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Small Molecule DnaK Modulators Targeting the β -Domain

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Abstract

The molecular chaperone DnaK is essential for the survival of bacterial pathogens in the hostile environment of the host. Hence, it is in principle a promising target for drug design but for which no current inhibitors are available apart from certain antimicrobial peptides. To this end, we have screened libraries of small molecules for their ability to interact with the substrate-binding domain of DnaK. The most promising hit from the screen was synthesized and along with its analogs subjected to further assays to determine their binding affinity and ability to interfere with bacterial growth. This work resulted in the identification of a number of compounds that bind with submicromolar affinity and capable of inhibiting *Yersinia pseudotuberculosis* growth more effectively than the previously characterized peptides.

Keywords

allostery; chaperone; DnaK; drug discovery; NMR

Chaperones provide crucial cellular functions across all domains of life (1). Hsp70 homologs are involved in such essential processes as protein folding, translocation, degradation, and even gene expression (2–7). The multiple homologs and the wide array of co-chaperones for Hsp70 underscore their importance and functional diversity (1,8,9). Hsp70s, including DnaK, the *Escherichia coli* Hsp70 (10–14) investigated here, have been extensively studied and have greatly increased our understanding of allostery. DnaK is composed of two structural domains (Figure 1A and B), an N-terminal nucleotide-binding ATPase domain (NBD) and a C-terminal substrate-binding domain (SBD), which can be further subdivided into an all β -strand region that binds substrate (β -domain) and an α -helical lid region that can close over bound substrate (11,12,15). DnaK cycles between two main states: an ATP-bound state with low affinity for substrate that is characterized by a tight interaction of the two domains, and an ADP-bound state that has high substrate affinity with less interaction between the domains (16–18). Structural and biochemical data have shown that residues from both domains (including loop L2,3 of the β -domain), as well as from the linker between them are required for communicating the nucleotide or substrate occupancy to the other respective domain (10,12,14,19,20).

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Chaperones are essential for survival under myriad stress conditions. Of particular interest for drug discovery are the essential roles of Hsp70 in pathogenic microorganisms growing intracellularly in a host (21,22), and in cancer cells proliferating under the stressful environment of a tumor (23). Recently, there has been much progress in targeting the Hsp90 class of chaperones with potential anti-cancer compounds (24,25). With the experience of protein kinase inhibitors in mind (26), screens for Hsp70 inhibitors have focused on compounds targeting the ATP-binding site. While this approach has been successful for Hsp90 (27), the binding of ATP to Hsp70 is drastically different from that of kinases (28). In protein kinases, most inhibitors take advantage of a hydrophobic pocket and a set of highly conserved hydrogen bond interactions available at the site. In contrast, the ATP-binding site of Hsp70 is rather hydrophilic, and most interaction energy between the nucleotide (ATP or ADP) and the protein is derived from the phosphate groups (29,30). As a consequence, high throughput screening approaches targeting the ATP-binding pocket of Hsp70s are unlikely to produce viable hits, although a recent structure-based design study was successful in identifying submicromolar affinity binders (31).

We have taken the novel approach of directly targeting the SBD of DnaK. Previous work on a class of antimicrobial peptides from insects that target DnaK via the SBD helps to validate our approach (32–35). These SBD-targeting peptides show significant efficacies against a series of clinical pathogens, including fluoroquinolone-resistant strains (35). Furthermore, at least one of the peptides did not bind to human Hsp70 or alter its function (32), suggesting that effective and safe antimicrobial drugs could arise from targeting the SBD of DnaK.

Using a construct of DnaK corresponding to the β -domain only (residues 393–507) for which solution structures with and without bound peptide substrate are known (36,37) and an NMR-based screen, we have identified a scaffold compound capable of binding the β -domain. Our results led to the identification of a compound series that occupies a binding pocket near a proposed allosteric site of the SBD, loop L2,3 (Figure 1A and B). We report structure–activity relationship (SAR) studies, *in silico* docking, isothermal titration calorimetry (ITC), and antimicrobial activity of the most promising compounds of the series. Given the dire need for new classes of antibiotics, we hope that these compounds can eventually stimulate further research in this area may lead to clinically useful drugs.

Materials and Methods

Protein expression and purification

The gene coding for the *E. coli* DnaK substrate-binding (β) domain (393–507) was amplified by PCR and subcloned into pET21a using the NdeI and BamHI cloning sites. The resulting protein contains 17 extra amino acid residues (MGSSHHHHHGLVPRGS) at the N-terminus. The protein was expressed in the *E. coli* strain BL21(DE3) pLysS and purified using Ni^{2+} affinity chromatography. Uniformly, ^{15}N -labeled DnaK was produced by growing the bacteria in M9 minimal media containing $^{15}\text{NH}_4\text{Cl}$ as the sole nitrogen source. The NMR samples were dissolved in 20 mM sodium phosphate buffer (pH 7.5) containing 90%/10% ($\text{H}_2\text{O}/\text{D}_2\text{O}$) or 99.5% D_2O . DnaK, human Hsp70, DnaJ, and GrpE were purchased from Stressgen (Ann Arbor, MI, USA).

Peptides

Apidaecin1a (GKPRPYSPRPTSHPRPIRV), pyrrolicorin (VDKGSYLPRP-TPPRPIYNRN), drosocin (GNNRPVYIPGPRPPHPRI), and the model substrate NRRLLTG were synthesized by the Medical College of Wisconsin (Milwaukee, WI, USA).

NMR-based screening

Spectra were acquired on a 600-MHz Bruker Avance spectrometer (Bruker BioSpin Corp., Billerica, MA, USA) equipped with a TCI cryoprobe. Ligand binding was monitored by comparing the aliphatic region of 1D ^{13}C -filtered ^1H NMR spectra of 20 μM protein solutions (containing 90%/10% $\text{H}_2\text{O}/\text{D}_2\text{O}$ or 99.5% D_2O buffered with 20 mM sodium phosphate, pH 7.5; $T = 300\text{ K}$) in the presence or absence of 80 μM mixtures of 10 compounds, and then individual compounds from mixtures that caused significant perturbations in the spectra were identified.

The binding affinity of each ligand was initially estimated with a single titration point according to the following equation:

$$K_d = \frac{[T_o] \times (1 - p) \times ([L_o] - [T_o] \times p)}{[T_o] \times p}$$

where T_o and L_o are total concentration of target protein and ligand, respectively. The parameter p represents the fractional population of bound versus free species at equilibrium, which for fast exchanging ligands is measured as:

$$p = \frac{\delta_{\text{obs}} - \delta_{\text{free}}}{\delta_{\text{sat}} - \delta_{\text{free}}}$$

where δ_{obs} is the observed protein chemical shift during the titration, and δ_{free} and δ_{sat} are the chemical shifts for the protein in the fully unbound and fully bound (saturated) states, respectively.

Isothermal titration calorimetry

Titration were performed using a VP-ITC instrument from MicroCal (Northampton, MA, USA). Full-length DnaK or β -domain were used at 10–100 μM in 20 mM sodium phosphate buffer (pH 7.4) and 5% dimethyl sulphoxide (DMSO). Compounds were used at 15-fold excess in the same buffer. Data were analyzed using MicroCal Origin software provided by the ITC manufacturer (MicroCal, Northampton, MA, USA).

Minimum inhibitory concentration measurements

Escherichia coli cultures were seeded in 96-well plates by mixing 90 μL aliquots of an $\text{OD}_{600} = 0.001$ culture with 10 μL of compound solutions at various concentrations and grown overnight. Optical density (OD) at 530 nm was measured using a Perkin Elmer Victor 2 Multilabel plate reader (PerkinElmer, Shelton, CT, USA). Testing was carried out in 50% Mueller-Hinton II broth (BBL) in H_2O . Wild-type *Yersinia pseudotuberculosis* YP126 (38) was grown overnight at 25 $^{\circ}\text{C}$ and approximately 10^6 bacteria were inoculated into each 1 mL of broth containing serial dilutions of the compound to be tested. After overnight incubation at 40 $^{\circ}\text{C}$, the OD was read at 600 nm and compared with the growth without inhibitor. The minimal inhibitory concentration (MIC) was read as the concentration producing greater than fourfold reduction in final OD_{600} .

Chemical synthesis

To a stirred solution of free amine (1.0 equiv.) and Et_3N (2.0 equiv.) in 5 mL dichloromethane (DCM) was added a solution of thiophene-2-carbonyl chloride (1.1 equiv.) in 5 mL DCM at $-30\text{ }^{\circ}\text{C}$ (Scheme 1). The resulting solution was stirred for 1 h and then allowed to warm to room temperature. After removal of the solvent, the residue was purified

by flash column chromatography in hexane-ethyl acetate or DCM-methanol to provide the correspondent product (yield 75–95%). Synthesis scale was 100 mg, for which about 0.5 mmol of starting material was required.

BI-88D5

N-(naphthalen-1-ylmethyl)thiophene-2-carboxamide ¹H NMR (CDCl₃, 600 MHz): δ 8.17 (s, 1H), 7.87 (s, 1H), 7.73 (s, 1H), 7.46 (m, 6H), 6.99 (s, 1H), 6.39 (s, 1H), 5.01 (s, 2H); HRESI-TOF-MS: calcd for C₁₆H₁₃NOS 268.0791 [M + H]⁺, found 268.0796.

BI-88D7

N-(1H-indol-5-yl)thiophene-2-carboxamide ¹H NMR (CDCl₃, 600 MHz): δ 8.19 (s, 1H), 7.92 (s, 1H), 7.71 (s, 1H), 7.62 (s, 1H), 7.52 (s, 1H), 7.33 (m, 2H), 7.23 (s, 1H), 7.12 (s, 1H), 6.54 (s, 1H); HRESI-TOF-MS: calcd for C₁₃H₁₀N₂OS 243.0587 [M + H]⁺, found 243.0592.

BI-88D9

N-(quinazolin-2-yl)thiophene-2-carboxamide ¹H NMR (CDCl₃, 600 MHz): δ 9.35 (s, 1H), 7.90 (m, 3H), 7.61 (m, 4H), 6.89 (s, 1H); HRESI-TOF-MS: calcd for C₁₃H₉N₃OS 256.0539 [M + H]⁺, found 256.0545.

BI-88D10

N-(benzo[*b*]thiophen-7-ylmethyl)thiophene-2-carboxamide ¹H NMR (CDCl₃, 600 MHz): δ 8.35 (s, 1H), 7.82 (m, 5H), 7.47 (s, 1H), 6.97 (s, 1H), 5.30 (s, 2H); HRESI-TOF-MS: calcd for C₁₄H₁₁NOS₂ 274.0355 [M + H]⁺, found 274.0361.

BI-88F6

N-(3,4-dichlorobenzyl)thiophene-2-carboxamide ¹H NMR (DMSO-*d*₆, 600 MHz): δ 9.11 (s, 1H), 7.79 (m, 2H), 7.60 (d, *J* = 7.2 Hz, 1H), 7.56 (s, 1H), 7.30 (d, *J* = 7.2 Hz, 1H), 7.16 (s, 1H), 4.43 (s, 2H); HRESI-TOF-MS: calcd for C₁₂H₉Cl₂NOS 285.9855 [M + H]⁺, found 285.9861.

BI-88F7

N-(2,6-dichlorobenzyl)thiophene-2-carboxamide ¹H NMR (DMSO-*d*₆, 600 MHz): δ 9.11 (s, 1H), 7.79 (m, 2H), 7.51 (s, 1H), 7.35 (m, 2H), 7.17 (s, 1H), 4.44 (s, 2H); HRESI-TOF-MS: calcd for C₁₂H₉Cl₂NOS 285.9855 [M + H]⁺, found 285.9860.

BI-88F8

N-(2-hydroxybenzyl)thiophene-2-carboxamide ¹H NMR (DMSO-*d*₆, 600 MHz): δ 8.96 (s, 1H), 8.09 (s, 1H), 8.03 (s, 1H), 7.78 (s, 1H), 7.39 (m, 2H), 7.28 (m, 2H), 7.14 (s, 1H), 4.42 (s, 2H); HRESI-TOF-MS: calcd for C₁₂H₁₁NO₂S 232.0438 [M – H][–], found 232.0440.

BI-88F9

N-(3,5-dichlorobenzyl)thiophene-2-carboxamide ¹H NMR (DMSO-*d*₆, 600 MHz): δ 8.67 (s, 1H), 7.79 (s, 1H), 7.75 (s, 1H), 7.51 (m, 2H), 7.38 (s, 1H), 7.11 (s, 1H), 4.66 (s, 2H); HRESI-TOF-MS: calcd for C₁₂H₉Cl₂NOS 285.9855 [M + H]⁺, found 285.9862.

BI-88F10

(4-(2-hydroxyethyl)piperazin-1-yl)(thiophen-2-yl)methanone ¹H NMR (DMSO-*d*₆, 600 MHz): δ 7.75 (s, 1H), 7.38 (s, 1H), 7.11 (s, 1H), 3.61 (m, 4H), 3.51 (m, 2H), 2.42 (m, 6H); HRESI-TOF-MS: calcd for C₁₁H₁₆N₂O₂S 241.1005 [M + H]⁺, found 241.1012.
Commercially available **BI-88B12** analogs were purchased from Chembridge Corp., (San

Diego, CA, USA). Structures for purchased and synthesized compounds are shown in Table 1.

Results and Discussion

Chemical screening and *in silico docking studies*

We have assembled a scaffold library composed of ~4000 compounds that were selected based upon their anticipated use as building blocks or scaffold components of further optimized molecules. The library has been acquired from three different sources (39), and chemical structures of the compounds have been deposited into PubChem (<http://pubchem.ncbi.nlm.nih.gov/>). In addition to this small molecule selection, we have also included a collection of 602 natural products (MicroSource, Gaylordsville, CT, USA) that was also screened by NMR.

To screen such a relatively high number of compounds with minimal protein and spectrometer time, we used simple 1D ^1H NMR experiments of the protein measured in the presence and absence of mixtures of potential ligands, observing the aliphatic region of the spectra ($\delta < 1$ ppm). This experiment requires relatively low protein concentrations (10–20 μM) and can be conducted in a relatively short time (30 min to 1 h by using a spectrometer operating at 600 MHz ^1H frequency and equipped with a cryoprobe). Briefly, chemical shifts were monitored in the presence and absence of mixtures of 10 compounds at a fourfold excess to the protein. Mixtures causing spectral perturbation above our threshold of 0.08 ppm were deconvoluted. The compound **BI-88B12** emerged as a hit from the screen (Figure 1C). Selected commercially available **BI-88B12** analogs were purchased and then qualitatively assessed for their ability to bind the β -domain of DnaK by the magnitude of the chemical shifts induced upon complexation on the $^{13}\text{C}^\delta, ^1\text{H}^\delta$ resonances of Leu484 (1:10 protein to ligand ratio, at 70 μM protein concentration) by 2D [^{13}C , ^1H] correlation spectra. Hit compounds with estimated K_d values of 200 μM or less were selected for further SAR studies including K_d determination by ITC. These data were further analyzed to design additional analogs for chemical synthesis ('Materials and Methods').

Based upon the NMR experiments and assays described below on the **BI-88B12** analogs three compounds of interest emerged. **BI-88B12** was the initial hit and causes the greatest NMR chemical shift change upon binding and was thus used as a comparison for all other compounds. **BI-88D7** (synthesis described in 'Materials and Methods') was the strongest binder as determined by ITC. The commercially available **BI-88E3** was the most potent inhibitor of microbial growth and thus the bulk of the discussion here will focus on **BI-88B12**, **BI-88D7**, and **BI-88E3**. Findings for all other compounds to come through the NMR screen with an estimated $K_d < 200 \mu\text{M}$ are summarized in Table 1.

After the initial identification of **BI-88B12**, chemical shift mapping studies with $^{13}\text{C}/^{15}\text{N}$ -labeled protein were performed. Based upon these experiments, we discovered that **BI-88B12** and its analogs do not bind to the substrate-binding pocket but rather to a pocket located around loop L2,3 on the opposite side of the SBD (Figures 1A, B and 2; Figure S1). Molecular docking studies then revealed the presence of a deep pocket centered between residues Leu484 and Pro419, which likely constitutes the binding site of these compounds (Figure 2). Residues in this region were previously reported to be essential for the allosteric communication between the substrate binding and the ATPase domains (10,12,14,19,20). Gold docking software (40) was used to predict the binding poses of compounds **BI-88B12** and **BI-88D7** near residues Leu484 and Pro419 (pdb: 1DKX) (41).

Most interestingly, residues in this pocket are among the least conserved between bacterial and mammalian Hsp70s (Figure 1D), furthering the potential of our compounds as non-toxic

antibiotics. Figure S2 further shows that the conservation of this site among bacterial DnaKs reaches across many other important bacterial pathogens, both Gram-negative and Gram-positive while differing significantly from mammalian sequences in this region.

Molecular modeling studies suggest that, despite the shared thiophene, the compounds might adopt two different positions. Both maintain a hydrogen bond with Gly482 but while **BI-88B12** places its thiophene ring outside the pocket, this moiety is inserted into the pocket by **BI-88D7**. For **BI-88B12**, the fluorobenzene group is inserted deep into the hydrophobic pocket as evidenced by the strongest perturbation of residues there. With its thiophene group in the pocket, the indole ring of **BI-88D7** is also able to interact with Asp481, located further out near the edge of the pocket. **BI-88D7** causes equal perturbation of both Leu484 and Asp481, possibly because of its larger size that allows it to bridge both residues. These results may explain the much lower K_d of **BI-88D7** as determined by ITC.

Isothermal titration calorimetry

Isothermal titration calorimetry was performed on the most promising binders from the first set of **BI-88B12** analogs (Figure 3C). These data were used in the design of a number of compounds which were not commercially available, including **BI-88D7**. As seen in Figure 3B and Table 1, the designed compounds exhibit increased binding affinity for DnaK by an order of magnitude compared with **BI-88B12**. The K_d s determined by ITC for **BI-88B12** and the earliest derivatives tested were in the range of ~10–70 μ M while many of the optimized compounds exhibited single digit to submicromolar range dissociation constants.

Using ITC, we also investigated how binding of a substrate mimic peptide or ATP affected the binding of a compound and vice versa. This thermodynamic information may help explain how the compounds bind and how they may be affecting allosteric communication between the domains. In the context of the full-length protein, which includes the NBD, SBD, and the α -helical lid domains as shown in Figure 1, the substrate mimic peptide was found to bind stronger by an order of magnitude if the protein was presaturated with compound (Table 2) with the increased affinity mostly entropically driven.

This is possibly because of bound compound causing hydrophobic surface area in the substrate-binding pocket to become exposed. Furthermore, the SBD is known to be highly dynamic (36), and if compound binding is able to reduce the conformational exchange of the SBD, the peptide should then be able to bind without the normal entropic penalty of binding to a highly dynamic apo-SBD. The priming of the SBD by these compounds may be an example of the model of Swain *et al.* (12), in which prevention of docking between the two domains by the interdomain linker, specifically residues 389–392 (VLLL), would leave the β -domain in a state of high substrate affinity.

Minimal inhibitory concentration

All compounds were first tested for their ability to limit cell growth of *E. coli*. Compounds were tested initially in mixtures of five over a range of concentrations. Mixtures that restricted growth were de-convoluted to determine efficacies of individual compounds (Figure 4A). Compound **BI-88E3** demonstrated the greatest ability to inhibit growth, followed closely by **BI-88D7** (Table 3). Both compounds were found to have similar MICs to apidaecin1a, the most effective peptide tested in this study (Table 3). The substrate mimic peptide NRLLLTG was not inhibitory in this assay up to a concentration of 100 μ M. We also found no inhibitory effect under our experimental conditions from pyrrocoricin, a result at odds with previous results (42). This difference may be possible because of the fact that we are using a different strain of *E. coli* as the peptide displayed tight binding by ITC.

As DnaK is required for growth under heat shock conditions, we tested the compounds' ability to inhibit growth of *E. coli* at 42 °C. Only apidaecin1a showed any increased efficacy at the higher temperature, and its MIC value was about twofold better (80 μ M at 37 °C to 40 μ M at 42 °C). To determine whether these compounds have potential against an intracellular pathogen, we conducted preliminary sensitivity testing using *Y. pseudotuberculosis* (Figure 4B; Table 3). Compound **BI-88E3** demonstrated a greater than 20-fold decrease in the MIC when tested over a temperature range of 25–40 °C. These results were consistent with inhibition of DnaK, as loss of DnaK function in bacteria is known to produce a temperature-dependent effect on growth.

Conclusions

By attempting to target the substrate-binding β -domain of DnaK, we have discovered compounds that bind not in the substrate-binding pocket but rather in a cavity involved in allosteric communication between protein domains. This series of compounds will help us gain further insight into the allosteric mechanism of DnaK by determining more precisely how compound binding affects substrate peptide and possibly ATP binding to DnaK and inhibit cell growth. Our preliminary data (not shown) indicate that many of the compounds inhibit both the ATPase and protein refolding activities of DnaK but more work is necessary. More importantly, we have identified a compound, **BI-88E3**, which is capable of inhibiting growth of *E. coli* and is even more potent than apidaecin1a against *Y. pseudotuberculosis*. Further work is needed to fully characterize the mode of action of the compounds in the presence of co-chaperones and unfolded proteins rather than a model peptide. However, we believe that the discovered compounds with submicromolar affinity for DnaK have the potential to be both biochemical tools for further understanding DnaK function and lead compounds for developing sorely needed antibacterial drugs with a novel target.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

DCM	dichloromethane
ITC	isothermal titration calorimetry
MIC	minimal inhibitory concentration
NBD	nucleotide-binding domain
SBD	substrate-binding domain

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Figure 1.

(A) Cartoon model of DnaK. Nucleotide binding domain (NBD) is shown in green. The substrate-binding domain (SBD) is composed of the β -domain (violet) and the α -helical lid (red). (B) Ribbon diagram of DnaK [1DKX³⁴] (blue and red) and 1HPM (43) (green) with key compound interacting residues identified. In addition, shown as a surface is the proposed binding site of the compounds. The linker between the ATPase and substrate-binding domains is added for clarity. (C) NMR spectra of the β -domain of DnaK. Black, apo; blue, β -domain + peptide; green, β -domain + compounds. In the presence of peptide, a new peak occurs at -0.15 ppm. In the presence of **BI-88B12**, the I483 peak shifts to 0.24 ppm, while it remains unchanged in the presence of peptide. (D) Sequence alignment of the predicted binding pocket of compounds.

Figure 2. Chemical shift and *in silico* docking studies. (A) Docked **BI-88B12** in the predicted binding pocket in the isolated β -domain. The colors correspond to the chemical shifts upon ligand binding shown in the graph in (B). (C, D) Docked **BI-88D7** and its corresponding chemical shift changes upon binding.

Figure 3.
Representative ITC titrations. (A) **BI-88B12** to β -domain. (B) **BI-88E3** to β -domain. (C) **BI-88D7** to full-length DnaK. (D) NRLLLTG to fDnaK saturated with **BI-88F10**.

Figure 4.

(A) MIC of inhibitors tested against *Escherichia coli* at 37 °C. Apidaecin1a is the most effective inhibitor of *E. coli* growth. Black, Apidaecin1a; diagonal cross hatching, **BI-88E3**; gray, **BI-88D7**; stippling, **BI-88B12**. (B) MIC of inhibitors tested against *Yersinia pseudotuberculosis* at 37 °C. Black, Apidaecin1a; horizontal cross hatching, **BI-88E3**; gray, **NRLLLLTG**.

Scheme 1.

Preparation of the thiophene-2-carbonyl amide derivatives.

Table 1

SBD or FL following values in the ITC column indicates to which protein the compound was titrated

BI88	Structure
B12	<chem>O=C(NCc1ccccc1F)c2cc3sc(C(=O)Nc4ccc5c(c3)ccc5[nH]4)cc2</chem>
E3	<chem>O=C(NCc1ccc(C)cc1)c2cc3sc(C(=O)Nc4ccc5c(c3)ccc5[nH]4)cc2</chem>
D7	<chem>O=C(NCc1ccc2c(c1)ccc[nH]2)c3cc4sc(C(=O)Nc5ccc6c(c4)ccc6[nH]5)cc3</chem>
F6	<chem>O=C(NCc1ccc(Cl)c(Cl)c1)c2cc3sc(C(=O)Nc4ccc5c(c3)ccc5[nH]4)cc2</chem>

B188	Structure
F8
F10
B1
D10
B3
B4

B188

Structure

F7

F9

D5

D9

B188	Structure
C5	<chem>BrC1=CC=CC=C1CN(C1=CC=CS1)C(=O)N</chem>

Table 2

ITC data for peptide, compound or ATP after being saturated with compound, peptide, or compound, respectively

	K_d (μM)	dH (cal/mol) approximately	dS [cal/(mol·T)]
NRLLLTG	33.4 ± 10.8	-10^4	$-20-0$
Peptide post- BI-88F6	0.6	-10^2	$0-20$
Peptide post- BI-88F8	1.2	-10^3	$0-20$
Peptide post- BI-88F10	7.9	-10^3	$0-20$
Peptide post- BI-88D7	2.2	-10^2	$20-40$
Peptide post- BI-88B1	0.2	-10^3	$20-40$
ATP	0.01	-10^3	$0-20$
ATP post- BI-88D10	2.5	-10^5	>40

Table 3

Sequences, structures and ATPase, MIC, and ITC data for peptides and compounds with best bacterial growth inhibition profiles

Compound	Structure	MIC (μM)	ITC K_d (μM)
Apidaecin1a	GNNRPVYIPGPRPPHPRI	80 ^a 40 ^b 50 ^c	0.6 (FL)
Pyrrhocoricin	VDKGSYLPRPTPPRIYNRN	>2000 ^a	7.6 (FL)
Drosocin	GKPRPYSPRPTSHPRPIRV	150 ^a	1.1 (FL)
Substrate mimic	NRLLLTTG	>2000 ^a >100 ^c	57.2 $\pm$ 28.5 (SBD) 33.4 (FL)
BI-88E3		250 ^{a,b} 400 ^d 25 ^c <12 ^e	25 (SBD)
BI-88D7		400 ^{a,b} 400 ^{c,d,e}	4.9 (SBD) 12.7 (FL)

^a *Escherichia coli* at 37 °C;

^b *E. coli* at 42 °C;

^c *Yersinia pseudotuberculosis* at 37 °C;

^d *Y. pseudotuberculosis* at 22 °C;

^e *Y. pseudotuberculosis* at 40 °C.