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Synthesis and Evaluation of Quinazolines as Inhibitors of the Bacterial Cell Division Protein FtsZ

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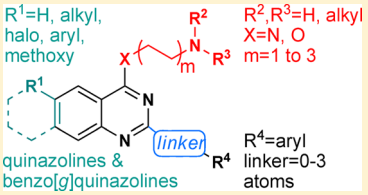
Supporting Information

ABSTRACT: The bacterial cell division protein FtsZ is one of many potential targets for the development of novel antibiotics. Recently, zantrin Z3 was shown to be a cross-species inhibitor of FtsZ; however, its specific interactions with the protein are still unknown. Herein we report the synthesis of analogues that contain a more tractable core structure and an analogue with single-digit micromolar inhibition of FtsZ's GTPase activity, which represents the most potent inhibitor of *Escherichia coli* FtsZ reported to date. In addition, the zantrin Z3 core has been converted to two potential photo-cross-linking reagents for proteomic studies that could shed light on the molecular interactions between FtsZ and molecules related to zantrin Z3.



KEYWORDS: *FtsZ, zantrin Z3, SAR, bacterial cell division*

Bacterial infections are increasingly difficult to combat as available antibiotics become less effective and bacteria evolve resistance to current therapies.[1] With more than two million illnesses annually attributed to resistant bacteria, and the diminishing timeline between drug implementation and resistance, we face a dire need for new antibiotics.[2] Oftentimes, available antibiotics are modified to prolong their use for a known target,[3,4] but a new protein or pathway offers an area of therapeutic research that has no known resistance. The bacterial divisome is a complex set of biochemical machinery that contains many proteins whose shape and function remain somewhat elusive. These proteins offer new areas of antibiotic study.[5,6] Temperature-sensitive filamenting protein Z (FtsZ) is highly conserved among bacterial species and is essential for cell division. Therefore, FtsZ is an exciting target for developing a broad spectrum antibiotic.[7] The homologous eukaryotic protein, tubulin, has been successfully targeted by small molecules such as taxol,[8,9] epothilone,[10] and many others to halt the division of cancer cells.[11−13] By analogy, an inhibitor of FtsZ could halt bacterial cell division and form the basis of a novel antibiotic.

There are many reported inhibitors of FtsZ that have shown the ability to cause filamentation in bacteria.[14−18] However, certain inhibitors were recently shown to have irreproducible activity or showed the filamentation phenotype but were not specific to FtsZ inhibition.[19,20] Two compounds were found to directly modulate FtsZ: PC190723 and zantrin Z3 (Figure 1). PC190723 has been shown to activate the GTPase activity of *Staphylococcus aureus* FtsZ.[21−23] On the other hand, zantrin Z3[24] (ZZ3, **1**) was the only compound to reliably inhibit *Bacillus subtilis* FtsZ (BsFtsZ) and *Escherichia coli* FtsZ (EcFtsZ). Although ZZ3 reliably inhibits FtsZ, its specific molecular interactions are unknown. Furthermore, very little

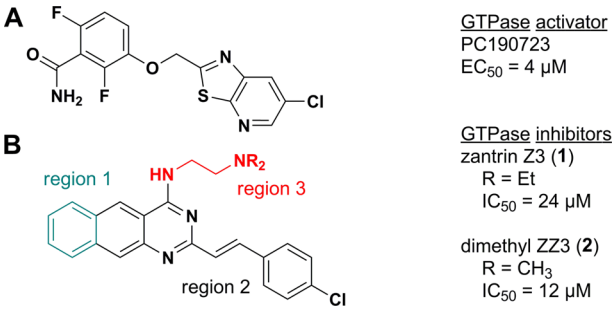


Figure 1. Small molecule modulators of FtsZ. (A) Activator PC190723.[19,23] (B) Zantrin Z3 (**1**) and its more potent analogue, dimethyl ZZ3 (**2**).[19] Three regions are identified in ZZ3 as synthetically accessible for further modification.

structural data is available for inhibitor-FtsZ interactions even though many crystal structures of the protein itself have been published. Despite only modest inhibitory activity in an absolute sense, zantrin Z3 remains the best known inhibitor of FtsZ's GTPase activity. Unlike tubulin, for which inhibitors act at nano- and picomolar concentrations, *all known FtsZ inhibitors act in the micromolar range*!

Preliminary modifications of **1** led to the discovery of **2**, which is twice as potent as its parent compound[19] and provided a benchmark for further structure−activity studies. ZZ3 has three regions where modifications are synthetically accessible. Substituted anthranilic acids were used to explore the binding interactions of the benzoquinazoline core by replacing the fused

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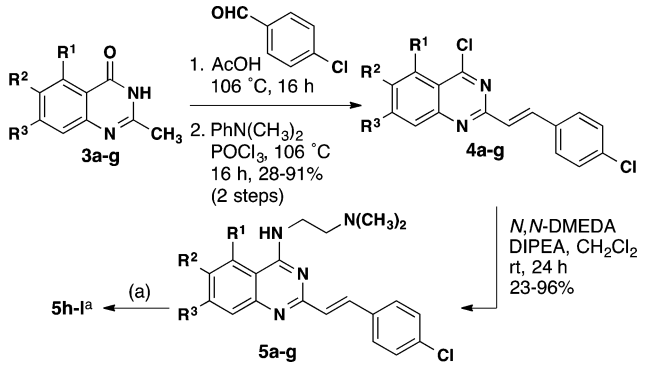
benzene ring with functional groups of varied sterics and electronics (Figure 1, region 1), while the styryl component was replaced by functional groups thought to be isosteric in region 2. Continued exploration of the amine fragment was achieved through S_NAr reactions. Further modifications to aryl halide analogues allowed for the creation of potential photo-cross-linkers to be used as small molecule probes for binding site identification of ZZ3 derivatives in FtsZ.

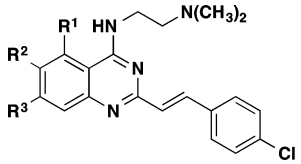
Early changes to **1** focused on reducing its size in hopes of finding a compound with greater "ligand efficiency." This term has emerged to describe the level of activity on a per-atom (or per-Dalton) basis, thus avoiding a tendency to achieve potency at the expense of drug-likeness.[25] The 4-quinazalone core was synthesized in one step from commercially available substituted anthranilic acids (**3a−g**), subjected to acid-mediated aldol condensation conditions and subsequently chlorinated with phosphorus(V) oxychloride to give quinazolines **4a−g** in moderate yields. Amination with *N*,*N*-dimethylethylenediamine and Hünig's base yielded **5a−g** in high yields (Scheme 1).[19]

Scheme 1. Synthesis of Dimethyl ZZ3 Analogues[a]



[a]Reagents and conditions: (a) R^2-B(OH)$_2$, dppf·PdCl$_2$(II), CH_3CN/H_2O (1:1), μW, 140 °C, 45 min, 12-48% yield. Compounds **5k−l** were synthesized similarly from **5g** in 24 and 20% yield, respectively.

Table 1. Region 1 Analogues of 2 and Related IC_{50}.



entry	R_1	R^2	R^3	IC_{50} (μM)
5a	H	H	H	>128
5b	CH_3	H	H	>128
5c	H	CH_3	H	24
5d	H	H	CF_3	>128
5e	H	CH_3O	CH_3O	100
5f	H	Br	H	30
5g	H	I	H	95
5h	H	phenyl	H	28
5i	H	4-pyridyl	H	30
5j	H	2-naphthyl	H	58
5k	H	2-thiophenyl	H	>128
5l	H	cyclohexenyl	H	49

Compounds **5a−g** were then subjected to an enzyme-coupled GTPase assay to measure their half-maximal inhibition concentration (IC_{50}).[26] Methyl and bromo substitution at the 6-position (R^2) exhibited the best inhibitory activity, leading to investigation of larger substituents at this position. Palladium-catalyzed cross-coupling reactions of aryl bromide **5f** afforded **5h−k** in acceptable yields.[27] Cyclohexenylboronic acid was similarly coupled in order to test a nonaromatic substituent (**5l**). Diminished reactivity of the alkenyl boronic ester required use of the 6-iodo precursor (**5g**) to boost the yield to an isolatable amount. None of the subsequent (**5h−k**) compounds showed better inhibitory activity compared to the 6-methyl analogue.

With the 6-position fixed as methyl, alteration and replacement of the styryl side chain (region 2) were examined. Compounds **8a−d** were synthesized in good yield as previously described in Scheme 1. 2-Amino-5-methylbenzoic acid was cyclized with potassium cyanate followed by sodium hydroxide and hydrochloric acid to form quinazolinedione **6**.[28] Chlorination and amination yielded **7** in good yield.[19] Boc-protection of **7**,[29] subsequent displacement of the chloride, and deprotection with trifluoroacetic acid resulted in benzylamine derivatives **8e** and **8f** (Figure 2).[30] Under the same deprotection conditions,

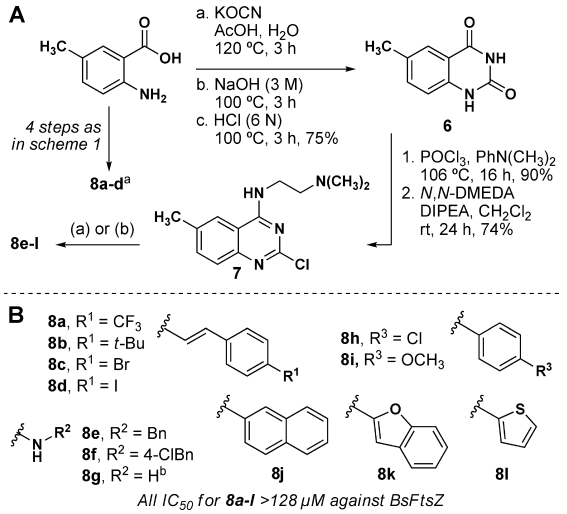


Figure 2. Synthesis and IC_{50} of compounds **8a−m**. (A) [a]Compounds **8a−d** were made in 4 steps as previously described in Scheme 1. Compounds **8e−g**: (1) Boc_2O, DMAP, Et_3N, THF, 25 °C, 24 h, 76%; (2) 4-RBnNH$_2$, DMSO, 90 °C, 16 h, 43−64%; (3) TFA, CH_2Cl_2, 0 °C to rt, 24 h, 100%. [b]Upon subjection to TFA, the 4-methoxybenzylamine derivative (not shown) yielded **8g** in 40% yield. (b) Compounds **8h−l** were synthesized by Suzuki couplings as described in Scheme 1 in 24−65%. (B) Region 2 analogues and their corresponding IC_{50}.

the 4-methoxybenzylamine derivative (not shown) yielded primary amine **8g**. Compound **7** was also subjected to Suzuki cross-couplings with dppf·PdCl$_2$(II) to give biaryl derivatives **8h−l**.[27] Unfortunately, none of the changes in region 2 demonstrated better activity than **1** or **2**.

The observation that major alterations to the styryl unit diminished activity suggested that an isosteric replacement was required. Amides, often considered isosteric to alkenes, required incorporation of a pendant carboxyl group. Fisher esterification of 2-amino-5-methylbenzoic acid yielded amino ester **9**.[31] Cyclization with the ethyl variant of Mander's reagent[32] followed by chlorination yielded **10**, which was subsequently aminated to provide esters **11a** and **11b** (Scheme 2). In order to directly compare the styryl moiety to an isostere,

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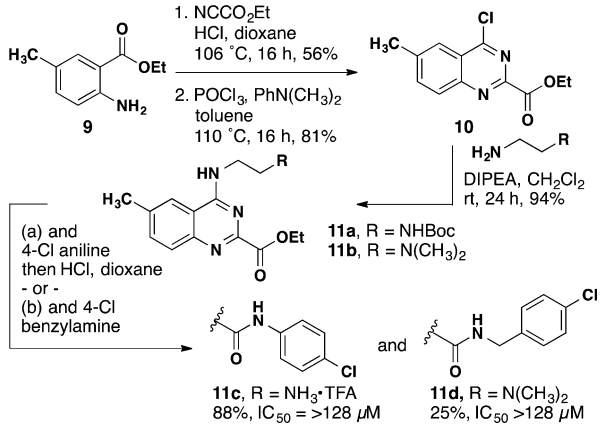
Scheme 2. Synthesis and IC_{50} of 11a–d[a]

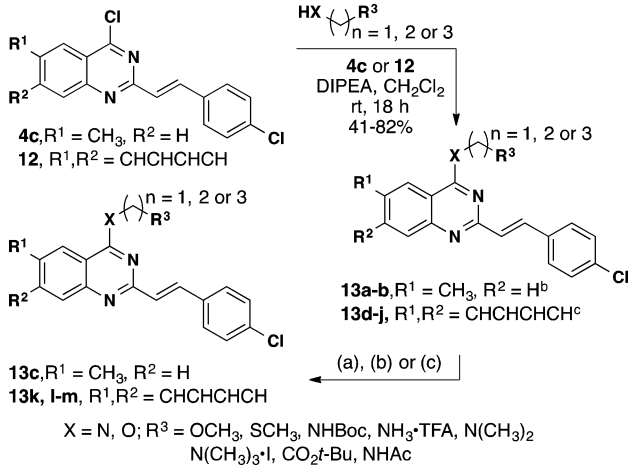


[a]Reagents and conditions: (a) **11c** (1) *n*-BuLi, 4-ClPhNH$_2$, DMPU THF, 0 °C, 20 min; **11a**, THF, rt to 80 °C, 1 h; 10%. (2) HCl/dioxane, 40 °C, 3 h, 88%. (b) **11b**, *i*-PrMgCl·LiCl, THF, rt, 12 h.

11a was converted to a benzamide analogue. The dimethylamine version of the 4-chlorobenzamide (not shown) proved difficult to isolate cleanly; fortunately, concurrent exploration of the amine fragment yielded a Boc-protected amine would reveal an ammonium salt with better inhibition than the corresponding dimethylamine (Table 2). Ester **11a** was converted to benzamide **11c** in 88% yield after deprotection. 4-Chlorobenzylamide **11d** was synthesized in 25% yield providing a less rotationally hindered analogue to parallel the styrene. Neither amide modification improved inhibitory activity relative to **1** and **2** despite incorporating the ammonium side chain in **11c**.

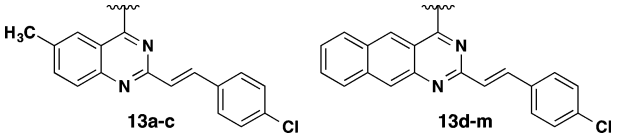
The final modifications examined involved altering or replacing the amino ethyl side chain (region 3). Compound **4c** was subjected to S_NAr reactions with nucleophiles containing single-point changes to generate analogues with different side chains at the 4-position of the quinazoline ring. Deprotection of **13b** with TFA gave **13c** in quantitative yield. These three compounds (**13a–c**, Scheme 3) suggested a

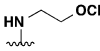
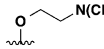
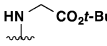
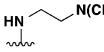
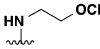
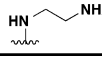
Scheme 3. Synthesis of Region 3 Analogues[a]



[a]Reagents and conditions: [b]**13a–b** were synthesized from **4c** in 41% yield each. [c]**13d–j** were synthesized similarly from **12** in 54-82% yield. (a) Compounds **13c** and **13l**: TFA, CH_2Cl_2, 0 °C to rt, 3 h, 80-100%. (b) Compound **13k**: CH_3I, CH_3OH, rt, 12 h, 21%. (c) Compound **13m**: Ac_2O, Et_3N, CH_2Cl_2, rt, 45 min, 31%.

Table 2. Region 3 Analogues of 5c and 2 and Related IC_{50}.



entry	amine	IC_{50} (μM)	entry	amine	IC_{50} (μM)
13a		>128	13g		65
13b		>128	13h		>128
13c		27	13i		>128
13d		26	13j		>128
13e		>128	13k		52
13f		>128	13l		9
			13m		>128

positively charged fragment is necessary for inhibition, although the ammonium substrate (**13c**) only showed marginal activity. Upon comparing the most potent inhibitors in the 2- and 3-ring systems (**5c** and **2**, respectively), we prepared several other substrates (Table 2, **13d–j**) by cyclizing 2-naphthoic acid and subsequently chlorinating as described in Scheme 1. Substitution with 2-(dimethylamino)ethanol resulted in **13d**, which showed moderate inhibition. Further substitutions with various amines gave **13e–j**. Deprotection of **13i** with TFA resulted in **13l**.[33] Further modification through acetylation[34] of **13l** to **13m** resulted in complete loss of inhibitory activity. While a positively charged "tail" retained some inhibitory activity (**13d** and **13g**), the quaternary ammonium side chain of compound **13k**, which was formed by alkylation with CH_3I,[35] is quite bulky and likely disrupts important binding interactions. Conversely, the benzoquinazoline ammonium substrate (**13l**) is now the first substrate to demonstrate activity in the single-digit micromolar range.

In addition to optimizing inhibitory activity through structure–activity relationships (SAR), knowledge of modifications that are tolerated has provided the structural basis for the design of photoaffinity reagents. A survey of potential cross-linkers revealed photoactivatable azides, benzophenones and diazirines, which upon irradiation could covalently link a ZZ3 derivative to FtsZ. Digestion and mass spectrometry analysis would lead to proteomic information that could be used to identify the binding-site of ZZ3. Benzophenones are historically reliable cross-linkers that are easily synthesized but run the risk of reduced binding due to the size of the phenyl ketone. The preceding observations suggested the phenylketone could be installed at the 6-position of the quinazoline core. A carbonylative Stille coupling with tributylphenylstannane and carbon monoxide readily converted aryl iodide **5g** to the corresponding benzophenone derivative (**14**, Figure 3) in 67% isolated yield. Unfortunately, this photo-cross-linker showed a loss of inhibitory activity with an IC_{50} of 85 μM. We then turned our attention to installing an aryl azide in region 3 as it is less sterically demanding and electronically similar to an aryl chloride. Aryl iodide **15**, which was synthesized from 2-aminonaphthoic acid in 4 steps as described in Scheme 1, was

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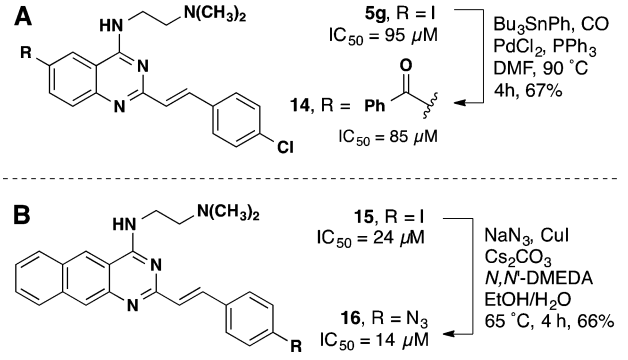

Figure 3. Synthesis and IC_{50} of photo-cross-linkers. (A) Benzophenone cross-linker **14**. (B) Azide cross-linker **16**.

subjected to copper-mediated azidonation[36] providing **16** in good yield. Compound **16** demonstrated similar inhibitory activity to **2**, which is important for ensuring specific site selection during photoactivation.

This SAR study has revealed three important features. First, the benzo[*g*]quinazoline of **1** and **2** can be replaced by the smaller quinazoline, provided that a small substituent is maintained at position 6 (e.g., **5c**, Table 1). Second, a smaller, and yet still positively charged, amino side chain provides the best activity to date (e.g., **13l**, Table 2). Finally, the 4-chlorostyryl fragment remains necessary for inhibitory activity; replacement with several different isosteres obliterates inhibitory activity. Although the IC_{50}s of the best compounds in this series are still in the micromolar range, this activity is comparable to the best inhibitors of FtsZ reported so far. More dramatic changes to the core are difficult to predict or design without direct structural data. Installing a reactive functional group for cross-linking studies may determine the binding site of this unique inhibitor. Although preliminary competition studies suggest zantrin Z3 does not bind to the GTP pocket (see Supporting Information), further illumination will require successful cross-linking studies or crystallography of the inhibitor-FtsZ complex. Although our SAR studies did not shed much light on specific interactions between the analogues and FtsZ, we discovered a derivative that could be pursued in photo-cross-linking studies as a new avenue for probing this small molecule−protein interaction. Azide **16** is under investigation as a potential photo-cross-linker.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and characterization data for all synthetic products and intermediates; FtsZ GTPase assay; GTP competition experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

FtsZ, temperature sensitive filamenting protein Z; ZZ3, zantrin Z3; BsFtsZ, *Bacillus subtilis* FtsZ; EcFtsZ, *Escherichia coli* FtsZ; AcOH, acetic acid; *N*,*N*-DMEDA, *N*,*N*-dimethylethylaminediamine; DIPEA, diisopropylethylamine; dppf, 1,1′-bis(diphenylphosphino)ferrocene; μW, microwave; IC_{50}, half-maximal inhibition concentration; μM, micromolar; *t*-Bu, *tert*-butyl; Ph, phenyl; Bn, benzyl; Boc, *tert*-butyl carbamate; TFA, trifluoroacetic acid; *n*-BuLi, *n*-butyllithium; DMPU, *N*,*N*′-dimethylpropyleneurea; Ac_2O, acetic anhydride; DMF, dimethylformamide; EtOH, ethanol; THF, tetrahydrofuran; *N*,*N*′-DMEDA, *N*,*N*′-dimethylethylaminediamine

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