FSASI “Chumakov FSC R&D IBP RAS” (Institute of Polyomielitis)

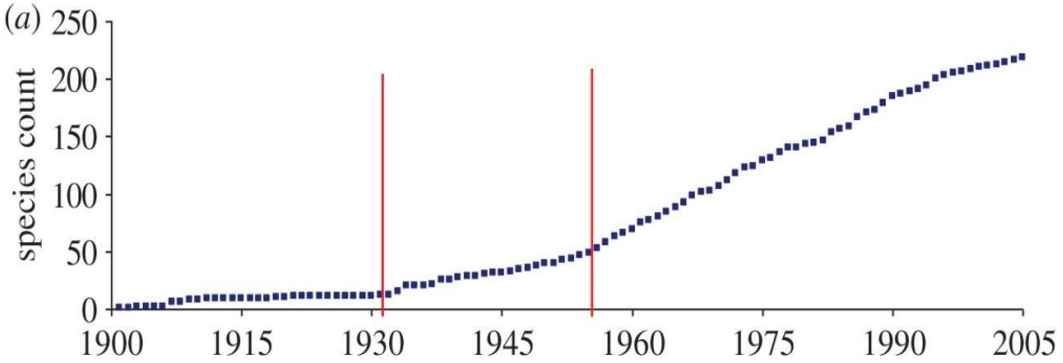
Competition and collaboration of *in silico* and *in vitro* screening in the search for new antiviral compounds

Dmitry Osolodkin

XXVIII Symposium on Bioinformatics and Computer-Aided Drug Discovery
Moscow, May 25, 2022

Natural Diversity of Viruses

International Committee on Taxonomy of Viruses (ICTV): 6 realms, 10 kingdoms, 17 phyla, 2 subphyla, 39 classes, 65 orders, 8 suborders, 233 families, 168 subfamilies, 2606 genera, 84 subgenera, **10434 species**



There are **219 virus species** that are known to be able to infect humans. The first of these to be discovered was yellow fever virus in 1901, and **three to four new species are still being found every year**.

M. Woolhouse *et al.*, Phil. Trans. R. Soc. B (**2012**) 367, 2864–2871 doi:10.1098/rstb.2011.0354

Viruses are believed to be the most abundant and diverse biological entities on our planet, with an estimated 10^{31} particles on Earth. The human virome is similarly vast and complex, consisting of approximately 10^{13} particles per human individual, with great heterogeneity.

G. Liang & F. D. Bushman, Nat. Rev. Microbiol. (2021) 19, 514-527 doi:10.1038/s41579-021-00536-5

GLOBAL VIROME PROJECT

There are an estimated 500,000+ undiscovered animal viruses capable of transmission to people

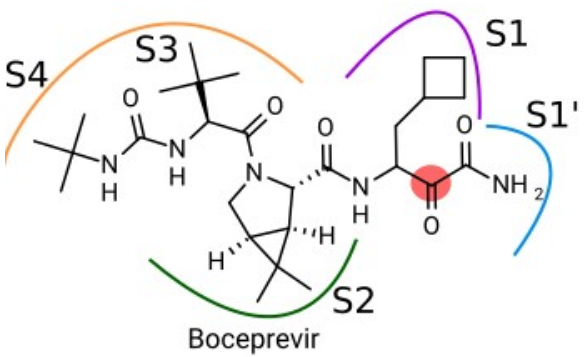
Diversity of Antiviral Drugs

- ~120 different antiviral drugs (including antibodies, biologicals, combinations, drugs approved at smaller jurisdictions)
- 11 different viruses: HIV, HBV, HCV, HCMV, HSV, VZV, IAV, RSV, HPV, VARV, EBOV, SARS-CoV-2
- Most common classes: nuscleo(s|t)ide analogues, peptidomimetics
- Lots of clinical limitations
- Repurposing options

Vaccination? Nice, **but** cold chain, biologicals only, prophylaxis only, antivaxxers, community immunity, efficiency issues, no guarantee, biosafety — is it easier at all?

Nirmatrelvir: Drug development should start before it is needed

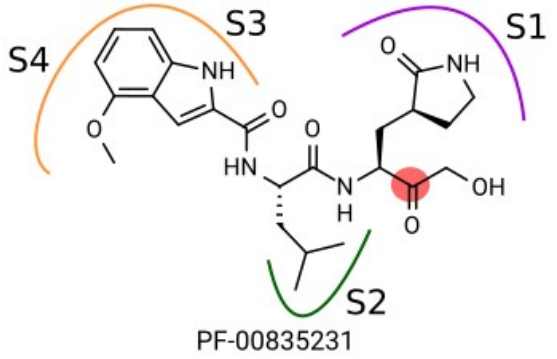
(and a textbook example of molecular similarity)



Boceprevir

- selective covalent inhibitor of HCV protease
- **approved in 2011, withdrawn from the market in 2015**
- one of the most common hits in drug repurposing programs against SARS-CoV-2

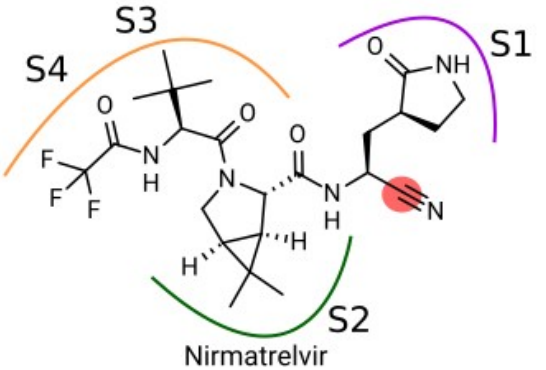
- K_i ~ 15 μM (Mpro, FRET)



PF-00835231

- selective covalent inhibitor of SARS-CoV(-2) Mpro protease
- intravenous administration of a prodrug
- **developed during the SARS-CoV outbreak (2003-2005), never progressed to clinical studies due to lack of cases**
- DOI: 10.1021/acs.jmedchem.0c01063
- DOI: 10.1101/2020.09.12.293498

- EC_{50} 0.06 ± 0.03 μM (SARS-CoV-2, Vero)
- K_i 0.27 ± 0.1 nM (Mpro, FRET)

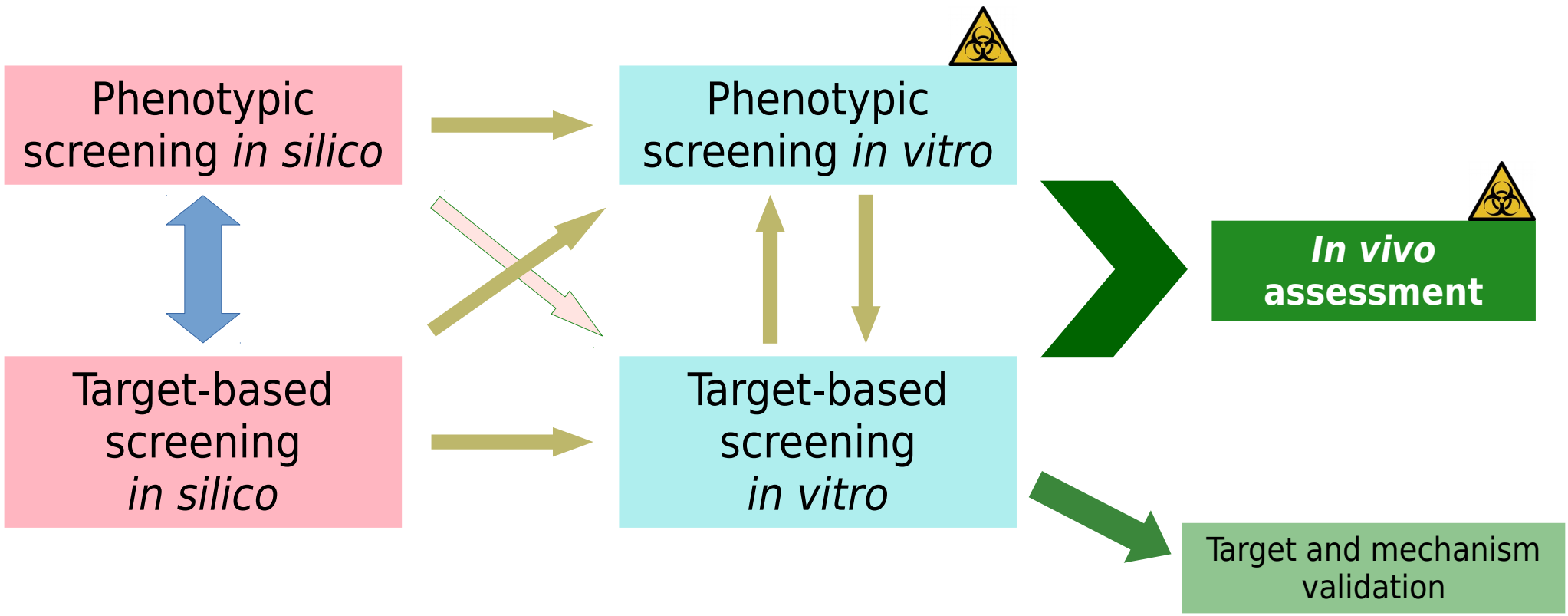


Nirmatrelvir

- selective covalent inhibitor of SARS-CoV-2 Mpro protease
- oral administration
- **first disclosed April 6, 2021**
- EUA in **December 2021** (Paxlovid — combination with ritonavir)
- DOI: 10.1126/science.abl4784

- EC_{50} 74.5 nM (SARS-CoV-2, Vero E6)
- K_i 3.11 nM (Mpro, FRET)

Complementary Approaches to Antiviral Drug Discovery



Data source curation in GIGO world

- Only 20% of early discovery data are of high confidence
- 0.3 to 1.0 log units errors are not uncommon
- As many variables as possible should be traced
- Mine and resurrect data from the past

Terry Stouch in one of ACS meeting talks

Raw data

Curated data

Search string issues

"Frederick A. Murphy, Life Member and former President of the International Committee on Taxonomy of Viruses (ICTV), once suggested to me that there are **three things one should not discuss in polite company: religion, politics, and taxonomy**. At first I thought he was joking, but I have come to realize he was not."

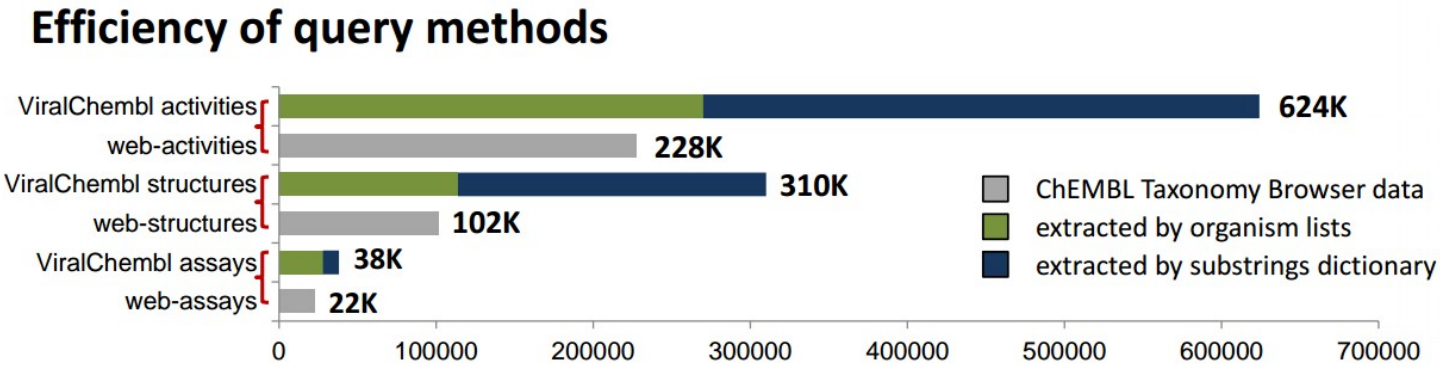
Charles H. Calisher
Life Member of ICTV
Arch Virol (2016) 161:1419–1422
DOI:10.1007/s00705-016-2779-x

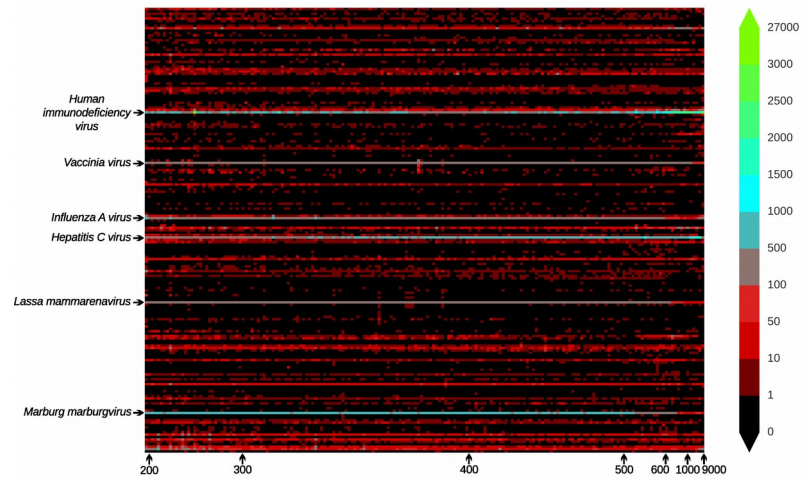
Hepatitis C virus type 2	Hepatitis C virus subtype 6a	Hepatitis C virus subtype 5a
Hepatitis C virus subtype 4a	Hepatitis C virus subtype 3a	Hepatitis C virus subtype 2b
Hepatitis C virus subtype 2a	Hepatitis C virus subtype 1b	Hepatitis C virus subtype 1a
Hepatitis C virus SA13	Hepatitis C virus S52	Hepatitis C virus JFH-1
Hepatitis C virus isolate HC-J4	Hepatitis C virus genotype 6	Hepatitis C virus genotype 5
Hepatitis C virus genotype 4	Hepatitis C virus genotype 3	Hepatitis C virus genotype 2
Hepatitis C virus genotype 1	Hepatitis C virus ED43	Hepatitis C virus (isolate NZL1)
Hepatitis C virus (isolate H77)	Hepatitis C virus (isolate H)	Hepatitis C virus (isolate Con1)
Hepatitis C virus (isolate BK)	Hepatitis C virus	

Hepatitis C virus → *Hepacivirus C*

Phenotypic screening *in silico:* ViralChEMBL

- ChEMBL antiviral activity data annotation procedure to ICTV taxonomy
- Antiviral activity database



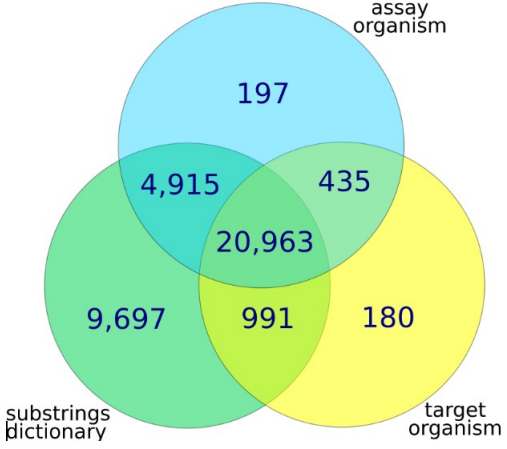


ViralChEMBL version 0.1

260 866 compounds

615 029 bioactivities

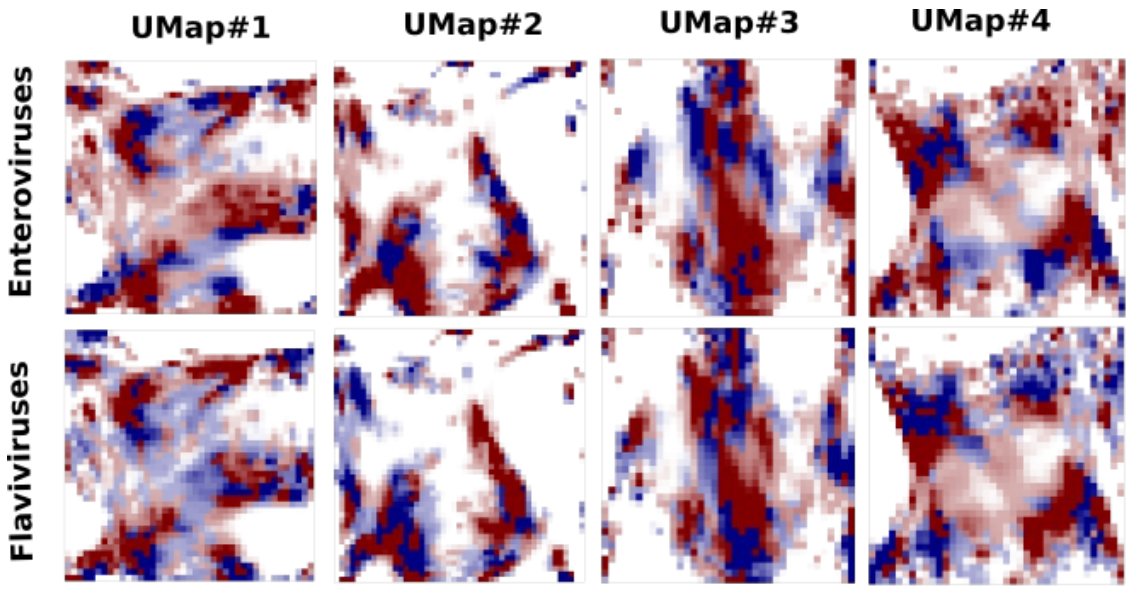
351 targets



A. A. Nikitina *et al.*, Database (2019) bay139

Phenotypic screening *in silico:* Chemical space

Generative Topographic Mapping (GTM)

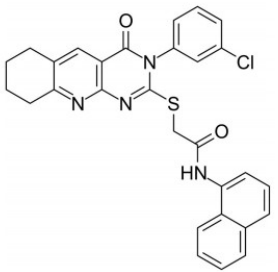
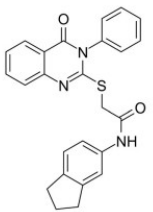
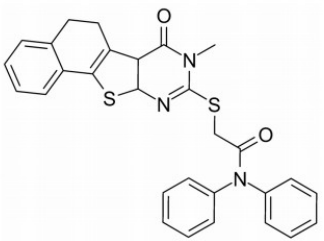
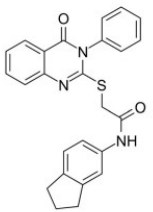


GTM: Strasbourg implementation (Prof. A. Varnek et al.)

- ISIDA fragment descriptors
- *Universal maps of* ChEMBL
- Training set compounds and screening library are projected on them
- Compounds with similar responsibility patterns are selected

Phenotypic screening *in vitro:* Experimental validation

Generative Topographic Mapping (GTM)

13		CHEMBL1406668		0.011	23.07	WNV	0.20 ± 0.16	> 50	> 50
14		CHEMBL1376916		0.012	26.57	WNV	0.6 ± 0.1	> 50	> 50

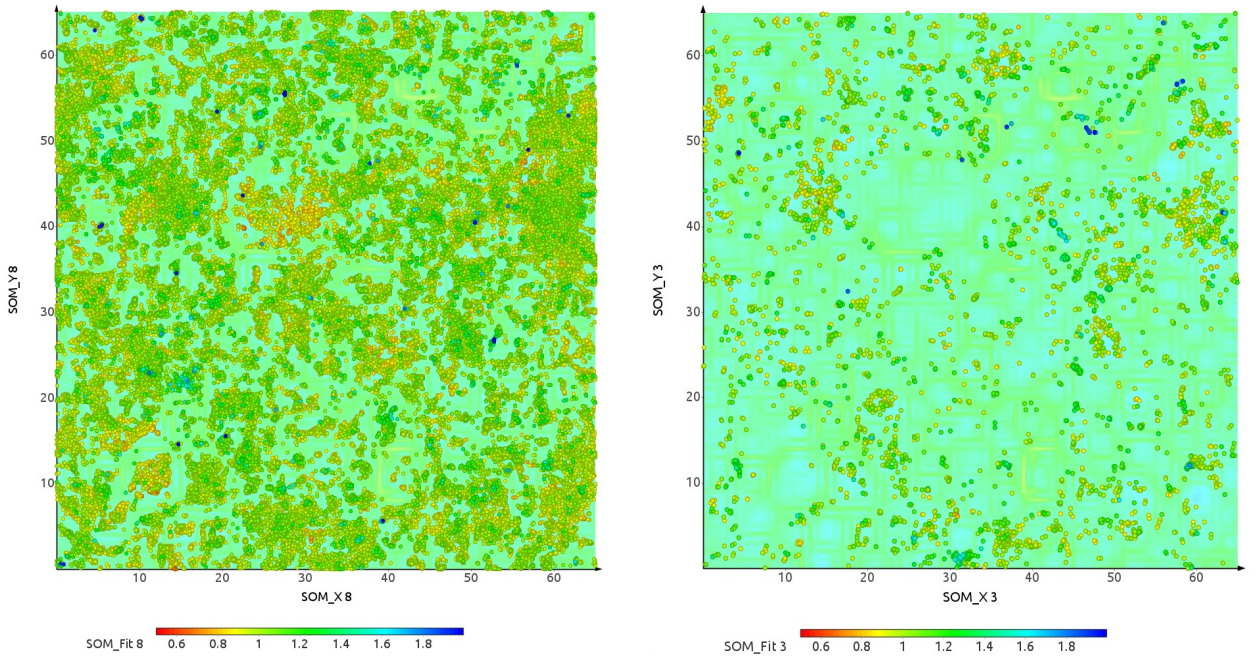
- 48 compounds purchased
- 46 tested for anti-TBEV activity
- 21 inactive
- 3 inconclusive
- 22 active (4 of them on 1 uM level of EC_{50})

50% hit rate!!

A. A. Orlov *et al.*, Mol. Inf. (2019) 38, 1800166

Phenotypic screening *in silico:* Chemical space

Self-Organizing Maps (SOM)



SOM: DataWarrior implementation

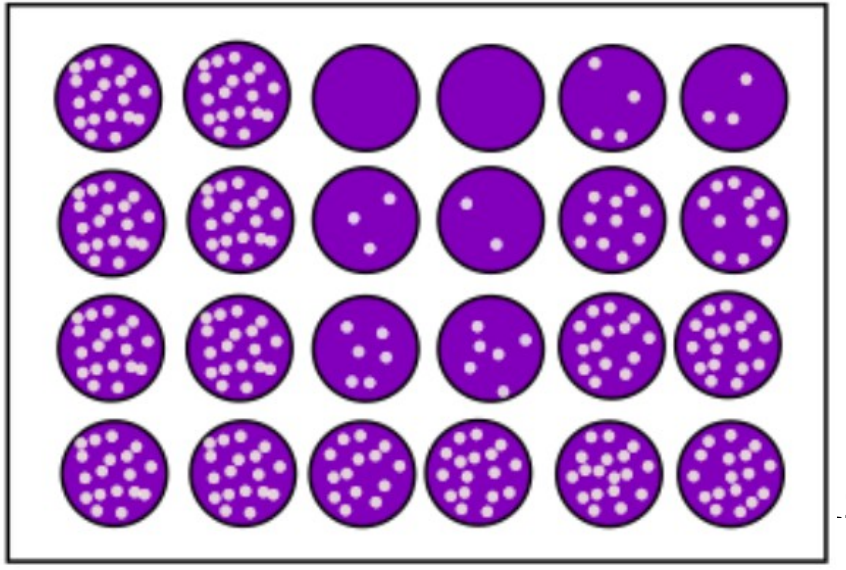
- *SkelSphere* fingerprints
- Target library is mapped
- Training set is projected onto this map
- Neighbourhood shenanigans

Phenotypic screening *in vitro:* Experimental validation

Self-Organizing Maps (SOM)

IBS INTERBIOSCREEN

- 194 compounds purchased and screened against TBEV anf YFV
- 55 anti-TBEV compounds
- 31 anti-YFV compounds
- 4 selective anti-YFV compounds
- 13 compounds with EC_{50} < 5 uM



Phenotypic sctreening *in vitro:* privileged structures

Bioorganic & Medicinal Chemistry Letters 27 (2017) 1267–1273

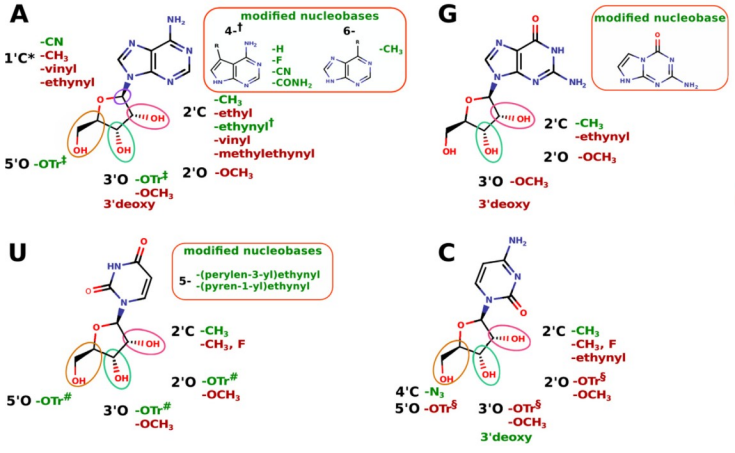
Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

New tools in nucleoside toolbox of tick-borne encephalitis virus reproduction inhibitors

Alexey A. Orlov [a,b,1], Mikhail S. Drenichev [c,1], Vladimir E. Oslovsky [c], Nikolay N. Kurochkin [c], Pavel N. Solyev [c], Liubov I. Kozlovskaya [a], Vladimir A. Palyulin [b], Galina G. Karganova [a], Sergey N. Mikhailov [c], Dmitry I. Osolodkin [a,b,*]



Bioorganic & Medicinal Chemistry Letters 30 (2020) 127100

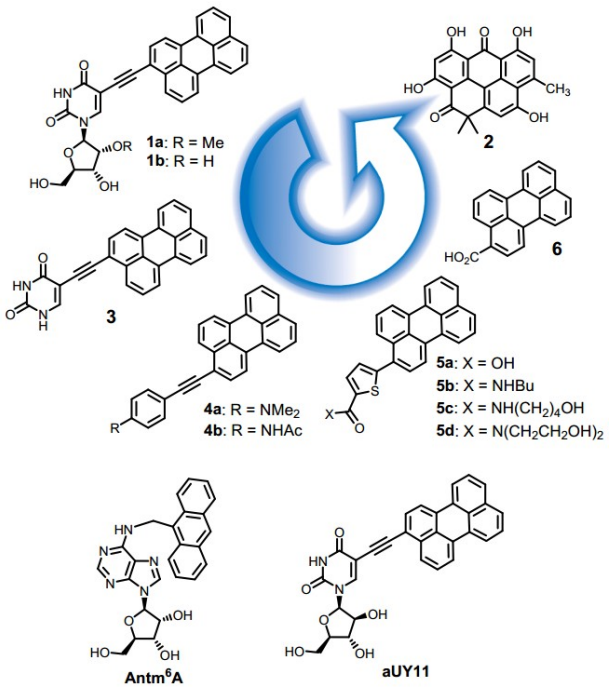
Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Simplistic perylene-related compounds as inhibitors of tick-borne encephalitis virus reproduction

Nikita A. Slesarchuk [a,b,c], Evgeny V. Khvatov [a,d], Alexey A. Chistov [a,c], Gleb V. Proskurin [a], Timofei D. Nikitin [a,b], Anastasiya I. Lazarevich [a,c], Angelina A. Ulanovskaya [a,c], Egor A. Ulashchik [f], Alexey A. Orlov [d], Artjom V. Jegorov [a], Alexey V. Ustinov [a,c,*], Anton P. Tyurin [a,c,g], Vadim V. Shmanai [i], Aydar A. Ishmukhametov [d,h], Vladimir A. Korshun [a,c,g], Dmitry I. Osolodkin [b,d,h,*], Liubov I. Kozlovskaya [d,h], Andrey V. Aralov [a]



European Journal of Medicinal Chemistry 220 (2021) 113467

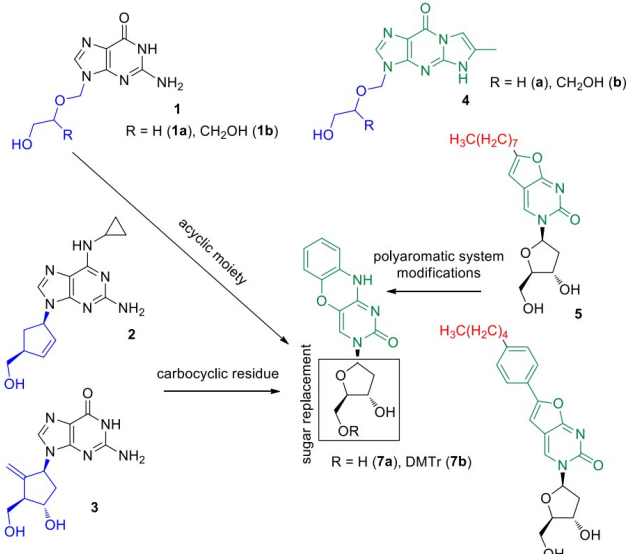
Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

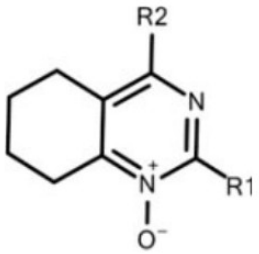
journal homepage: http://www.elsevier.com/locate/ejmech

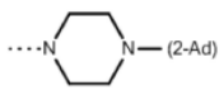
Phenoxazine nucleoside derivatives with a multiple activity against RNA and DNA viruses

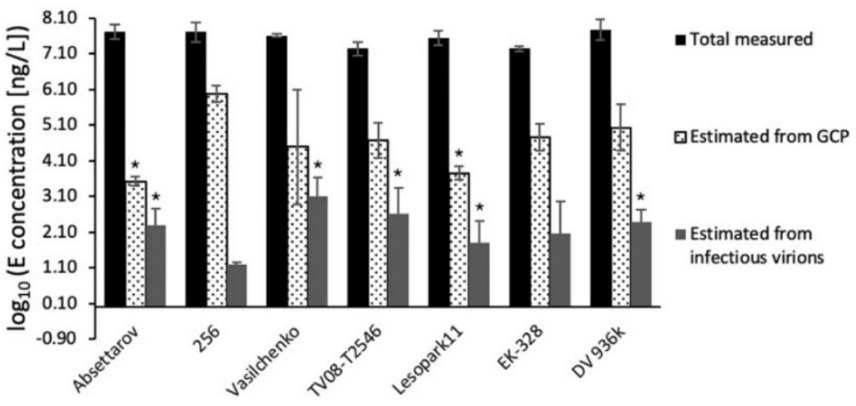
Liubov I. Kozlovskaya [a, b], Viktor P. Volok [a], Anna A. Shtro [c], Yulia V. Nikolaeva [c], Alexey A. Chistov [d], Elena S. Matyugina [e], Evgeny S. Belyaev [f], Artjom V. Jegorov [d], Robert Snoeck [g], Vladimir A. Korshun [d], Graciela Andrei [g], Dmitry I. Osolodkin [a, b], Aydar A. Ishmukhametov [a, b], Andrey V. Aralov [d, *]



Antiviral activity spectrum



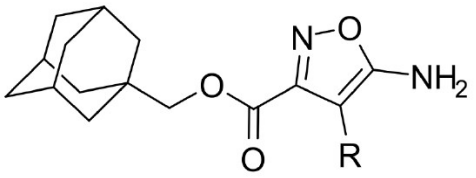
Code	R1	R2	Subtype/Strain/EC_{50} (mean ± SD, μM) Eu: Absettarov	Eu: 256	Sib: Vasilchenko	Sib: TV08-T2546	Sib: Lesopark11	Sib: EK-328	FE: 205KGG	FE: DV 936k
7a	Me	NHBu	31 ± 5	>50	>50	18 ± 4	>50	>50	>50	18 ± 3
7c	*t*-Bu	$NHCH_2Ph$	>50	>50	>50	>50	>50	>50	>50	>50
7o	Me	$NH(2\text{-}OH\text{-}C_6H_4)$	8 ± 3	21 ± 2	3.4 ± 0.2	4.0 ± 0.4	6.5 ± 0.1	12 ± 1	7.0 ± 0.2	7.4 ± 0.3
7t	Et	$NH(CH_2)_2$(1-Ad)	35 ± 2	>50	>50	25 ± 3	>50	>50	>50	>50
7u	*t*-Bu	$NH(CH_2)_2$(1-Ad)	6 ± 2	>50	13 ± 2	7 ± 2	6.0 ± 1.5	9.7 ± 0.7	6.9 ± 0.8	6.5 ± 0.6
7w	*t*-Bu	$NH(CH_2)_2$(2-Ad)	6 ± 3	>50	16 ± 2	11.4 ± 0.9	4.4 ± 0.2	7.5 ± 0.6	8.3 ± 0.4	5 ± 2
7y	Me	NHCH(1-Ad)Ph	8 ± 3	>50	16 ± 2	15 ± 2	23 ± 4	15.0 ± 0.2	10.9 ± 0.5	14.7 ± 0.1
7z	*t*-Bu	NHCH(1-Ad)Ph	4 ± 1	26 ± 2	4.3 ± 0.3	3.3 ± 0.4	9 ± 1	10.1 ± 0.9	4.1 ± 0.3	4.3 ± 0.1
7ab	*t*-Bu		23 ± 6	>50	>50	29 ± 1	35 ± 5	39 ± 2	37 ± 1	12 ± 2



- TBEV Strain 256: identical to Absettarov by E protein sequence, but all compounds are much less active
- Contains much more decoy particles: non-infectious, immature, destroyed

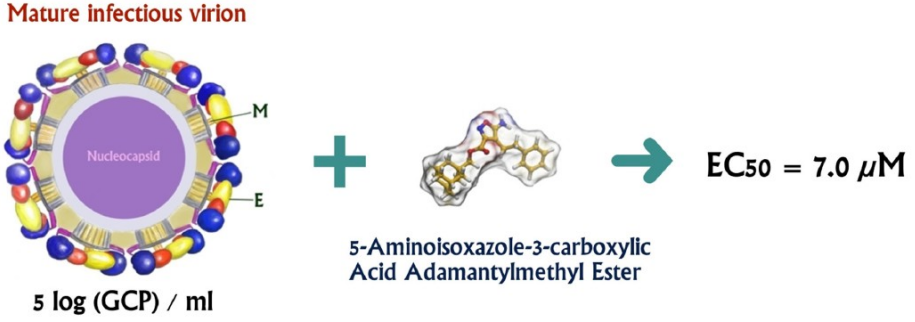
E. V. Dueva *et al.*, Antivir. Chem. Chemother. (2020) 28, 2040206620943462

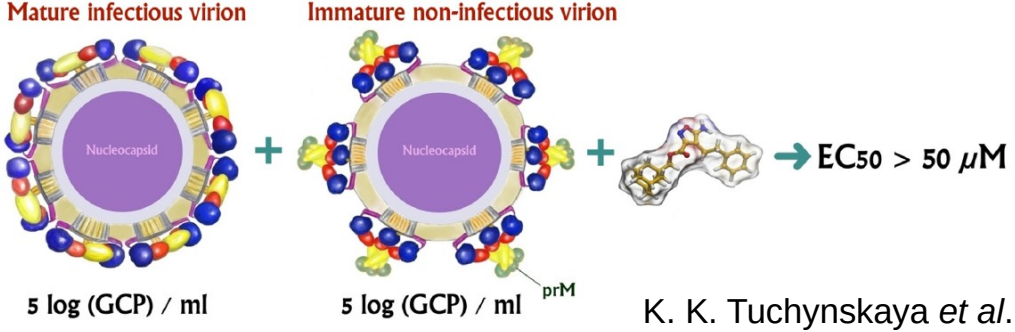
Influence of immature virions



R = H (**4j**), CH_2CH_2Ph (**4o**)

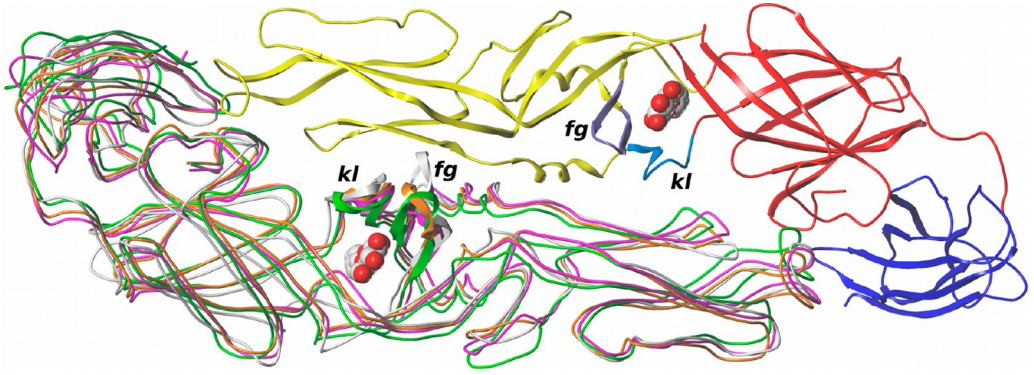
Virus sample (strain, maturity, dose)	4j EC_{50}, μM	4o EC_{50}, μM
EK-328, immature, 6 log(GCP) ml^{-1}	>50	>50
EK-328, immature, 6,7 log(GCP) ml^{-1}	>50	>50
EK-328, mature, 5 log(GCP) ml^{-1}	**39.56±0.4**	**7.00±1.4**
Absettarov, mature, 3 log(GCP) ml^{-1}	**1.7±0.1***	**3.7±0.2*a**

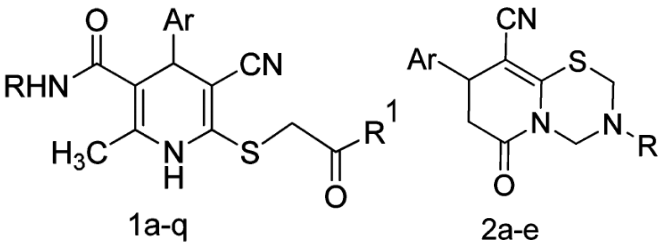




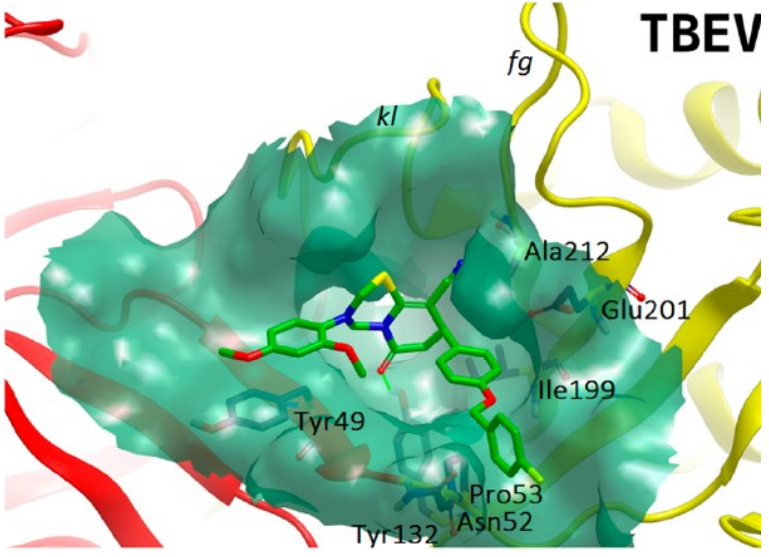
K. K. Tuchynskaya *et al*., J. Gen. Virol. (2021) 102, 001658

Target-based screening *in silico:* TBEV E protein molecular docking



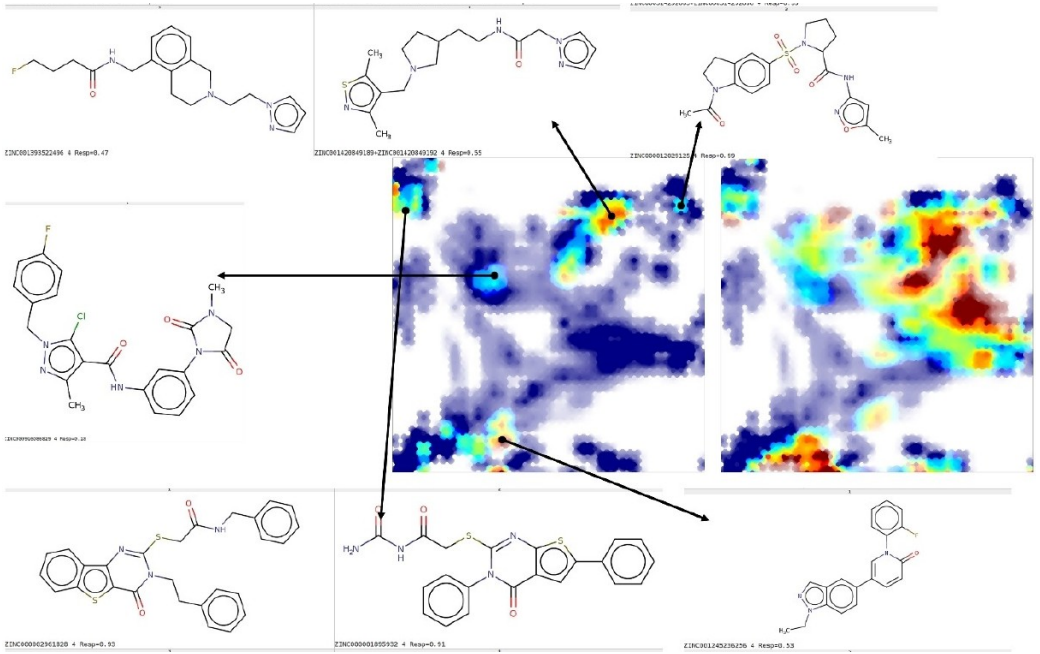


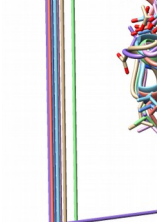
compd	Ar	R	R^1	CC_{50}, μM (acute)	CC_{50}, μM (chronic)	IC_{50}, μM TBEV	IC_{50}, μM POWV	IC_{50}, μM OHFV
1a	2-furyl	4-$EtOC_6H_4$	4-$nBuC_6H_4NH$	64	14	2.5 ± 0.5	>10	>10
1b	2-furyl	4-$H_2NSO_2C_6H_4$	4-EtC_6H_4NH	>250[a]	153	>10	>10	10 ± 7.8
1c	2-furyl	4-ClC_6H_4	4-EtC_6H_4NH	26	27	>10	>10	>10
1d	2-furyl	4-EtC_6H_4	4-EtC_6H_4NH	57	33	>10	>10	>10
1e	2-furyl	2-MeC_6H_4	2-naphthyl-NH	>250	>250	>10	>10	5.3 ± 0.1
1f	2-furyl	Ph	3,4-$Me_2C_6H_3$	>250	19	>10	>10	3.2 ± 0.8
1g	2-furyl	2,6-$Me_2C_6H_3$	2-benzothiazolyl-NH	>250	7	>10	>10	7.1 ± 0.1
1h	2-furyl	2-benzothiazolyl	2-benzothiazolyl-NH	>250	52	>10	>10	2.5 ± 0.9
1i	2-furyl	2-benzothiazolyl	4-*i*PrC_6H_4NH	>250	29	>10	>10	2.5 ± 0.1
1j	5-Me-2-furyl	2-$MeOC_6H_4$	4-BrC_6H_4NH	248	34	>10	>10	3.7 ± 0.4
1k	2-thienyl	Ph	Ph	>250	17	>10	>10	>10
1l	2-thienyl	2-$MeOC_6H_4$	4-MeC_6H_4NH	>250	97	>10	>10	5.5 ± 0.9
1m	Ph	4-ClC_6H_4	3-MeC_6H_4NH	>250	41	2.0 ± 0.4	>10	>10
1n	Ph	4-ClC_6H_4	4-$PhOC_6H_4NH$	>250	38	2.8 ± 0.6	>10	>10
1o	Ph	4-ClC_6H_4	2-naphthyl-NH	111	20	>10	>10	>10
1p	2-FC_6H_4	2-MeC_6H_4	4-$MeOC_6H_4NH$	>250	89	>10	>10	7.2 ± 0.5
1q	4-HO-3-$MeOC_6H_3$	Ph	4-EtC_6H_4NH	114	31	>10	>10	1.8 ± 0.4
2a	4-(4-$ClC_6H_4CH_2O$)C_6H_4	2,4-$(MeO)_2C_6H_3$		109	39	0.07 ± 0.02	1.3 ± 0.1	>10
2b	3-$BnOC_6H_4$	2-$EtOC_6H_4$		>250	116	2.6 ± 0.4	2.2 ± 0.3	>10
2c	3-$BnOC_6H_4$	4-EtC_6H_4		>250	236	>10	>10	>11.9
2d	4-$BnOC_6H_4$	4-*n*BuC_6H_4		>250	35	1.9 ± 0.4	>10	>10
2e	4-BnO-3-$MeOC_6H_3$	4-$MeOC_6H_4$		>250	53	0.09 ± 0.01	>10	>10



D. I. Osolodkin *et al.*, ACS Med. Chem. Lett. (2013) 4, 869-874

Virtual screening of SARS-CoV-2 main protease inhibitors

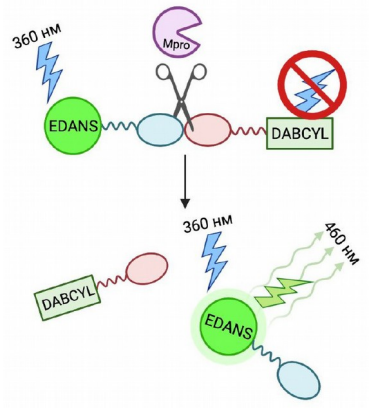


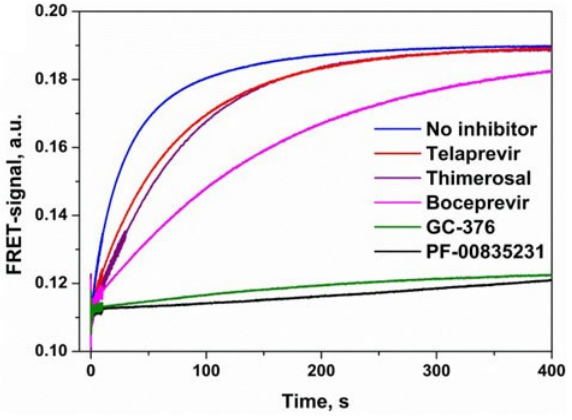


800M *GTM* **439** *docking* **17 classes** *$* **10** *in vitro* **2**

D. Horvath *et al.*, Mol. Inf. (2020) 39, 2000080
M. Y. Zakharova *et al.*, Front. Pharmacol. *(2021)* 12, 773198

In vitro screening of Mpro inhibitors



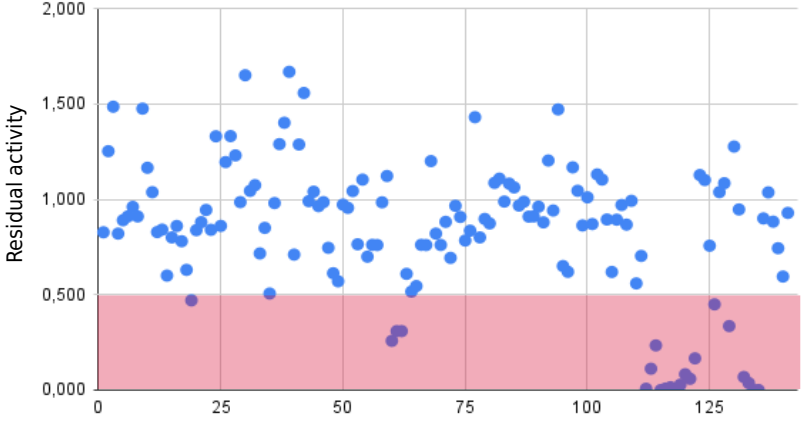


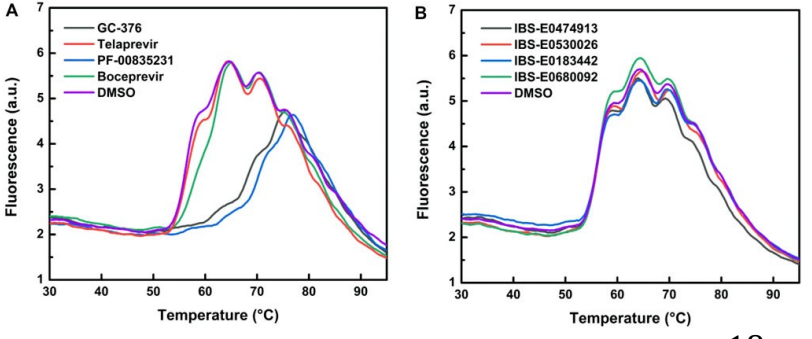
Inhibitor	K_i exp., uM	K_i lit., uM
PF-00835231	0.0057	0.00027
GC-376	0.066	0.06
Boceprevir	1.2	1.18
Telaprevir	8.9	18 (IC_{50})
Disulfiram	0.1	0.3
Thiomersal	0.6	0.6
IBS-E0680092	20.7	—
IBS-E0183442	26.3	—

Activity distribution for assessed compounds

Inactives (125)

Actives (21)





M. Y. Zakharova *et al.*, Front. Pharmacol. *(2021)* 12, 773198

Outlook

- We have developed *in vitro* and *in silico* screening approaches with a specific attention to discovery of antivirals;
- Applicability of the approaches to the practically relevant problems is demonstrated;
- New antiviral chemotypes discovered;
- Mechanism of action studies for new antivirals are underway, including the structural virology studies.

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