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Development of sulfahydantoin derivatives as β-lactamase inhibitors

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ABSTRACT

Sulfahydantoin-based molecules may provide a means to counteract antibiotic resistance, which is on the rise. These molecules may act as inhibitors of β-lactamase enzymes, which are key in some resistance mechanisms. In this paper, we report on the synthesis of 6 novel sulfahydantoin derivatives by the key reaction of chlorosulfonyl isocyanate to form α-amino acid derived sulfamides, and their cyclization into sulfahydantoins. The synthesis is rapid and provides the target compounds in 8 steps. We investigated their potential as β-lactamase inhibitors using two common Class A β-lactamases, TEM-1 and the prevalent extended-spectrum TEM-15. Two compounds, **3** and **6**, show substantial inhibition of the β-lactamases with IC_{50} values between 130 and 510 μM and inferred K_i values between 32 and 55 μM.

Bacterial resistance to antibiotics is one of the most serious worldwide healthcare problems.[1,2] This situation is mainly caused by the widespread use and misuse of currently available antibiotics[3] and the slow development of new ones.[4] One of the oldest and most widely used antibiotic families is the β-lactams. However, there is considerable resistance to these drugs. This is especially problematic with multidrug-resistant gram-negative bacteria such as extended-spectrum β-lactamase-producing *Enterobacteriaceae,* which costs over 1 billion dollars in healthcare in the USA annually.[5] Bacteria resist β-lactams by different mechanisms, but the most prevalent for gram-negative bacteria is the production of β-lactamase enzymes that hydrolyze the β-lactam heterocycle.[6] There are two groups of those hydrolyzing enzymes: the serine β-lactamases (Classes A, C and D), and the metallo-β-lactamases (Class B).[7]

Over the years, one successful approach to overcome that type of resistance has been to combine β-lactam antibiotics with β-lactamase inhibitors to protect the drug.[8] However, multiple types of β-lactamase, which bacteria have usually developed through mutations, render the currently used inhibitors less effective. Commonly used inhibitors, such as clavulanic acid, sulbactam, and tazobactam, mostly target Class A and some Class D serine-dependent β-lactamases.[9] In addition, these compounds contain a β-lactam heterocycle similar to the antibiotics they are protecting. Recently, three new inhibitors have been introduced to the market. Those compounds have the advantage of having different heterocyclic structures. Avibactam and relebactam belong to the diazabicyclooctane family, whereas vaborbactam belongs to the boronate family.[4] Even with these new structures, the wide range of β-lactamases that exist is not well covered. Therefore, even more new inhibitors need to be found; ones that have different core structures to minimize the emergence of resistance mechanisms, while maximizing the antibacterial arsenal.

A potential chemical entity to achieve this goal is the sulfahydantoin heterocycle. Derivatives of this 5-membered ring have already been shown to inhibit serine-dependent proteases, such as the human neutrophil elastase.[10,11] This suggests that it could also be active against Class A, C or D serine β-lactamases. In addition, the sulfahydantoin's ability to react with nucleophiles can be fine-tuned by changing the substituent on the N5 of the heterocycle.[12] Surprisingly, sulfahydantoins themselves have not been studied as β-lactamase inhibitors. The few examples found that include the heterocycle in potential inhibitor candidates all include a β-lactam ring as the effector group.[13,14]

In previous studies, our group developed a synthetic pathway in solution and on a solid support to efficiently prepare chiral sulfahydantoins from amino acids in order to insert them in peptide sequences.[15] Building upon this approach, we synthesized a variety of sulfahydantoin compounds in solution, starting from various natural

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and unnatural amino acids, and screened them as potential β-lactamase inhibitors.[16] These previous works demonstrated that sulfahydantoins bearing a benzyl substituent at position C4 and N5 showed weak inhibition of TEM-1, a well-known β-lactamase. Therefore, we focused the present investigation on sulfahydantoin compounds starting from unnatural substituted L-phenylalanines. Herein, we report on the synthesis of six novel chiral sulfahydantoins (Fig. 1) and our investigation of their potential as inhibitors of the prominent TEM-1 and TEM-15 Class A serine β-lactamases.

Compounds **1**–**6**, shown in Fig. 1, were prepared starting from 4-fluoro-L-phenylalanine **7** and 4-bromo-L-phenylalanine **8**. The synthesis is summarized in Scheme 1 and details are available in the Supplementary material (SM). The starting amino acid (**7** or **8**) was protected by the formation of a methyl ester using thionyl chloride in methanol to obtain compounds **9** and **10**, respectively, with yields > 95%. The key sulfamide group was introduced using *N*-Boc-protected chlorosulfonamide prepared in situ from chlorosulfonyl isocyanate (CSI) and *t*-butyl alcohol and then mixed with **9** or **10** in basic conditions to cleanly obtain **11** and **12** with isolated yield of 92% and 96%, respectively. An allyl group was added with a Mitsunobu reaction using allyl alcohol and diisopropyl azodicarboxylate (DIAD). The reaction selectively added the allyl group onto the *N*-Boc nitrogen, as needed, to obtain **13** and **14** with yields of 40% and 60%, not optimized.

To allow the upcoming cyclization, the Boc moiety was removed using trifluoroacetic acid giving *N*-allyl compounds **15** and **16** with 82% and 72% yields, respectively. The key cyclization step to obtain **17** or **18** was performed with sodium methoxide in anhydrous methanol at reflux for only 1 h to minimize the reopening of the heterocycle. This key step gives **17** in 76% yield and **18** in 88% yield. Indeed, longer reaction times led to the nucleophilic opening of the sulfahydantoin cycle and lower yields. Noteworthy of mention, the cyclization conditions used do not lead to epimerization as demonstrated previously.[17] Indeed, the synthesis of sulfahydantoin constrained L-Phe-D-Ala dipeptide using the same conditions was shown to proceed without epimerization at the C4 chiral center. With these key intermediates in hand, three derivatives each of **17** and **18** were prepared using a standard S_N2 reaction with the N5 as the nucleophile and K_2CO_3 as the base. Compounds **19**, **20**, and **21** were obtained by mixing **17** with 4-nitrobenzyl bromide, 4-methoxybenzyl chloride, and 4-bromobenzyl bromide, respectively. Compounds **22**, **23**, and **24** were obtained by mixing **18** with the same alkylating agents, with yields ranging from 60 to 93%.

To obtain the final desired compounds (**1**–**6**), an ozonolysis was performed followed directly by an oxidation using potassium peroxymonosulfate (Oxone). These two steps yielded the final crude compounds **1**–**6**, which were purified by High-Performance Liquid Chromatography (HPLC) to obtain purities ≥ 95%. The isolated yields for the last two steps and the purification were from 8 to 51%. The low yields were mainly caused by losses during the HPLC purification. The 6 novel chiral sulfahydantoins were fully characterized. The sulfahydantoin ring was readily confirmed by the ^1H and ^{13}C NMR spectra, and the exact mass was determined by high resolution mass spectrometry (HRMS). Full spectroscopic data are reported in the SM. The overall yields vary from 3% to 11%.

The inhibition activity of sulfahydantoins **1**–**6** was investigated using two clinically relevant β-lactamases. The first was TEM-1, one of the most widespread β-lactamases.[18] The second was TEM-15, the Glu104-Lys/Gly238Ser double mutant of TEM-1. Those mutations, individually and combined, have emerged following the clinical application of cephalosporins and are among the most common substitutions in TEM-1.[19] TEM-15 was thus selected as a representative, prevalent extended-spectrum β-lactamase that inactivates third generation cephalosporins.[20,21] These two enzymes were obtained by overexpression in *E. coli*[22] followed by a subsequent purification.[23] The purity of the enzymes used in the following tests was > 83%, as determined by SDS-PAGE resolution.

The six novel sulfahydantoins were initially tested as inhibitors of TEM-1 using a previously described protocol.[24] The assay was performed by measuring the decrease in the hydrolysis of CENTA (300 μM), a chromogenic substrate of β-lactamases. This concentration is ~ 8 × the Michaelis-Menten constant (K_m) of TEM-1 for CENTA (36 μM) and has been determined to provide sufficient turnover while allowing for clear observation of inhibition.[24] Sulfahydantoins **1**–**6** were used at 1 mM for screening against 116 and 56 nM of TEM-1 and TEM-15 respectively. Additional details can be found in the SM.

The activity of TEM-1 is mainly unchanged or even slightly augmented in the presence of compounds **1**, **2**, **4** or **5** at a concentration of 1 mM as compared to the control. Activity ranged from 92 to 124% (Table 1). However, compounds **3** and **6** show an inhibition of TEM-1 with a percentage of activity dropping to 34 ± 7% and 10 ± 8% respectively at 1 mM concentration. Further tests with compounds **3** and **6**, the most potent inhibitors of TEM-1, were performed to assess their inhibition potential for TEM-15. Compound **3** shows less inhibition of TEM-15 than TEM-1, with a percentage of activity of 53 ± 7% (Table 1). On the other hand, in the presence of compound **6** at 1 mM, no signal was detectable, demonstrating that the activity of TEM-15 is completely inhibited by sulfahydantoin **6**.

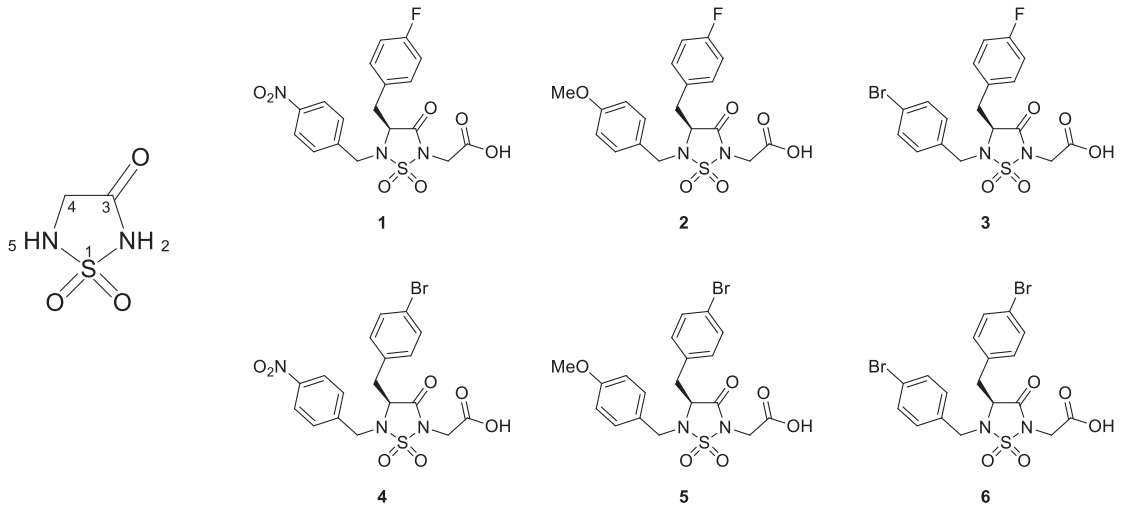
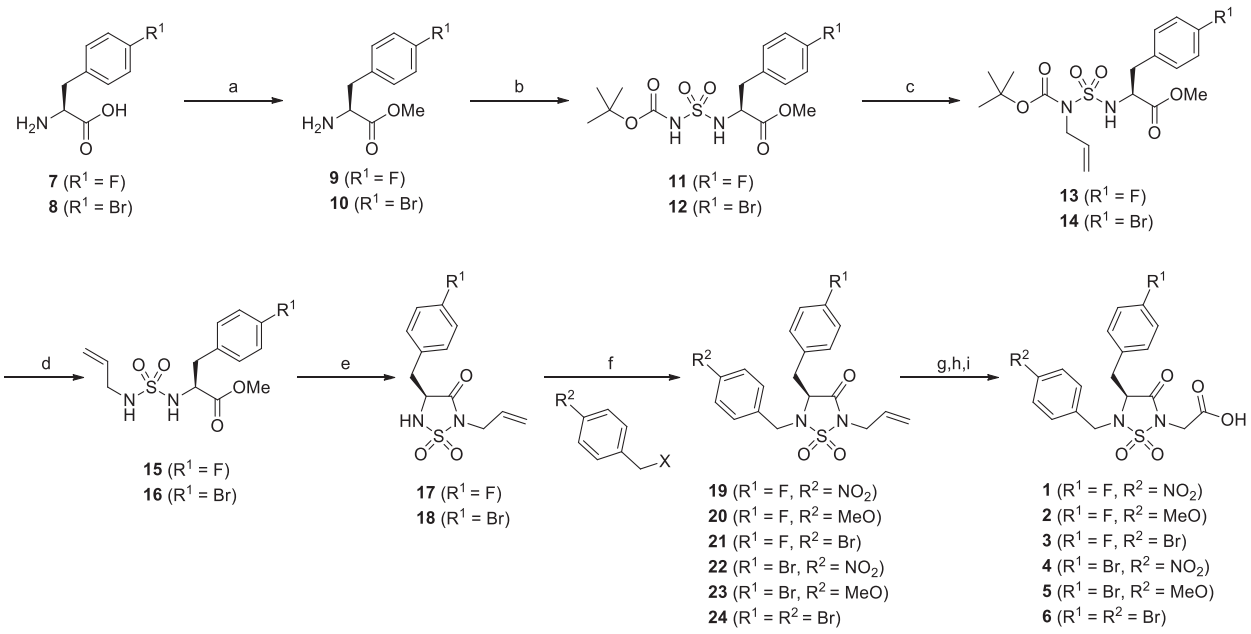


Fig. 1. Generic structure of the sulfahydantoin heterocycle and structures of sulfahydantoin derivatives investigated in the present study as potential β-lactamase inhibitors.



Scheme 1. Synthesis of sulfahydantoin derivatives **1**–**6**. a) $SOCl_2$ (2 equiv), MeOH, 0 °C to reflux, 2 h, ≥ 95%. b) CSI (1 equiv), *t*-BuOH (1.1 equiv), Et_3N (2.2 equiv), CH_2Cl_2, 0 °C to r. t., 1 h, 92–96%. c) PPH_3 (1.1 equiv), allyl alcohol (1.1 equiv), DIAD (1.1 equiv), THF, 0 °C to r. t., 2 h, 40–60%. d) TFA/CH_2Cl_2 1:2, 0 °C to r. t., 2 h, 72–82%. e) MeONa (2.5 equiv), MeOH, reflux, 1 h, 76–88%. f) benzyl halide (3 equiv), K_2CO_3 (20 equiv), acetone, r. t., 3 h to overnight, 60–93%. g) O_3, CH_2Cl_2, –78 °C, 5 min. h) DMS (40 equiv), CH_2Cl_2, –78 °C to r. t., overnight. i) Oxone (2.5 equiv), DMF, r. t., 24 h, 8 to 51% (for g, h and i).

Table 1
TEM-1 and TEM-15 hydrolysis of CENTA in presence of sulfahydantoins **1** to **6** at 1 mM.

	% of activity[a]	
	TEM-1	TEM-15
1	101 ± 12	–
2	124 ± 11	–
3	34 ± 7	53 ± 7
4	100 ± 8	–
5	92 ± 8	–
6	10 ± 8	N.D.[b]

[a]Data are given as mean activity relative to the positive control (absence of inhibitor) ± standard deviation of at least three replicates.
[b]N.D. = not detectable.

To further characterize compounds **3** and **6**, the IC_{50} value was determined. These assays were done using two-fold dilutions and calculated by fitting a dose–response curve. The results (Table 2) show an IC_{50} value of 510 μM for **3** with TEM-1 and values of 300 μM and 130 μM for **6** with TEM-1 and TEM-15, respectively. These IC_{50} values, although modest, indicate a good potential for the development of improved β-lactamase inhibitors based especially on compound **6**, which shows a good inhibition capacity for the extended-spectrum TEM-15.

From the IC_{50} values, the inhibition constant (K_i) can be extrapolated[25] with TEM-1 using K_m of CENTA (36 μM).[24] These values were calculated for three main types of inhibition mechanism, and are reported in Table 3. Based on a previous study from Groutas et al., in which related sulfahydantoins competitively inhibited a serine protease, the human neutrophil elastase,[10] it is plausible that inhibitors **3** and **6** act via the same mechanism. However, further experiments are needed to confirm the mode of inhibition.

Table 2
IC_{50} values of compounds **3** and **6** with TEM-1 or TEM-15.

	IC_{50} (μM)[a]	
	TEM-1	TEM-15
3	510 ± 120	–
6	300 ± 70	130 ± 10

[a]Data are represented as mean ± standard deviation of at least three replicates.

Table 3
Inferred K_i values for compounds **3** and **6** with TEM-1.

Compound	Mode of inhibition	K_i (μM)
3	Competitive	55
	Uncompetitive	455
	Noncompetitive	510
6	Competitive	32
	Uncompetitive	268
	Noncompetitive	300

Infering a competitive binding mechanism, we obtain K_i values of 55 μM and 32 μM for compounds **3** and **6,** respectively, with TEM-1. Overall, these values confirm that the core structures of **3** and **6** are promising scaffolds, which offer a good potential for the development of new and improved β-lactamase inhibitors, exploiting the sulfahydantoin heterocycle.

In summary, we reported on the synthesis of six novel chiral sulfahydantoin derivatives starting from readily available amino acids. While most synthetic steps proceeded with high yields, the overall yields were modest as a result of the low-yielding last reaction step and the difficult final purification. Nevertheless, highly pure sulfahydantoins **1**–**6** were obtained, fully characterized, and investigated for their potential as β-lactamase inhibitors. Compounds **3** and **6** showed modest activity levels against the clinically widespread β-lactamase TEM-1 with IC_{50} values of 510 μM and 300 μM, and inferred K_i values of 55 μM and 32 μM, respectively. In addition, sulfahydantoin **6** exhibited a more potent inhibition against the extended-spectrum β-lactamase TEM-15, with an IC_{50} of 130 μM. Interestingly, the inferred K_i values of compounds **3** and **6** are in the micromolar range according to the plausible hypothesis that they act as competitive inhibitors of the Class A β-lactamases studied. Further investigations are required to validate and better understand

their mechanism of action and to explore more potent analogs. Nevertheless, the results demonstrate that the sulfahydantoin scaffold shows good potential for developing new and improved β-lactamase inhibitors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127781.

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