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Anti-mycobacterial activity of a bis-sulfonamide

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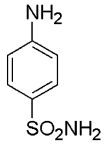
Abstract—A bis-arylsulfonamide, **7**, has been identified that exhibits growth inhibition of *Mycobacterium smegmatis* at less than 25 μg/mL, but has no such activity against *Escherichia coli* or *Staphylococcus aureus*. A closely related bis-arylsulfonamide (**8**) was much less active, but was the only other compound among 54 arylsulfonamides tested with detectable growth inhibition of *M. smegmatis*.

Since the introduction of prontosil over 70 years ago, sulfa drugs have been widely used to treat a broad spectrum of infectious microorganisms. The bioactive component of prontosil, sulfanilamide (**1**), inhibits 6-hydroxymethyl-7,8-dihydropteroate synthase (DHPS) selectively limiting folate synthesis in prokaryotes and lower eukaryotes thus disrupting the integrity of their DNA synthesis.[1] The evolution of drug resistance in infectious microorganisms underpins an immense and ongoing need for new therapies to treat infectious diseases. The identification of new and novel chemical entities with activity against cells infected with the microorganism is a crucial component of this anti-infective drug development pathway.[2] Although sulfonamides are not specifically incorporated into current tuberculosis treatment, many sulfur-,[3,4] sulfonyl-,[4–6] and sulfonamide-containing[7,8] compounds are active against mycobacteria. This prompted us to screen our growing library of triazole-based sulfonamides (e.g., **2**) for anti-mycobacterial activity. Herein, we report identification of a novel bis-sulfonamide with anti-microbial activity against *Mycobacterium smegmatis* that is relatively inert toward common bacterial and fungal strains.



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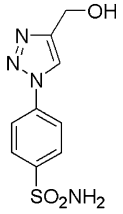

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The syntheses of the bis-sulfonamides **7** and **8** are shown in Scheme 1. The propargyl derivatives **4** and **5** were synthesized from 4-carboxybenzene sulfonamide (**3**) by esterification (EDC/HOBT, 38%) and amide coupling (HBTU/HOBT, 82%), respectively.[9] Compounds **7** and **8** were prepared by reaction of **4** and **5** with the azido arylsulfonamide **6** using the 'click-tailing' approach described previously for the construction of a diverse library of glycosyl triazole-linked arylsulfonamides.[9]

Using Cu(I) catalysis the ester-linked bis-sulfonamide **7** (42%) and amide-linked analog **8** (48%) were each obtained cleanly from **6**.[9–11] Yields were less than ideal, owing to poor product solubility during purification. As both **4** and **7** are potentially labile to cellular esterases, compounds **2** and **3** were included in the biological screening as control compounds to identify if these esters were behaving as prodrugs.

Along with a commercial sample of sulfanilamide **1** and 4-carboxybenzene sulfonamide **3**, synthetic compounds

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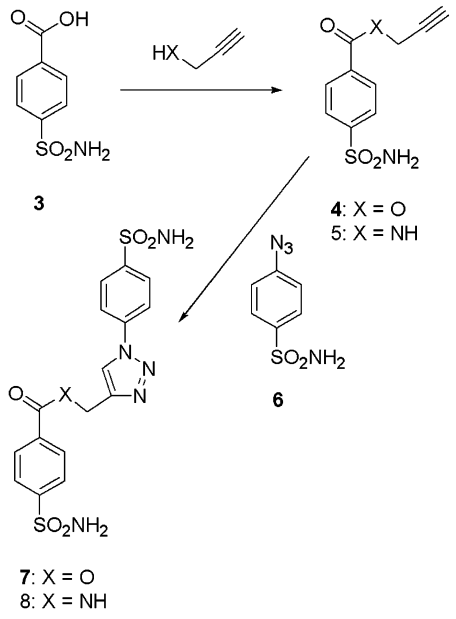

Scheme 1. Synthesis of bis-sulfonamides.

2, **4**–**8** were tested for anti-microbial activity against five microorganisms: *M. smegmatis*, *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, and *Vibrio harveyi*. Each compound was assessed at five doses (100, 25, 6.25, 1.56, and 0.39 μg/mL) and scored for % growth inhibition of the microorganisms (average of two-independent determinations).[12] Bioassay results are presented in Table 1.

The results in Table 1 demonstrate that only the bis-sulfonamides **7** and **8** were able to inhibit the growth of *M. smegmatis* at concentrations of 100 μg/mL or less. The ester **7** had activity comparable to some current antitubercular agents (aminoglycosides such as kanamycin, a Class B antitubercular drug, are active against most mycobacterial strains in the range of 4–16 μg/mL) and had only modest growth inhibition of the yeast strain, *C. albicans*. *M. smegmatis* is used as a surrogate host to perform virulence studies of *Mycobacterium tuberculosis*.[13,14] None of these sulfonamides displayed dose-dependent growth inhibition of any of the three bacterial strains tested: *E. coli*, *S. aureus*, and *V. harveyi*. In this regard, the selective anti-microbial activity displayed by the bis-arylsulfonamide **7** is remarkable as previous work on 35 sulfanilamides showed comparable inhibitory effects against both *E. coli* and *M. smegmatis*.[15] Among the 54 arylsulfonamides screened in this study, bis-arylsulfonamide **7** was the *only* compound with significant activity against *M. smegmatis* at concentrations of less than 100 μg/mL.

Table 1. Minimum inhibitory concentration (MIC_{50}) values in μg/mL versus various microorganisms

Compound	*Mycobacterium smegmatis*	*Candida albicans*	Bacterial strains
1	>100	NA	NA
2	>100	NA	NA
3	>100	NA	NA
4	>100	NA	NA
5	>100	NA	NA
6	NA	NA	NA
7	<25[a]	~100[b]	NA
8	~100	NA	NA
Ampicillin[c]	—	—	500
Amphotericin	—	25	—
Streptomycin	500	—	—

Bacterial strains tested: *Escherichia coli*, *Staphylococcus aureus*, *Vibrio harveyi*; NA, not active.

[a] 68% growth inhibition at 25 μg/mL (92% at 100 μg/mL).

[b] 36% growth inhibition at 25 μg/mL (49% at 100 μg/mL).

[c] The three controls, ampicillin, amphotericin, and streptomycin, were all used at dose levels that guaranteed 100% mortality of the test organism.

In vivo ester hydrolysis of **7** would give rise to **2** and **3**, neither of which had any activity against *C. albicans*, and much less activity against *M. smegmatis* (~20% growth inhibition at 100 μg/mL). The increased activity of the ester **7** versus the amide **8** may be due to greater flexibility of the linker in the former. Calculations of the log *P* values for the ester ($\log P = 0.52$) indicate that it is more hydrophobic than the amide ($\log P = -0.654$).[16] This would undoubtedly improve membrane passage of **7** through the waxy mycobacterial exterior thus increasing its bioavailability relative to the amide.

Compound **7** may be exploiting a unique feature in the active site periphery of mycobacterial DHPS or it may be acting on a novel cellular target unique to mycobacteria. Sulfonamides display increasingly well-understood pharmacological effects most notably the potent inhibition of the carbonic anhydrase catalyzed hydration of carbon dioxide to give bicarbonate and a proton: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$.[17,18] Recently it has been shown that both *M. smegmatis* and *C. albicans* encode for carbonic anhydrases and that this enzyme is critical for the growth of these pathogens.[19,20] Structure–activity work to identify the role of the two arylsulfonamide moieties is underway. These investigations will be reported in due course.

Acknowledgments

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(1-(4-Sulfamoylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl-4-sulfamoylbenzoate (**7**): ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.53 (s, 2H, OCH_2), 7.50 (br s, 2H, SO_2NH_2), 7.54 (br s, 2H, SO_2NH_2), 7.93–8.01 (m, 4H, *Ar* CH), 8.12–8.16 (m, 4H, *Ar* CH), 9.06 (s, 1H, triazole CH); ^{13}C {^{1}H} NMR (100 MHz, DMSO-d_6): δ 58.90 (OCH_2), 121.15 (*Ar* CH), 124.06 (triazole CH), 126.77 (*Ar* CH), 128.18 (*Ar* CH), 130.78 (*Ar* CH), 123.66 (*Ar* C), 139.15 (*Ar* C), 143.90 (*Ar* C), 144.67 (triazole C), 148.95 (*Ar* C), 165.17 (C=O); ESIMS: *m*/*z* $[M-H]^-$ 436.2.
4-Sulfamoyl-*N*-((1-(4-sulfamoylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)benzamide (**8**): ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.62 (d, $^3J_{CH-NH}$ = 5.6 Hz, 2H, $NHCH_2$), 4.76 (br s, 4H, $SO_2NH_2 \times 2$), 7.89–8.12 (m, 8H, *Ar* CH), 8.80 (s, 1H, triazole CH), 9.32 (t, $^3J_{NH-CH}$ = 5.6 Hz, 1H, NH); ESIMS: *m*/*z* $[M-H]^-$ 435.3.
4-(4-(Hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (**2**): ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.60 (d, 2H, $^3J_{CH-OH}$ = 5.2 Hz, 2H, CH_2OH), 5.34 (t, $^3J_{OH-CH}$ = 5.2 Hz, 1H, OH), 7.48 (br s, 2H, SO_2NH_2), 7.97–8.12 (m, 4H, *Ar* CH), 8.76 (s, 1H, triazole CH); ESIMS: *m*/*z* $[M-H]^-$ 253.2.
12. Procedure for anti-bacterial screening. Compounds were tested against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *V. harveyi* (strain C071). Anti-microbial assays were all conducted in a similar fashion with all manipulations for *S. aureus* performed in PYE media (peptone 10 gL^{-1}, yeast extract 5 gL^{-1}, and NaCl 5 gL^{-1}) and manipulations of *E. coli* and *V. harveyi* performed in LB media (BD, Brisbane, Australia). A single colony was inoculated into 20 mL of sterile growth media and grown overnight. This culture was then diluted 1000-fold into fresh media the following day. Assays were carried out in 96-well microtiter plates in an assay volume of 200 μL—made up of culture (198 μL) and test compound in DMSO (final DMSO concentration of 1% v/v). Controls that contained no test compound (DMSO 1% v/v) and antibiotic (500 μg/mL ampicillin—as a no growth control) were also assayed. All assays were performed in duplicate and incubated overnight at 37 °C.
Procedure for anti-fungal screening. Compounds were tested against *C. albicans*. Test cultures were prepared by inoculating 10 mL of YM broth with a loop of fresh *C. albicans* culture. This culture was grown for 18 h at 37 °C to give a culture with an optical density (OD) of 0.3 at 595 nm, corresponding to a cell density of 5×10^9 cells/mL. A 100 μL aliquot of this culture was added to 500 mL of fresh YM broth and well mixed. Assays were carried out in 96-well microtiter plates in an assay volume of 200 μL—made up of culture (198 μL $\sim 2 \times 10^5$ cells) and test compound in DMSO (final DMSO concentration of 1% v/v). Controls that contained no test compound (DMSO 1% v/v) and antibiotic (25 μg/mL amphotericin B—as a no growth control) were also assayed. All assays were performed in duplicate and incubated for 48 h at 37 °C.
Procedure for anti-mycobacterial screening. Compounds were tested against *Mycobacterium smegmatis* (ACM916). Test cultures were prepared by inoculating *M. smegmatis* from precultures into approximately 20 mL of Middlebrook broth in a 50 mL falcon tube. The culture was vortexed briefly and placed in an incubator at 37 °C, and 120 rpm for 3 days. This culture was then sonicated until all cells were thoroughly dispersed and then diluted 1:20 into Middlebrook broth to a final volume of 300 mL. Assays were carried out in 96-well microtiter plates in an assay volume of 200 μL made up of culture (195 μL) and test compound in DMSO (final DMSO concentration of 2.5% v/v). Controls that contained no test compound (DMSO 2.5% v/v) and antibiotic (50 mg/mL streptomycin solution—for a no growth control) were also assayed. All assays were performed in duplicate. An initial absorbance reading was taken at 595 nm and the plates incubated in a humidified incubator at 37 °C for 96 h. At the end of this period plates were removed form the incubator and allowed to equilibrate to room temperature (25 °C).
For all anti-microbial assays optical densities (ODs) were recorded at 595 nm at the start and end of the assay incubation periods. The test compound activity was calculated as a percent of control according to the following equation: Activity % of control = $100 \times ([T_{0(\text{sample})} - T_{\text{end(sample)}}] - [T_{0(\text{neg ctl})} - T_{\text{end(neg ctl)}}])/([T_{0(\text{pos ctl})} - T_{\text{end(pos ctl)}}] - [T_{0(\text{neg ctl})} - T_{\text{end(neg ctl)}}])$.
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