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Synthesis and antibacterial activity of novel and potent DNA gyrase inhibitors with azole ring

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Abstract—The 4-piperidyl moiety and the pyrazole ring in 1-(3-chlorophenyl)-5-(4-phenoxyphenyl)-3-(4-piperidyl)pyrazole **2**, which has previously shown improved DNA gyrase inhibition and target-related antibacterial activity, were transformed to other groups and the in vitro antibacterial activity of the synthesized compounds was evaluated. The selected pyrazole, oxazole and imidazole derivatives showed moderate inhibition against DNA gyrase and topoisomerase IV with similar IC_{50} values (IC_{50} = 9.4–25 μg/mL). In addition, many of the pyrazole, oxazole and imidazole derivatives synthesized in this study exhibited potent antibacterial activity against quinolone-resistant clinical isolates and coumarin-resistant laboratory isolates of Gram-positive bacteria with minimal inhibitory concentration values equivalent to those against susceptible strains.
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1. Introduction

The emergence and spread of multidrug resistant Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant enterococci (VRE), have made treatment of infectious diseases difficult and have over the last decades become a serious medical problem. As pathogenic bacteria continuously evolve mechanisms of resistance to currently used antibacterial agents, the discovery of novel and potent bactericides is the best way to overcome bacterial resistance and develop effective therapies. Unfortunately, finding novel antibacterial agents has been difficult with the oxazolidinones, identified in 1980, being the last example to successfully reach the clinic.[1]

Bacterial DNA gyrase is a proven target for antibacterial chemotherapy.[2] Quinolones, such as sparfloxacin[3] (SPFX, Fig. 1), inhibit bacterial DNA gyrase and topoisomerase IV and cause bacterial cell death. Besides the quinolones, other naturally occurring bacterial DNA gyrase inhibitors, such as the coumarins, which include novobiocin (NB, Fig. 1), clorobiocin and coumermycin

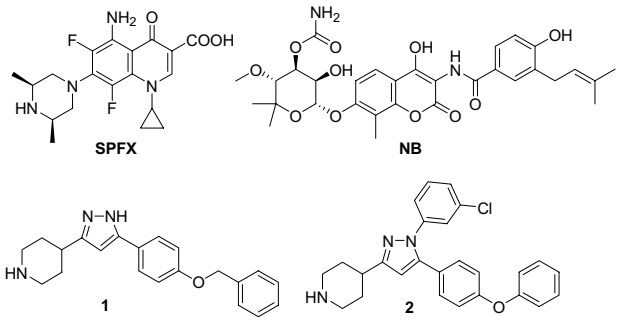

Figure 1.

Keywords: DNA gyrase inhibitor; Pyrazole, oxazole and imidazole derivatives; Multidrug resistant strains.
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A_1 have also been known as antibacterial agents.[4] The coumarins inhibit ATPase activity of DNA gyrase by competing with ATP for binding to the B subunit of the enzyme. However, no pharmaceutically useful drug has so far been derived from the coumarins. Although many efforts have been dedicated to finding potent antibacterial agents that can overcome bacterial resistance, promising lead structures of DNA gyrase inhibitors have not been found.[5–10]

Using a new screening system for specific inhibitors of chromosome partitioning in *Escherichia* (*E.*) *coli*[11,12] we have previously reported that one of our hit compounds (compound **1**, Fig. 1) having a piperidine ring represents a new class of bacterial DNA gyrase inhibitors that have potent antibacterial activity against *Staphylococcus* (*S.*) *aureus* and *Enterococcus* (*E.*) *faecalis*.[13] We have also demonstrated that compound **2** (Fig. 1), a derivative of **1**, shows improved DNA gyrase inhibition and target-related antibacterial activity.[11] Moreover compound **2** exhibits the same minimal inhibitory concentration (MIC) values against clinically isolated multidrug resistant Gram-positive bacteria as against drug susceptible strains.

Our aim in this study was to optimize the piperidine moiety and pyrazole ring of compound **2** and find new potent DNA gyrase inhibitors with antibacterial activity against MRSA, PRSP and VRE. Here we report the synthesis and structure–activity relationships (SARs) of a series of pyrazole and other five-membered heterocyclic derivatives.

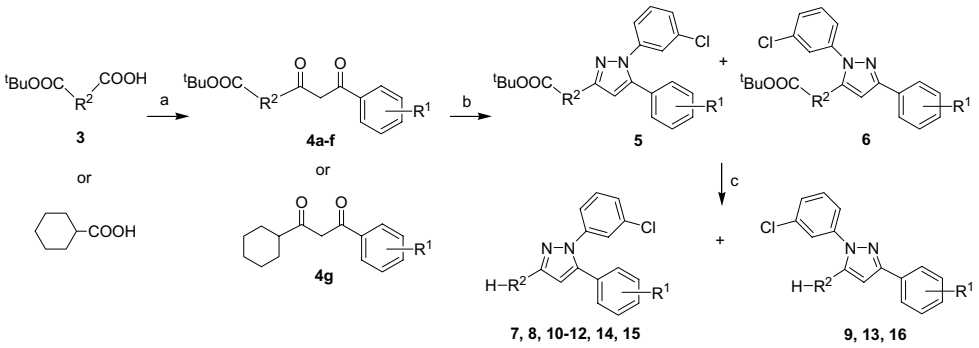
2. Chemistry

The novel pyrazole derivatives **8**–**16** were synthesized as shown in Scheme 1. The requisite intermediate 1,3-diketones **4a**–**f** were prepared by coupling reaction of the *N*-(*tert*-butoxycarbonyl) amino acids **3** with various benzophenone derivatives in THF using 1,1′-carbonyldi-1*H*-imidazole (CDI) and lithium hexamethyldisilazide (LHMDS).[14,15] Condensation of **4a**–**f** with 3-chlorophenylhydrazine hydrochloride with aqueous NaOH gave a mixture of the 1-(3-chlorophenyl)pyrazoles **5** and **6**. Cleavage of the protecting group under acidic conditions, followed by separation using CHP-20P (reverse phase) column chromatography and/or recrystallization gave the 3-substituted 1-(3-chlorophenyl)pyrazoles **8**, **10**–**12**, **14**, **15** and the regioisomers **9**, **13**, **16** as acid salts. Compound **7** having a cyclohexyl group at the 3-position instead of the 4-piperidyl group of **2** was synthesized from cyclohexanecarboxylic acid and 4-phenoxyacetophenone in a similar manner to that described above. The corresponding 5-substituted isomers of 1-(3-chlorophenyl)pyrazole derivatives **7**, **10**, **11** and **14** were not isolated.

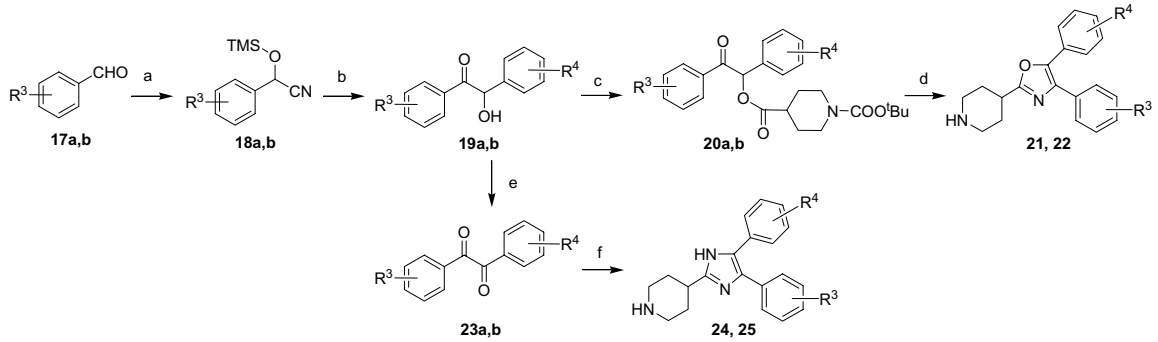
The position of 3-chlorophenyl group in **7**–**16** was determined on the basis of nuclear Overhauser effects (NOE) experiments. For example, in compound **8**, a correlation of NOE was observed between the protons of the phenyl moiety on R^1-Ph and the *ortho* protons of the 3-chlorophenyl moiety. On the other hand, in compound **9**, a correlation of NOE was observed between the 2-H protons of the 3-piperidyl moiety on R^2 and the *ortho* protons of the 3-chlorophenyl moiety.

The novel oxazole derivatives **21** and **22** and the imidazole derivatives **24** and **25** were synthesized as shown in Scheme 2. The 2-trimethylsilyloxyacetonitrile **18a** was prepared by condensation of 4-phenoxybenzaldehyde (R^3 = 4-phenoxy) with trimethylsilylcyanide and zinc iodide in CH_2Cl_2.[16] Coupling reaction of **18a** with 3-chlorobenzaldehyde (R^4 = 3-chlorophenyl) using LHMDS in THF, followed by treatment with aqueous H_2SO_4 afforded the 2-hydroxyethane-1-one **19a**. Reaction of **19a** with 1-(*tert*-butoxycarbonyl)piperidine-4-carbxylic acid in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP) gave the ester **20a**, which was treated with NH_4OAc in AcOH to afford the desired **21**.[17] In a similar manner to that described above, **22** was obtained from 3-chlorobenzaldehyde (R^3 = 3-chloro) as starting material via **18b**–**20b** (R^4 = 4-benzyloxy).

The imidazole analogues **24** and **25** were obtained by condensation of **23a**,**b**, which were prepared by oxidation of **19a**,**b** using $CuSO_4$ in aqueous pyridine, with 1-(*tert*-butoxycarbonyl)-4-formylpiperidine in the presence of NH_4OAc in AcOH.[18]



Scheme 1. General synthesis of the 1-(3-chlorophenyl)pyrazole derivatives. Reagents: (a) i. CDI, THF, ii. R^1-$PhCOCH_3$, LHMDS, THF; (b) 3-chlorophenylhydrazine·HCl, aq NaOH, EtOH; (c) HCl, EtOH or TFA, CH_2Cl_2.



Scheme 2. Synthesis of the oxazole and imidazole derivatives. Reagents: (a) TMSCN, ZnI, CH_2Cl_2; (b) i. R^4-CHO, LHMDS, THF, ii. aq H_2SO_4, MeOH; (c) 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid, EDC, DMAP, CH_2Cl_2; (d) NH_4OAc, AcOH; (e) $CuSO_4$, pyridine, H_2O; (f) 1-(*tert*-butoxycarbonyl)-4-formylpiperidine, NH_4OAc, AcOH.

3. Results and discussion

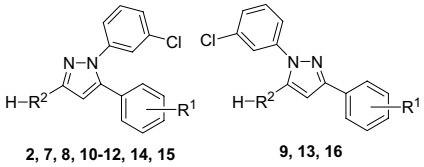
3.1. Antibacterial activity

To investigate the effect of basic amine appended on the pyrazole ring on the antibacterial activity, the 4-piperidyl moiety in **2** was transformed to other groups (**7**–**14**) and the in vitro antibacterial activity of the compounds obtained was evaluated against two Gram-positive bacteria, that is a drug susceptible strain, *S. aureus* FDA 209P and a multidrug resistant strain, *S. aureus* KMP9 (MRSA), and against two Gram-negative bacteria that is a susceptible strain, *E. coli* NIHJ JC-2 and a multidrug efflux pump mutant, *E. coli* W3110 *ΔacrA*. For comparison, the in vitro antibacterial activity of sparfloxacin and novobiocin has also been evaluated.

As shown in Table 1, the cyclohexyl derivative **7** having no basic amine did not exhibit any antibacterial activity. This result suggests that basic amines in the R^2 moiety appended on the pyrazoles are essential for antibacterial activity. The 3-piperidyl analogue **8** showed 2-fold more potent antibacterial activity against the four strains of bacteria than the corresponding 4-piperidyl derivative **2**. However, the 2-piperidyl analogue **10** showed four- to eightfold less potent antibacterial activity against *S. aureus* than the 4-piperidyl derivative **2** and the 3-piperidyl derivative **8**. These results suggest that the position of NH group in the R^2 moiety is important for antibacterial activity.

Compound **11** with an acyclic substituent, *N*-methylaminopropyl, exhibited twofold and fourfold more potent

Table 1. Antibacterial activity of the pyrazole derivatives



Compd	R^1	H-R^2	MIC (μg/mL)			
			S. aureus		*E. coli*	
			FDA 209P	KMP9[a]	NIHJ JC-2	W3110 *ΔacrA*[b]
2	4-PhO	4-Piperidyl	4	4	32	4
7	4-PhO	*c*-Hexyl	>128	>128	>128	>128
8	4-PhO	3-Piperidyl	2	2	16	2
9	4-PhO	3-Piperidyl	4	4	32	16
10	4-PhO	2-Piperidyl	16	16	>128	8
11	4-PhO	$CH_3NHCH_2CH_2CH_2$	2	2	8	2
12	4-PhO	CH_3NHCH_2	4	4	16	2
13	4-PhO	CH_3NHCH_2	8	8	>128	4
14	4-PhO	$PhCH_2NHCH_2$	64	128	>128	>128
15	3-$PhCH_2O$	4-Piperidyl	2	2	32	1
16	3-$PhCH_2O$	4-Piperidyl	4	4	32	2
1	—	—	64	64	128	64
SPFX	—	—	0.125	128	0.032	0.004
NB	—	—	0.25	0.25	64	0.5

[a] Multidrug resistant *S. aureus*.
[b] A multidrug efflux pump mutant.

antibacterial activity against *S. aureus* and *E. coli* NIHJ JC-2, respectively, than the lead compound **2**. Compound **12** having another acyclic substituent, *N*-methylaminomethyl, showed the same or twofold more potent antibacterial activity against the four strains of bacteria studied compared to compound **2**. However the *N*-benzylaminomethyl derivative **14** showed 16–32-fold less potent antibacterial activity against *S. aureus* than compound **2**. The SARs of these acyclic substituent suggest that contrary to cyclic amines, the position of NH group in the R^2 moiety is not important for antibacterial activity. It is also noted that a small group, such as a methyl on NH retains strong potency whereas a bulky group, such as benzyl weaken the potency.

The 3-benzyloxyphenyl pyrazole **15** revealed twofold more potent antibacterial activity against *S. aureus* and the same potency against *E. coli* NIHJ JC-2 as compound **2**. When regioisomers of the synthesized compounds were compared, the 3-aminoalkyl-1-(3-chlorophenyl)pyrazoles **8**, **12** and **15** showed slightly more potent antibacterial activity than the corresponding 5-aminoalkyl-1-(3-chlorophenyl)pyrazoles **9**, **13** and **16**. Among all pyrazole analogues synthesized in this study, compounds **8**, **11** and **15** had comparatively strong antibacterial activity against the four strains of bacteria studied.

Next, the effects of a transformation of the pyrazole ring of compound **2** on the antibacterial activity were examined (Table 2). The oxazole and imidazole derivatives configured with three substituents in the structure of the pyrazole derivatives **2** and **15** (i.e., 4-piperidyl, 3-chlorophenyl and a substituted phenyl) were synthesized. The oxazole derivative **21** and **22** showed the same antibacterial activity against *S. aureus* and less potent antibacterial activity against *E. coli* NIHJ JC-2 compared to the corresponding pyrazole derivatives **2** and **15**.

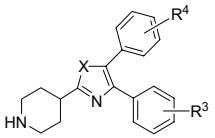
On the other hand, the imidazole derivatives **24** and **25** showed similar antibacterial activity against *S. aureus* and four- to eightfold more potent antibacterial activity against *E. coli* NIHJ JC-2 compared to the corresponding pyrazole derivatives **2** and **15**. From these results, it is assumed that the imidazole ring induces potent antibacterial activity against not only Gram-positive bacteria but against Gram-negative bacteria. Since the imidazole derivatives showed nearly the same antibacterial activity against *E. coli* NIHJ JC-2 and W3110 *ΔacrA*, it is presumed that these derivatives are not affected by bacterial outer membrane pump.

The SARs of the pyrazole derivatives described in this study can be summarized as follows: a basic amine structure on the R^2 moiety of the pyrazoles is essential for antibacterial activity. In addition, the position of the nitrogen atom in the cyclic amine and the size of the substituents on the nitrogen atom of the acyclic amine are important for potent antibacterial activity. Although no significant difference in the antibacterial activity against *S. aureus* among the three types of azole, the imidazole derivatives had more potent antibacterial activity against *E. coli* NIHJ JC-2 than the corresponding pyrazoles. On the whole, the order of antibacterial activity for these five-membered ring derivatives can be summarized as follows: imidazole > pyrazole > oxazole.

3.2. Inhibitory effects against DNA gyrase and topoisomerase IV

To elucidate the mechanism by which the pyrazole, oxazole and imidazole derivatives induce their antibacterial activity, the inhibitory activity of selected compounds (**15**, **22** and **25**) against DNA gyrase and topoisomerase IV isolated from *E. coli* was examined.[19,20] As shown in Table 3, the IC_{50} values of the initial lead compound **1** against DNA gyrase and topoisomerase IV were >128 and 128 μg/mL, respectively. Compounds **15**, **22** and **25** having the same three substituents (i.e., 4-piperidyl, 3-chlorophenyl and 3-benzyloxyphenyl) showed moderate inhibition against the two enzymes (IC_{50} = 9.4–25 μg/mL). The oxazole derivative **22** and the imidazole derivative **25** showed twofold more potent inhibitory activity against DNA gyrase than the pyrazole derivative **15**. On the other hand, compounds **22** and **25** showed an inhibitory activity against topoisomerase IV almost similar to that of the pyrazole derivative **15**.

Table 2. Antibacterial activity of the oxazole and imidazole derivatives



Compd	X	R^3	R^4	MIC (μg/mL)			
				S. aureus		*E. coli*	
				FDA 209P	KMP9[a]	NIHJ JC-2	W3110 *ΔacrA*[b]
21	O	4-PhO	3-Cl	4	4	128	4
22	O	3-Cl	3-$PhCH_2O$	2	2	64	4
24	NH	4-PhO	3-Cl	2	4	8	2
25	NH	3-Cl	3-$PhCH_2O$	2	2	4	2

[a] Multidrug resistant *S. aureus*.
[b] A multidrug efflux pump mutant.

Table 3. Inhibitory effects of selected compounds against DNA gyrase and topoisomerase IV

Compd	IC_{50} (μg/mL)[a]	
	Gyrase[b]	Topo IV[c]
1	>128	128
15	18.8	25
22	9.4	25
25	9.4	18.8
SPFX	0.25	6.9
NB	0.25	3.5

[a] Isolated from *E. coli*.
[b] DNA gyrase supercoiling activity.
[c] Topoisomerase IV decatenation activity.

There was a good correlation between the MICs and the IC_{50}s (Tables 1 and 2), suggesting that inhibition of the DNA gyrase and topoisomerase IV by the pyrazole, oxazole and imidazole derivatives suppresses bacterial cell growth. While sparfloxacin ratio of inhibition of DNA gyrase and topoisomerase IV was more than 20, that of the pyrazole, oxazole and imidazole derivatives ranged between 1.3 and 2.7. These results suggest that the pyrazole, oxazole and imidazole derivatives would not be easily tolerated by bacteria.

3.3. Effect against multidrug resistant Gram-positive bacteria

To examine whether the pyrazole, oxazole and imidazole derivatives are effective against multidrug resistant Gram-positive bacteria, MIC values of selected compounds against quinolone-resistant clinical isolates and coumarin-resistant laboratory isolates of Gram-positive bacteria were determined and compared with those of sparfloxacin and novobiocin. The strains of bacteria used for MIC values determination were: *S. aureus* KMP9 (MRSA; a sparfloxacin-, clarithromycin- and ampicillin-resistant strain), *S. aureus* N175 (a novobiocin-resistant strain) derived from RN4220,[21] *Streptococcus* (*S.*) *pneumoniae* KT2524 (PRSP; a sparfloxacin-, clarithromycin- and ampicillin-resistant strain) and *Enterococcus* (*E.*) *faecium* KU1778 (VRE; a sparfloxacin-, clarithromycin-, ampicillin- and vancomycin-resistant strain).

As shown in Table 4, MIC values of sparfloxacin against the susceptible strains (MSSA, PSSP and VSE) were 0.125, 0.125 and 0.25 μg/mL, respectively, and those against MRSA, PRSP and VRE were 128, 4 and 64 μg/mL, respectively. MIC values of novobiocin against *S. aureus* RN4220 and *S. aureus* N175 derived from RN4220 were 0.125 and 4 μg/mL, respectively. On the other hand, the pyrazole derivatives **8**, **11** and **15**, the oxazole derivative **22** and the imidazole derivative **25** revealed almost the same antibacterial activity against both susceptible and resistant Gram-positive bacteria. Although sparfloxacin and novobiocin ratio of MIC values against susceptible and resistant strains was more than 32, that of the pyrazole, oxazole and imidazole derivatives ranged between 1 and 2.

The pyrazole, oxazole and imidazole derivatives were also effective against quinolone- and coumarin-resistant Gram-positive bacteria with compounds **8**, **11**, **15**, **22** and **25** showing a more potent antibacterial activity against clinically isolated quinolone-resistant Gram-positive bacteria than sparfloxacin, and compounds **22** and **25** revealing the same antibacterial activity against laboratory isolated coumarin-resistant Gram-positive as novobiocin. Compounds **8** and **15** demonstrated the most potent antibacterial activity against susceptible and resistant Gram-positive bacteria with across-the-board MIC values of 1–4 μg/mL.

It is therefore clear that the pyrazole, oxazole and imidazole derivatives have potent antibacterial activity against both susceptible and quinolone- and coumarin-resistant Gram-positive bacteria. Additionally, the results above suggest that the mode of action of these analogues against DNA gyrase and topoisomerase IV is different from that of the quinolones or the coumarins.

4. Conclusions

In this study, we have described the synthesis and SARs of new pyrazole, oxazole and imidazole analogues. Among the pyrazole analogues, compounds **8** and **11** had comparatively strong antibacterial activity, whereas

Table 4. Antibacterial activity of selected pyrazole derivatives against susceptible and resistant Gram-positive bacteria

Organism[a]	MIC (μg/mL)						
	8	**11**	**15**	**22**	**25**	SPFX	NB
S. aureus FDA 209P (MSSA)[b]	2	2	2	2	2	0.125	0.25
S. aureus KMP9 (MRSA)[c]	2	2	2	2	2	128	0.25
S. aureus RN4220[b]	—	—	—	4	4	0.125	0.125
S. aureus N175[d]	—	—	—	4	4	0.125	4
S. pneumoniae ATCC49619 (PSSP)[b]	1	4	2	4	4	0.125	0.5
S. pneumoniae KT2524 (PRSP)[e]	2	4	2	4	4	4	0.5
E. faecium ATCC19434 (VSE)[b]	4	8	4	16	4	0.25	2
E. faecium KU1778 (VRE)[f]	2	4	4	8	2	64	2

[a] SPFX: sparfloxacin, CAM: clarithromycin, ABPC: ampicillin, VCM: vancomycin.
[b] Susceptible strain.
[c] SPFX-, CAM-, ABPC-resistant strain.
[d] *S. aureus* N175 (GyrB; R144I) was derived from RN4220 with selection of novobiocin.
[e] SPFX-, CAM-, ABPC-resistant strain.
[f] SPFX-, CAM-, ABPC-, VCM-resistant strain.

among the oxazole and imidazole analogues, compounds **22** and **25** showed comparatively strong antibacterial activity. These selected compounds **8**, **11**, **22** and **25** possessed potent antibacterial activity against not only susceptible strains, but also against multidrug resistant strains. In addition, **8**, **11**, **22** and **25** showed a more potent antibacterial activity against clinically isolated quinolone-resistant Gram-positive bacteria than sparfloxacin. Compounds **22** and **25** revealed the same antibacterial activity against laboratory isolated coumarin-resistant Gram-positive bacteria compared to novobiocin.

Though the inhibitory activity of sparfloxacin against DNA gyrase was more than 20-fold that against topoisomerase IV, the pyrazole, oxazole and imidazole derivatives synthesized in this study showed almost the same inhibitory activity against both enzymes. These results suggest that the pyrazole, oxazole and imidazole derivatives would not be easily resisted by bacteria. We are pursuing further modifications of the pyrazole, oxazole and imidazole scaffold to obtain more potent inhibitors of both DNA gyrase and topoisomerase IV.

5. Experimental

5.1. Spectroscopic measurements

All melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. ^{1}H NMR spectra were taken at 200 MHz on a Varian Gemini spectrometer, at 300 MHz on a JEOL spectrometer, or at 400 MHz on a Varian Mercury-400 spectrometer in DMSO-d_6 or $CDCl_3$. All chemical shift values are reported as ppm (δ) values with tetramethylsilane as internal standard. The abbreviations used are as follows: s; singlet, d; doublet, t; triplet, q; quartet, m; multiplet, and br; broad. Mass spectra were obtained on a JEOL JMS-SX 102A QQ for fast atom bombardment mass spectra (FABMS) or a Hitachi M-1000 LC APCI mass spectrometer for atmospheric pressure chemical ionization mass spectra (APCIMS). For elements analysis, C, H and N were analyzed using a CE instruments EA1110 CHN elements analyzer, and F, Cl and Br were analyzed using a Yokogawa IC-7000 ion chromatograph. All analytical results were within ±0.4% of the theoretical values obtained by a given formula.

5.2. Starting material

The following compounds **3** were commercially available from Aldrich or Watanabe Chemical Industries, Ltd, Japan: 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid, 1-(*tert*-butoxycarbonyl)piperidine-3-carboxylic acid, 1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid, *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine and *N*-(*tert*-butoxycarbonyl)-*N*-benzylglycine. The following compounds were synthesized according to published procedures: *N*-(*tert*-butoxycarbonyl)-*N*-methyl-γ-aminobutylic acid[22] and 1-(*tert*-butoxycarbonyl)-4-formylpiperidine.[23]

5.3. General procedure for 1,3-diketones 4a–g

To a solution of lithium hexamethyldisilazide (11 mmol) in THF (30 mL) was added benzophenone derivatives (10 mmol) in THF (10 mL) at −78 °C under argon atmosphere, and the resulting yellow suspension was stirred for 30 min at the same temperature (solution A). A solution of 1,1′-carbonyldi-1*H*-imidazole (CDI, 16.2 g, 10 mmol) and an *N*-(*tert*-butoxycarbonyl) amino acid derivatives **3** [e.g., 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid] (10 mmol) in THF (30 mL) was stirred for 45 min at room temperature (solution B). The resulting solution B was added dropwise to solution A over 30 min at −78 °C. After removal of the cold bath, the reaction mixture was warmed to room temperature and stirred for 12 h. To this reaction mixture was added 10% aqueous citric acid (30 mL), and the resulting solution was taken up in AcOEt (100 mL), washed with brine, dried over $MgSO_4$, and concentrated to dryness. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$) to give **4** as an oil.

5.3.1. *tert*-Butyl 3-[1,3-dioxo-3-(4-phenoxyphenyl)-1-propyl]piperidine-1-carboxylate (4a). 56% Yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (s, 9H), 1.61–1.67 (m, 3H), 2.31–2.36 (m, 2H), 2.94–2.98 (m, 2H), 4.09 (br, 2H), 6.46 (s, 1H), 6.99 (d, 2H, J = 8.7 Hz), 7.04–7.06 (m, 2H), 7.15–7.20 (m, 1H), 7.35–7.40 (m, 2H), 7.83 (d, 2H, J = 8.7 Hz), 16.02 (br, 1H); HRMS calcd for 424.2124, found 424.2127.

5.3.2. *tert*-Butyl 2-[1,3-dioxo-3-(4-phenoxyphenyl)propyl]piperidine-1-carboxylate (4b). 29% Yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, 9H), 1.65–1.69 (m, 3H), 1.93–1.97 (m, 1H), 2.43–2.51 (m, 1H), 2.82–3.07 (m, 2H), 3.90–4.13 (m, 2H), 6.17 (s, 1H), 6.96 (d, 2H, J = 8.8 Hz), 7.01–7.04 (m, 2H), 7.12–7.17 (m, 1H), 7.27–7.37 (m, 2H), 7.85 (d, 2H, J = 8.8 Hz), 15.95 (br, 1H); HRMS calcd for 424.2124, found 424.2119.

5.3.3. 1-[(*tert*-Butoxycarbonyl)-*N*-methyl]amino-6-(4-phenoxyphenyl)hexane-4,6-dione (4c). 29% Yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 9H), 1.81–1.95 (m, 2H), 2.42 (t, 2H, J = 7.3 Hz), 2.86 (s, 3H), 3.29 (t, 2H, J = 7.0 Hz), 6.13 (s, 1H), 6.99 (d, 2H, J = 8.8 Hz), 7.02–7.08 (m, 2H), 7.16–7.21 (m, 1H), 7.36–7.41 (m, 2H), 7.86 (d, 2H, J = 8.8 Hz), 16.08 (br, 1H); HRMS calcd for 412.2124, found 412.2119.

5.3.4. 1-[(*tert*-Butoxycarbonyl)-*N*-methyl]amino-4-(4-phenoxyphenyl)butane-2,4-dione (4d). 49% Yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H), 2.99 (s, 3H), 4.03 (s, 2H), 6.12 (s, 1H), 7.01 (d, 2H, J = 8.7 Hz), 7.04–7.23 (m, 3H), 7.39–7.42 (m, 2H), 7.83 (d, 2H, J = 8.7 Hz), 15.98 (br, 1H); HRMS calcd for 383.1732, found 383.1735.

5.3.5. 1-[*N*-Benzyl-(*tert*-butoxycarbonyl)]amino-4-(4-phenoxyphenyl)butane-2,4-dione (4e). 33% Yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H), 3.93 (s, 2H), 4.58 (s, 2H), 6.03 (s, 1H), 6.99 (d, 2H, J = 8.6 Hz), 7.05–7.08 (m, 2H), 7.17–7.42 (m, 8H), 7.79 (d, 2H,

$J = 8.6$ Hz), 16.16 (br, 1H); HRMS calcd for 460.2124, found 460.2129.

5.3.6. *tert*-Butyl 4-[3-(3-benzyloxyphenyl)-1,3-dioxo-1-propyl]piperidine-1-carboxylate (4f). 68% Yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (s, 9H), 1.45–1.60 (m, 2H), 1.73–1.76 (m, 2H), 2.28–2.38 (m, 1H), 2.62–2.70 (m, 2H), 3.98–4.08 (m, 2H), 4.95 (s, 2H), 6.05 (s, 1H), 6.98 (m, 1H), 7.19–7.42 (m, 8H), 16.13 (br, 1H); HRMS calcd for 437.2202, found 437.2204.

5.3.7. 1-Cyclohexyl-3-(4-phenoxyphenyl)propane-1,3-dione (4g). 69% Yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20–1.51 (m, 5H), 1.69–1.95 (m, 5H), 2.30 (tt, 1H, $J = 11.4$ Hz, $J = 3.1$ Hz), 6.12 (s, 1H), 7.00 (d, 2H, $J = 8.9$ Hz), 7.05–7.08 (m, 2H), 7.15–7.20 (m, 1H), 7.35–7.42 (m, 2H), 7.86 (d, 2H, $J = 8.9$ Hz), 15.95 (br, 1H); HRMS calcd for 323.1647, found 323.1645.

5.4. General procedure for 1-(3-chlorophenyl)pyrazole derivatives 8–16

(a) A solution of 1,3-diketone derivatives **4** (10 mmol), 3-chlorophenylhydrazine hydrochloride (3.58 g, 20 mmol) and 2 mol/L NaOH (19 mmol) in EtOH (30 mL) was heated to reflux for 12 h and cooled to 0 °C. To this reaction mixture was added 10% aqueous citric acid (30 mL) and the resulting solution was taken up in AcOEt (100 mL), washed successively with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The residue was purified by silica gel column chromatography ($MeOH/CHCl_3$) to give a mixture of the 1-(3-chlorophenyl)-3-[*N*-(*tert*-butoxycarbonyl)]aminoalkylpyrazoles **5** and the 1-(3-chlorophenyl)-5-[*N*-(*tert*-butoxycarbonyl)]aminoalkylpyrazoles **6**.

(b) A solution of the mixture of **5** and **6** (10 mmol) and CF_3COOH (0.5 mL) in CH_2Cl_2 (30 mL) was stirred for 4 h at room temperature. The reaction mixture was concentrated to dryness, and the resulting residue was separated using CHP-20P (reverse phase, Mitsubishi Chemical Co., Ltd, Japan; CH_3CN/H_2O) column chromatography and/or recrystallization from suitable solvents to give the 3-aminoalkyl-1-(3-chlorophenyl)pyrazole derivatives and the 5-aminoalkyl-1-(3-chlorophenyl)pyrazole derivatives.

5.4.1. 1-(3-Chlorophenyl)-5-(4-phenoxyphenyl)-3-(3-piperidyl)pyrazole hydrochloride (8). 38% Yield from **4a**, prepared by lyophilization. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.68–1.86 (m, 3H), 2.11–2.14 (m, 1H), 2.85–2.92 (m, 1H), 3.04–3.10 (m, 1H), 3.17–3.29 (m, 2H), 3.49–3.51 (m, 1H), 6.63 (s, 1H), 6.99 (d, 2H, $J = 8.8$ Hz), 7.04 (d, 2H, $J = 7.6$ Hz), 7.15–7.20 (m, 2H), 7.24 (d, 2H, $J = 8.8$ Hz), 7.38–7.43 (m, 5H), 8.81–9.19 (m, 2H); MS (APCI$^+$), *m/z* 430 (MH$^+$). Anal. Calcd for $C_{26}H_{24}ClN_3O{\cdot}HCl{\cdot}2H_2O$: C, 62.15; H, 5.82; N, 8.36; Cl, 14.11. Found: C, 62.38; H, 5.70; N, 8.32; Cl, 14.12.

5.4.2. 1-(3-Chlorophenyl)-3-(4-phenoxyphenyl)-5-(3-piperidyl)pyrazole hydrochloride (9). 3% Yield from **4a**, prepared by lyophilization. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.64–2.04 (m, 4H), 2.83–3.09 (m, 2H), 3.17–3.51 (m, 3H), 6.96 (s, 1H), 7.04–7.22 (m, 5H), 7.39–7.78 (m, 6H), 7.85 (d, 2H, $J = 8.3$ Hz), 8.76–9.16 (m, 2H); MS (APCI$^+$), *m/z* 430 (MH$^+$). Anal. Calcd for $C_{26}H_{24}ClN_3O{\cdot}1.2HCl{\cdot}2H_2O$: C, 61.55; H, 5.77; N, 8.24; Cl, 15.30. Found: C, 61.55; H, 5.60; N, 8.17; Cl, 15.12.

5.4.3. 1-(3-Chlorophenyl)-5-(4-phenoxyphenyl)-3-(2-piperidyl)pyrazole hydrochloride (10). 8% Yield from **4b**, mp 245–248 °C (acetone–Et_2O). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.56 (br, 1H), 1.76–1.94 (m, 3H), 2.11–2.15 (m, 2H), 3.08 (br, 2H), 4.27 (br, 1H), 7.07–7.09 (m, 4H), 7.16–7.26 (m, 2H), 7.38–7.48 (m, 2H), 7.55–7.69 (m, 4H), 7.79–7.82 (m, 2H), 9.31 (br, 1H), 9.37 (br, 1H); MS (APCI$^+$), *m/z* 430 (MH$^+$). Anal. Calcd for $C_{26}H_{24}ClN_3O{\cdot}1.25HCl{\cdot}0.75H_2O$: C, 63.86; H, 5.51; N, 8.59; Cl, 16.31. Found: C, 63.96; H, 5.29; N, 8.58; Cl, 16.02.

5.4.4. 1-(3-Chlorophenyl)-3-[3-(*N*-methyl)aminopropyl]-5-(4-phenoxyphenyl)pyrazole hydrochloride (11). 53% Yield from **4c**, prepared by lyophilization. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.99–2.09 (m, 2H), 2.52–2.56 (m, 3H), 2.73 (t, 2H, $J = 7.3$ Hz), 2.98 (br, 2H), 6.54 (s, 1H), 6.98–7.01 (m, 2H), 7.04 (d, 2H, $J = 8.4$ Hz), 7.16–7.21 (m, 2H), 7.26 (d, 2H, $J = 8.4$ Hz), 7.35–7.45 (m, 5H), 9.03 (br, 2H); MS (APCI$^+$), *m/z* 418 (MH$^+$). Anal. Calcd for $C_{25}H_{24}ClN_3O{\cdot}1.25HCl{\cdot}0.75H_2O$: C, 63.19; H, 5.66; N, 8.84; Cl, 16.41. Found: C, 63.13; H, 5.80; N, 8.76; Cl, 16.23.

5.4.5. 1-(3-Chlorophenyl)-3-(*N*-methylaminomethyl)-5-(4-phenoxyphenyl)pyrazole fumarate (12). 14% Yield from **4d**, mp 145–148 °C (*i*-PrOH–Et_2O). ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.48–2.54 (m, 3H), 4.05 (br, 2H), 6.49 (s, 2H), 6.72 (s, 1H), 6.98–7.08 (m, 4H), 7.15–7.28 (m, 4H), 7.38–7.46 (m, 5H), 9.87 (br, 3H); MS (APCI$^+$), *m/z* 390 (MH$^+$). Anal. Calcd for $C_{23}H_{20}ClN_3O{\cdot}C_4H_4O_4$: C, 64.10; H, 4.78; N, 8.31; Cl, 7.01. Found: C, 64.11; H, 4.76; N, 8.31; Cl, 6.91.

5.4.6. 1-(3-Chlorophenyl)-5-(*N*-methylaminomethyl)-3-(4-phenoxyphenyl)pyrazole hydrochloride (13). 57% Yield from **4d**, mp 184–187 °C (CH_3CN). ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.48–2.53 (m, 3H), 4.25 (m, 2H), 7.05–7.31 (m, 6H), 7.42 (d, 2H, $J = 8.4$ Hz), 7.47–7.76 (m, 4H), 7.83 (d, 2H, $J = 8.4$ Hz), 9.93 (br, 2H); MS (APCI$^+$), *m/z* 390 (MH$^+$). Anal. Calcd for $C_{23}H_{20}ClN_3O{\cdot}0.95HCl{\cdot}0.10H_2O$: C, 64.80; H, 5.00; N, 9.86; Cl, 16.22. Found: C, 64.53; H, 4.91; N, 10.00; Cl, 16.07.

5.4.7. 3-(*N*-Benzylaminomethyl)-1-(3-chlorophenyl)-5-(4-phenoxyphenyl)pyrazole hydrochloride (14). 25% Yield from **4e** as an oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.21 (s, 2H), 4.24 (s, 2H), 6.91 (s, 1H), 7.00–7.10 (m, 4H), 7.16–7.26 (m, 4H), 7.37–7.49 (m, 8H), 7.54–7.60 (m, 2H), 9.99 (br, 2H); HRMS calcd for 466.1686, found 466.1693.

5.4.8. 5-(3-Benzyloxyphenyl)-1-(3-chlorophenyl)-3-(4-piperidyl)pyrazole hydrochloride (15). 19% Yield from **4f**, mp 90–93 °C (acetone–Et_2O). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.84–2.03 (m, 2H), 2.13–2.21 (m, 2H),

2.96–3.10 (m, 3H), 3.31–3.34 (m, 2H), 5.05 (s, 2H), 6.59 (s, 1H), 6.76–6.82 (m, 1H), 6.94–7.18 (m, 3H), 7.25–7.44 (m, 9H), 9.02 (br, 1H), 9.18 (br, 1H); MS (APCI$^+$), *m/z* 444 (MH$^+$). Anal. Calcd for $C_{27}H_{26}ClN_3O{\cdot}HCl{\cdot}0.50H_2O$: C, 66.26; H, 5.77; N, 8.59; Cl, 14.49. Found: C, 66.37; H, 5.84; N, 8.55; Cl, 14.79.

5.4.9. 3-(3-Benzyloxyphenyl)-1-(3-chlorophenyl)-5-(4-piperidyl)pyrazole hydrochloride (16). 41% Yield from **4f**, mp 135–138 °C (CH_3CN). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.75–1.98 (m, 4H), 2.85–2.99 (m, 2H), 3.07–3.32 (m, 3H), 5.17 (s, 2H), 6.88 (s, 1H), 6.97–7.04 (m, 1H), 7.31–7.70 (m, 12H), 9.11 (br, 2H); MS (APCI$^+$), *m/z* 444 (MH$^+$). Anal. Calcd for $C_{27}H_{26}ClN_3O{\cdot}1.40HCl{\cdot}0.25H_2O$: C, 64.92; H, 5.63; N, 8.41; Cl, 17.03. Found: C, 65.16; H, 5.45; N, 8.57; Cl, 16.90.

5.4.10. 1-(3-Chlorophenyl)-3-(cyclohexyl)-5-(4-phenoxyphenyl)pyrazole (7). A solution of 1-cyclohexyl-3-(4-phenoxyphenyl)propane-1,3-dione (2.11 g, 6.54 mmol) and 3-chlorophenylhydrazine hydrochloride (2.34 g, 13.1 mmol) in EtOH (20 mL) was heated to reflux for 12 h and cooled to 0 °C. To this reaction mixture was added 10% aqueous citric acid (20 mL) and the resulting solution was taken up in AcOEt (70 mL), washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated to dryness. The residue was then purified by silica gel column chromatography (AcOEt/*n*-hexane) to give 1.15 g (41%) of **7** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21–1.48 (m, 5H), 1.70–1.91 (m, 6H), 6.48 (s, 1H), 7.00–7.18 (m, 5H), 7.28–7.53 (m, 6H), 7.81 (d, 2H, J = 8.8 Hz); HRMS calcd for 429.1734, found 429.1730.

5.5. Synthesis of 2-hydroxyethane-1-one 19a,b

5.5.1. 2-(3-Chlorophenyl)-1-(4-phenoxyphenyl)-2-hydroxyethane-1-one (19a). (a) To a solution of **17a** (6.33 g, 31.3 mmol) in CH_2Cl_2 (30 mL) was added dropwise trimethylsilylcyanide (3.11 g, 31.3 mmol) at 0 °C and added zinc iodide (15 mg) at the same temperature. The mixture was stirred for 48 h at room temperature. The solvent was evaporated and the residue was purified by silica gel column chromatography ($CHCl_3$) to give **18a** as an oil, which was used in the next step without further purification.

(b) To a solution of 1 mol/L lithium hexamethyldisilazide (25 mmol) in THF (50 mL) was added dropwise **18a** (7.44 g, 25 mmol) in THF (15 mL) at −78 °C under argon atmosphere. The yellow suspension was stirred for 30 min at the same temperature. To the resulting solution was added dropwise 3-chlorobenzaldehyde (3.51 g, 25 mmol) in THF (20 mL) at the same temperature. After removal of the cold bath, the reaction mixture was raised to room temperature and stirred for 12 h. To this reaction mixture was added saturated aqueous NH_4Cl (60 mL), and the solution was taken up in AcOEt (100 mL), washed with brine, dried over $MgSO_4$, and concentrated to dryness. The residue was dissolved in MeOH (30 mL) and was added 5% aqueous H_2SO_4 (15 mL). The reaction mixture was stirred for 30 min at room temperature. The resulting solution was taken up in Et_2O (50 mL), washed with 0.5 mol/L NaOH (15 mL), dried over $MgSO_4$, and concentrated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$) to give 2.01 g (19% from **17a**) of **19a** as an oil. ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.61 (d, 1H, J = 6.0 Hz) 5.84 (d, 1H, J = 6.0 Hz), 6.94 (d, 2H, J = 8.6 Hz), 7.01–7,42 (m, 9H), 7.88 (d, 2H, J = 8.6 Hz); MS (APCI$^+$), *m/z* 339 (MH$^+$).

5.5.2. 2-(3-Benzyloxyphenyl)-1-(3-chlorophenyl)-2-hydroxyethane-1-one (19b). In a similar manner to that described above, **19b** was prepared from **17b** via **18b** in 78% overall yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.40 (d, 1H, J = 6.1 Hz), 5.05 (s, 2H), 5.85 (d, 1H, J = 6.1 Hz), 6.89–6.94 (m, 2H), 7.23–7.51 (m, 9H), 7.70–7.90 (m, 2H); MS (APCI$^+$), *m/z* 353 (MH$^+$).

5.6. Synthesis of oxazole derivatives 21, 22

5.6.1. 5-(3-Chlorophenyl)-4-(4-phenoxyphenyl)-2-(4-piperidyl)oxazole fumarate (21). A solution of **19a** (1.60 g, 4.72 mmol), 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (1.19 g, 5.18 mmol), 1-ethyl-3[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC, 0.92 g, 5.18 mmol) and 4-dimethylaminopyridine (DMAP, 0.58 g, 4.72 mmol) in CH_2Cl_2 (40 mL) was stirred for 6 h at room temperature. To this reaction mixture was added 10% aqueous citric acid (30 mL) and the resulting solution was taken up in $CHCl_3$ (50 mL), dried over $MgSO_4$ and concentrated to dryness. The residue was dissolved in AcOH (40 mL) and was added NH_4OAc (0.91 g, 11.8 mmol). The reaction mixture was heated to reflux for 6 h and cooled to room temperature. The solvent was evaporated, and the residue was purified by silica gel column chromatography (2:1 $CHCl_3$/MeOH) and recrystallization from EtOH–Et_2O to give 1.16 g (51% from **19a**) of **21**, mp 145–148 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.62–1.74 (m, 1H), 1.91–2.02 (m, 2H), 2.22–2.26 (m, 1H), 2.83–2.92 (m, 1H), 2.99–3.07 (m, 1H), 3.18–3.32 (m, 3H), 6.48 (s, 2H), 6.63 (br, 2H), 7.04–7.10 (m, 4H), 7.16–7.21 (m, 1H), 7.42–7.51 (m, 5H), 7.54–7.61 (m, 3H), 8.12 (br, 1H); HRMS calcd for 430.1448, found 430.1454.

5.6.2. 5-(3-Benzyloxyphenyl)-4-(3-chlorophenyl)-2-(4-piperidyl)oxazole fumarate (22). In a similar manner to that described above, **22** was prepared from **19b** via **20b** in 44% overall yield, mp 123–126 °C ($CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.67 (br, 3H), 1.81–1.93 (m, 2H), 2.10–2.14 (m, 2H), 2.74–2.81 (m, 2H), 2.97–3.05 (m, 1H), 3.19–3.23 (m, 2H), 5.00 (s, 2H), 6.95–6.97 (m, 1H), 7.15–7.18 (m, 2H), 7.23–7.39 (m, 10H), 7.50–7.70 (m, 2H); MS (APCI$^+$), *m/z* 445 (MH$^+$). Anal. Calcd for $C_{27}H_{25}ClN_2O_2Cl{\cdot}C_4H_4O_4$: C, 66.37; H, 5.21; N, 4.99; Cl, 6.32. Found: C, 66.06; H, 4.86; N, 4.93; Cl, 6.43.

5.7. Synthesis of imidazole derivatives 24, 25

5.7.1. 5-(3-Chlorophenyl)-4-(4-phenoxyphenyl)-2-(4-piperidyl)imidazole difumarate (24). (a) A solution of $CuSO_4$ (1.35 g, 8.50 mmol) in pyridine (5 mL) and H_2O (1 mL) was heated to reflux for 10 min and cooled to

room temperature. To the resulting solution was added **19a** (1.44 g, 4.25 mmol) and reheated to reflux for 1 h and cooled to room temperature. The solvent was evaporated and the residue was taken up Et_2O (20 mL) and H_2O (10 mL). The organic layer was separated and dried over $MgSO_4$, and concentrated to dryness. The residue was purified by silica gel column chromatography (3:1 $CHCl_3$/*n*-hexane) to give **23a** as an oil, which was used in the next step without further purification.

(b) A solution of **23a** (0.65 g, 1.93 mmol), 1-(*tert*-butoxycarbonyl)-4-formylpiperidine (0.41 g, 1.93 mmol) and NH_4OAc (1.19 g, 1.54 mmol) in AcOH (15 mL) was stirred for 4 h at 90 °C. The solvent was evaporated, and the residue was taken up in $CHCl_3$ (20 mL), washed with saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. The solvent was evaporated. The residue was purified by CHP-20P (3:1 CH_3CN/H_2O) column chromatography to give 0.22 g (12% from **19a**) of **24**, mp 93–95 °C (EtOH–Et_2O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.89–2.02 (m, 2H), 2.10–2.16 (m, 2H), 2.95–3.06 (m, 3H), 3.30 (br, 5H), 3.32–3.36 (m, 2H), 6.51 (s, 4H), 7.00–7.06 (m, 4H), 7.13–7.18 (m, 1H), 7.24–7.49 (m, 9H); HRMS calcd for 429.1608, found 429.1599.

5.7.2. 5-(3-Benzyloxyphenyl)-4-(3-chlorophenyl)-2-(4-piperidyl)imidazole dihydrochloride (25). In a similar manner to that described above, **25** was prepared from **19b** via **23b** in 24% overall yield, mp 140–141 °C (CH_3CN). ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.06–2.34 (m, 4H), 2.97–3.16 (m, 2H), 3.39–3.53 (m, 3H), 5.11 (s, 2H), 7.03 (d, $J = 8.0$ Hz, 1H), 7.09–7.21 (m, 2H), 7.35–7.64 (m, 11H), 9.24 (br, 3H); MS (APCI$^+$), *m/z* 444 (MH$^+$). Anal. Calcd for $C_{27}H_{26}ClN_3OCl{\cdot}2HCl{\cdot}1.30H_2O$: C, 60.02; H, 5.71; N, 7.78; Cl, 19.68. Found: C, 59.65; H, 5.33; N, 7.75; Cl, 19.71.

5.8. Microbiology

5.8.1. In vitro antibacterial activity. The minimal inhibitory concentration (MIC) of each test-compound was determined by a microdilution method according to guidelines of the National Committee for Clinical Laboratory Standard.

5.8.2. Enzyme inhibition. DNA gyrase supercoiling and topoisomerase IV decatenation assays using *E. coli* enzymes were carried out by the methods of Sato et al.[19] and Peng and Marians,[20] respectively.

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