Synthesis, Structure and Antibacterial Activity of Potent DNA Gyrase Inhibitors: N’-Benzoyl-3-(4-Bromophenyl)-1H-Pyrazole-5-Carbohydrazide Derivatives

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Abstract
A total of 19 novel (3a–3s) N’-benzoyl-3-(4-bromophenyl)-1H-pyrazole-5-carbohydrazide analogs were designed, synthesized, and evaluated for biological activities as potential DNA gyrase inhibitors. The results showed that compound 3k can strongly inhibit Staphylococcus aureus DNA gyrase and Bacillus subtilis DNA gyrase (with IC50 of 0.15 µg/mL and 0.25 µg/mL, respectively). Structure-activity relationships were also discussed based on the biological and docking simulation results.

Introduction
Over the past decade, bacterial DNA gyrase has drawn much attention as a selected target for finding potent antibacterial agents [1–6]. DNA gyrase is mainly inhibited by quinolones and coumarins, some of which are widely used for the treatment of bacterial infectious diseases (e.g., ciprofloxacin) [7–11]. However, because of side effects, no pharmacologically useful drug has so far been derived from the coumarins. Recently, multidrug-resistant Gram-positive bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA), penicillin-resistant Streptococcus pneumoniae (PRSP), and vancomycin-resistant enterococci (VRE), have become a serious medical problem [12]. Since most of these multidrug-resistant bacteria are also quinolone-resistant ones, it is important to find a new class of DNA gyrase inhibitors to solve this problem.

Many pyrazole derivatives are well acknowledged to possess a wide range of antibacterial bioactivities [13–18]. Tanitame et al. [12] have found compound 1 (Figure 1a) as potent and selective inhibitor of DNA gyrase. For the sake of simplicity, here the DNA gyrase and DNA gyrase inhibitors as antibacterial agents. Docking simulations were performed using the X-ray crystallographic structure of the DNA gyrase of Staphylococcus aureus, which was shown in Figure 2, in complex with the most potent inhibitor to explore the binding model of the compound at the enzyme active site.

Results and Discussion
Chemistry
The synthetic route to target compounds (3a–3s) is shown in Figure 3. The synthesis of ester 1 was carried out using a literature method [22], by reaction of commercially available bromoacetoephene and dimethyl oxalate in the presence of sodiumhydride with excellent yield. Treatment of 1 with anhydrous hydrazine [23] yielded pyrazolehydrazide 2 which can be condensed with various substituted benzoic acids under standard conditions [24] provided the desired DNA gyrase inhibitors (3a–3s) with good yield.

All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were full accordance with their depicted structures.

In vitro antibacterial assay
The activities of synthesized compounds were tested against Bacillus subtilis ATCC 6633, Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 6538 which may be causal agents of some serious infections in
humans using MH medium (Mueller-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs of the compounds against four bacteria are presented in Table 2. Also included are the activities of reference compounds kanamycin. The results revealed that some of the synthesized compounds exhibited significant antibacterial activity, especially against *B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538.

The compounds 3j and 3d showed antibacterial activities against *B. subtilis* with the MIC of 1.12, 3.66 μg/mL, respectively, comparable to that of positive control penicillin. Compound 3k with MIC value of 0.78 μg/mL exhibited promising antibacterial activities against *B. subtilis* which were even better than that of the

### Table 1. Chemical structures of 3a–3s.

| compound | R¹ | R² | R³ |
|----------|----|----|----|
| 3a       | H  | H  | F  |
| 3b       | H  | H  | Cl |
| 3c       | H  | H  | Br |
| 3d       | H  | H  | CH₃ |
| 3e       | H  | H  | CH₂O |
| 3f       | H  | H  | NO₂ |
| 3g       | H  | F  | H  |
| 3h       | H  | Cl | H  |
| 3i       | H  | Br | H  |
| 3j       | H  | CH₃ | H  |
| 3k       | H  | CH₂O | H  |
| 3l       | H  | NO₂ | H  |
| 3m       | F  | H  | H  |
| 3n       | Cl | H  | H  |
| 3o       | Br | H  | H  |
| 3p       | CH₃ | H  | H  |
| 3q       | CH₂O | H  | H  |
| 3r       | NO₂ | H  | H  |
| 3s       | H  | H  | H  |

Figure 1. Structure of compound 1 and N'-benzoyl-3-(4-bromophenyl)-1H-pyrazole-5-carbohydrazide analogs and common structural characteristic of compound 1.

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Figure 2. Crystal structure of *Staphylococcus aureus* DNA gyrase co-complexed with inhibitor.

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commercial penicillin. The compounds 3d, 3e, 3j, 3k, 3p and 3s showed moderate antibacterial activities against *P. aeruginosa* with MIC of 12.50 μg/mL. Besides, compound 3s also showed moderate antibacterial activities against *E. coli* with MIC of 12.50 μg/mL.

From the structure-activity relationships presented in Table 2, it can be concluded that some N-9-benzoyl-3-(4-bromophenyl)-1H-pyrazole-5-carbohydrazide derivatives showed good activity against Gram positive strains (*B. subtilis* ATCC 6633 and *S. aureus* ATCC 65385), but most of the derivatives displayed poor activity against Gram negative strain (*P. aeruginosa* ATCC 27853 and *E. coli* ATCC 35218).

Among all the synthetic compounds, we found a law between the compounds and antibacterial activity of *S. aureus*. In general, compounds with electronic-donating substituents (methyl or methoxy) on the benzene ring showed more potent inhibitory activities than compounds only contained electronic-withdrawing substituents (halogen) on the benzene ring. Exceptionally compound 3q with methoxy on the benzene ring exhibited lower antibacterial activity compared with other compounds. The position of the same substituents on benzene ring also influenced the activities. For example, the order of the activities is that substituent at the meta position > substituent at the para position > substituent at the ortho position.

### DNA gyrase inhibitory assay

To elucidate the mechanism by which the pyrazole derivatives induce antibacterial activity, the inhibitory activities of selected compounds were examined against DNA gyrase isolated from *B. subtilis* and *S. aureus*. As shown in Table 3, compound 3k with potent antibacterial activities strongly inhibited *S. aureus* DNA gyrase and *B. subtilis* DNA gyrase (with IC<sub>50</sub> of 0.15 μg/mL against *S. aureus* DNA gyrase, 0.25 μg/mL against *B. subtilis* DNA gyrase). There was a good correlation between the MICs and the IC<sub>50</sub> (Tables 2 and 3), indicating that inhibition of the DNA gyrase by the pyrazole derivatives caused inhibition of bacterial cell growth. Bacterial topoisomerase inhibitors sometimes have poor selectivity against human topoisomerase, for example, the compound 3s showed the same activities against *S. aureus* and *B. subtilis* with the MIC of 10.56 μg/mL, but it showed different

### Table 2. Antimicrobial activity of the synthesized compounds.

| Compounds | Minimum inhibitory concentrations (μg/mL) |
|-----------|------------------------------------------|
|           | *B. subtilis* | *S. aureus* | *P. aeruginosa* | *E. coli* |
| 3a        | 12.52        | 15.00       | 50               | >50       |
| 3b        | 17.12        | 15.00       | 50               | >50       |
| 3c        | 10.21        | 13.79       | 50               | >50       |
| 3d        | 12.58        | 3.66        | 12.50            | 50        |
| 3e        | 10.12        | 6.68        | 12.50            | >50       |
| 3f        | 25.00        | 18.03       | >50              | >50       |
| 3g        | 25.00        | 13.00       | 50               | >50       |
| 3h        | 6.25         | 12.56       | 50               | >50       |
| 3i        | 12.58        | 12.11       | 50               | >50       |
| 3j        | 5.32         | 1.12        | 12.50            | 50        |
| 3k        | 3.12         | 0.78        | 12.50            | 50        |
| 3l        | 10.78        | 10.78       | 50               | >50       |
| 3m        | 25.00        | 22.10       | >50              | >50       |
| 3n        | 12.24        | 16.97       | >50              | >50       |
| 3o        | 23.12        | 20.99       | >50              | >50       |
| 3p        | 12.15        | 8.08        | 12.50            | 50        |
| 3q        | 20.58        | 18.71       | >50              | >50       |
| 3r        | 50           | 25.00       | >50              | >50       |
| 3s        | 10.56        | 10.56       | 12.50            | 12.50     |
| Penicillin| 1.56         | 1.56        | 6.25             | 6.25      |

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### Table 3. Inhibitory effects of the selected title compounds against DNA gyrase.

| Compounds | IC<sub>50</sub>(μg/mL) |
|-----------|-------------------------|
|           | *S. aureus* DNA gyrase  | *B. subtilis* DNA gyrase |
| 3d        | 1.50                    | 2.60                     |
| 3e        | 3.40                    | 12.25                    |
| 3j        | 0.13                    | 3.25                     |
| 3k        | 0.15                    | 0.25                     |
| 3p        | 5.21                    | 0.50                     |
| 3s        | 3.25                    | 1.00                     |
| Novobiocin | 0.25                   | 0.5                      |

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inhibition against the *S. aureus* DNA gyrase and *B. subtilis* DNA gyrase (IC\textsubscript{50} = 3.25 μg/mL, 1.00 μg/mL respectively).

In addition, we did activity correlation analysis between antibacterial and anti DNA gyrase. They were positively correlated with \( R^2 = 0.8097 \).

**Molecular docking study of synthetic compounds**

To help understand the SARs observed at the DNA gyrase and guide further SAR studies, we proceeded to examine the interaction of compound 3k with DNA gyrase (PDB code: 3G75). All docking runs were applied the CDOCKER protocol in Discovery Studio 3.1 (Discovery Studio 3.1, Accelrys, Inc., San Diego, CA). The obtained results were presented in the group of pictures. Figure 4 and 5 showed the binding mode of compound 3k interacting with DNA gyrase and the docking results revealed that two amino acids ARG 144, GLY 85 and ARG 84 located in the binding pocket played vital roles in the conformation with compound 3k, which were stabilized by two \( \pi \)-cation bonds and four hydrogen bonds that showed in 2D diagram. One \( \pi \)-cation bond with 5.50 Å was formed between amino acid AGR84 and benzene ring of compound 3k; one \( \pi \)-cation bond, of which its length was 3.97 Å, was formed by the pyrazole ring and ARG84. The nitrogen atom on pyrazole ring provided one hydrogen bond with ARG144 (N...H-N: 2.20 Å, 143.72°). The other hydrogen bonds were formed between GLY 85 and carbonyl oxygen (N...H-O: 2.24 Å, 120.69°), amino hydrogen (O-H: 2.44 Å, 119.96°) and hydrogen atom on pyrazole ring (O-H: 2.12 Å, 127.68°).

In addition, the enzyme surface model was showed in Figure 6, which revealed that the molecule was well embedded in the active pocket. Docking result along with the antibacterial activity date, suggested that compound 3k was a potential inhibitor of DNA gyrase. The docking calculations of the other compounds were also depicted in Table 4.

The difference in the target compounds is just their substituent, and therefore, their binding modes are substantially identical.
Since they are substantially the same, the difference exists in the substituent. Some substituents can form better interactions, so that the combination is enhanced. Some affect the activity by influencing peripheral electronic arrangement. Overall, these are reflected in the binding energy of this parameter. In support of this, we did an activity correlation analysis between docking calculations and anti DNA gyrase activity. They were positively correlated with $R^2 = 0.8045$.

Crystal structures of compounds 3n

Crystals of compound 3n were obtained from methanol solution. Figure 7 shows a perspective view of the monomeric unit with the atomic numbering scheme, and Figure 8 depicts the intramolecular and intermolecular hydrogen bonds. Crystallographic data, details of data collection and structure refinement parameters are listed in Table 5. The hydrogen bond lengths and bond angles are given in Table 6.

![Figure 7. Molecular structure of compound 3n with atomic numbering scheme.](doi:10.1371/journal.pone.0069751.g007)

![Figure 8. Crystal packing of the compound 3n.](doi:10.1371/journal.pone.0069751.g008)

Single crystal of 3n (0.32 mm x 0.27 mm x 0.25 mm) was mounted on a D-8 venture diffractometer equipped with graphite-monochromated MoKα ($\lambda = 0.71073 \text{ Å}$) radiation. For 3n, a total of 8021 reflections were collected, of which 3148 were unique with $R_{int} = 0.073$ and 1686 observed reflections with $I > 2\sigma(I)$ were used in the succeeding structure calculations. The final cycle of refinement of full matrix least-squares was converged to $R = 0.0994$ and $wR = 0.2662$. The highest and lowest residual peaks in the final difference Fourier map are 0.66 and $-0.41$ e/Å$^3$, respectively.

In the crystal structure of compound 3n, there are two benzene rings in the molecule. C(1), C(2), C(3), C(4), C(5) and C(6) form the first plane with the mean deviation of 0.0145 Å, defined as plane I; Similarly, C(12), C(13), C(14), C(15), C(16) and C(17) forms the second plane with the mean deviation of 0.0021 Å, defined as plane II. The dihedral angle between plane I and plane II is 55.5°. Besides, there is one pyrazole ring in the molecule, C(7), C(8), C(9), N(2) and N(1) form the third plane with the mean deviation of 0.0051 Å, defined as plane III. The dihedral angle between plane I

**Table 4. The docking calculation of the synthesized compounds (3a–3s).**

| Compound | -CDOCKER_ENERGY  |
|----------|-----------------|
| 3a       | 17.3167         |
| 3b       | 17.4755         |
| 3c       | 17.7234         |
| 3d       | 20.0936         |
| 3e       | 19.7220         |
| 3f       | 14.3571         |
| 3g       | 17.7452         |
| 3h       | 18.1927         |
| 3i       | 18.4996         |
| 3j       | 20.6595         |
| 3k       | 22.7154         |
| 3l       | 19.3675         |
| 3m       | 10.8604         |
| 3n       | 16.1455         |
| 3o       | 11.6705         |
| 3p       | 17.5309         |
| 3q       | 14.0062         |
| 3r       | 6.4841          |
| 3s       | 19.4014         |

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and assayed for their antibacterial (molecular docking information. These compounds were evaluated derivatives (3a–3s) were designed and synthesized based on the molecular docking information. These compounds were evaluated and assayed for their antibacterial (B. subtilis ATCC 6633, E. coli ATCC 35218, P. aeruginosa ATCC 27853 and S. aureus ATCC 6538) activities by MTT method. The results show that compound 3k possess potent antibacterial activity and can strongly inhibit S. aureus DNA gyrase and B. subtilis DNA gyrase, with IC50 of 0.13 µg/ml against S. aureus DNA gyrase, 0.25 µg/ml against B. subtilis DNA gyrase. The data of antibacterial activity and molecular docking are positively correlated with R value of 0.8045. The antibacterial activity and anti DNA gyrase inhibitory activity are also positively correlated as well, which has the R value of 0.8097.

**Experimental**

**Chemistry**

All chemicals and reagents used in the current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points (uncorrected) were determined on a XT4MP apparatus (Taite Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and 1H NMR spectra were collected on a Bruker DPX300 spectrometer at room temperature with TMS and solvent signals allotted as internal standards. Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument, and were within ±0.4% of the theoretical values.

**Synthesis of ethyl 4-(4-bromophenyl)-2,4-dioxobutanoate (1)**

To a suspension of sodium methylate (5.4 g, 100 mmol) in methanol (5 mL) at 0 °C was added tetrahydrofuran (50 mL) slowly. To this cold mixture was added a solution of 1-(4-bromophenyl) ethanone (9.9 g, 50 mmol) and dimethyl oxalate (8.76 g, 60 mmol) in tetrahydrofuran (150 mL) dropwise. The mixture was allowed to warm to room temperature, stirred overnight and filtered. The residue was purified through washing several times to yield compound 1 as a yellow solid.

**Synthesis of 3-(4-bromophenyl)-1H-pyrazole-5-carbohydrazide (2)**

Hydrazine hydrate (5.32 mL, 200 mmol) was added to a suspension of 1 (3.96 g, 20 mmol) in EtOH (250 mL) and the mixture was refluxed overnight. The precipitated white solid was filtered, washed with EtOH and dried under vacuum to yield compound 2 as a white solid.

**General procedure for the preparation of target compounds 3a–3s**

A stirred solution of compound 2 (0.1 mol) in CH2Cl2 (50 mL) was treated with the appropriate substituted benzoic acid, EDC.HCl (0.15 mol), HOBT (0.05 mol) and refluxed overnight. Then purification with recrystallisation afforded the corresponding compound.

**Bioassay conditions**

**In vitro antibacterial activity.** The antibacterial activity of the synthesized compounds was tested against B. subtilis, E. coli, P. aeruginosa and S. aureus using MH medium (Mueller-Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs (minimum inhibitory concentrations) of the test compounds were determined by a colorimetric method using the dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide). A stock solution of the synthesized compound (100 µg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified

| Table 5. Crystallographic data, details of data collection and structure refinement parameters. |
|-----------------------------------------------|
| compound | 3n |
| Empirical formula | C17H12BrClN4O2 |
| Formula weight | 419.66 |
| Crystal system | Triclinic |
| Space group | P-1 |
| a (Å) | 4.6083(12) |
| b (Å) | 4.6083(12) |
| c (Å) | 7.0246(18) |
| α (°) | 95.804(8) |
| β (°) | 92.071(8) |
| γ (°) | 95.433(8) |
| V (Å³) | 858.4(4) |
| Z | 2 |
| D calc/g cm⁻³ | 1.624 |
| θ range (°) | 2.3–25.5 |
| F(000) | 420 |
| Reflections collected/unique | 8021, 3148 |
| Data/restraints/parameters | 1686/0/226 |
| Absorption coefficient (mm⁻¹) | 2.569 |
| R₁/WR₁ (|<|2θ (|<|6) | 0.0994/0.2380 |
| R₁/WR₁ (all date) | 0.1762/0.2662 |
| GOOF | 1.106 |
| doi:10.1371/journal.pone.0069751.t005 |

and plane III is 17.0°, the dihedral angle between plane II and plane III is 68.2°.

In addition, a connection in terms of structural aspects between theoretical results from docking calculations and X-ray data for compound 3n is also explored. Figure 9 showed the binding mode of compound 3n interacting with DNA gyrase. The main bond lengths and bond angles of docking calculations and X-ray data are given in Table 7 and Table 8. There fitting degree are 0.9597 and 0.8565, respectively.

**Conclusion**

Using the structure-based drug design concept, a series of new N'-benzoyl-3-(4-bromophenyl)-1H-pyrazole-5-carbohydrazidederivatives (3a–3s) were designed and synthesized based on the molecular docking information. These compounds were evaluated and assayed for their antibacterial (B. subtilis ATCC 6633, E. coli ATCC 35218, P. aeruginosa ATCC 27853 and S. aureus ATCC 6538) activities by MTT method. The results show that compound 3k possess potent antibacterial activity and can strongly inhibit S. aureus DNA gyrase and B. subtilis DNA gyrase, with IC50 of 0.13 µg/ml against S. aureus DNA gyrase, 0.25 µg/ml against B. subtilis DNA gyrase. The data of antibacterial activity and molecular docking are positively correlated with R value of 0.8045. The antibacterial activity and anti DNA gyrase inhibitory activity are also positively correlated as well, which has the R value of 0.8097.

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| Table 6. Hydrogen Bond Lengths (Å) and Bond Angles (°) of compound 3n. |
|-----------------------------------------------|
| D–H...A | d(D–H) | d(H...A) | d(D–A) | □ DHA |
| N(3)…H(3)…N(2) | 0.86 | 2.36 | 2.720(12) | 105 |
| N(4)…H(4)…O(2) | 0.86 | 2.02 | 2.732(11) | 140 |
| C(5)…H(5)…O(1) | 0.93 | 2.54 | 3.409(15) | 156 |
| C(5)…H(5)…N(1) | 0.93 | 2.60 | 2.922(15) | 101 |
| doi:10.1371/journal.pone.0069751.t006 |
A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37°C for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (phosphate buffered saline 0.01 mol/L, pH 7.4, Na₂HPO₄·12H₂O 2.9 g, KH₂PO₄ 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm.

### Enzyme inhibition

*S. aureus DNA gyrase supercoiling.* The S. aureus DNA gyrase were purified by the F. Blanche [25] from a crude extract of

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**Table 7.** Bond lengths from docking calculations and X-ray data for compound 3n.

| Bond     | Bond lengths (Å) | Docking data | X-ray data |
|----------|------------------|--------------|------------|
| C2-Br1   | 1.878            | 1.902        |
| C4-C7    | 1.488            | 1.472        |
| C7-C8    | 1.403            | 1.376        |
| C8-C9    | 1.401            | 1.389        |
| C9-N2    | 1.341            | 1.310        |
| N1-N2    | 1.355            | 1.314        |
| N1-C7    | 1.365            | 1.355        |
| C9-C10   | 1.384            | 1.483        |
| C10-O1   | 1.225            | 1.174        |
| C10-N3   | 1.343            | 1.357        |
| N3-N4    | 1.236            | 1.372        |
| N4-C11   | 1.347            | 1.329        |
| C11-O2   | 1.225            | 1.218        |
| C11-C12  | 1.474            | 1.484        |
| C17-C12  | 1.749            | 1.708        |

**Table 8.** Bond Angles from docking calculations and X-ray data for compound 3n.

| Angle    | Bond Angles (°) | Docking data | X-ray data |
|----------|-----------------|--------------|------------|
| C2-C1-Br1| 120.48          | 121.6        |
| C3-C4-C7 | 121.78          | 121.3        |
| C5-C4-C7 | 119.97          | 120.6        |
| C9-C10-O1| 120.59          | 121.5        |
| C9-C10-N3| 115.53          | 113.5        |
| C10-N3-N4| 121.63          | 119.4        |
| O1-C10-N3| 123.88          | 124.9        |
| O2-C11-C12| 124.01        | 123.2        |
| C11-C12-C13| 119.60        | 119.4        |
| C11-C12-C17| 122.43        | 122.5        |
| C16-C17-C12| 115.88        | 119.1        |
S. aureus and cultivated with medium B, which was composed of 10 g of polypeptone, 2 g of yeast extract, 6 g of Na₂HPO₄, 2 g of KH₂PO₄, 1.2 g of (NH₄)₂SO₄, 0.2 g of MgSO₄, 4 g of glucose per liter of distilled water. Supercoiling and decatenation were performed according to F. Blanche [25].

**B. subtilis DNA gyrase supercoiling.** The B. subtilis DNA gyrase were purified by the methods of E. Orr. [26]: Cells were suspended in an equal volume of 25 mM HEPES-KOH (pH 8.0)-100 mM KCl and stored frozen at −70°C. The frozen cell suspension was thawed and diluted with an equal volume of 25 mM HEPES-KOH (pH 8.0-0.4 M sucrose-20 mM magnesium acetate-1 mM dithiothreitol-5 mM PMSF). All operations were performed at 0–4°C. Lysozyme was added to a final concentration, and the mixture was incubated for 2.5 h. One-third volume of 2 M KCl-1.5% Brij was added, and the incubation was continued for 15 min. The lysate was then centrifuged for 90 min at 4°C. The supernatant was adjusted to a KCl concentration of 100 mM KCl and stored frozen at −80°C. Antimicrob Agents Chemother 39: 163–169.

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**Experimental protocol of docking study**

Automated docking studies were carried out using Discovery Studio (version 3.1) as implemented through the graphical user interface DS-CDOCKER protocol [27].

The three-dimensional structures of the aforementioned compounds were constructed using Chem. 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2009)], then they were energetically minimized by using MOPAC with 100 iterations and minimum RMS gradient of 0.10. The Gasteiger-Huckel charges of ligands were assigned. The crystal structures of DNA gyrase (PDB code: 3G75) complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). For enzyme preparation, the hydrogen atoms were added with the pH of the protein in the range of 6.5–8.5. CDOCKER is an implementation of a CHARMM based molecular docking tool using a rigid receptor. It includes the following steps:

(1) A series of ligand conformations are generated using high temperature molecular dynamics with different random seeds.

(2) Random orientations of the conformations are generated by translating the center of the ligand to a specified position within the receptor active site, and making a series of random rotations. A softened energy is calculated and the orientation is kept when it is less than a specified limit. This process repeats until either the desired number of low-energy orientations is obtained, or the test times of bad orientations reached the maximum number.

(3) Each orientation is subjected to simulated annealing molecular dynamics. The temperature is heated up to a high temperature then cooled to the target temperature. A final energy minimization of the ligand in the rigid receptor using non-softened potential is performed.

(4) For each of the final pose, the CHARMM energy (interaction energy plus ligand strain) and the interaction energy alone are figured out. The poses are sorted according to CHARMM energy and the top scoring (most negative, thus favorable to binding) poses are retained. The whole DNA gyrase domain defined as a receptor and the site sphere was selected based on the ligand binding location of B-482, then the B-482 removed and the ligands prepared by us was placed during the molecular docking procedure. CHARMM was selected as the force field. The molecular docking was performed with a simulated annealing method. The heating steps were 2000 with 700 of heating target temperature. The cooling steps were 5000 with 300 cooling target temperature. Ten molecular docking poses saved for each ligand were ranked according to their dock score function. The pose with the highest -CDOCKER energy was chosen as the most suitable pose.

**X-ray crystallography**

Single crystal X-ray diffraction data was collected on a Bruker D-8 venture diffractometer at room temperature (293 K). The X-ray generator was operated at 50 KV and 35 mA using Mo Kα radiation (λ=0.71073 Å). The data was collected using SMART software package. The data were reduced by SAINT-PLUS, an empirical absorption correction was applied using the package SADABS and XPREP were used to determine the space group. The crystal structure was solved by direct methods using SIR92 and refined by full-matrix least-squares method using SHELXL97 [28,29]. All non-hydrogen atoms were refined anisotropically and hydrogen atoms have been refined in the riding mode on their carrier atoms wherever applicable.

**Supporting Information**

File S1 Experimental protocols, NMR data (1H and 13C), Mass spectrometry data (MS and HRMS) and Melting points data of compounds. The connection between theoretical results from docking calculations and X-ray data for compound 3n (graph 1 and graph 2). Correlations among antibacterial, anti DNA gyrase and CDOCKER-ENERGY (graph 3 and graph 4).

**Author Contributions**

Conceived and designed the experiments: JS PCL HLZ. Performed the experiments: JS RJY JM. Analyzed the data: JS PCL YY HLZ. Contributed reagents/materials/analysis tools: YY HLZ. Wrote the paper: JS HLZ.
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