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N-acylsulfonamide: a valuable moiety to design new sulfa drug analogues

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Sulfonamides are the oldest class of antibiotics discovered more than 80 years ago. They are still used today despite the appearance of drug resistance phenomena that limit their prescription. Since the discovery and use of the first sulfa drugs, many analogues have been synthesized in order to obtain new active molecules able to circumvent bacterial resistance. Structurally similar to sulfonamide, the *N*-acylsulfonamide group arouses interest in the field of medicinal chemistry due to their specific physico-chemical properties. We report here the synthesis and antibacterial / antibiofilm activities of 18 sulfa drug analogues comprising an *N*-acylsulfonamide moiety. These derivatives were obtained efficiently by sulfo-click reactions between readily available thioacid and sulfonyl azide synthons.

Introduction

All antibacterial agents have known a loss of clinical utility due to the apparition of drug resistance.¹ The sulfonamide class of antibiotics (*i.e.* sulfa drugs),²⁻⁴ as the pioneer drugs with a selective effect on bacteria used as soon as 1935 (Prontosil, Fig. 1)⁵ were the first victim of this phenomena. Sulfa drugs inhibit the dihydropteroate synthase (DHPS), a key enzyme in the folate pathway of bacteria. The DHPS catalyses the condensation of 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPP) with *p*-aminobenzoic acid (PABA) in the production of the 7,8-dihydropteroate folate intermediate.^{6,7} Many research groups are still working to develop new effective and less toxic sulfa drugs able to circumvent the resistance developed by bacteria over the years.⁸⁻¹²

Structurally close to its sulfonamide parent, the *N*-acylsulfonamide group exhibits an additional carbonyl that has the potential to establish new interactions with the amino acids of the DHPS active site. Furthermore, interest in *N*-acylsulfonamide derivatives has grown in recent years in different fields of medicinal chemistry.¹³ It is attested by the rising number of drugs and lead molecules comprising an *N*-acylsulfonamide moiety recently identified showing biological activity against various target such as voltage-gated

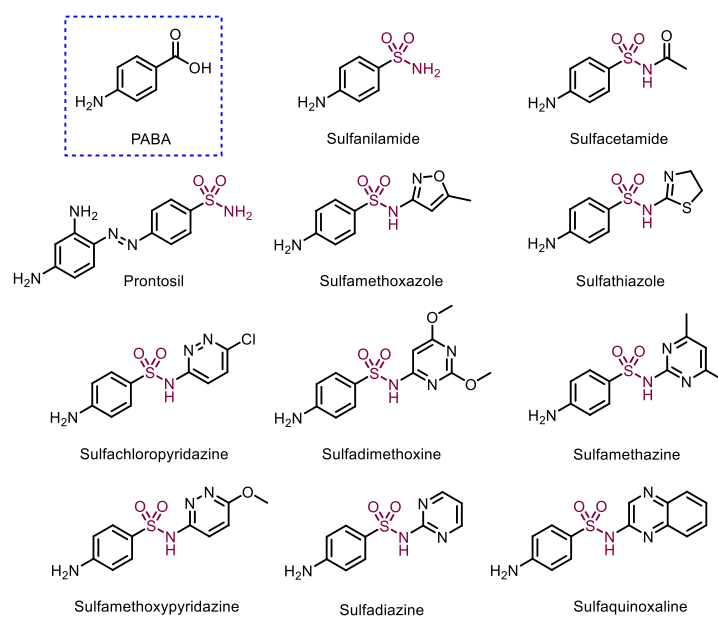


Fig. 1 Structures of the natural substrate (PABA) of the DHPS and examples of known sulfonamide antibiotics (sulfa drugs).

sodium channels for pain treatment^{14,15} or Bcl-2¹⁶ and MMP2¹⁷ protein targets for cancer therapy. Moreover, *N*-acylsulfonamide derivatives arouse a specific interest as bioisosteres of carboxylic acid compounds.¹⁸⁻²⁰ Indeed, they exhibit similar abilities to form a network of H-bond and comparable pK_a (around 3.5-4.5) that makes them particularly interesting in the context of DHPS inhibitors, considering the presence of the carboxylic acid function present in PABA.

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Besides, one of the sulfa drugs that is used for the treatment of skin, eye or urinary tract infections, but also acne and seborrheic dermatitis is actually an *N*-acylsulfonamide derivative (*i.e.* sulfacetamide, Fig. 1).^{21, 22} Sulfa drugs generally include a para amino sulfonamide moiety which could be substituted by an aromatic or heteroaromatic group to modulate the pharmacological parameters and the toxicity of the compounds (Fig 1). In this context, we investigated how the replacement of the sulfonamide by an *N*-acylsulfonamide would impact the biological activities of this class of inhibitors. Two series of compounds bearing either the natural para amino substituent or its acetylated analogue were synthesized using the sulfo-click reaction and their biological activities against four different bacterial strains were evaluated.

Results and discussion

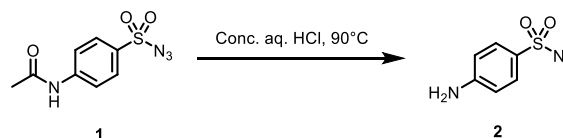
Synthesis of *N*-acylsulfonamide analogues using the sulfo-click reaction

The most conventional method to generate *N*-acylsulfonamides is through the acylation of the parent sulfonamide.²³⁻²⁵ Many methodologies have been developed to increase the efficacy of the reaction due to the low reactivity of the sulfonamide.²⁶⁻³² Despite interesting improvements, these procedures involve prolonged reaction time, harsh conditions or the need of additive to increase the reactivity of the involved substrates.

The sulfo-click reaction between a sulfonyl azide and a thioacid represents a valuable alternative to access *N*-acylsulfonamide derivatives with high efficiency.^{33, 34} We recently explored this reaction for the generation of 5'-bioconjugated nucleosides^{35, 36} and the synthesis of 4'-(*N*-acylsulfonamide) modified nucleosides as SARS-CoV-2 RNA cap N7-guanine-methyltransferase nsp14 inhibitors.³⁷ This catalyst-free reaction proved to be fast and efficient under aqueous conditions with an extremely simple implementation. Considering the importance of sulfa drugs in the context of the fight against bacterial infections, we decide to use the advantageous characteristics of the sulfo-click reaction to synthesize 17 original *N*-acylsulfonamide derivatives along with the well-known sulfacetamide. Quantitative conversions and high isolated yields were obtained in 10 minutes, using NaHCO₃ as the only additional reagent. Subsequently, we

evaluated their biological activity against four pathogenic bacterial strains.

Foremost, the implementation of the sulfo-click reaction requires the use of sulfonyl azide derivatives. We decided to work with commercially available 4-acetamidobenzenesulfonyl azide **1** that can be hydrolyzed in conc. aq. HCl to form the 4-aminobenzenesulfonyl azide **2** (scheme 1).³⁸ The choice of these synthons was guided by an objective to synthesize a series comprising the same structure as the natural substrate (4-aminobenzene) and a parent hydrophobic structure allowing comparative studies from the synthetic and the biological point of view.

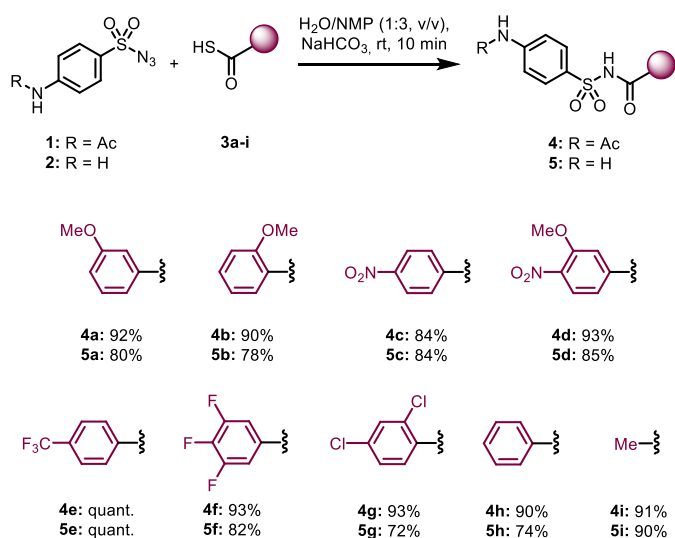


Scheme 1 Synthesis of the 4-aminobenzenesulfonyl azide **2** from commercially available 4-acetamidobenzenesulfonyl azide **1**.

Concerning the thioacid partners, we have chosen to work with compounds **3a-i** (Scheme 2) which are commercially available with the exception of compounds **3d,f**. However, most of these thioacids are sold by custom synthesis companies at prohibitive prices. Thus, thioacids **3a-g** were obtained in a single step from carboxylic acid derivatives using a modified version of the procedure described by Kanai and co-workers on peptides using AcSK as sulfur source in the presence of a catalytic amount of Ac₂S (0.2 eq.) in DMF (see ESI for detailed protocols).

Then, the sulfonyl azides **1** and **2** and the thioacid derivatives **3a-i** were engaged in sulfo-click reactions under aqueous conditions [\sim 100 mM thioacid derivatives **3a-i** in a 1:3 H₂O/NMP mixture with the addition of NaHCO₃] to access *N*-acylsulfonamide derivatives **4a-i** and **5a-i** (Scheme 2). We were pleased to observe that all reactions were complete within 10 minutes as confirmed by RP-HPLC analyzes whether using a sulfonyl azide bearing a 4-electron-withdrawing (**1**) or -donating (**2**) substituent. We consequentially obtained high isolated yields, the losses of material being due to RP-chromatographic purification followed by lyophilization processes.

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Scheme 2 Scope of the new sulfa drugs synthesized.

Moreover the performance of the sulfo-click reaction did not suffer of the use of aryl thioacids bearing activating or deactivating groups. The high efficiency of the sulfo-click reaction observed for the synthesis of the compounds **4a-i** and **5a-i** demonstrates the interest of our methodology over classical protocols that often require several steps and moderate overall yields.³⁹⁻⁴²

Antibacterial activity of *N*-acylsulfonamide analogues

All 17 *N*-acylsulfonamide derivative analogues were screened *in vitro* for their potential antibacterial activity against 2 representative Gram-negative (*e.g.* *Escherichia coli* and *Pseudomonas aeruginosa*) and 2 Gram-positive (*e.g.* *Bacillus subtilis* and *Staphylococcus aureus*) bacteria by comparing them with the sulfacetamide (**5i**).⁴ These bacteria have been chosen for their implications either in human health or in food poisoning.

First, minimum inhibitory concentration (MIC) values have been determined against the four bacterial strains selected.

No activity was observed with the series bearing a para-aminoacetyl group **4a-i**. This result confirms that the free para amino substituent present on the natural substrate PABA is essential for the bioactivity targeting the bacterial DHPS. However, some interesting results have been obtained with the para amino series (**5a-i**, Table 1). The sulfacetamide **5i** exhibits, as expected, significant activity against both *E. coli* and *B. subtilis* confirming the efficiency of this peculiar sulfa drug on both Gram-negative and Gram-positive bacteria strains. Concerning the original compounds synthesized, only three (**5a**, **5b** and **5h**) displayed an inhibitory activity on the bacterial growth of *E. coli* and *B. subtilis* strains. Remarkably, this bactericidal activity was comparable to the one observed with the sulfacetamide. Some interesting structural information can be deduced from these results. Indeed, the compound **5h** bearing a non-substituted aromatic ring exhibit a MIC of 32 $\mu\text{g mL}^{-1}$ against *E. coli* and a MIC of 128 $\mu\text{g mL}^{-1}$ against *B. subtilis*. While the addition of various electron-withdrawing substituents (*i.e.* trifluoromethyl, nitro, chloro, fluoro) implies a significant decrease in biological activity (compounds **5c-g**), the presence of a methoxy electron-donating group on compounds **5a** and **5b** induce an increase in the inhibition of bacterial growth while increasing the steric hindrance around the aromatic ring. Knowing the relationship between the aryl π -electron density and the strength of the resulting noncovalent π -stacking interactions,⁴³ it appears that the presence of electron-withdrawing substituents on the aromatic ring that increases the strength of their potential interactions, prevent them from being active. Noteworthy, a significant difference in activity was observed depending on the position of the methoxy group. If placed at the ortho position, compound **5a** shows significant activity against *E. coli* (MIC = 32 $\mu\text{g mL}^{-1}$) but not *B. subtilis*, while conversely compound **5b** carrying the methoxy group at the meta position exhibits activity against *B. subtilis* (MIC = 64 $\mu\text{g mL}^{-1}$) but not against *E. coli*. This demonstrates the structural differences that exist between the active sites of the DHPS of the different bacterial strains.

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Table 1 MIC of *N*-acylsulfonamide derivative analogues against representative Gram-negative and Gram-positive bacteria

Compounds	MIC ($\mu\text{g mL}^{-1}$) ^a			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>
5a	64	>256	256	>256
5b	256	>256	64	256
5c	>256	>256	256	>256
5d	>256	>256	>256	>256
5e	>256	>256	>256	>256
5f	>256	>256	>256	>256
5g	>256	>256	>256	>256
5h	32	>256	128	>256
Sulfacetamide (5i)	32	>256	128	256

^aMICs were determined in triplicate according to CLSI recommendations using broth microdilution method. Compounds were dissolved in DMSO at 25.6 mg mL^{-1} and diluted in cation-adjusted Mueller Hinton broth to obtain concentration ranging from 1 to $256 \mu\text{g mL}^{-1}$. Sulfacetamide was used as a control.

Inhibition of biofilm formation by *N*-acylsulfonamide analogues

Bacteria can form a biofilm which works as a barrier to protect them from threats, such as host immunity system or antibiotics. In addition, a biofilm provides the basis for bacteria to spread and colonize new niches.^{44, 45} Consequently, molecules exhibiting antibiofilm activity represent an interesting alternative to fight bacterial infections. Thus, we studied the potential of the three most active molecules synthesized to inhibit the growth of biofilms at $\frac{1}{2}$ MIC in *E. coli* and *P. aeruginosa*, the first serving as a model and the second being known to be a particularly robust strain (Fig. 2).⁴⁶ The *N*-acylsulfonamide derivative **5h** bearing a non-substituted aromatic ring inhibited the biofilm formation of *E. coli* and *P. aeruginosa* by about 30% ($P=0.0005$) and 14% ($P=0.0108$), respectively (Fig. 2). Concerning the methoxy substituted phenyl group, **5b** exhibited a moderate antibiofilm activity in *E. coli* (-25%; $P=0.0027$) and a low effect on *P. aeruginosa* biofilm formation (-14%; $P=0.0105$). Finally, compound **5a** showed the best results with 42% ($P<0.0001$) and 23% ($P<0.0001$) of biofilm growth inhibition of *E. coli* and *P. aeruginosa*, respectively. By comparison, the sulfacetamide induced lower inhibition indicating a specific effect of compound **5a** on the biofilm growth of the two assayed strains.

The significant results obtained in these two studies concerning the antimicrobial activity and the biofilm growth

inhibition of compounds **5a**, **5b** and **5h** demonstrate the potential use of *N*-acylsulfonamide group as anti-infective agents.

Conclusions

In summary, taking advantage of our previous work we applied the sulfo-click reaction to sulfonyl azides and thioacids moiety to access two series of sulfa drug analogues. The procedure allowed quantitative conversion of the substrates and high isolated yields in less than 10 min under aqueous conditions using NaHCO_3 as the only additional reagent, which represents a major improvement over the methods previously described. 17 *N*-acylsulfonamide derivatives along with the sulfacetamide (**5i**) were obtained and evaluated as potential antibacterial compounds as sulfa drug analogues. Three compounds (**5a**, **5b** and **5h**) exhibited antibacterial activity about the same order as the sulfacetamide **5i**. Additionally, compound **5a** revealed significant inhibition of biofilm formation of *E. coli* and *P. aeruginosa*. Although interesting, these results did not seem to indicate a crucial role of the *N*-acylsulfonamide moiety on the biological activities evaluated. Nevertheless, the efficient methodology presented herein may open the way to the easy synthesis of new series of *N*-acylsulfonamide derivatives exhibiting better antibacterial and antibiofilm activities.

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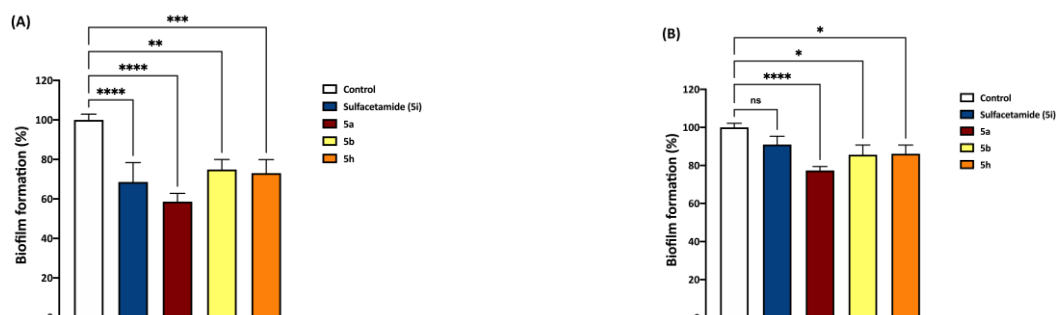


Fig. 2 Effect of compounds **5a**, **5b**, **5h** and **5i** on biofilm formation by *E. coli* (A) and *P. aeruginosa* (B). Statistics were achieved by ordinary one-way ANOVA followed by Dunnett multiple-comparison test. Asterisks indicate values that are significantly different as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, not significantly different

Author Contributions

R.A.: investigation, methodology, visualization, validation. A.T.: investigation, methodology, data curation, writing-review and editing, supervision. M.B.: investigation, methodology, validation. O.L.: data curation, writing-review and editing, supervision. F.D.: resources, writing-review and editing. G.C.: conceptualization, supervision, investigation, validation, visualization, funding acquisition, project administration, writing - original draft. M.S.: conceptualization, writing-review and editing, supervision, project administration, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

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