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Facile access to 4'-(N -acylsulfonamide) modified nucleosides and evaluation of their inhibitory activity against SARS-CoV-2 RNA cap N 7-guanine-methyltransferase nsp14

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Romain Amador, Adrien Delpal, Bruno Canard, Jean-Jacques Vasseur, Etienne Decroly, et al.. Facile access to 4'-(N -acylsulfonamide) modified nucleosides and evaluation of their inhibitory activity against SARS-CoV-2 RNA cap N 7-guanine-methyltransferase nsp14. *Organic & Biomolecular Chemistry*, 2022, 20 (38), pp.7582-7586. 10.1039/d2ob01569b . hal-03801035

HAL Id: hal-03801035

<https://cnrs.hal.science/hal-03801035>

Submitted on 6 Oct 2022

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COMMUNICATION

Facile access to 4'-(*N*-acylsulfonamide) modified nucleosides and evaluation of their inhibitory activity against SARS-CoV-2 RNA cap *N7*-guanine-methyltransferase nsp14

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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***N*-acylsulfonamides possess an additional carbonyl function compared to their sulfonamide analogues. Due to their unique physico-chemical properties, interest in molecules containing the *N*-acylsulfonamide moiety and especially nucleoside derivatives is growing in the field of medicinal chemistry. The recent renewal of interest in antiviral drugs derived from nucleosides containing a sulfonamide function has led us to evaluate the therapeutic potential of *N*-acylsulfonamide analogues. While these compounds are usually obtained by a difficult acylation of sulfonamides, we report here the easy and efficient synthesis of 20 4'-(*N*-acylsulfonamide) adenosine derivatives *via* the sulfo-click reaction. The target compounds were obtained from thioacid and sulfonyl azide synthons in excellent yields and were evaluated as potential inhibitors of the SARS-CoV-2 RNA cap *N7*-guanine-methyltransferase nsp14.**

The *N*-acylsulfonamide moiety appears in an increasing number of pharmaceutical compounds for a wide range of therapeutic applications.¹⁻⁶ Characterized by an additional carbonyl group that could increase binding interactions compared to its sulfonamide parent, the *N*-acylsulfonamide group has also been used as a surrogate for carboxylic acids and phosphates.⁷⁻⁹ The particular physico-chemical properties of the *N*-acylsulfonamide functionality are evidenced by the significant presence of this moiety in a wide variety of enzyme inhibitors such as methionyl-tRNA synthetase (MetRS),¹⁰ asparagine synthetase (AS),^{11, 12} anthranilyl-CoA synthetase,¹³ pantothenate synthetase¹⁴ or carbonic anhydrases (Fig. 1A).^{15, 16} Interestingly, several potent antiviral agents carrying an *N*-acylsulfonamide group and targeting the NS3 or NS3-4A protease of hepatitis C virus have also been described.¹⁷⁻¹⁹

Recently, our group reported the synthesis of 5'-*N*-sulfonamide adenosine derivatives that showed significant inhibition of SARS-CoV-2 (*N7*-guanine)-methyltransferase (*N7*-MTase) nsp14 by acting as a competitor of *S*-adenosyl-L-methionine (SAM), the methyl donor in the *N7*-cap RNA methylation.²⁰ Interestingly, out of 39 synthesized compounds, 20 diversely substituted arylsulfonamide adenosines were shown to inhibit the enzyme nsp14 in the submicromolar range (Fig. 1B). Closely related nsp14 inhibitors derived from naphthalene and benzopyridine 5'-*N*-sulfonamide adenosines were also recently described.²¹

Considering the various benefits of the *N*-acylsulfonamide moiety, here we investigated how replacing the sulfonamide by an *N*-acylsulfonamide would modulate the biological properties of these inhibitors. Traditionally, *N*-acylsulfonamide derivatives are synthesized by direct acylation of the corresponding sulfonamide. Numerous methods have been developed over the years to increase the low efficiency of the reaction that is consequent to the limited reactivity of the sulfonamide.²²⁻²⁸ Although improvements have been achieved, their specific application to nucleoside derivatives proved to be inefficient as demonstrated by the work of Widlanski and co-workers.²⁹ Therefore, they developed an alternative method starting from 5'-carboxylic acid *N*⁶-Bz protected adenosine that was condensed with sulfonamides using DCC / DMAP as coupling reagents (Fig. 1C).²⁹ Moderate to good yields were obtained under anhydrous conditions with extended reaction time (2-4 days). Alternatively, Ito's group used arylsulfonyl isocyanates in the presence of an organic base under anhydrous conditions (Fig. 1C).³⁰

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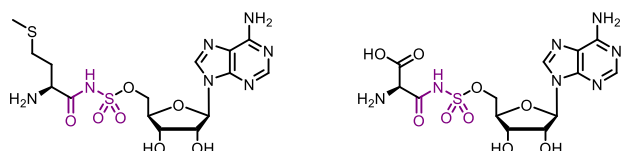
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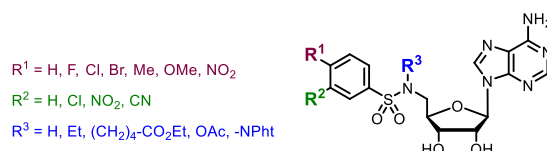
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DOI: 10.1039/x0xx00000x

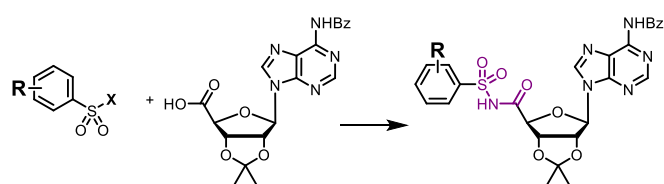
COMMUNICATION

A Examples of bioactive *N*-acylsulfonamides $IC_{50} = 0.5 \text{ nM}$ on *Giardia* MetRS $K_i^* = 728 \text{ nM}$ on human AS

B Sulfonamide inhibitors of SARS-CoV-2 N7-MTase nsp14

 $R^1 = \text{H, F, Cl, Br, Me, OMe, NO}_2$ $R^2 = \text{H, Cl, NO}_2, \text{CN}$ $R^3 = \text{H, Et, (CH}_2\text{)}_4\text{-CO}_2\text{Et, OAc, -NPh}$ $IC_{50} < 1 \mu\text{M}$

C Previous works

Widlanski et al. ■ DCC / DMAP ■ anhydrous conditions ■ 2-4 days reaction time
X: $-\text{NH}_2$ Ito et al. ■ Organic base ■ anhydrous conditions ■ 30 min reaction time
X: $-\text{N}=\text{C}=\text{O}$

D This work

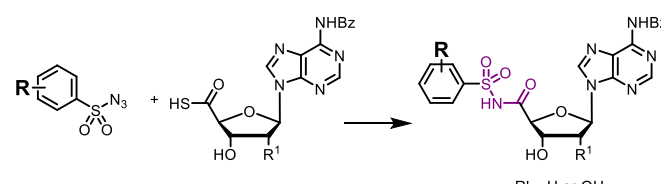
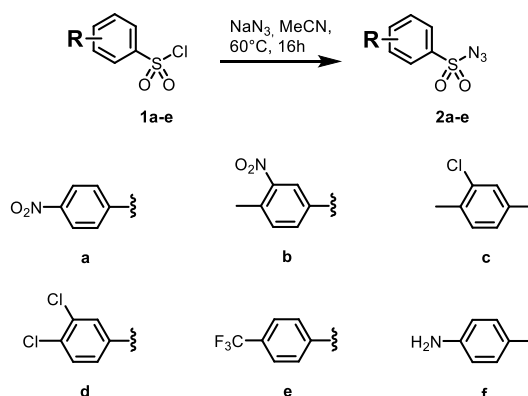
■ NaHCO_3 ■ aqueous conditions ■ <10 min reaction time

Fig. 1 A Examples of bioactive compounds comprising an *N*-acylsulfonamide moiety. B Sulfonamide inhibitors of SARS-CoV-2 N7-MTase nsp14 previously reported.²⁰ C Previous approaches for the synthesis of 4'-(*N*-acylsulfonamide) adenosine derivatives.^{29, 30} D This work describing an easy and efficient access to the target compounds *via* the sulfo-click reaction.

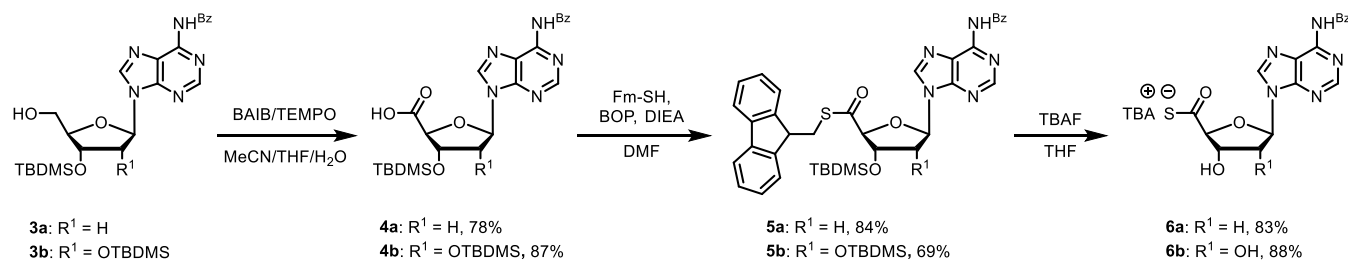
In this context, we have recently exploited the sulfo-click reaction between 4'-thioacid nucleosides and various sulfonyl azides for preparing 5'-bioconjugated nucleosides.^{31, 32} The reaction that leads to the formation of an *N*-acylsulfonamide in aqueous media was found to be efficient, rapid and biocompatible under catalyst-free conditions. Given the simplicity with which this method allows access to *N*-acylsulfonamides, we describe here the synthesis and biological evaluation of 20 original 4'-(*N*-acylsulfonamide) adenosine derivatives. These compounds were obtained with quantitative conversions in 10 min and excellent isolated yields using NaHCO_3 as the only additional reagent (Fig. 1D).

Foremost, the syntheses of sulfonyl azides **2a-e** were performed in a single step starting from commercially available sulfonyl chlorides **1a-e**.³³ The latter were reacted with sodium azide in MeCN at 60°C for 16 h to ensure complete conversion (Scheme 1). After a work-up to eliminate the salts present in the crude mixture, the isolated sulfonyl azides were sufficiently pure to be used without further purification. Compound **2f** was synthesized by deprotection of commercially available 4-acetamidobenzenesulfonyl azide in concentrated aq. HCl.³⁴ While only T, C, G and U derivatives were described in our earlier study, we successfully prepared the corresponding 4'-thioacid adenosine derivatives **6a,b** in 3 steps with good yields.³¹ Specifically, the *N*⁶-Bz-5'-OH nucleosides **3a,b** were oxidized to the carboxylic acids **4a,b**



Scheme 1. Synthesis of sulfonyl azide derivatives **2a-e**. Compound **2f** was obtained following a previously described procedure.³⁴

using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) in the presence of Bis(acetoxy)iodobenzene (BAIB) as a co-oxidant.³⁵ Then, the thioesters **5a,b** were synthesized *via* a coupling reaction with 9-fluorenylmethylthiol. The 4'-thioacid adenosine derivatives **6a,b** were obtained after treatment with TBAF in THF, leading to the desired compounds as tetrabutylammonium (TBA) thiocarboxylate salts. The sulfonyl azides **2a-f** and the 4'-thioacid adenosines **6a,b** were then engaged in sulfo-click reactions under aqueous conditions [100 mM thioacid nucleoside in a 34:66 $\text{H}_2\text{O}/\text{NMP}$ mixture with the

Scheme 2. Synthesis of thioacid adenosine derivatives **6a,b**

addition of NaHCO₃) to yield *N*-acylsulfonamide derivatives **7a-d** and **8a-g** (Scheme 3). In this work, both electron-enriched and electron-deficient aryl sulfonyl azides were used. Remarkably, all reactions were complete in less than 10 minutes as confirmed by RP-HPLC analyses. In a final step, the benzoyl protective group was quantitatively removed by treatment with aq. NH₃, leading to unprotected adenosine derivatives **9a-c** and **10a-f** (Scheme 3). The total conversion observed during both the sulfo-click reaction and the final deprotection led to high isolated yields demonstrating the efficiency of the implemented synthetic method and its advantages over previously reported methodologies.^{29, 30} These compounds were subsequently evaluated at 5 μM for their ability to inhibit the SARS-CoV-2 *N7*-MTase nsp14, which

is responsible for methylation of the RNA cap structure (Table 1). In addition, we aimed to determine the influence of the presence of a benzoyl protecting group on adenine and the importance of the ribose 2'-OH. We used a radioactive MTase assay (filter binding assay)³⁶ to assess the inhibition of purified SARS-CoV-2 nsp14 *N7*-MTase²⁰ by each derivative. Sinefungin was used as positive control for its well-known ability to inhibit MTases.³⁷ The inhibition data were not up to our expectations, with only 6 compounds (**7c**, **7d**, **8a**, **9c**, **10a** and **10b**) displaying moderate inhibitory activity at 5 μM (11-40%). However, some structural information can be deduced. Compared to their sulfonamide counterparts that showed IC₅₀ < 0.5 μM,²⁰ the double-substituted

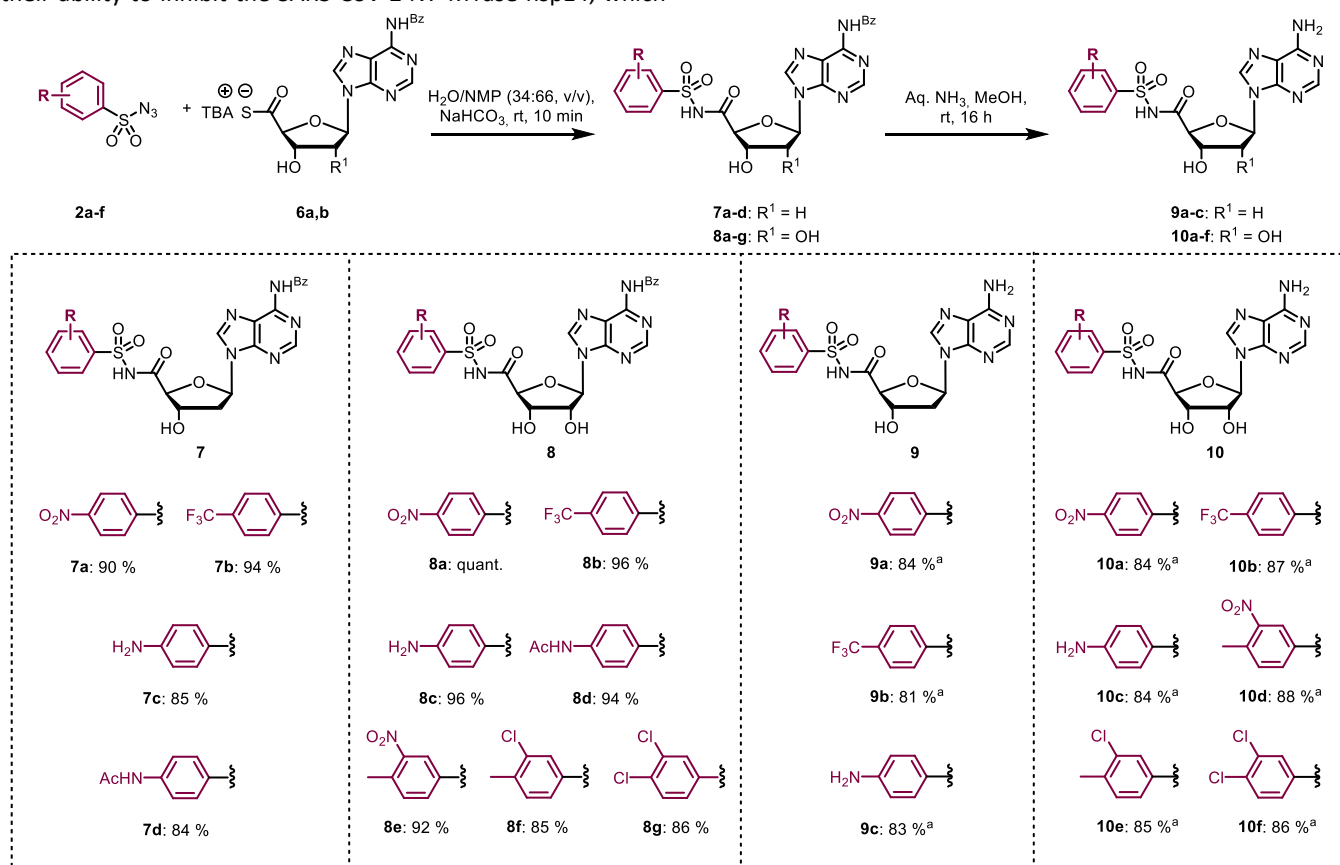
Scheme 3 Scope of the 20 adenosine derivatives synthesized. ^aIsolated yields over two steps.

Table 1 Screening for inhibitory activity of sinefungin and compounds **7a-d**, **8a-g**, **9a-c** and **10a-f** at 5 μ M on SARS-CoV-2 N7-MTase nsp14

Compounds	Percentage of inhibition of SARS-CoV-2 nsp14	Compounds	Percentage of inhibition of SARS-CoV-2 nsp14
Sinefungin	99	8g	n.i.
7a	n.i.	9a	n.i.
7b	7	9b	6
7c	11	9c	14
7d	39	10a	17
8a	40	10b	14
8b	n.i.	10c	n.i.
8c	n.i.	10d	n.i.
8d	7	10e	n.i.
8e	n.i.	10f	n.i.
8f	n.i.		

^aValues are the mean of three independent experiments. The MTase activity was measured using a filter binding assay. Assays were carried out in reaction mixture [40 mM Tris-HCl (pH 8.0), 1 mM DTT, 1 mM MgCl₂, 2mM SAM and 0.1 mM ³H-SAM] in the presence of 0.7 mM GpppAC₄ synthetic RNA and incubated at 30°C with SARS-CoV-2 nsp14 (50 nM). Compounds were previously dissolved in 100% DMSO at 10 mM. n.i.: no inhibition detected.

derivatives **8e**, **8f**, **8g**, **10d**, **10e** and **10f** featuring 3-nitro-4-methyl, 3-chloro-4-chloro and 3-chloro-4-methyl substituents on the phenyl ring, respectively had no impact on nsp14 inhibition activity, regardless of whether the base was protected or not. These results suggest that the presence of the additional carbonyl on compounds bearing two substituents on the phenyl ring may prevent the molecule from inserting into the MTase active site by steric hindrance. Regarding the compounds with only one substituent at the *para* position of the phenyl ring, no clear trend emerges from the observed results. Indeed, in the 2'-deoxy-adenosine and adenosine series and whether the base was protected or not, only modest inhibitory activities were observed, **8a** exhibiting the best activity (40%). Compared to **7a**, this result highlights the importance of the 2'-OH function due to the H-bonds established with the Asp352 of the MTase as previously reported.³⁸ Concerning the unprotected derivatives only modest activities were achieved with compounds **10a** (17%) and **10b** (14%) without being able to establish a true structure-activity relationship among this series. Overall, the presence of an additional carbonyl function did not increase the interactions between the substrates described in this work and the surrounding amino-acids accessible within the active site of the MTase. Low inhibition activities were observed overall, probably due to unfavorable steric hindrances. Studies concerning the role of the 2'OH and the benzoyl protective group led to contradictory interpretations.

In summary, we applied the sulfo-click reaction to 4'-thioacid adenosine with various aromatic sulfonyl azides. The implemented protocol allowed total conversion of the reactants and high isolated yields within 10 min in aqueous conditions using NaHCO₃ as the only additive which represents a major improvement over the methods described so far. 20 *N*-acylsulfonamide derivatives were obtained and evaluated as potential inhibitors of the SARS-CoV-2 N7-MTase nsp14. Beyond the unsatisfactory results obtained for the inhibition of nsp14, the methodology developed in this work allows the efficient synthesis of 4'-(*N*-acylsulfonamide) nucleosides that

disclose new opportunities for the synthesis of potentially bioactive compounds.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors thank the Agence Nationale de la Recherche (ANR "TALAN"-ANR-19-CE07-0004-01) and the European Union's Horizon 2020 Research and Innovation program under grants no. 101003627 (the SCORE project) and no. 101005077 (the CARE project) (E.D., A.D) for financial support. The GAVO consortium is gratefully acknowledged.

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