Dihydroorotate dehydrogenase inhibitors containing carboxylic acid bioisosters with superior broad-spectrum antiviral activity for next pandemic preparedness

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Background

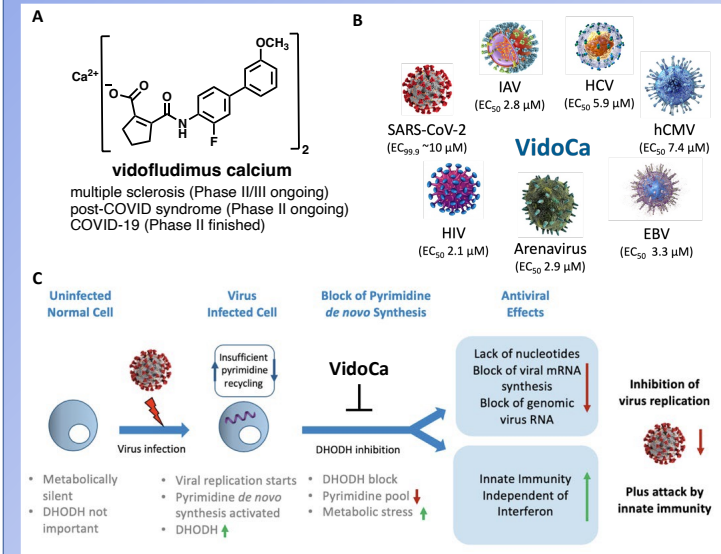


Fig. 1: **Antiviral and immunomodulatory activity of vidofludimus calcium (VidoCa).** (**A**) Chemical structure of VidoCa with clinical study status [1,2]. (**B**) VidoCa inhibits replication of DNA, RNA, and retroviruses in the lower micromolar range *in vitro*, including strong anti-Epstein-Barr virus (EBV) activity. (**C**) Dihydroorotate dehydrogenase (DHODH) that catalyzes the rate-limiting step of *de novo* pyrimidine synthesis represents a promising target for development of host-directed antivirals. VidoCa specifically targets DHODH, thereby inhibiting viral replication and reactivation. VidoCa displays a unique dual antiviral mode of action. First, it directly affects viral replication by depletion of the cellular pyrimidine pool. Second, VidoCa indirectly induces an antiviral state by inducing interferon-independent innate immune responses.

Starting point

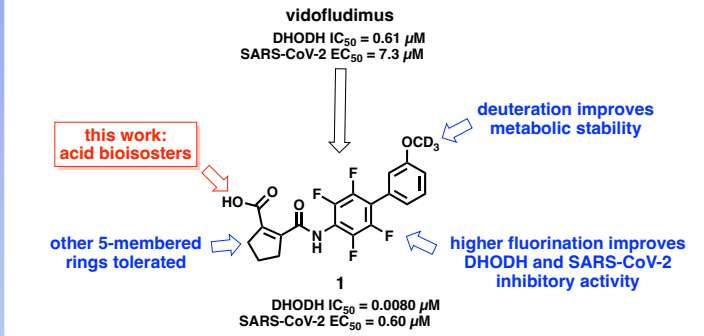
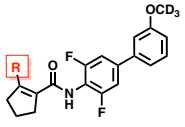


Fig. 2: **Improved carboxylic acids as starting point.** By modification of other parts of the VidoCa scaffold more potent and stable carboxylic acid derivatives were obtained [3]. In this project, carboxylic acids were replaced by bioisosters, which can be easily synthesized from its acid precursor.

Finding a suitable bioisoster



ID	R	DHODH IC50 [µM]	SARS-CoV-2/Caco-2 EC50 [µM]	CC50 [µM]	SI
2		0.064	10.0	>100	>10
2a		1.7	9.6	>100	>10
2b		9.3	5.9	>100	>17
2c		0.31	18	>100	>5.6
2d		3.7	2.8	28	10
2e		40% @100 µM	24	>100	>4.1
2f		0.19	1.6	>100	>62
2g		1.1	4.3	>100	>23
2h		0.050	32	>100	>3.1
2i		0.047	>50	>100	2

Fig. 3: **DHODH and SARS-CoV-2 activity of carboxylic acid bioisosters.** Only *N*-methoxy-amide **2f** showed acceptable inhibition in both the biochemical DHODH and cellular SARS-CoV-2 assay compared to carboxylic acid **2**. Even polar elements seem to be tolerated there, as exemplified with **2g**. In contrast, replacement by polar *N*-(methylsulfonyl)- or *N*-sulfamoyl-amide was only tolerated on DHODH. The (optionally deuterated) *N*-methoxyamide group was therefore used in the further optimization process.

Combination with other beneficial elements

ID	structure	DHODH IC50 [µM]	SARS-CoV-2 EC50 [µM] / CC50 [µM] / SI	mPK (5 mpk ♀) Cmax [µM] / t1/2z [h] / AUC0-∞ [µM*h]
Vido		0.61	7.3 / >100 / >14	2.1 / 1.7 / 5.8
2f		0.13	1.6 / >100 / >62	1.7 / 1.3 / 3.0
*d*3-2f		0.19	1.6 / >100 / >62	6.1 / 3.2 / 26
3a		0.0063	0.31 / >100 / >320	5.0 / 1.5 / 8.8
3b		0.022	0.33 / >100 / >300	160 / 10 / 870
3c		0.0014	0.0020 / >100 / >50000	15 / 2.1 / 54
3d		0.0022	0.00098 / >100 / >100000	8.1 / 1.4 / 20

Fig. 4: **Inhibitory activity and mouse cassette PK data for advanced *N*-methoxyamide derivatives.** Deuteration of the *N*-methoxy residue can lead to a significant improvement of oral exposure in mice (**2f** to ***d*3-2f**). Addition of further fluorine residues in the central phenyl moiety improves DHODH and SARS-CoV-2 inhibitory activity (**3a**). The introduction of an ether element into the cyclopentenyl moiety (**3b**) dramatically improves oral exposure in mice, albeit with some loss of activity on DHODH. Replacing this element with an aromatic system such as thiophene (**3b**) now dramatically improves SARS-CoV-2 inhibitory activity. Introduction of a nitrogen towards thiazole **3d** provided a sub-nanomalar inhibitor on SARS-CoV-2 without eliciting cytotoxic effects in Caco-2 and other cells, yielding a selectivity index (SI) of >100000.

Antiviral testing

- early lead **2f** showed only moderate efficacy in an *in-vivo* SARS-CoV-2 replication model in K18-hACE mice mice similar as described for **VidoCa** and cpd. **1** [3,4]
- optimized DHODH inhibitors **3b**-**3d** potently restrict SARS-CoV-2 replication at nanomolar to subnanomolar concentrations in human airway epithelial cells infected with SARS-CoV-2
- in *ex vivo*-cultivated human lung tissue **3d** inhibits influenza virus replication and reduces the release of pro-inflammatory cytokines

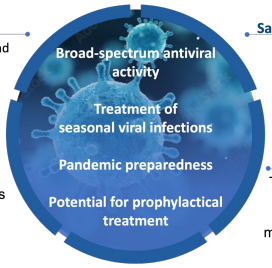
virus	VidoCa EC50 [nM]	3b EC50 [nM]	3d EC50 [nM]
HAdV5	6400	350	0.4
HCMV	7000	670	–
MPXV [5]	700	58	0.5
SARS-CoV-2	7300	330	1.0
CoV-229E	–	170	3.4
CoV-OC43	–	170	4.1
ZIKV	–	470	44
IAV	2200	460	3.0
MeV	–	300	3.1
RSV	–	20	–
HIV	2100	8.7	0.001

Fig. 5: **Broad spectrum antiviral activity.** Cpd. **3b** and **3d** demonstrated a pronounced inhibitory activity against several viruses from the Baltimore groups I, IV, V and VI including enveloped, non-enveloped, DNA, RNA, and a retrovirus with comparable EC50 values. This highlights the broad- spectrum antiviral activity of this compound class and its potential for further clinical development as an antiviral medication (Institute of Molecular Virology, University Ulm; Institute of Virology, University Erlangen-Nürnberg).

HAdV: human adenovirus C5, HCMV: human cytomegalovirus, MPXV: monkeypox virus, CoV: human coronavirus, ZIKV: Zika virus, IAV: influenza A virus, MeV: measles virus, RSV: respiratory syncytial virus, HIV-1: human immunodeficiency virus type 1.

Conclusions and outlook

- **The (optionally deuterated) *N*-methoxyamide group can serve as carboxylic acid bioisoster, exhibiting improved target engagement and pharmaceutical properties compared to its progenitor vidofludimus calcium (VidoCa)**
- **An advanced derivative potently restricts replication of different (respiratory) viruses in the single-digit nanomolar range *in vitro***



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