19-21 GIUGNO ROMA
UNIVERSITA' CATTOLICA DEL SACRO CUORE CENTRO CONGRESSI EUROPA
RESEARCH and CARE: FROM BENCH, TO BEDSIDE, TO COMMUNITY
Promosso da SIMIT
16° CONGRESSO NAZIONALE ICAR Italian Conference on AIDS and Antiviral Research
P 216
POSTER 4 YOU ICAR 2024

Evaluation of broad-spectrum piperazine-based compounds able to inhibit flavivirus and/or SARS-CoV-2 replication in a live virus assay

Camilla Biba[1], Ilenia Varasi[1], Palmira Alessia Cavallaro[2], Federica Giammarino[1], Niccolò Bartolini[1], José Manuel Vega-Pérez[3], Fernando Iglesias-Guerra[3], Antonella Leggio[2], Margarita Vega-Holm[3], and Ilaria Vicenti[1]

[1] *Department of Medical Biotechnologies, UOC Microbiology and Virology, University of Siena, Siena University Hospital, Siena, Italy*
[2] *Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036, Arcavacata di Rende (CS), Italy*
[3] *Department of Organic and Medicinal Chemistry, Faculty of Pharmacy, University of Seville, E-41071 Seville, Spain*

Introduction

- No specific antiviral therapy is available for some viruses belonging to the flaviviruses genus such as Zika or Dengue viruses (ZIKV, DENV).
- Currently, only 2 drugs (Nirmatrelvir, NRM and Remdesivir RDV) are available against SARS-CoV-2.
- The aim of this work was to evaluate the *in vitro* activity of a set of newly synthesized compounds (CMPs) against ZIKV, DENV and wild-type (WT) SARS-CoV-2 virus variant.

Study Design

- The CMPs investigated in this study were designed against the NS3/4A of HCV employing piperazine ring as central core and using a privileged structure-based approach for the functionalization of both nitrogens
- Two families of piperazine-derived small molecules were designed with the 2-phenyl piperazine (1° family, CMPs 1-29) or the unsubstituted piperazine (2° family, CMPs 30-51)

Methods

- The CMPs were tested in a live virus cell-based assay to determine their antiviral activity.
- Once assessed the 50% cytotoxic drug concentration (CC_{50}), lung A549 ACE-2 TMPRSS-2 (A549-AT) and hepatoma Huh7 human cell lines were treated with scalar non-toxic doses of each CMP and tested with SARS-CoV-2 (A549-AT) and ZIKV/DENV (Huh7) viral stocks at 0.001 MOI.
- Each experiment was performed in 2 independent runs including a mock infection control, a virus control and 2 reference CMPs (NRM for SARS-CoV-2 and sofosbuvir, SOF for flaviviruses, *figure 1*).
- The inhibitory activity of each CMP was determined by measuring the expression ZIKV/DENV envelope by immunodetection (ELISA assay) and by measuring cytopathic effect quantified by luminescence for WT SARS-COV-2.
- Results were expressed as half-maximal inhibitory concentration (IC_{50}), and the selectivity index (SI) was defined as the ratio between CC_{50} and IC_{50}.

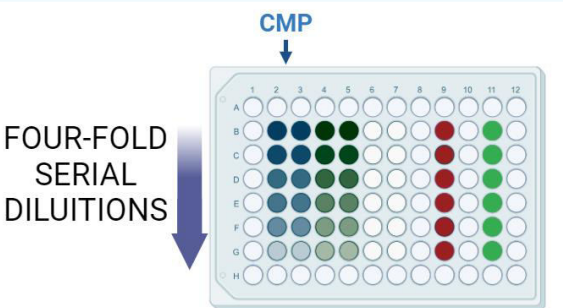

Figure 1. *In vitro antiviral assay to determine the activity of the CMPs against WT SARS-COV-2, ZIKV and DENV. Serial dilutions of the CMPs, starting from the first not-toxic dose, were treated with a fixed amount of virus at 0.001 MOI.*

Results

		CC_{50} µM		IC_{50} µM			SI		
Compound	Family	Huh7	A549-AT	ZIKV Huh7	DENV Huh7	SARS-CoV-2 A549-AT	ZIKV	DENV	SARS-CoV-2 WT
24	1°	200,0	400,0	2,5 ± 1,4	NT	NT	80,0	NT	NT
26	1°	24,0	22,0	9,6 ± 4,0	NT	NT	2,5	NT	NT
34	2°	400,0	109,0	27,9 ± 5,3	NA	NA	14,3	NT	NT
35	2°	182,0	95,4	48,1 ± 0,3	67,9 ± 5,4	NA	3,8	2,7	NT
37	2°	400,0	200,0	38,7 ± 0,7	NA	NA	10,3	NT	NT
38	2°	53,0	21,0	5,1 ± 0,0	NA	NA	10,4	NT	NT
39	2°	155,7	84,0	13,2 ± 0,4	33,8 ± 3,3	NA	11,8	4,6	NT
41	2°	400,0	188,0	4,6 ± 0,7	11,7 ± 2,2	NA	87,5	34,3	NT
42	2°	400,0	28,6	1,6 ± 0,0	3,8 ± 0,7	NA	250,0	105,5	NT
43	2°	400,0	400,0	159,0 ± 48,3	NA	NA	2,5	NT	NT
44	2°	400,0	400,0	43,7 ± 3,6	NA	NA	9,2	NT	NT
49	2°	400,0	151,1	3,2 ± 0,9	NA	NA	127,0	NT	NT
50	2°	400,0	169,9	2,7 ± 0,7	NA	22,5 ± 1,5	148,1	NT	7,6
NRM	/	NT	40,0	NT	NT	0,04 ± 0,0	148,1	NT	1000,0
SOF	/	400,0	NT	3,7 ± 0,9	4,3 ± 1,4	NT	108,0	93,0	NT

Table 1. *Half-maximal cytotoxic concentration (CC_{50}) and half-maximal inhibitory concentration (IC_{50}) and selectivity index (SI) against Zika (ZIKV), Dengue (DENV) and WT SARS-CoV-2 virus of piperazine based compounds as determined in a live virus cell-based assay. NRM: Nirmatrelvir; SOF: Sofosbuvir; NA: Not active; NT: not tested.*

- The median CC_{50} of the 1° family was 61.5 [22.7-200.0] µM in Huh7 and 138 [36.5-200.0] µM in A549-AT (*Table 1*).
- The 2° family showed a median CC_{50} of 400.0 [112.9-400.0] µM in Huh7 and 129.9 [89.4-400.0] in A549-AT.
- CMPs **24** and **26** were active only against ZIKV in the low micromolar range (IC_{50} 2.5±1.4 and 9.6± 4 µM, respectively; SI 80.0 and 2.5, respectively).
- CMP **50** showed broad activity against ZIKV (IC_{50} 2.7±0.7 µM; SI =148.1) and SARS-CoV-2 (IC_{50} 22.5±1.5 µM, SI= 7.6).
- Nine CMPs were active against ZIKV (IC_{50} 13.2 [3.2-43.7] µM, SI 11.8 [9.2-127]) and four of them (35, 39, 41 and 42) were active also against DENV (mean IC_{50} 29.3±14.3 µM; mean SI 36.8±24.0).
- Globally, compounds with an IC_{50} <15 µM progressed to enzymatic assay and docking studies to establish the interactions with the active site of the enzyme.

Conclusion

- CMP **50** displayed broad-spectrum activity vs. WT SARS-CoV-2 and ZIKV.
- Despite the anti-ZIKV activity of CMP **50** was higher than SOF, its anti-SARS-CoV-2 activity was 500-fold lower than NRM. However, its low molecular complexity will allow further structure-based optimization.
- CMPs **41**, **42** and **49** inhibited ZIKV with IC_{50} <5 µM displaying similar profile with respect to SOF. Among them, **42** showed a higher SI than SOF, both for ZIKV and DENV.
- Considering the lack of options for the treatment of ZIKV and DENV infections, these piperazine based compounds are promising for the development of a new class of pan anti flavivirus agents.