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N-Benzyl-3-sulfonamidopyrrolidines as Inhibitors of Cell Division in *E. coli*

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Abstract

A new small molecule inhibitor of bacterial cell division has been discovered using a high-throughput screen in *E. coli*. Although the lead screening hit (**534F6**) exhibited modest inhibition of the GTPase activity of FtsZ (20±5% at 100 μM primary target for bacterial cell division inhibitors, several analogs caused potent bacterial growth inhibition with negligible antagonism of FtsZ GTPase activity. A library of analogs has been prepared and several alkyne-tagged photoaffinity probes have been synthesized for use in experiments to elucidate the primary target of this compound.

The emergence of antibiotic-resistant strains of bacteria has prompted a worldwide effort to seek new avenues for fighting infectious disease.[1] Most antibiotics discovered to date target a narrow range of biochemical processes in bacteria.[2] FtsZ, the prokaryotic analog of tubulin,[3] has been examined as a potential new target for antimicrobial chemotherapy. Although FtsZ has been the primary target for small molecules that inhibit bacterial cell division,[4] it is likely that other proteins essential for bacterial cytokinesis can also be targeted.[5] A high-throughput screen has recently been developed to identify compounds that cause lethal cell filamentation in *E. coli*.[6,7] This screen revealed new inhibitors of FtsZ and at the same time yielded several compounds that caused cell filamentation without inducing the SOS response or without significantly inhibiting the GTPase activity of FtsZ. Herein we describe our preliminary SAR studies of **534F6**, an *N*-benzyl-3-sulfonamidopyrrrolidine

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(Figure 1) and our initial preparation of photoaffinity reagents for the identification of this compound's protein target(s).

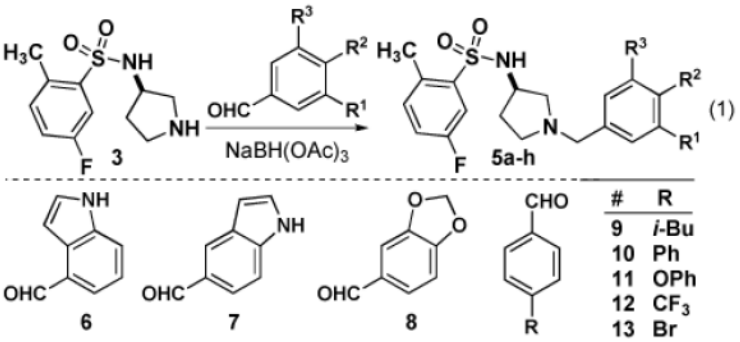
534F6 displayed weak inhibition of FtsZ GTPase (20±5% at 100 μM), did not affect steady-state FtsZ polymerization as assayed by high-speed sedimentation, and induced SOS-independent *E. coli* cell filamentation (data not shown). Despite a certain degree of similarity to sulfonamide antibiotics, such as sulfamethoxazole, compound **534F6** exhibited markedly different effects on *E. coli*. Sulfamethoxazole showed modest lethality (MIC > 80μM) and did not cause *E. coli* (AcrAB efflux pump knockout strain DRC 39[4d]) to filament. Based on these observations, we set out to develop a library synthesis of **534F6** in an effort to optimize potency and eventually determine the protein target of this compound.

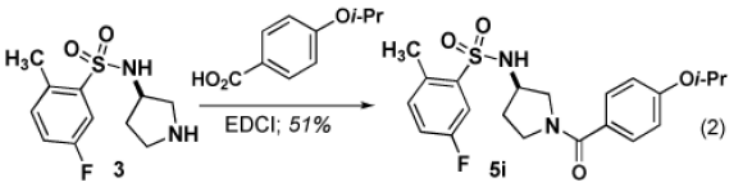
We began by investigating the SAR of **534F6**. Since the configuration of this compound was unknown, we prepared each of the two enantiomers as a test for the influence stereochemistry on activity. Although no synthesis of **534F6** had previously been reported, we were able to convert commercially available (*S*)-**1** and (*R*)-**1** in to (*S*)- and (*R*)-**534F6**, respectively, in three

Steps (Scheme 1). We were delighted to find that the (R)-enantiomer caused lethal *E. coli* filamentation with an MIC of 10 μM, whereas the (S)-enantiomer neither induced cell filamentation nor killed *E. coli* up to 80 μM. This result suggests that **534F6** is reasonably selective in its interaction with its target or targets.

Using our synthetic route to **534F6**, we were able to prepare an initial series of analogs to establish the influence of the *N*-benzyl substituent. Intermediate **3** was condensed with a series of aromatic aldehydes to produce a series of analogs featuring different ortho-, meta-, and para substituents (eq 1). As Table 1 shows, introduction of fused rings or polar para substituents was deleterious to the activity of these compounds. Replacement of the isopropxy group with either the isosteric isobutyl group, a phenyl ring, or a phenoxy group retained activity. Replacement of the benzyl amine with a benzoyl amide greatly diminished the antimicrobial activity of this compound (eq 2).

Based on these preliminary results, we developed a solid phase synthesis of 210 analogs of **534F6**, mindful of the importance of the *N*-benzyl substituent. Since the





substituent at the para position had proven to be crucial for activity, we elected to keep this structural feature invariant. We replaced the isopropoxy group of **534F6** with a hydroxyethyl group as a point of attachment to solid phase synthesis resin.[8] Approximately 35 mg of resin were employed in Irori[tm] kans and the synthesis was tracked using 2D-barcoding.[9] The first step of the synthesis was a reductive amination with a protected amine that would later be functionalized. We initially explored phthaloyl (phth) and tetrachlorophthaloyl (tcph) protecting groups for the primary amine, but found the former to be preferable once the conditions were adjusted to account for the precipitated phthalyl hydrazide. In order to explore the structural elements that might contribute to activity, we prepared protected amine cores **A1**-**A7**. These were condensed onto the aldehyde starting material in six different reaction batches, then pooled for deprotection, and split for attachment of the sulfonyl groups. A series of sulfonyl chlorides were employed in the last step to yield a total of 210 compounds after cleavage. The compounds were tested for growth inhibition and cell filamentation in *E. coli*. None showed improved activity and the majority appeared to be less potent than **534F6**. Although we expected diminished activity from the hydroxyethoxy substituent, we had hoped that an optimized core and sulfonyl substituent would compensate.

We next focussed on a narrower selection of core structures with a single benzyl substituent. Amine core structures **A2**, **A4**, and **A6** were selected and each was *N*-benzylated using *p*-isobutylbenzyaldehyde. These three amines were converted in parallel to the corresponding sulfonamides using excess quantities of sulfonyl chlorides **B1**-**B15** and a scavenger resin for removal of the excess reagent.[10] These 45 compounds were tested and compound **14** was found to be the most potent, with an MIC of 10 μM. Examination of the *E. coli* culture treated with 5 μM **14** showed extensive filamentation (fig. 2A). Compound **15**, featuring the same sulfonamide and benzyl groups on a different core, exhibited an MIC of 20 μM, but little filamentation was observed at 10 μM (fig. 2B).

In a parallel effort, we have prepared several derivatives of **534F6** for use in target identification. We designed several compounds that would serve as photoaffinity reagents to modify their protein targets.[11] In addition, we incorporated terminal alkyne substituents as chemical tags that would allow us to separate the modified target from the cellular lysate (scheme 3).[12]

We initially explored the possibility of incorporating a benzophenone group in the para position of the *N*-benzyl substituent. The requisite 4-formyl benzophenone (**18**) was prepared using the palladium coupling reported by Winkel.[13] Reductive amination of **18** yielded **19**. This compound's weak activity (MIC >80 μM) prompted us to installing a photoreactive group.

Our next compound was designed to use an aryl azide as the photoreactive group. **21a** was prepared by reductive amination of **20**. This compound was converted to sulfonamide **22a**, which was carried on to alkyne **23a**. Sulfonamidopyrrolidine **23a** exhibited an MIC of <12 μM, alkyne did not affect the activity. Encouraged by this result, we proceeded with the

synthesis of **23b** by a parallel synthetic route. This synthesis was enabled by the ligand- and copper-free Sonagashira reaction reported Examination by Verkade,[14] which avoids of reduction the and 5 cycloaddition μM of the aryl azide. Compund-**23b** exhibited an MIC of >40 μM, of a lipophilic group at this site for activity. The activity little of **23a** established the viability of an alkyne on sulfonamide portion of the molecule.

The activities of **23a** and **23b** encouraged us to explore the possibility of a hybrid of these two compounds with **534F6**. Sulfonamide **25** was prepared in two steps from *N*-Boc-(S)-3-aminopyrrolidine (**20**). The Boc group was removed and the 3-nitro-4-isopropoxy benzyl group was installed by reductive amination.[15] The nitro group was reduced to the corresponding aniline, which was then diazotized and displaced with azide. After deprotection with TBAF, compound **29** was examined for antimicrobial activity and found to have an MIC of >64 μM. Although sulfonamide only slightly lowered the activity of **23a** relative to **5a**, it is apparent that the combined effect of the ortho azide and the alkyne greatly diminishes the activity of **29**. We are currently preparing an affinity matrix with **23a** using “clickable” agarose in hopes of pulling down the protein target from a cell lysate of *E. coli.*

In summary, we have discovered a new compound (**534F6**) that appears to inhibit bacterial cell division without inhibiting FtsZ as the primary target. Initial attempts to prepare alkyne tagged photoaffinity reagents have revealed regions of the molecule that are not suitable for structural variation. We are currently examining a variety of alternative approaches for identifying the target of this compound.

Acknowledgments

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10. PS-N(i-Pr)$_2$ = PL-DIPAM MP, 150-300 μM, 1.6 N/g (Varian/Polymer Laboratories). PS(NHCH$_2$CH$_2$)$_2$-NH$_2$ = PL-DETA MP, 150-300 μm, 4.5 mmol N/g (Varian/Polymer Laboratories).
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15. The requisite aldehyde is prepared in one step (91% yield) from 4-hydroxy-3-nitrobenzaldehyde, *i*-PrBr, and K_2CO_3 in CH_3CN by heating to 150 °C with microwave irradiation.

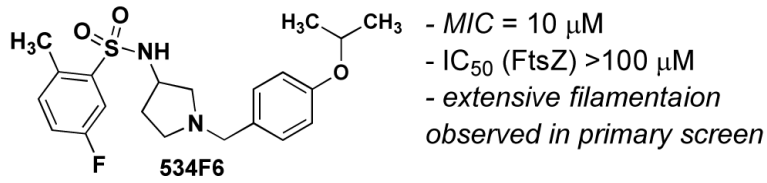


Figure 1.
Lead compound (534F6) from phenotypic HTS for compounds that induce lethal filamentation in *E. coli*.

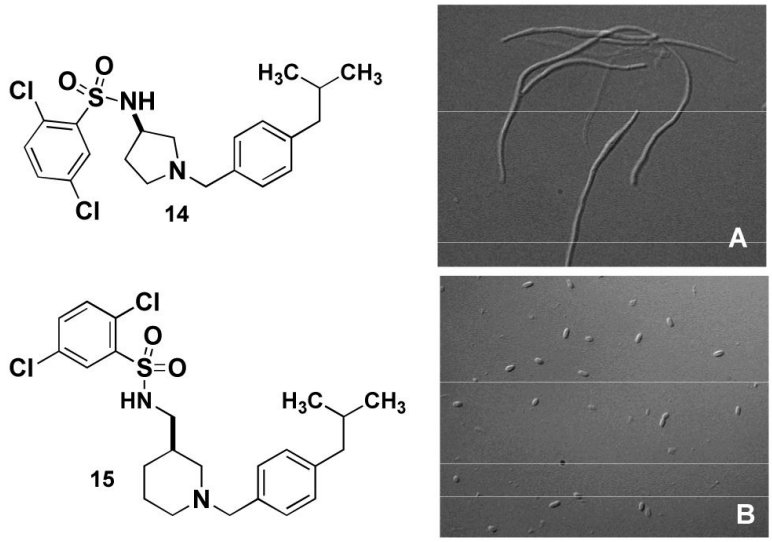
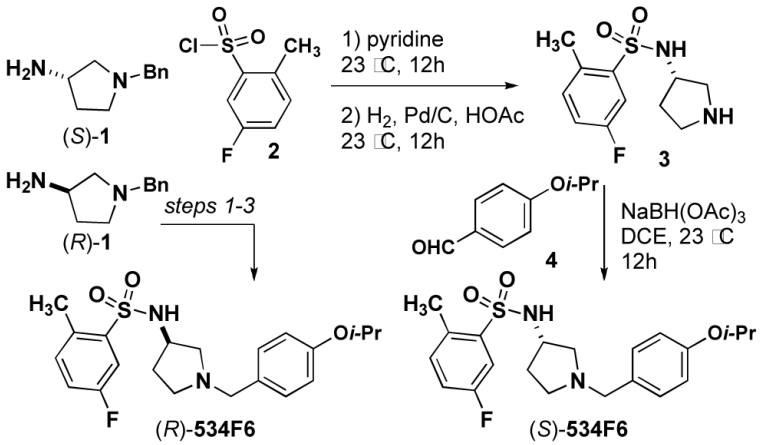
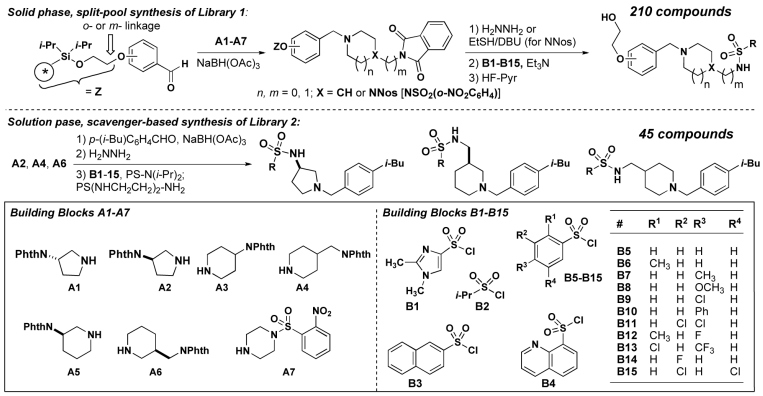


Figure 2.
E. coli DRC39 treated for 15h with A) 5 μM **14** **15**.

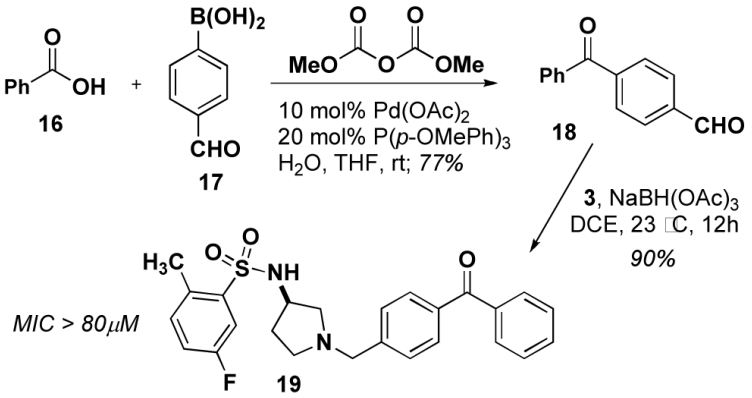


Scheme 1.
Synthesis of the enantiomers of **534F6**



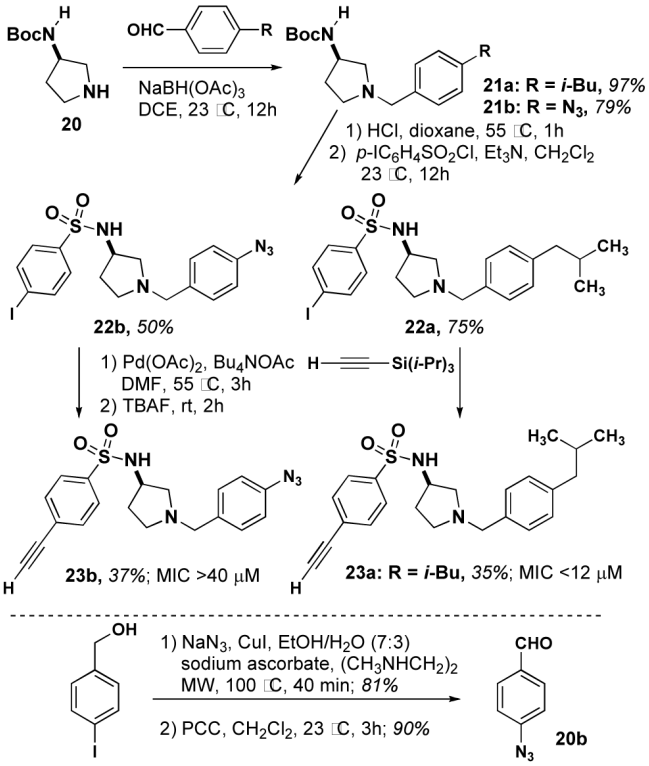
Scheme 2.

Solid- and solution-phase synthesis of analogs of 534F6.



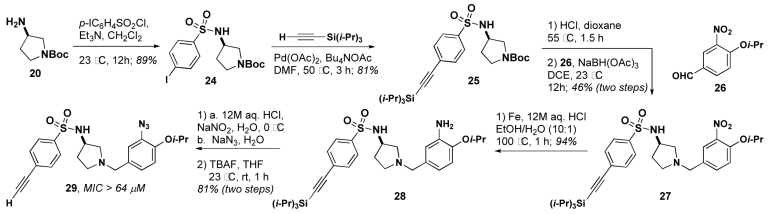
Scheme 3.

Synthesis of **19**, a benzophenone derivative of **534F6**.



Scheme 4.

Synthesis of **23a** and **23b**, alkyne-tagged derivatives of **5a**.



Scheme 5.
Synthesis of **29**, an alkyne-tagged and azide-appended derivative of **534F6**.

Table 1

GTPase Inhibition and antimicrobial activities of **5a-i**.

entry	aldehyde	Product (%yield)	FtsZ GTPase inhibition at 100μM[a], %	MIC[b], μM
1	**9**	**5a** (87)	13.7	<10
2	**10**	**5b** (90)	9.4	<10
3	**11**	**5c** (93)	12.3	20
4	**8**	**5d** (86)	14.6	>80
5	**7**	**5e** (83)	24.9	>80
6	**12**	**5f** (72)	26.9	>80
7	**6**	**5g** (83)	29.9	>80
8	**13**	**5h** (94)	31.5	>80
9	**-**	**5i**	31.9	>80

[a] 4 μM FtsZ was used in the GTPase assays (see ref. 4d); Values shown are % of the GTPase activity in DMSO-containing control reactions.

[b] AcrAB efflux pump-deficient *E. coli* DRC 39 was used (see ref. 4d).