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Synthesis of acyclic analogues of adenosine sulfonamides and their activity against RNA cap guanine *N*7-methyltransferase of SARS-CoV-2†

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***N*-Arylsulfonamide-based adenosine analogues were previously shown to be potent inhibitors of SARS-CoV-2 RNA cap guanine *N*7-methyltransferase nsp14. Here, we synthesized three series of *N*-arylsulfonamide acyclic analogues of adenosine as bisubstrates of nsp14. Most of these acyclic compounds were barely active at 50 μM against this cap *N*7-methyltransferase.**

The RNA cap guanine *N*7-methyltransferase (MTase) of SARS-CoV-2 has been validated as an antiviral target as mutations in the *N*7-MTase catalytic site have been shown to impair SARS-CoV replication.[1] Indeed, reverse genetics experiments identified two amino acid substitutions abrogating virus viability and a mutation producing a crippled phenotype in several betacoronaviruses. *N*7-MTase is thus a critical enzyme for betacoronavirus replication. The CoV *N*7-MTase nsp14 is the first enzyme involved in the enzymatic cascade of viral RNA cap methylations and is responsible for *N*7-methylation of the cap guanosine, which is essential for viral RNA translation into proteins. The *N*7-methylation of the cap structure is followed by its 2′-*O*-methylation of the first RNA transcribed nucleotide by the nsp10/nsp16 complex.[2,3]

In recent years, we and other groups have followed a rational drug design approach to synthesize competitive inhibitors and have identified a number of potent nanomolar *N*7-MTase nsp14 inhibitors that mimic SAM/SAH derivatives.[4–11] Some SAR data supported by molecular docking led to the elucidation of a bisubstrate-based mechanism of action and was used to optimize the design of bisubstrate inhibitors.[7,9,10] These inhibitors were designed as compounds that occupy both the methyl donor (*S*-adenosylmethionine, SAM) binding pocket and the RNA cap binding pocket of the SARS-CoV-2 *N*7-MTase. In our previous work and that of Chen and Nencka's groups,[7,9,10] the most efficient bisubstrate inhibitors were composed of an *N*-arylsulfonamide core, a pharmacophore found in many biologically active compounds, and occupying the RNA cap binding site, attached to the 5′ position of an adenosine or a derivative, mimicking that of SAM (Fig. 1). The *N*-arylsulfonamide core has been shown to account for much of the compound's high affinity for SARS-CoV-2 nsp14, resulting in potent inhibition.

In this study, we designed molecules with the essential *N*-arylsulfonamide core, and the sugar scaffold has been removed, leading to acyclic nucleosides. The glycosidic linkage (cleavable by purine nucleoside phosphorylases) of our previous adenosine-derived inhibitors is replaced by an acyclic alkyl chain that could stabilize the compounds against metabolic degradation (Fig. 1). Moreover, the abstraction of 2′- and 3′-hydroxyls could improve the lipophilicity of these analogues. Similar replacement of the ribose moiety has already been used many years ago in antiviral research.[12–14] One of the most medically important acyclic nucleosides is acyclovir, an acyclic guanosine mimic with high activity and specificity against HSV-1 and HSV-2.[15] It has been shown that the flexibility of the acyclic alkyl chain can optimize interactions with the target enzyme binding sites. Adefovir and tenofovir are two other well-known phosphonate carrying acyclic nucleosides. These two adenosine mimetics have been reported as potent antiviral drugs against HIV and HBV.[16,17] In addition, numerous examples on the synthesis and activity of nucleoside analogues with acyclic sugar mimic as antiviral or anticancer agents have been widely reported.[18–26] In this work, we propose the possibility of preventing the *N*7 methylation of SARS-CoV-2 RNA cap structure thereby limiting the viral RNA translation into protein through the inhibitory action of acyclic nucleosides on the *N*7- MTase nsp14. Thus, we synthesized three series of novel acyclic analogues of adenosine sulfonamides containing a butyl chain (**1–9**), an *N*-ethyloxymethyl

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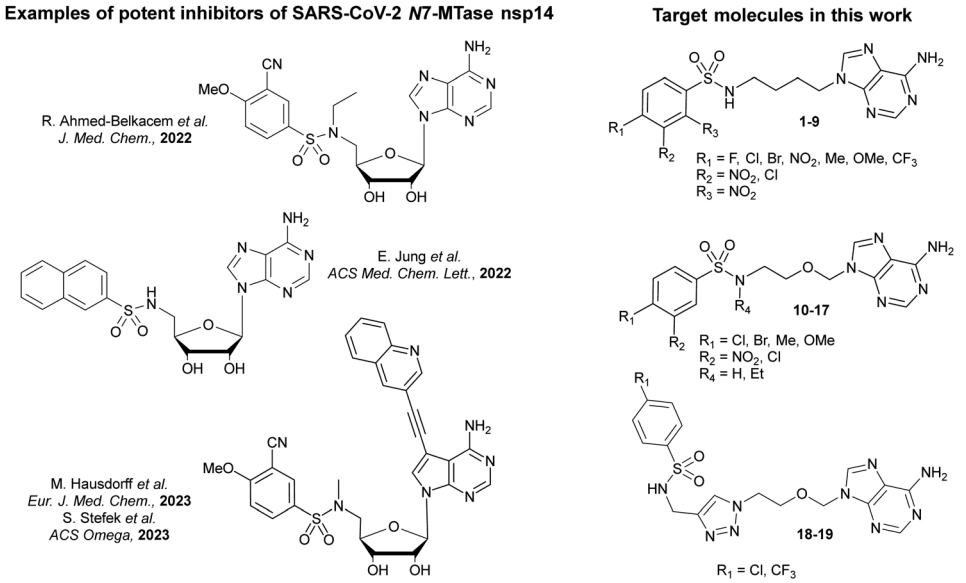


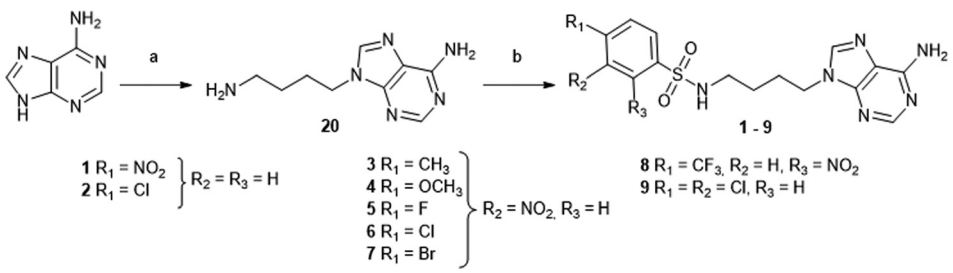
Fig. 1 Examples of previous *N*-arylsulfonamide-based adenosine analogues showing low nanomolar inhibitory activity against SARS-CoV-2 *N*7-methyltransferase nsp14 (left panel).[6,7,9,10] Chemical structures of the target molecules as acyclic analogues of adenosine sulfonamides **1–19** (right panel).

chain (**10–17**) or a 1,2,3-triazolylethyloxymethyl linker between the *N*-arylsulfonamide core and adenine (**18–19**).

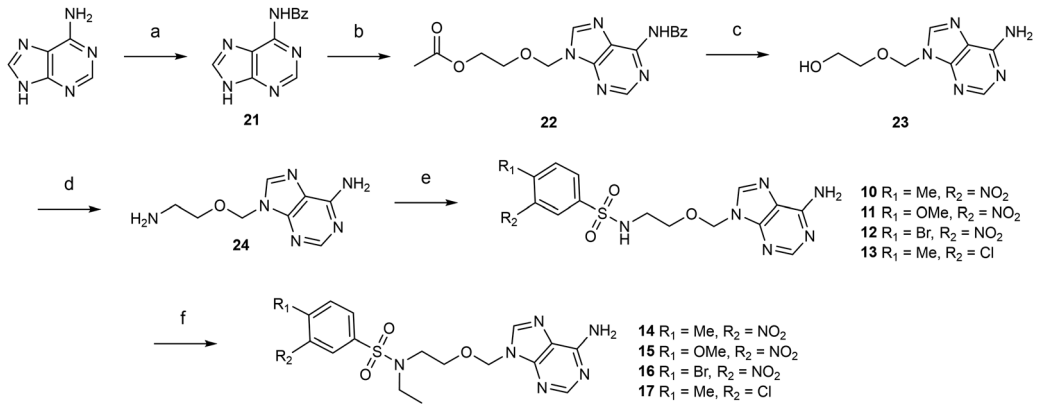
Molecular docking of compound **1** showed a suitable overlay with SAH in SARS-CoV-2 nsp14 (PDB ID: 7R2V, resolution 2.53 Å, data not shown) (Scheme 1). As we had shown the relevance of the *p*-OMe substituent in combination with *m*-NO_2 on the *N*-ethyl-*N*-arylsulfonamide ring,[6] compound **15** was docked into nsp14 and the overlay of its adenosine moiety with the SAH present in the SARS-CoV-2 nsp14 structure was confirmed (ESI†). It is noteworthy that even in the absence of the ribose, the acyclic nucleoside adopts a conformation that preserves hydrogen interactions with Arg310 and Asn386. In addition, the π–π stacking interaction previously reported between the phenylsulfonamide core and the Phe426 is retained. However, this occurred as a ''parallel displaced'' rather than a ''face-to-face'' interaction observed with the previous nucleoside inhibitors.[6,9] Finally, the *p*-substituents (OMe, Me, F, Cl, Br) always fit correctly into the hydrophobic pocket formed by Phe506, Phe401 and Tyr420. Overall, these findings supported the synthesis and evaluation of these novel acyclic nucleosides.

The first target molecules were acyclic nucleosides **1–9**, which contain a 4-carbon chain between the *N*9-adenine and the sulfonamide function, instead of ribose. The synthesis started with an alkylation step on adenine to introduce the butyl chain. Adenine was deprotonated by potassium carbonate in DMF and alkylated with *N*-(4-bromobutyl)phthalimide to give an intermediate *N*-protected acyclic nucleoside (Scheme 1). A further hydrazine treatment afforded compound **20** with 60% yield[27] and the resulting primary amine was reacted with various commercially available arylsulfonyl chlorides to give the *N*-arylsulfonamide-containing acyclic nucleosides **1–9**.[28]

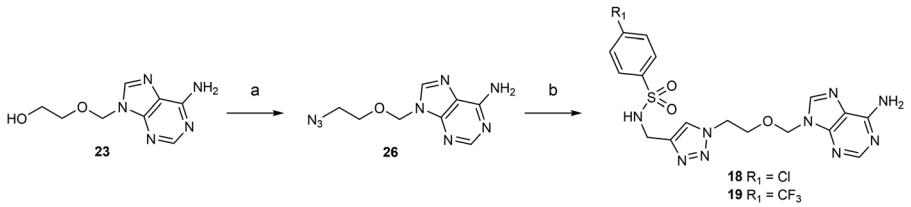
Next, we designed acyclic adenosine analogues **10–17**, in which the sugar was replaced by an aliphatic ethyloxymethyl linker decorated with various arylsulfonamides that mimic the cap guanosine. These compounds were prepared in 5 steps from adenine (Scheme 2). After protection of the *N*6-amino function of adenine with a benzoyl group,[29] the alkylation of *N*6-benzoyl adenine **21** was performed using 1,3-dioxolane and acetic anhydride, affording intermediate **22** in 55% yield.[24] The acetyl group at the extremity of the ethyloxymethyl chain and the *N*-benzoyl group were then cleaved in basic medium and the resulting hydroxyl in **23** was converted to primary amine using Mitsunobu conditions, giving **24** in quantitative yield.[30] The free amine of **24** was finally reacted with various



Scheme 1 Synthesis of acyclic adenosine analogues **1–9**. *Reagents and conditions*: (a) (i) *N*-(4-bromobutyl)phthalimide, K_2CO_3, DMF, 70 °C, 18 h; (ii) hydrazine hydrate (50–60%), EtOH, 65 °C, 2 h, 60%.[27] (b) Arylsulfonyl chloride, Et_3N, DMF, 3 h, 34–81%.



Scheme 2 Synthesis of acyclic adenosine analogues **10–17**. *Reagents and conditions*: (a) BzCl, pyridine, 100 °C, 3 h then 25 °C, 18 h, 62%. (b) 1,3-dioxolane, acetic anhydride, TMSOTf, MeCN, 25 °C, 15 min, 55%. (c) NH_4OH/MeOH 3/1 (v:v), 25 °C, 18 h, quant. (d) (i) Phtalimide, DIAD, PPh_3, THF, 25 °C, 2.5 h; (ii) hydrazine hydrate (50–60%), EtOH, 65 °C, 2 h, quant. (e) Arylsulfonyl chloride, Et_3N, DMF, 3 h, 61–74%. (f) Ethyl *p*-toluenesulfonate, KI, K_2CO_3, DMF, 50 °C, 16 h, 47–54%.



Scheme 3 Synthesis of acyclic adenosine analogues **18–19**. *Reagents and conditions*: (a) (i) DPPA, DBU, 1,4-dioxane, 25 °C, 16 h; (ii) NaN_3, 15-C-5, 100 °C, 4 h, 74%. (b) *N*-arylsulfonamide-*N*-propargyl reagents,[11] $CuSO_4$, sodium ascorbate, H_2O/dioxane, 0 °C, 3 h, 74% (for *p*-Cl), 69% (for *p*-CF_3).

arylsulfonyl chlorides to provide eight *N*-arylsulfonamide-containing acyclic nucleosides **10–17**.

Acyclic nucleosides **18** and **19** containing a 1,2,3-triazole ring were the last target compounds to be synthesized from **23** and *N*-propargyl-*N*-arylsulfonamide reagents previously prepared in-house (Scheme 3).[11] First, an azide group was introduced using diphenyl phosphoryl azide (DPPA) in 1,4-dioxane reacting with **23** to give **26** with 74% yield. Then, a copper-catalyzed azide–alkyne cycloaddition in the presence of $CuSO_4$ and sodium ascorbate afforded acyclic nucleoside analogues **18** and **19** in 74% yield and 69% yield, respectiveley.[31]

All synthesized compounds **1–19** were screened for inhibitory activity of SARS-CoV-2 *N*7-MTase nsp14 using a radioactive MTase assay. Briefly, the nsp14 protein was incubated together with **1–19** at 5 μM and 50 μM concentrations and the [^{3}H]-radiolabeled methyl transferred from the SAM methyl donor onto the cap structure of a short synthetic RNA substrate ($GpppAC_4$) was measured by a filter binding assay.[32] All acyclic nucleoside analogues show no inhibitory activity at 5 μM concentration, and most of them are barely active at 50 μM against the *N*7-MTase nsp14, as enzyme activity remains above 50% at this concentration. Replacement of the ribose between the adenine nucleobase and the *N*-arylsulfonamide moieties is clearly detrimental for the inhibitory activity of these acyclic analogues, mimics of our previous nsp14 inhibitors (0.146 μM $<$ IC_{50} $<$ 14.1 μM).[6] However, two acyclic compounds **1** and **4** with a butyl chain display a modest inhibitory effect at 50 μM on *N*7- MTase (*N*7-MTase activity $<$ 35%). Overall, the butyl chain seems to be less detrimental than ethyloxymethyl or triazole-containing linkers for the inhibitory activity of these acyclic adenosine sulfonamides against SARS-CoV-2 N7-MTase nsp14. The lack of activity could be explained by the high flexibility of the alkyl chain, which might prevent the simultaneous binding of adenine in the SAM binding pocket and the arylsulfonamide ring in the cap binding pocket observed when ribose is present in the nucleoside inhibitors.[6,9]

Conclusions

We have successfully prepared three series of acyclic nucleoside sulfonamides as analogues of *N*-arylsulfonamide-based adenosines, which had been found to be potent inhibitors of SARS-CoV-2 RNA cap *N*7-methyltransferase nsp14. Replacement of the ribose moiety by a butyl, ethyloxymethyl or triazolylethyloxymethyl linker impaired the inhibitory activity of these acyclic compounds. However, these results are valuable for SAR and will be useful for further design of nsp14 inhibitor bisubstrates.

Author contributions

R. A.-B.: conceptualization, investigation, methodology, validation, writing – original draft. A. D.: investigation, methodology, validation. B. C.: resources, writing – review & editing. J.-J. V.: resources,

supervision, writing – review & editing. E. D.: data curation, supervision, validation, funding acquisition, project administration, writing – review & editing. F. D.: funding acquisition, project administration, supervision, validation, writing – review & editing.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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