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From Oxetane to Thietane: Extending the Antiviral Spectrum of 2′-Spirocyclic Uridines by Substituting Oxygen with Sulfur

Sandrine Grosse,*,‖ Abdellah Tahri,‖ Pierre Raboisson, Yannis Houpis, Bart Stoops, Edgar Jacoby, Jean-Marc Neefs, Marnix Van Loock, Olivia Goethals, Peggy Geluykens, Jean-François Bonfanti, and Tim H. M. Jonckers

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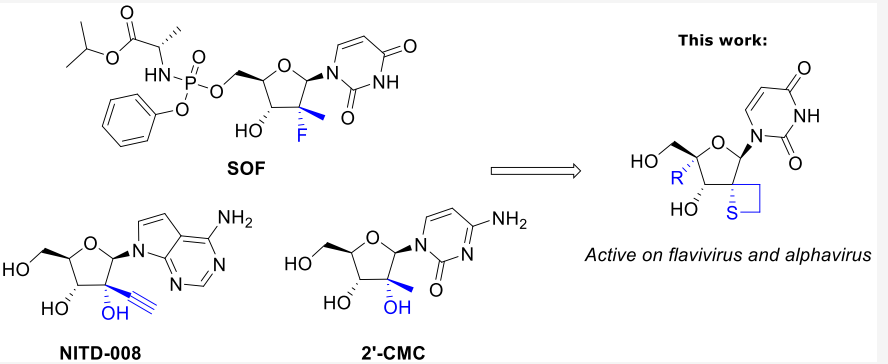

ABSTRACT: In continuation of our efforts of finding novel nucleoside inhibitors for the treatment of viral diseases, we initiated a discovery research program aimed at identifying novel nucleos(t)ide inhibitors for emerging diseases like Dengue and Chikungunya. Based on the previously reported 2′-spiro-oxetane uridine derivatives active against Hepatitis C Virus (HCV), we envisaged its sulfur analogue as an interesting congener both from a synthetic as well as biological point of view. Surprisingly, we found the 2′-spirothietane uridine derivatives not only to be active against HCV and Dengue virus (DENV), viruses belonging to the flavivirus family, but also to demonstrate activity against alphaviruses like Chikungunya virus (CHIKV) and Sindbis virus (SINV).

KEYWORDS: *Nucleos(t)ide, thietane, spirocycle, Hepatitis C, Dengue, Chikungunya*

Synthetic nucleosides and nucleotide analogues represent an important class of antiviral, antiparasitic, and anticancer therapeutics that have been widely applied in clinical practice.[1] For example, Sofosbuvir, a 4′-modified nucleoside, often used in combination with other antivirals, has played a key role in the fight against Hepatitis C (HCV).[2−4] Lately nucleosides derivatives have regained great interest from the scientific community with the recent approval of Remdesivir and Molnupiravir for SARS COV-2 treatment.[5−7] While the current focus on finding treatment options against COVID-19 is understandable, there are other pathogens of concern for which no effective therapies exist. Infections caused by dengue virus (DENV) or chikungunya virus (CHIKV) are expected to spread on a global scale,[8] and reliable treatment options for patients suffering from these diseases are needed. NITD-008, an adenine derivative bearing a 2′-ethynyl fragment, has demonstrated antiviral activity against DENV in cell based assays[9] as did 2′-C-methyl cytidine (2′-CMC).[10]

To exert any antiviral activity, most nucleoside analogues need to be converted into the bioactive nucleoside triphosphate (NTP) metabolite which acts in a competitive manner to natural NTPs that are used by viral polymerases to synthesize *de novo* RNA or DNA. Incorporation of these unnatural nucleosides results in chain elongation termination which prevents the formation of newly generated viral particles.[11]

Structural modification of the ribose moiety of nucleosides is a well-documented approach that has been demonstrated to be a common tool to modulate biological properties of nucleoside analogues.[12−14] Such strategies even led to approved antiviral drugs.[15]

It has been shown that ribose modifications can include spirocyclization at various positions[16−18] which is confirmatory for the increasing prominence of spirocyclic scaffolds in drug discovery programs.[19,20] The use of small rings like cyclo-

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propyl, oxetane, or azetidine provides access to denser, more rigid molecular structures which often confer beneficial properties compared to their nonspirocyclic analogues.

Thietane, the sulfur analogue of the well-known oxetane is an important structural motif found in natural products and pharmacologically active ingredients.[21] A 4′-5′ spirothietane modified sugar analogue was disclosed by Roy et al.[22,23]

Aiming to identify novel antiviral active nucleoside analogues and extending on our previous work[24−28] we investigated further modifications of the 2′-region of uridine with original spiro-thietane motifs (Figure 1).[29] To the best of our knowledge, no 2′-spirothietane nucleosides have been reported so far.

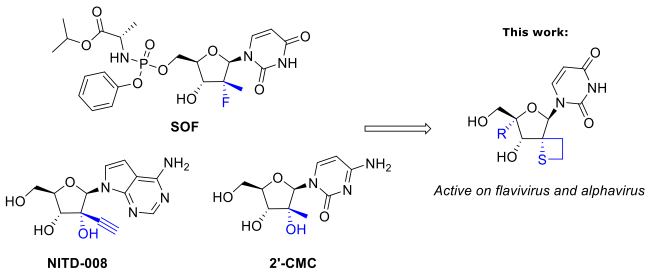
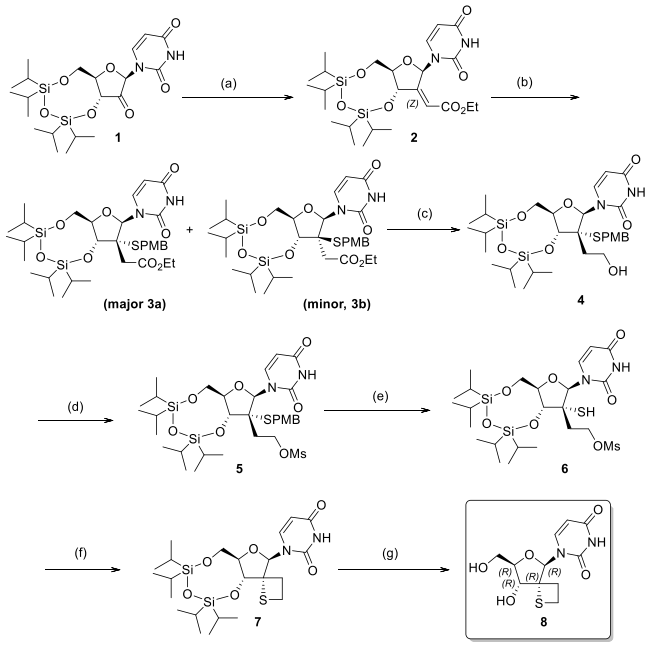


Figure 1. 2′-Spirothietane ribose derivatives active on flavivirus and alphavirus.

Our synthetic approach toward 2′-spirothietane uridine derivatives started from the previously reported ketone **1** (Scheme 1).[24] The key step in the sequence was the Michael reaction of the α,ß-unsaturated ester **2** with (4-methoxyphenyl)methanethiol leading to intermediates **3a** and **3b** with the desired *S*-α-isomer being formed predominantly. Subsequently, compound **5** was obtained in good yield after reduction of **3a** followed by mesylation of the primary alcohol. Mercury mediated deprotection of the para methoxybenzyl group, followed by intramolecular cyclization in basic conditions afforded compound **7** in a moderate yield of 46% over 2 steps. Finally, deprotection of the hydroxyl groups led to the novel 2-spirothietane nucleoside **8**, a key intermediate.

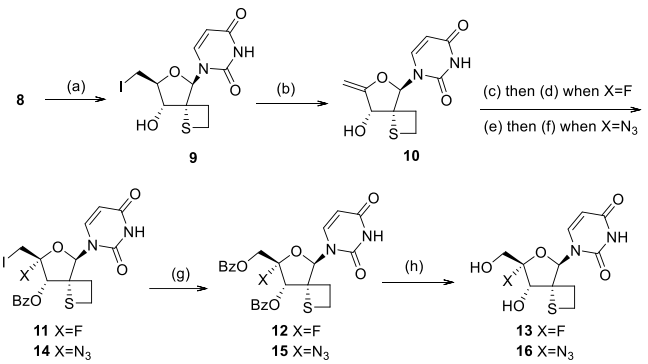
This synthetic protocol allowed us to obtain multigram quantities of **8** and set us up to further extend our exploration of this new 2′-spirothiethane scaffold. The synthetic approach to install a 4′-F substituent was based on initial work reported by Moffat et al.[30] which was also extensively exploited by others[31−34] (Scheme 2). First, compound **8** was reacted with iodine in the presence of triphenylphosphine followed by an elimination step leading to 4′,5′-unsaturated nucleoside **10**. Iodofluorination of compound **10** using *N* -iodosuccinimide (NIS) and $Et_3N(HF)_3$, yielded 4′-fluoro-5′-iodo derivative **11** in 62% yield. After benzoylation of the 3′-hydroxy group, displacement of the iodine by means of sodium benzoate afforded the double protected nucleoside **12** which after treatment with ammonia led to the fully deprotected 4′-F, 2′-spirothietane nucleoside **13** in good yield. The 4′-azido nucleoside **16** was obtained in a similar manner utilizing sodium azide in step 3. Nucleoside prodrug approaches like "Protides/phosphoramidates" are well-documented. Such technologies are aimed at circumventing any problematic first phosphorylation which is often observed with unnatural derivatives.[35] 5′-Phosphoramidate analogues **17−20** were obtained using standard conditions as mixtures of P-stereo-isomers[30] (Scheme 3). While stereoselective synthetic methods are reported, we opted for a nonstereoselective synthesis for initial evaluation. In the case of **18**, preparative SFC (Supercritical Fluid Chromatography) yielded the enantiopure phosphoramidates **18a** (*Rp) and **18b** (*Sp) of which the stereochemistry at the P-atom was assigned arbitrarily.

Scheme 1. Synthesis Access to Spirothietane Nucleoside 8[a]



[a]Conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$, ^tBuOK, THF, −15 °C to RT (1 h), then **1** in THF 0 °C to 15 °C (1 h) then RT (5 h) 83%. (b) (4-methoxyphenyl)Methanethiol, KHMDS; THF, −40 to 20 °C (2 h), 50% (**3a**) 12% (**3b**). (c) $LiAlH_4$, Et_2O, 0 to 20 °C (16 h) 60%. (d) MsCl, pyridine, 25 °C (16 h), 78%. (e) $Hg(OAc)_2$, CF_3CO_2H, PhOH, 0 °C (1 h), then DTT, 0 °C (10 min). (f) NaH, THF, 0 to 25 °C (16 h), 46% over 2 steps. (g) TBAF, THF, RT (2 h), 86%.

Scheme 2. Synthetic Access to 4′-Functionalized Spirothietane Nucleosides[a]



[a]Conditions: (a) I_2, $P(Ph)_3$, NMI, THF, 25 °C, 4 h. (b) MeONa, MeOH, reflux, 2 h, 55% over 2 steps. (c) $Et_3N(HF)_3$, THF/ACN, NIS, −15 °C (1 h). (d) Et_3N, DMAP, THF, BzCl, 0 to 25 °C (3 h), 62%. (e) (1) $BnEt_3NCl$, NaN_3, CH_3CN, RT (16 h) then **10**, NMM, I_2, THF, 0 °C to RT (5 h), 99%. (f) BzCl, Et_3N, DMAP, THF, RT (2 h), 81%. (g) BzONa, 15-crown-5, DMF, 120 °C (18 h), 59% when X = F, 47% when X = N_3. (h) NH_3, MeOH, RT, overnight, 65% when X = F, 84% when X = N_3.

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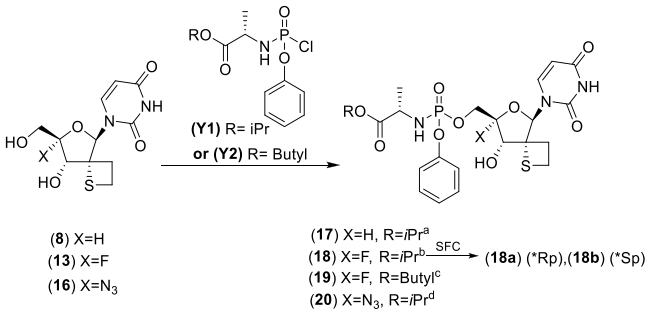
Scheme 3. 5′-Phosphoramidate Nucleosides Synthesis[a]



[a]Conditions: (a) **Y1**, DCM, NMI, 20°C (16 h), 12%. (b) ^{t}BuMgCl, THF, −5°C (45 min.) then **Y1**, −5°C (2 h) then RT (overnight), 38%. (c) **Y2**, NMI, DCM, (overnight), 17%. (d) **Y1**, NMI, DCM, RT (20 h), 22%.

Cell-based antiviral activity of phosphoramidates **17**−**20** was evaluated (Table 1). The non-4′-substituted thietane analogue **17** demonstrated broad antiviral activity against CHIKV, HCV, and DENV without apparent cytotoxicity up to 100 μM. Interestingly, the corresponding 4′-fluoro analogue **18** showed a similar extended antiviral profile, with a marked improvement on CHIKV inhibition. In contrast, the 4′-azido derivative **20** displayed reduced potency against DENV and CHIKV. No significant difference in activity was noted between the pure diastereomers **18a** and **18b**. Phosphoramidate **19**, having a butyl chain replacing the terminal isopropyl group in **18**, demonstrated similar potencies. Interestingly, phosphoramidates **18**, **18a**, **18b**, and **19** also demonstrated antiviral activity against Sindbis virus (SINV), another virus from the alphaviruses family. This illustrates again the extended antiviral profile of the 2′-spirothietane 4′-F-modified nucleosides. Noteworthy is the fact that **17** and **18** having a sulfur-ring surprisingly show improved activity when compared with their corresponding oxetane analogues.[13]

In order to rationalize the broad spectrum antiviral activity of the compounds, we modeled the binding interaction of the thietane-2′-spirocyclic uridine triphosphate **23** and the corresponding oxetane compound **24** (Figure 2) into the

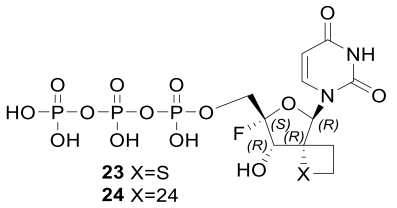
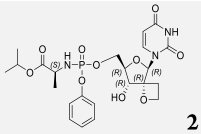
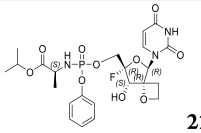


Figure 2. Structure of triphosphates **23** and **24**.

crystal structure available for the HCV NS5B and compared the primary sequences for DENV NS5, CHIKV NSP4 and SINV NSP4 for the ligand binding domain and especially the thietane and oxetane microenvironment (Figure 3).

The modeling was based on the HCV NS5B crystal structure in complex with sofosbuvir diphosphate and a symmetrical RNA primer template.[36] The thietane and oxetane-2′-spirocyclic system was built in MOE2020.0101 software based on the sofosbuvir 2′-fluoro-2′-methyl ribose coordinates (PDB 4WTG). The γ-phosphate group was modeled based on the coordinates of the Norwalk virus crystal structure complex with a cytidine triphosphate (PDB 3BSO).[36,37] The canonical

Table 1. Antiviral Activity against CHIKV, SINV, HCV, and DENV, and Cytotoxicity in Huh-7 cells for 2′-Thietane Analogues and the 2′-Oxetane Reference Nucleosides[a]

COMPOUND	CHIKV° EC_{50} (μM) [n]	SINV EC_{50} (μM) [n]	HCV° EC_{50} (μM) [n]	DENV° EC_{50} (μM) [n]	TOX^ CC_{50} (μM) [n]
17	22.4±16 [4]	NA	1.17±0.44 [5]	26.1±9.3 [5]	>100
18	3.25±1.2 [4]	6.19 [1]	2.58±1.4 [6]	28.3±15.6 [8]	>100
18a	4.6±2.3 [3]	5.93 [1]	2.1±1.4 [3]	42±22 [3]	>100
18b	3.18±1.5 [3]	5.82 [1]	2.2 [1]	29.3±14.1 [7]	>100
19	2.87 [1]	4.64 [1]	1.92 [1]	29±14 [5]	>100
20	>100 [3]	NA	2.8±1.5 [3]	>100 [8]	>100
 **21**	>100 [2]	NA	21.4±5.3 [5]	>50±0 [4]	>100
 **22**	>100 [3]	NA	>100 [4]	>100 [6]	>100

[a]Data represent mean values of [n] independent experiments. °Mean values generated with two different compound dispensing methods. ^Identical results were obtained in two different toxicity assays (2-day and 3-day assay) in Huh-7 cells. CC_{50}, 50% cytotoxic concentration; EC_{50}, 50% effective concentration. Reference compounds behaved as expected in the different antiviral assays.

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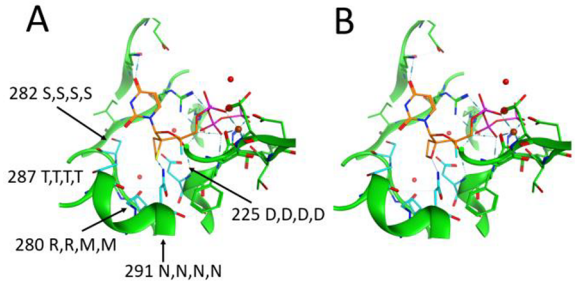

Figure 3. Model of the thietane-2′-spirocyclic uridine triphosphate compound **23** (Panel A) and corresponding oxetane compound **24** (Panel B) into the crystal structure available for the HCV NS5B (PDB 4WTG). Selected residues within a 5 Å contact sphere of the ligand are shown. Carbon atoms of the ligand are colored in orange. Highlighted in cyan are the contact residues of the thietane/oxetane rings. These residues are numbered according to the HCV NS5B sequence, and sequence conservation is indicated: the first letter is the amino acid in HCV NS5B, the second letter is the amino acid in DENV NS5, the third letter is the amino acid in CHIKV NSP4, and the fourth letter is the amino acid in SINV NSP4.

RNA-dependent RNA polymerase architecture resembles a right-hand with palm, fingers and thumb domains surrounding the active site. Polymerase catalytic motifs A-E in the palm and motifs F-G in the fingers are shared by all viral RNA-dependent RNA polymerase with sequence and/or structural conservations.[38] The analysis of the putative interactions shows that ASN291 (Motif B) is a key contact residue with a H-bond to the sulfur atom of the thietane ring (acceptor donor distance 3.147 Å).[39]

The hydrophobic thietane ring is surrounded by the nonpolar carbon atoms of the side chains of ASP225 (Motif A) and ARG280, SER282, and THR287 (all Motif B). The analysis of the sequence conservation shows that the nature of these residues and their surroundings are highly conserved also in DENV NS5, CHIKV NSP4 and SINV NSP4 which might thus provide a rationale for the conservation of binding. The inactivity or lower potency of the oxetanes compared to the thietanes can possibly be due to the stronger interaction of the side chain N−H of ASN 291 to the sulfur atom of the thietane ring.[39] The acceptor−donor distance is in the oxetane complex, 3.324 Å, and thus is longer than that in the thietane complex. The C_3 OH group interacts via H-bonds with the N−H group of ASP225 and the β-phosphate group. The C_4 fluorine atom points toward the side chain NH_2 group of ASN291. These observations provide a potential rationale for the measured antiviral activity. However, in the absence of any crystal structure, this remains hypothetical.

Phosphoramidate **18** was evaluated in a mouse PK experiment to demonstrate the formation of the corresponding NTP **23** upon subcutaneous and/or oral administration. As can be seen from the Figure 4, efficient formation of NTP **23** was shown in the liver. No prodrug was observed in the plasma or the liver after 7 h. The highest liver concentrations of NTP were obtained after subcutaneous dosing of compound **18.**

To further increase our understanding of these results, the metabolic stability in mouse and human liver microsomes as well as *in vitro* stability of compound **18** in simulated gastric fluid (SGF), fasted state simulated intestinal fluid (fassif) and plasma (human and mouse) were evaluated (Tables 2 and 3). Phosphoramidate **18** demonstrated poor metabolic stability both in mouse and human liver microsomes, indicating a fast liver mediated metabolism. While being highly stable in SGF ($t_{1/2}$ > 6 h, 93 ± 5% of compound remaining after 6 h) and human plasma, compound **18** also showed high instability in fassif and mouse plasma. Results suggest fast turnover of the prodrug in plasma which is not surprising knowing that phosphoramidate esters are prone to extensive metabolization in rodent plasma given the high abundance of carboxylesterases in this medium.[40] The combination of the instabilities and limited oral absorption potentially explains differences between oral and subcutaneous dosing. Complementary studies would be required to identify if additional metabolic pathways are involved.

Route	C_{max} (ng/mg)	t_{max} (h)	AUC_{last} (h*ng/g)	t_{last} (h)
PO	637	2	3050	7
SC	2060	2	10000	7

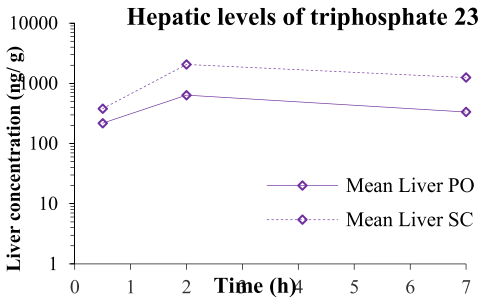

Figure 4. PK profile in mouse liver after subcutaneous or oral administration of phosphoramidate **18**. Samples of plasma and liver were collected. Liver was analyzed for the prodrug **18** and triphosphate **23**. Plasma samples were only analyzed for prodrug. [Dose 50 mg/kg, Dose Volume 10 mL/kg, Fed Mice Male, Vehicle HP-ß-CD 20% (solution)]. LLOQ [PO] = 90.1−126 ng/g, LLOQ [SC] = 91.8−124] ng/g.

Table 2. Stability of Phosphoramidate 18 in Different Media

medium	$t_{1/2}$ (h)
fassif	0.2
SGF	>6
mouse plasma	<0.4
human plasma	>30

Table 3. Metabolic Stability of Phosphoramidate 18 in Mouse and Human Liver Microsomes

	Clint (μL/min/mg protein)
mouse	>347
human	>347

Several novel 2′-spirothietane uridine analogues were found to be inhibitors of pathogens belonging to different viral families (flavivirus and alphavirus). Preliminary docking is in line with the observed activities. Finally, initial PK confirmed the formation of active triphosphate in the liver.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00372.

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Methods for biological and DMPK assay; modeling details; experimental procedures for synthesis and characterization of the compounds generated in this study (PDF)

Polymerase HCV CHIK DENV sequence alignment (XLSX)

AUTHOR INFORMATION

Corresponding Author

Sandrine Grosse − *Janssen Research and Development, 2340 Beerse, Belgium;* orcid.org/0000-0003-4385-2206; Email: sgrosse@its.jnj.com

Authors

Abdellah Tahri − *Janssen Research and Development, 2340 Beerse, Belgium*

Pierre Raboisson − *Janssen Research and Development, 2340 Beerse, Belgium;* Present Address: Galapagos, General De Wittelaan L11, A3, 2800 Mechelen, Belgium

Yannis Houpis − *Janssen Research and Development, 2340 Beerse, Belgium;* Present Address: Van Eycklei 15, 2018, Antwerp, Belgium

Bart Stoops − *Janssen Research and Development, 2340 Beerse, Belgium*

Edgar Jacoby − *Janssen Research and Development, 2340 Beerse, Belgium*

Jean-Marc Neefs − *Janssen Research and Development, 2340 Beerse, Belgium*

Marnix Van Loock − *Janssen Global Public Health, 2340 Beerse, Belgium*

Olivia Goethals − *Janssen Global Public Health, 2340 Beerse, Belgium*

Peggy Geluykens − *Charles River, Discovery, 2340 Beerse, Belgium*

Jean-François Bonfanti − *Janssen Research and Development, Chaussée du Vexin, 27100 Val de Reuil, France;* Present Address: Galapagos, 102 Avenue Gaston Roussel, 93230 Romainville, France

Tim H. M. Jonckers − *Janssen Research and Development, 2340 Beerse, Belgium*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.2c00372

Author Contributions

‖S.G. and A.T. contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

HCV, Hepatitis C Virus; DENV, Dengue Virus; CHIKV, Chikungunya Virus; SINV, Sindbis Virus; NTPs, nucleoside triphosphates; NIS, *N*-iodosuccinimide; SFC, supercritical fluid chromatography; RT, room temperature; PK, pharmacokinetics; NA, not available; LLOQ, lower limit of quantification

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