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Design and synthesis of indolo[2,3-*a*]quinolizin-7-one inhibitors of the ZipA–FtsZ interaction

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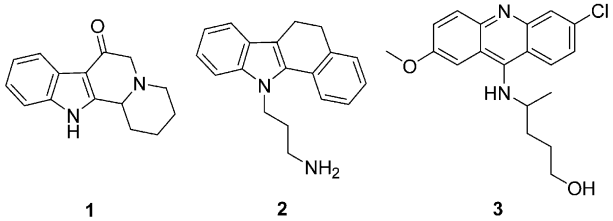
Abstract—The binding of FtsZ to ZipA is a potential target for antibacterial therapy. Based on a small molecule inhibitor of the ZipA–FtsZ interaction, a parallel synthesis of small molecules was initiated which targeted a key region of ZipA involved in FtsZ binding. The X-ray crystal structure of one of these molecules complexed with ZipA was solved. The structure revealed an unexpected binding mode, facilitated by desolvation of a loosely bound surface water.

The prokaryotic tubulin analogue, FtsZ, and the membrane-anchored protein, ZipA, are essential for *Eschercia coli* cell division. ZipA links FtsZ to the cytoplasmic membrane, forming part of the septal ring that mediates cell division. Overexpression or depletion of ZipA creates non-septate filaments, disrupting cell division in *E. coli*.[1] Accordingly, agents that inhibit the ZipA–FtsZ interaction are potentially useful in treating bacterial infections in humans and in animals. Consequently, the discovery of small-molecule inhibitors of the ZipA–FtsZ interaction was chosen as a goal of our anti-infective research program.[2]

Prior to screening of the entire corporate compound collection, the members of a set of structurally diverse, lead-like compounds were assayed for their interaction with ZipA. 1,2,3,4,12,12b-Hexahydro-indolo[2,3-*a*]quinolizin-7-one (**1**) was found to inhibit ZipA–FtsZ binding by surface plasmon resonance and in a fluorescence polarization assay.[3] The crystal structure of **1** bound to ZipA was solved at 2.0 Å resolution and showed that **1** occupies a hydrophobic cavity on the surface of ZipA necessary for the binding of the FtsZ peptide.[4] The strongest contribution to binding appears to be the hydrophobic effect, as the molecule displaces about 600 Å^2 worth of low entropy water from the site. A smaller enthalpic contribution to binding stems from both van der Waals interactions and a π-stacking interaction between the hexahydroquinolizinone ring system and a phenylalanine sidechain within the cavity.



1 **2** **3**

A series of searches of our corporate compound collection for compounds similar to **1** yielded a number of compounds of interest. Among these were the dihydrocarbazole **2**[5] and the acridine **3**[6] which, while

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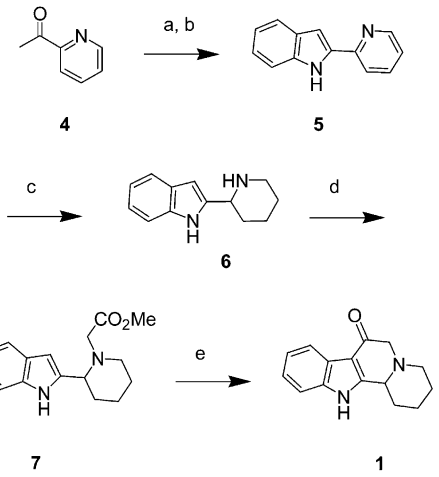
less potent inhibitors than **1**, also yielded high quality X-ray structures of their complex with ZipA. The indole nucleus of **2** bound in an essentially identical position to that of **1** but had the added feature of the amine side chain accessing a neighboring pocket of ZipA which is not involved in the binding of FtsZ. The acridine ring system of **3** also bound to a similar region of ZipA but the longer side chain not only entered the neighboring pocket but also made a hydrogen bonding interaction with a histidine residue His46. We therefore sought to prepare a series of compounds which would retain the superior binding of **1** to the shallow hydrophobic pocket but would also bear side-chains which would emulate the interaction of the second pocket displayed by **2** and **3**.[7]

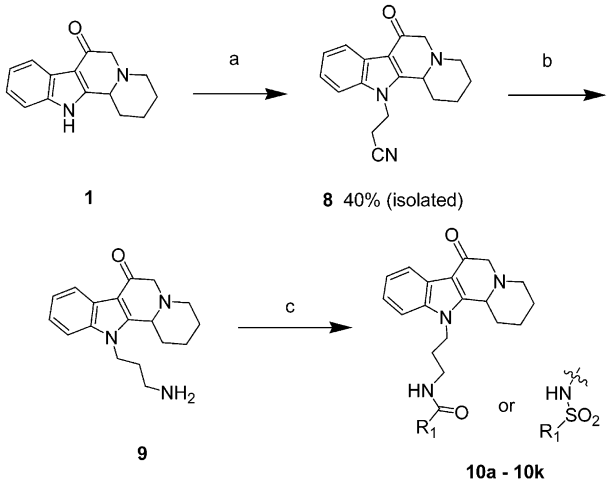
Indoloquinolizinone **1**[8] was prepared following the published route to the 9-methyl substituted 1,2,3,4,6,7,12,12b-octahydro-7-oxo-indolo[2,3-*a*]quinolizine[9] (Scheme 1). Phenylhydrazine was condensed with 2-acetyl pyridine in ethanol and the resultant hydrazone was cyclized in polyphosphoric acid at 130 °C to afford the 2-pyridin-2-yl-1*H*-indole **5**. The pyridine ring of **5** was reduced with hydrogen over platinum in acetic acid. The saturated product **6** was alkylated on nitrogen with ethyl bromoacetate and the resulting ester was cyclized in methanesulfonic acid at 55 °C to afford **1**.

Derivatives of **1** were prepared by the following methods. In Method A (Scheme 2), **1** was alkylated with acrylonitrile in the presence of lithium bis(trimethylsilyl)amide at 150 °C under microwave irradiation[10] to afford the nitrile **8**. The nitrile was reduced by hydrogenation over Raney nickel in a methanolic solution of ammonia at 50 PSI H_2. The resulting amine (**9**) was acylated in parallel with acid chlorides and sulfonyl chlorides to give amides and sulfonamides **10a–k**.[11] Products were individually purified by reverse phase HPLC and checked for purity and integrity by LC-MS.

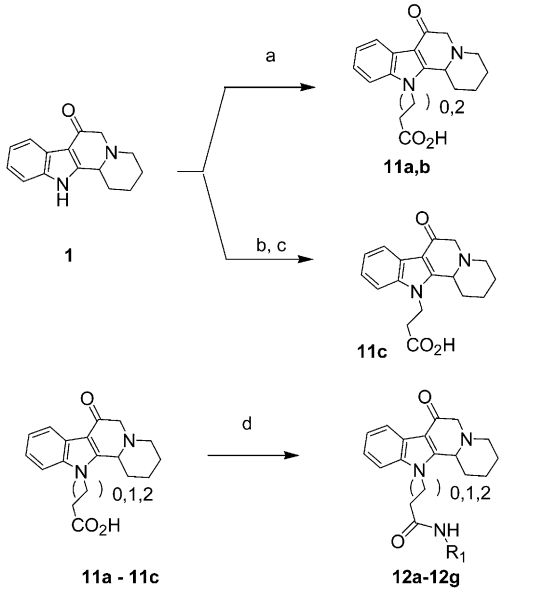
In Method B (Scheme 3), **1** was alkylated with methyl 4-bromobutyrate or with methyl bromoacetate in the presence of potassium hydroxide in DMSO at 180 °C under microwave irradiation to give the carboxylic acids **11a** and **11b** having chain lengths of 1 and 3 methylene units. The carboxylic acids were coupled with amines to form amides using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI) as a coupling reagent. Similarly, **1** was alkylated with methyl acrylate in the presence of potassium *tert*-butoxide at 150 °C under microwave irradiation and the methyl proprionate intermediate was hydrolyzed with acid to give the propionic acid derivative **11c**. In the same way this carboxylic acid intermediate was also coupled with amines to afford amides.



Scheme 1. Synthesis of Compound **1**. Conditions: (a) phenylhydrazine, EtOH; (b) PPA, 130 °C, (81% for two steps); (c) H_2, 50 psi, PtO_2, AcOH (82%); (d) methyl bromoacetate, Na_2CO_3, *n*-Bu_4N + I- (100%); (d) $MeSO_3H$, 55 °C (79%).



Scheme 2. Synthesis of Compounds **10a–k**. Conditions: (a) acrylonitrile, LiHMDS, toluene, 150 °C, 17 min, microwave irradiation; (b) H_2, 50 psi, Raney nickel, NH_3, MeOH (65%); (c) R_1COCl or R_1SO_2Cl, Na_2CO_3, CH_2Cl_2–H_2O.



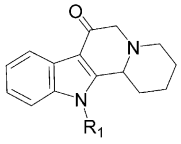
Scheme 3. Synthesis of compounds **12a–g**. Conditions: (a) methyl bromoacetate or methyl 4-bromobutyrate, KOH, DMSO, 180 °C, 2–17 min, microwave irradiation; (b) methyl acrylate, KO*t*-Bu, toluene, 150 °C, 17 min, microwave irradiation; (c) 6N HCl; (d) R_1NH_2, EDCI, DIEA, CH_2Cl_2–H_2O.

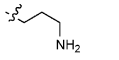
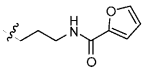
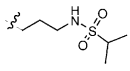
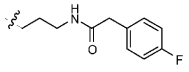
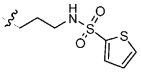
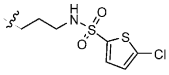
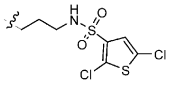
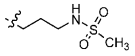
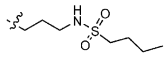
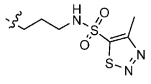
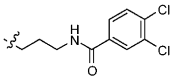
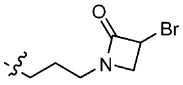
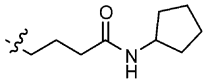
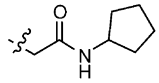
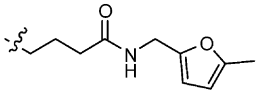
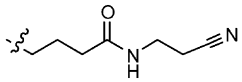
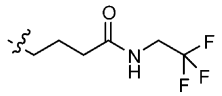
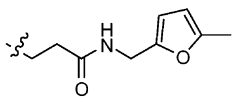
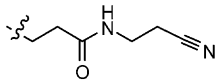
Inhibition of the ZipA–FtsZ interaction was assayed on a Biacore 3000 (Biacore Inc., Piscataway, NJ) using the peptide CEPDWLDIPAFLRKAD-NH2 immobilized on a CM5 sensor chip via thiol coupling as described by the manufacturer. This peptide is an N-terminal cysteine labeled version of the C-terminal 16 amino acid residues of *E. coli* FtsZ. A tryptophan has been substituted for tyrosine in the fifth position in order to lower the K_D of the peptide binding to ZipA approximately three-fold. The K_D in 5% DMSO was approximately 5 μM and ZipA was used at this concentration in the assays.[12] Compounds were tested at 1 mM concentration. Results for selected compounds are collected in Table 1. Compounds were also tested for antibacterial activity against a panel of Gram-positive and Gram-negative bacteria, including *E. coli*.[13] The minimal inhibitory concentration was found to be greater than or equal to 200 μM for all compounds tested against all species in the panel.

Table 1 shows that despite significant variation in the shape and length of the sidechains attached to the indoloquinolizinone scaffold, no significant improvement in the inhibition of the interaction of ZipA with the modified FtsZ 16-mer was observed. Consequently, elucidation of the structure of the ZipA/inhibitor complex was felt to be necessary in order for us to determine how best to make progress on the optimization of this series.

The compound **10b** was co-crystallized with ZipA[14] and the crystal structure was solved using the molecular replacement method (AMORE)[15] with ZipA monomer as a search model. The complex was crystallographically refined with CNS,[16] and the process of the refinement and rebuilding was repeated cyclically several times until most of the water molecules could be fitted into the density. The refined models (with no inhibitor added)

Table 1. Inhibition of ZipA–FtsZ N-terminal 16-mer association observed using an optical biosensor system[a]



Example	R_1	% Inhibition at 1 mM
1	H	39
9		47
10a		38
10b		46
10c		33
10d		25
10e		11
10f		12
10g		41
10h		25
10i		30
10j		8
10k		38
12a		24
12b		31
12c		23
12d		39
12e		21
12f		32
12g		22

[a] Conditions: Compounds were tested at 1 mM concentration in the presence of 5 μM ZipA in 5% DMSO.

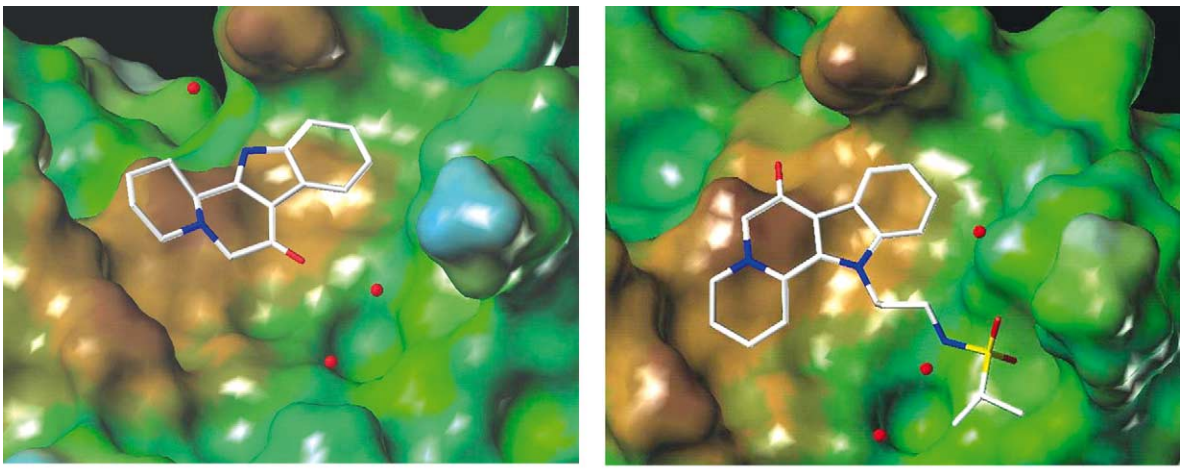


Figure 1.

were then used to calculate the 3Fo-2Fc and Fo-Fc maps, which showed clear electron density for the bound compounds. The final model for the ZipA/**10b** complex contained 2 protein molecules, one inhibitor molecule, and 250 molecules of water. No electron density that could be attributed to the inhibitor bound to the second ZipA monomer was observed. The corresponding refinement values obtained were $R_{\text{factor}} = 22.6\%$ and $R_{\text{free}} = 27.9\%$.

The crystal structure shows that, while **10b** occupies the same hydrophobic area on ZipA as **1** [Fig. 1(a)], the scaffold is inverted in the binding domain and the sulfonamide chain extends in the exact opposite direction as was expected [Fig. 1(b)]. The key hydrophobic interaction between the hydrophobic hexahydroquinolizinone and the active site Phe269 is maintained. Additionally, the sulfonamide nitrogen atom makes a hydrogen bond with a bound water molecule that is also present in the FtsZ peptide–ZipA structure.[4] The primary association of the bound water with the protein is with the backbone carbonyl of Asn247. The *iso*-propylsulfonyl group acts to shield the water molecule, isolating it from the bulk water which surrounds the protein. In retrospect, this binding mode might have been anticipated, as the FtsZ peptide binds in exactly the same region as does **10b**. Because the hydrophobic effect is non-specific and dominant for the interaction of **1** with the protein, **10b** was able to find alternate, more potent modes within the binding site.

The key interactions between **1** and the FtsZ binding domain on ZipA are hydrophobic. Hydrophobic interactions are not strongly directional and consequently compounds in this lead series bound in unexpected ways, making structure based design difficult. Even though potency is largely unaffected by the additional hydrogen bonding capacity of these chains, compound **10b** is inverted in the binding cavity. This scaffold reorientation illustrates the powerful balance between electrostatics that orient the molecule and the desolvation costs that prevent significant potency gains from those hydrogen bonds in this relatively open site. Waters are bound in both the site and the targeted region adjacent to it. Models for compound **10b** outside the site require significant rearrangement of the waters, whereas the crystal structure reveals a mode in which the water network is largely undisturbed. In addition, the molecule of water that makes a hydrogen bond with compound **10b** is mostly shielded from bulk water. This shielding should lower the local dielectric around the water, enhancing the water's binding to the protein. Thus, the water effectively becomes a structural component of the protein. Given these considerations, locking inhibitors into a specific orientation appeared possible, opening the door to structure-based potency optimization. We will report the design and synthesis of these subsequent series of ZipA inhibitors in a forthcoming publication.

Acknowledgements

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11. Na_2CO_3 (0.034 g, 0.32 mmol) in water (0.3 mL) was added to a solution of propylamine **9** (0.024 g, 0.08 mmol) in 0.3 mL CH_2Cl_2. The reaction was shaken for 1 min. Furoyl chloride (0.021 g, 0.16 mmol) was added and the reaction was mixed at ambient temperature overnight. The reaction was diluted with 2 mL CH_2Cl_2 and washed with approximately 0.8 mL aqueous $NaHCO_3$. The organic phase was removed and the solvent was evaporated. The residue was purified by RP-HPLC (Gilson Semi-Preparative HPLC system with Unipoint Software v. 1.71, Phenomenex C_{18} Luna column, 21.2×100 mm, 5 μ particle size, water–acetonitrile solvent system with added 0.05% NH_4OH buffer, at 22.5 mL/min) to give 14.3 mg (36.5 μmol) of **10a** [LC/MS (Hewlett Packard 1100 MSD with ChemStation Software, Keystone Aquasil C_{18} 50 mm×2 mm column, 5μ particle size, at 40 °C, 10 mM NH_4OAc–acetonitrile solvent system at 0.8 mL/min flow rate, 254 nm DAD detection, API-ES scanning mode, fragmentor 70 mV): *m*/*z* 392 (M + H); retention time 1.96 min].
12. *E. coli* ZipA(185-328) was passed over the immobilized peptide in Biacore's HBS-EP buffer (10 mM Hepes, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% P20) with DMSO added to a final concentration of 5%. The running buffer was the same. The sample was also run over a blank channel in which a mock thiol coupling with no peptide had been performed and the signal from the blank was subtracted from that with the peptide. Compounds were added to ZipA, filtered, and then binding to the peptide was determined. Percent inhibition is calculated from the average resonance signal for the sensogram collected with inhibitor present and the sensogram collected in the absence of inhibitor.
13. 'Methods for Dilution of Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically', Approved Standards: M7-A5, Vol. 20, National Committee for Clinical Laboratory Standards, Wayne, PA. Bacterial species in the panel included *S. aureus* (ATCC 29213), *E. faecalis*, *S. pneumoniae*, *B. subtilis*, *H. influenzae* (ATCC 49247), *M. catarrhalis*, and *E. coli*.
14. 2 mM of **10b** was added to ZipA (20 mg/mL), so that the final mixture contained 2:1 compound versus the protein. Co-crystals of approximate dimensions 0.5×0.7×0.15 mm^3 were grown by vapor diffusion from 2 μL droplets consisting of 1 μL of protein solution and 1 μL of precipitant (20% PEG 6000, 0.1 M MES pH 6.0). To produce high quality crystals, streak seeding with the native crystals as seeds was applied. Crystals belonging to space group $P2_1$ (a = 52.45 Å, b = 38.85 Å, c = 71.52 Å, b = 106.3 deg) containing two ZipA molecules per asymmetric unit were obtained. Prior to data collection, the crystals were flash cooled in liquid nitrogen at 100 K using 25% ethylene glycol as a cryoprotectant. The 2.1 Å data were collected in house using RAXIS IV imaging plate system. The data were again integrated using DENZO and then scaled and merged with SCALEPACK.[17]
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