21	70 ±3 IC <sub>50</sub> =53 ±3 LE=0.22
5	 <p>007-242-697 ZINC000019718669</p>	nsp14	49 ±16	66 ±9	52 ±4
6	 <p>029-999-750 ZINC000096114839</p>	nsp14	28 ±7	17 ±5	83 ±6
7	 <p>045-937-707 ZINC000952973746</p>	nsp14	27 ±9	38 ±6	41 ±1
8	 <p>004-649-205 ZINC000012821397</p>	nsp14	27 ±5	15 ±5	-5 ±2
9	 <p>023-306-621 ZINC000072333096</p>	nsp14	25 ±10	33 ±12	17 ±4
10	 <p>038-393-864 ZINC000218615582</p>	nsp16	21 ±3	51 ±4	55 ±2
11	 <p>006-348-599 ZINC000030974583</p>	nsp16	19 ±1	45 ±11	48 ±8

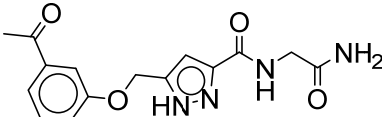
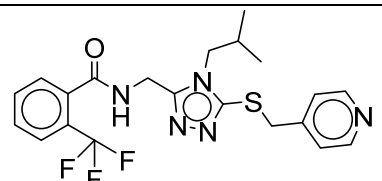
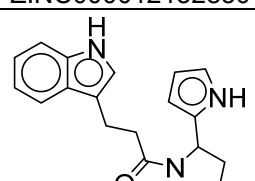
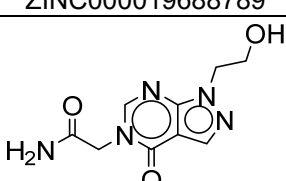
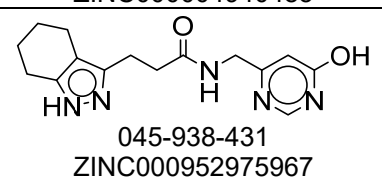
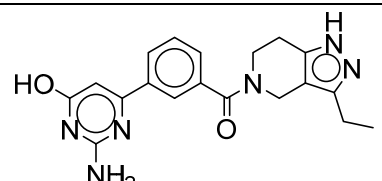
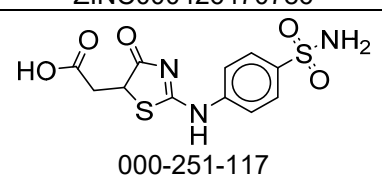
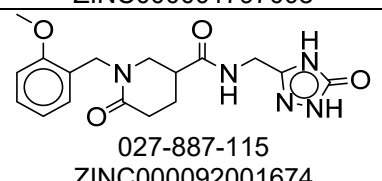
12	 000-511-203 ZINC000008994271	nsp16	19 ±10	39 ±3	-3 ±6
13	 045-910-851 ZINC000680227150	nsp14	19 ±7	23 ±5	not tested
14	 046-744-664 ZINC1772709012	nsp16	16 ±13	32 ±8	not tested
15	 046-750-782 ZINC1772809556	nsp14	15 ±9	5 ±8	not tested
16	 021-745-257 ZINC000072406141	nsp14	14 ±7	31 ±8	not tested
17	 009-255-069 ZINC000032754717	nsp14	14 ±6	-24 ±9	not tested
18	 038-397-244 ZINC000252528037	nsp14	13 ±2	36 ±3	not tested

19		nsp14	12 ±4	36 ±10	45 ±7
010-686-097 ZINC000021941344					
20		nsp14	12 ±5	9 ±8	not tested
020-196-938 ZINC000071682020					
21		nsp14	11 ±10	24 ±5	not tested
045-941-016 ZINC000952966781					
22		nsp16	11 ±3	39 ±8	not tested
019-887-754 ZINC000067754517					
23		nsp14	11 ±6	17 ±6	not tested
000-846-375 ZINC000018085229					
24		nsp16	10 ±9	7 ±4	not tested
007-880-423 ZINC000021649685					
25		nsp14	10 ±6	-8 ±4	not tested
007-610-022 ZINC000009617688					

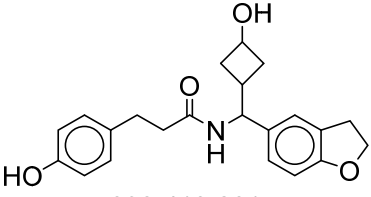
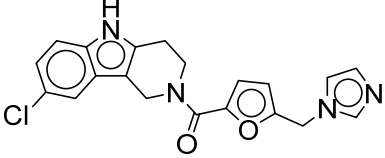
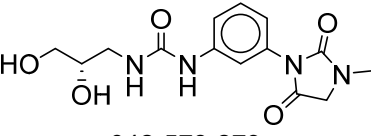
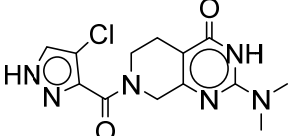
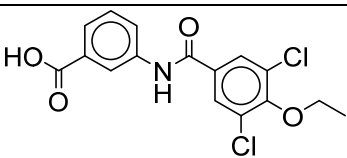
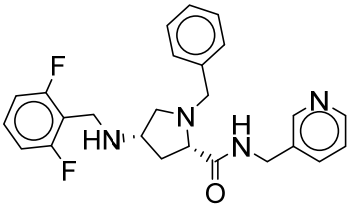
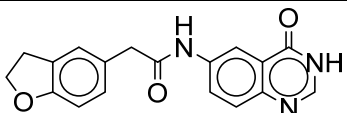
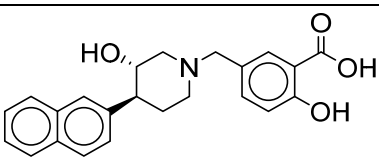
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028-851-132 ZINC000096124096					
27		nsp16	9 ±3	32 ±7	not tested
010-695-959 ZINC000033268761					
28		nsp14	9 ±4	11 ±5	not tested
002-297-972 ZINC000016448507					
29		nsp14	9 ±5	40 ±8	61 ±4
005-070-505 ZINC000012755219					
30		nsp16	9 ±6	24 ±4	21 ±6
027-822-894 ZINC000091693861					
31		nsp16	9 ±3	40 ±6	22 ±6
035-370-066 ZINC000215108606					
32		nsp14	8 ±3	37 ±9	not tested
004-007-966 ZINC000019078629					
33		nsp16	8 ±5	-4 ±3	42 ±2
028-736-920					

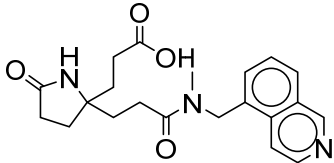
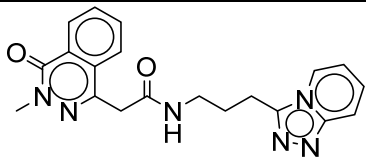
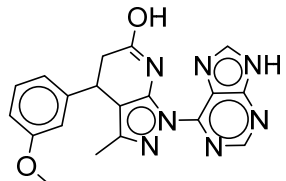
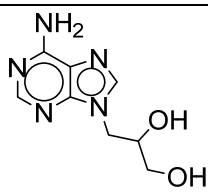
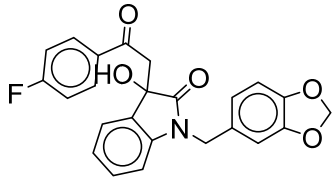
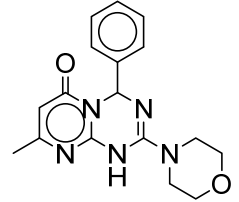
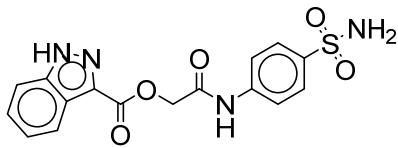
ZINC000095521577					
34		nsp16	7 ±6	17 ±7	not tested
000-770-854 ZINC000020502712					
35		nsp14	6 ±2	15 ±2	not tested
046-170-871 ZINC1548405210					
36		nsp14	5 ±6	15 ±10	not tested
044-561-472 ZINC000077301924					
37		nsp14	5 ±5	17 ±11	not tested
005-137-833 ZINC000011821342					
38		nsp14	5 ±2	-15 ±6	not tested
000-859-258 ZINC000012895535					
39		nsp16	5 ±4	-68 ±6	not tested
028-855-794 ZINC000096115284					
40		nsp14	5 ±2	19 ±7	-46 ±2
030-055-667 ZINC000097636636					

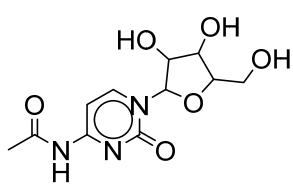
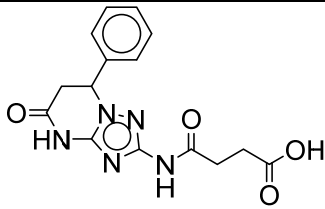
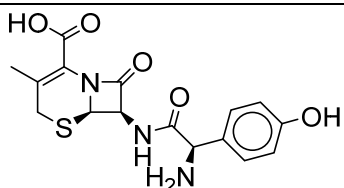
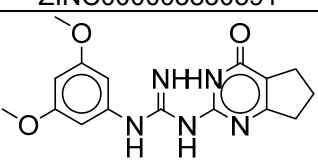
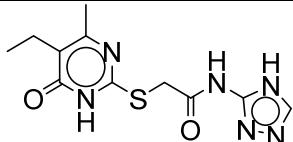
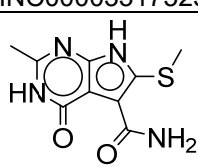
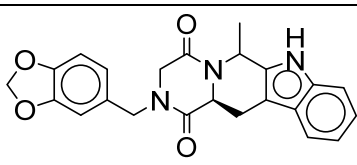
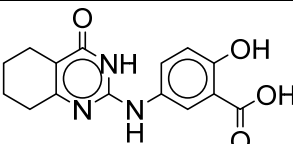
41	 000-856-143 ZINC000009340377	nsp16	5 ±4	2 ±5	not tested
42	 044-430-985 ZINC000604419204	nsp14	5 ±11	-3 ±5	not tested
43	 006-828-073 ZINC000035326963	nsp16	4 ±8	-2 ±6	not tested
44	 044-563-263 ZINC000019345667	nsp14	4 ±1	22 ±6	not tested
45	 004-060-271 ZINC000003404544	nsp14	3 ±2	6 ±8	not tested
46	 044-674-956 ZINC000534574506	nsp16	3 ±3	17 ±8	not tested
47	 038-401-110 ZINC000219517301	nsp14	3 ±4	28 ±8	not tested

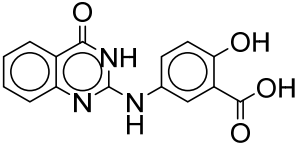
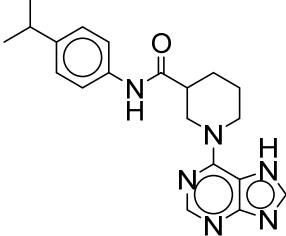
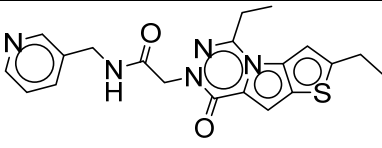
48		nsp16	2 ±2	-1 ±3	not tested
	019-899-381 ZINC000067920483				
49		nsp16	2 ±8	19 ±6	not tested
	005-103-886 ZINC000012152850				
50		nsp14	2 ±0	-10 ±1	not tested
	009-042-162 ZINC000019688789				
51		nsp16	2 ±0	6 ±6	not tested
	003-162-096 ZINC000004940485				
52		nsp16	2 ±7	-24 ±4	not tested
	045-938-431 ZINC000952975967				
53		nsp14	1 ±6	8 ±5	not tested
	042-563-901 ZINC000426470786				
54		nsp16	0 ±2	-35 ±10	not tested
	000-251-117 ZINC000001797008				
55		nsp16	0 ±7	22 ±12	44 ±8
	027-887-115 ZINC000092001674				



56	 038-410-201 ZINC000220681335	nsp14	0 ±2	10 ±5	not tested
57	 021-759-980 ZINC000072429175	nsp14	0 ±4	6 ±5	not tested
58	 042-578-273 ZINC000426716892	nsp16	-1 ±2	30 ±2	not tested
59	 020-232-114 ZINC000072172639	nsp16	-1 ±2	29 ±5	not tested
60	 002-245-502 ZINC000004649202	nsp16	-1 ±3	14 ±5	not tested
61	 005-074-359 ZINC000020999776	nsp14	-2 ±4	8 ±5	not tested
62	 035-814-299 ZINC000170622294	nsp16	-2 ±2	-6 ±3	not tested
63	 016-617-317 ZINC000065452571	nsp14	-3 ±8	-2 ±5	not tested

64	 038-408-691 ZINC000220483024	nsp16	-5 ±3	31 ±6	16 ±4
65	 028-605-864 ZINC000095478169	nsp16	-6 ±2	-53 ±8	not tested
66	 006-807-837 ZINC000020745418	nsp14	-8 ±5	-7 ±2	not tested
67	 001-002-015 ZINC000000370772	nsp16	-9 ±12	-34 ±9	not tested
68	 001-824-709 ZINC000000783765	nsp16	-10 ±3	-3 ±4	not tested
69	 002-997-525 ZINC000104279901	nsp16	-14 ±2	-33 ±4	not tested
70	 005-328-022 ZINC000005360401	nsp16	-15 ±7	3 ±1	not tested

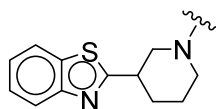
71	 000-395-882 ZINC000001078602	nsp16	-15 ±5	-9 ±1	not tested
72	 002-580-688 ZINC000000095711	nsp16	-16 ±0	-77 ±13	not tested
73	 002-507-346 ZINC000003830391	nsp16	-18 ±6	-8 ±1	not tested
74	 002-997-627 ZINC000009935252	nsp16	not tested**	not tested**	not tested**
75	 007-980-293 ZINC000033175254	nsp16	not tested**	not tested**	not tested**
76	 035-884-006 ZINC000006694156	nsp16	not tested**	not tested**	not tested**
77	 001-737-468 ZINC000002102009	nsp16	not tested**	not tested**	not tested**
78	 021-780-054 ZINC000073647922	nsp16	not tested**	not tested**	not tested**

79	 021-780-059 ZINC000073647929	nsp16	not tested**	not tested**	not tested**
80	 000-842-477 ZINC000009059943	nsp14	not tested**	not tested**	not tested**
81	 007-669-585 ZINC000012088756	nsp14	not tested**	not tested**	not tested**

\* NMR and MS analysis indicated that sample contains mixture of compounds

\*\* compound not soluble in DMSO

Table S2. Enzymatic potency, ligand efficiency of commercially available ZINC23398144 hit analogues



No	Compound (MolPort ID) (ZINC ID)	Percent inhibition at 100 $\mu$ M, % ( $IC_{50}$ , $\mu$ M) (Ligand Efficiency (LE), kcal/mol)		
		nsp14	nsp16	Human
1	 009-161-166 ZINC000023398144	57 $\pm$ 8 $IC_{50}$ =77 $\pm$ 3 LE=0.23	39 $\pm$ 7 $IC_{50}$ =197 $\pm$ 9 LE=0.21	21 $\pm$ 2 $IC_{50}$ =315 $\pm$ 5 LE=0.20
2	 046-188-339	31 $\pm$ 3	66 $\pm$ 12	not tested
3	 046-114-939	15 $\pm$ 4	37 $\pm$ 13	not tested
4	 021-760-906	10 $\pm$ 2	28 $\pm$ 6	not tested
5	 042-575-680	8 $\pm$ 1	33 $\pm$ 9	not tested
6	 045-938-897	7 $\pm$ 2	-29 $\pm$ 5	not tested
7	 005-679-672	6 $\pm$ 0	-59 $\pm$ 2	not tested
8	 	5 $\pm$ 3	15 $\pm$ 8	not tested

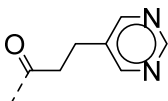
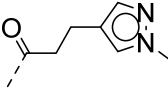
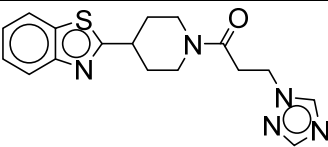
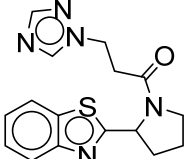
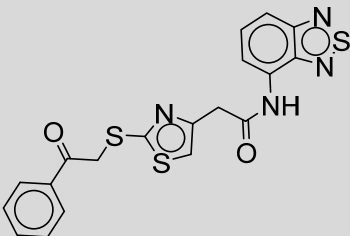
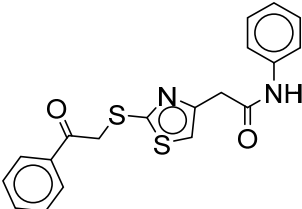
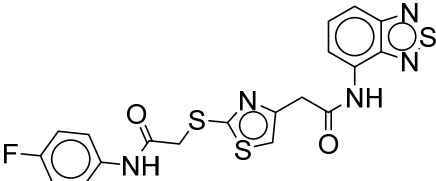
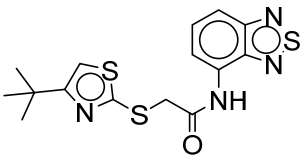
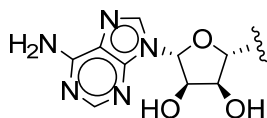
046-705-372				
9		$5 \pm 3$	$-45 \pm 13$	not tested
046-723-388				
10		$5 \pm 3$	$-113 \pm 16$	not tested
046-706-934				
11		$2 \pm 1$	$-114 \pm 6$	not tested
009-436-708				
12		$2 \pm 0$	$1 \pm 1$	not tested
009-447-345				

Table S3. Enzymatic potency, ligand efficiency of commercially available ZINC38661771 hit analogues

No	Compound (MolPort ID) (ZINC ID)	Percent inhibition at 100 $\mu$ M, % (IC <sub>50</sub> , $\mu$ M) (Ligand Efficiency (LE), kcal/mol)		
		nsp14	nsp16	Human
1	 009-724-498 ZINC000038661771	58 $\pm$ 7 IC <sub>50</sub> =118 $\pm$ 4 LE=0.19	69 $\pm$ 8 IC <sub>50</sub> =51 $\pm$ 2 LE=0.21	63 $\pm$ 5 IC <sub>50</sub> =58 $\pm$ 3 LE=0.21
2	 005-296-365	27 $\pm$ 2	-17 $\pm$ 4	not tested
3	 009-741-602	25 $\pm$ 2	45 $\pm$ 5	24 $\pm$ 7
4	 009-639-090	16 $\pm$ 1	43 $\pm$ 7	61 $\pm$ 9

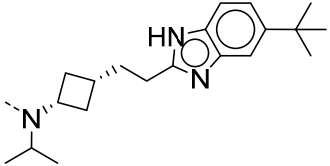
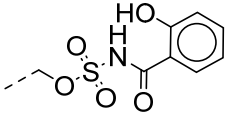
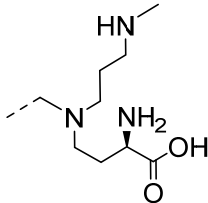
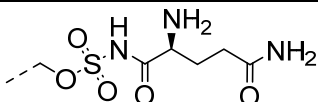
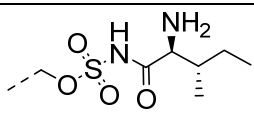
\* Only 3 ZINC38661771 analogues were purchased due to limited chemical space covered by commercially available compounds – majority of analogues varied with the placement of minor functional groups, mostly halogen atoms, at the terminal benzene ring.

Table S4. Enzymatic potency and ligand efficiency of commercially available SAM analogues



No	Compound (MolPort ID) (ZINC ID)	Percent inhibition at 100 $\mu$ M, % ( $IC_{50}$ , $\mu$ M) (Ligand Efficiency (LE), kcal/mol)		
		nsp14	nsp16	Human
1	 Sinefungin 044-183-244 ZINC000004217451	$IC_{50}=0.45$ $\pm 0.02$ LE=0.32	$IC_{50}=0.86$ $\pm 0.04$ LE=0.30	$IC_{50}=316$ $\pm 15$ LE=0.18
2	 S-(5'-Adenosyl)-L-homocysteine (SAH) 003-925-198 ZINC000004228232	$IC_{50}=0.14$ $\pm 0.01$ LE=0.33	$IC_{50}=0.28$ $\pm 0.03$ LE=0.32	$IC_{50}=1.7$ $\pm 0.3$ LE=0.28
3	 001-834-574 ZINC000004228245	$IC_{50}=1.4$ $\pm 0.1$ LE=0.40	$IC_{50}=1.02$ $\pm 0.05$ LE=0.41	$IC_{50}=1.8$ $\pm 0.2$ LE=0.39
4	 004-959-541 ZINC000003861767	$IC_{50}=1.5$ $\pm 0.2$ LE=0.27	$IC_{50}=1.14$ $\pm 0.06$ LE=0.28	$IC_{50}=2.80$ $\pm 0.14$ LE=0.26
5	 046-857-324 ZINC000473112262	$IC_{50}=3.1$ $\pm 0.3$ LE=0.28	$IC_{50}=2.46$ $\pm 0.12$ LE=0.28	$IC_{50}=5.8$ $\pm 0.3$ LE=0.26
6	 003-934-134 ZINC000048222133	$58 \pm 2$ $IC_{50}=53 \pm 3$ LE=0.19	$42 \pm 6$ $IC_{50}=105 \pm 6$ LE=0.17	$87 \pm 1$ $IC_{50}=25.3$ $\pm 1.3$ LE=0.20
7	 047-543-936 ZINC000017654909	$62 \pm 2$ $IC_{50}=75 \pm 4$ LE=0.14	$65 \pm 7$ $IC_{50}=75 \pm 4$ LE=0.14	$98 \pm 2$ $IC_{50}=22.8$ $\pm 1.2$ LE=0.16
8	 	$32 \pm 6$	$54 \pm 10$	$56 \pm 1$



039-336-807 ZINC000004475116				
9		30 ±4	68 ±7	not tested
035-706-611 ZINC000100074350				
10		28 ±2	14 ±3	not tested
046-417-911 ZINC000028567519				
11		22 ±5	1 ±5	not tested
046-594-122 ZINC000584905214				
12		20 ±3	50 ±11	89 ±8
046-683-328 ZINC000036477981				
13		8 ±5	34 ±6	not tested
046-416-871 ZINC000013488353				

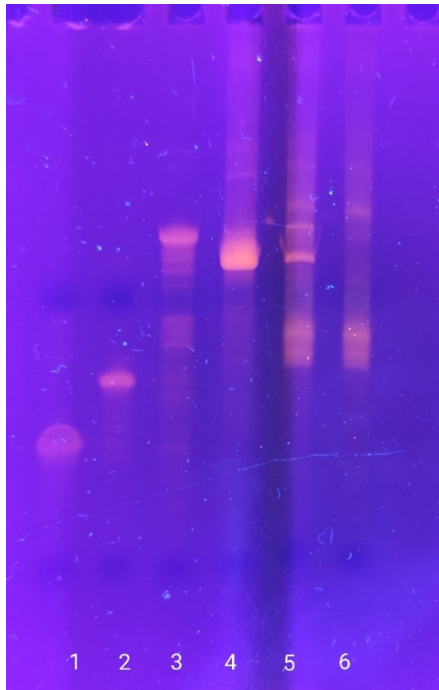


Figure S1. Analysis of RNA transcripts by 20 % polyacrylamide denaturing gel electrophoresis.

1. – 5.8 kDa standard
2. – 8.3 kDa
3. – 19.3 kDa
4. – DNA template
5. - m7G(5')ppp(5')Co25 co-transcriptionally capped RNA, before DNase digestion
6. - m7G(5')ppp(5')Co25 co-transcriptionally capped RNA, after DNase digestion, desalted