Synthesis and Antibacterial Activity of a Novel Series of Potent DNA Gyrase Inhibitors. Pyrazole Derivatives

Akihiko Tanitame,*,† Yoshihiro Oyamada,‡ Keiko Ofuji,† Mika Fujimoto,‡ Noritaka Iwai,§ Yoichi Hiyama,† Kenji Suzuki,† Hideaki Ito,‡ Hideo Terauchi,† Motoji Kawasaki,† Kazuo Nagai,# Masaaki Wachi,§ and Jun-ichi Yamagishi‡

Chemistry Research Laboratories, Pharmacology and Microbiology Research Laboratories, Dainippon Pharmaceutical Co., Ltd., 33-94, Enoki, Suita, Osaka 564-0053, Japan, Department of Bioengineering, Tokyo Institute of Technology, 4259, Nagatsuda, Midori-ku, Yokohama 226-8501, Japan, and Department of Biological Chemistry, Chubu University, 1200 Matsumoto, Kasugai, Aichi 487-8501, Japan

Received August 14, 2003

We have previously found that a pyrazole derivative **1** possesses antibacterial activity and inhibitory activity against DNA gyrase and topoisomerase IV. Here, we synthesized new pyrazole derivatives and found that 5-[(*E*)-2-(5-chloroindol-3-yl)vinyl]pyrazole **16** possesses potent antibacterial activity and selective inhibitory activity against bacterial topoisomerases. Many of the synthesized pyrazole derivatives were potent against clinically isolated quinolone- or coumarin-resistant Gram-positive strains and had minimal inhibitory concentration values against these strains equivalent to those against susceptible strains.

Introduction

Over the past decade, bacterial DNA gyrase has drawn much attention as a selected target for finding potent antibacterial agents. Accordingly, a number of synthetic quinolone antibacterial agents have been developed and are now widely used for treatment of bacterial infectious diseases. Quinolones inhibit DNA gyrase and topoisomerase IV and cause bacterial cell death.[1] In particular, fluoroquinolones, such as sparfloxacin (SPFX, Figure 1), have been shown to be highly successful inhibitors of bacterial DNA gyrase.[2] Besides the quinolones, naturally occurring bacterial DNA gyrase inhibitors such as the coumarins, which include novobiocin (NB, Figure 1), are also known as antibacterial agents.[3,4] The coumarins inhibit ATPase activity of DNA gyrase by competing with ATP for binding to the subunit B of the enzyme. However, because of side effects, no pharmaceutically 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. Since most of these multidrug-resistant bacteria are also resistant to treatment with the quinolones, it is important to find a new class of DNA gyrase inhibitors to solve this problem. Although many efforts have been dedicated to finding potent antibacterial agents that can overcome bacterial resistance, promising lead structures of DNA gyrase inhibitors with novel mechanisms of action have not been found.[5]

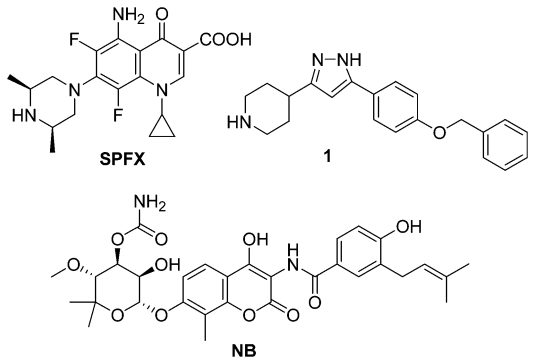


Figure 1.

We have previously developed a new screening system for specific inhibitors of chromosome partitioning in *E. coli*.[6] This assay system can detect the production of bacterial anucleate cells (chromosomeless cells) caused by inhibition of chromosome partitioning as development of blue color around paper disks containing the inhibitors. To generate a new class of DNA gyrase inhibitors, we randomly screened our chemical library on this assay system and found several new DNA gyrase inhibitors. Among them, the pyrazole derivative **1** (Figure 1) was identified as an interesting lead candidate. Compound **1** showed the same minimal inhibitory concentration value (MIC) against clinically isolated multidrug resistant Gram-positive bacteria as against susceptible strains. However, the antibacterial activity of **1** was weak (MIC = 64 μg/mL) because it only slightly inhibited DNA gyrase and topoisomerase IV (IC_{50} = 128 μg/mL). Our aim in this study was to optimize the lead compound **1** and to find new, potent DNA gyrase inhibitors with antibacterial activity against MRSA, PRSP, and VRE. We report herein the synthesis and structure−activity relationships of a series of pyrazole derivatives.

* To whom correspondence should be addressed. Phone: +81-6-6337-5906. Fax: +81-6-6338-7656. E-mail: akihiko-tanitame@dainippon-pharm.co.jp.

† Chemistry Research Laboratories, Dainippon Pharmaceutical Co., Ltd.

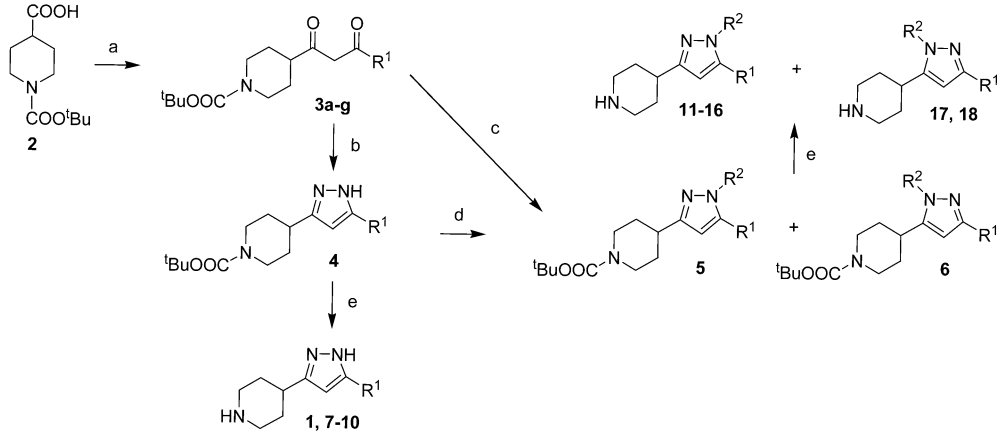
‡ Pharmacology and Microbiology Research Laboratories, Dainippon Pharmaceutical Co., Ltd.

§ Tokyo Institute of Technology.

Chubu University.

10.1021/jm030394f CCC: $27.50
Published on Web 06/05/2004

Scheme 1. General Synthesis of 3- and 5-(4-Piperidyl)pyrazole Derivatives[a]



[a] Reagents: (a) (i) CDI/THF, (ii) R^1COCH_3/LDA or LHMDS; (b) $NH_2NH_2 \cdot H_2O$/EtOH; (c) R^2 $NHNH_2$/EtOH; (d) $R^2B(OH)_2$/$Cu(OAc)_2$/pyridine/CH_2Cl_2; (e) HCl/EtOH or TFA/CH_2Cl_2.

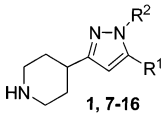
Chemistry

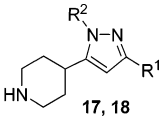
The 3- and 5-(4-piperidyl)pyrazole derivatives **1** and **7**−**18** were synthesized as shown in Scheme 1. The requisite intermediate 1,3-diketones **3a**−**g** were prepared by coupling reaction of the (1-*tert*-butyloxycarbonyl)isonipecotic acid **2** with various methyl ketones in THF using *N*,*N*′-carbonyldiimidazole and a base (e.g., lithium diisopropylamide or lithium hexamethydisilazide).[7] Cyclization of the 1,3-diketones **3a**−**g** with hydrazine hydrate in EtOH gave the 1-unsubstituted 3-[4-(1-*tert*-butyloxycarbonyl)piperidyl]pyrazole **4**, which furnished the 1-unsubstituted 3-(4-piperidyl)pyrazoles **1** and **7**−**10** as acid salts by removal of the protecting group under acidic conditions (method A). The 1-substituted 3- and 5-[4-(1-*tert*-butyloxycarbonyl)piperidyl]-pyrazoles **5** and **6** were obtained as a mixture by two different routes, i.e., either condensation of **3a**−**g** with phenyl- or alkylhydrazines (method B) or phenylation of **4** using substituted phenylboronic acids with cupric acetate and pyridine in CH_2Cl_2 (method C).[8] Cleavage of the protecting group in the mixture of **5** and **6** under acidic conditions, followed by separation using CHP-20P (reverse phase) column chromatography and/or recrystallization gave the 1-substituted 3-(4-piperidyl)pyrazole derivatives **11**−**16** and the 1-substituted 5-(4-piperidyl)-pyrazole derivatives **17** and **18** as acid salts. The other 1-substituted 5-(4-piperidyl)pyrazole derivatives, except for **17** and **18**, were not isolated. The regiochemistry of **11**−**18** was determined on the basis of nuclear Overhauser effect (NOE) experiments.

Results and Discussion

To verify whether the lipophilicity of the synthesized pyrazole derivatives affects their antibacterial activity, we evaluated the in vitro antibacterial activity of the 3-(4-piperidyl)pyrazoles **7**−**16** and the 5-(4-piperidyl)-pyrazoles **17** and **18** against two Gram-positive and two Gram-negative bacteria (Table 1). First, transformation of the 4-benzyloxyphenyl moiety of the lead compound **1** was examined (**7**−**10**). The 4-methoxyphenyl derivative **7** did not show any antibacterial activity. However, the 4-phenoxyphenyl derivative **8** and the 2-naphthyl derivative **9** showed the same antibacterial activity as **1**. The (*E*)-2-(3,4-dichlorophenyl)vinyl derivative **10** revealed 16-fold more potent antibacterial activity against *S. aureus* and was 16-fold more potent against *E. coli* than **1**. The MIC value of **10** against *E. coli* NIHJ JC-2 was almost similar to that against *E. coli* K901. From these results, it was considered that the (*E*)-2-(3,4-dichlorophenyl)vinyl substituent on the R^1 moiety induces potent antibacterial activity against not only Gram-positive bacteria but also Gram-negative bacteria.

Table 1. Antibacterial Activity of the Synthesized Pyrazole Derivatives





Compd	R^1	R^2	MIC (μg/mL)			
			S.a.1	S.a.2	E.c.1	E.c.2
1	4-PhCH$_2$O-Ph	H	64	64	128	64
7	4-MeO-Ph	H	>128	>128	>128	>128
8	4-PhO-Ph	H	64	64	128	64
9	2-naphtyl	H	64	64	128	64
10		H	4	4	8	4
11	4-PhCH$_2$O-Ph	Me	32	32	128	64
12	2-naphtyl	3-Cl-Ph	4	4	32	4
13	4-PhO-Ph	3-Cl-Ph	4	4	32	4
14	4-PhO-Ph	PhCH$_2$	4	4	64	4
15		3-Cl-Ph	4	4	64	4
16		3-Cl-Ph	1	1	8	4
17	4-PhCH$_2$O-Ph	Me	64	64	128	64
18		3-Cl-Ph	8	8	128	8
SPFX			0.125	128	0.032	0.004
NB			0.25	0.25	64	0.5

[a] S.a.1, *S. aureus* FDA 209P; S.a.2, *S. aureus* KMP9 (MRSA); E.c.1, *E. coli* NIHJ JC-2; E.c.2, *E. coli* K901 (a multidrug efflux pump mutant).

Second, the effects of different substituents on the nitrogen atom of the pyrazole ring were investigated (**11**−**14**). The 1-methylpyrazole derivative **11** and the 1-unsubstituted pyrazole derivative **1** showed almost similar antibacterial activity against the four strains of bacteria studies. The 1-(3-chlorophenyl)pyrazole derivatives **12** and **13** and the 1-benzyl derivative **14**, however, showed 16-fold more potent antibacterial activity against *S. aureus* and 2- to 16-fold more potent antibacterial activity against *E. coli* than the parent compound **1**. Thus, lipophilic substituents, such as a phenyl (**12**, **13**) or a benzyl (**14**) appended on the nitrogen atom of the pyrazole ring, gave good antibacterial activity, while small substituents, such as a hydro-

Table 2. Antibacterial Activity of Selected Pyrazole Derivatives against Susceptible and Resistant Strains

	MIC (μg/mL)				
organism[a]	**1**	**12**	**16**	SPFX	NB
S. aureus FDA 209P[b]	64	4	1	0.125	0.25
S. aureus KMP9 (MRSA)[c]	64	4	1	128	0.25
S. aureus RN4220[b]	64	8	2	0.125	0.125
S. aureus N175[d]	64	8	2	0.125	4
S. pneumoniae ATCC49619[b]	64	8	2	0.125	0.5
S. pneumoniae KT2524 (PRSP)[c]	64	8	2	4	0.5
E. faecalis ATCC29212[b]	64	8	2	0.25	2
E. faecalis KU1777 (VRE)[e]	64	8	2	32	2

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

gen (**1**) or a methyl (**11**), ended in less potent antibacterial activity.

As for the 5- and 3-vinyl derivatives (**15**, **16**, **18**), the MIC values of the 5-[(*E*)-2-(2,6-dichlorophenyl)vinyl]-pyrazole **15** against the four strains of bacteria studied were almost similar to those of **13** or **14**. However, the 5-[(*E*)-2-(5-chloroindol-3-yl)vinyl]pyrazole **16**, having a 3-chlorophenyl moiety, showed more potent antibacterial activity than **15**. Although **18** had 8-fold more potent antibacterial activity against *S. aureus* and *E. coli* K901 than the parent compound **1**, its antibacterial activity against *E. coli* NIHJ JC-2 was similar to that of **1**. From the MIC values of **8** and **13**, and those of **10** and **15** against *E. coli* NIHJ JC-2 and *E. coli* K901, it seems that the 1-(3-chlorophenyl)pyrazoles **13** and **15** were pumped out of the bacteria by a bacterial outer membrane pump. However, because **16** showed the most potent antibacterial activity against the four strains of bacteria, it is believed that this compound was not affected by the bacterial outer membrane pump and that it penetrated well through the bacterial membrane. The 5-[(*E*)-2-(2,6-dichlorophenyl)vinyl]pyrazole **15** showed slightly more potent antibacterial activity than the 3-[(*E*)-2-(2,6-dichlorophenyl)vinyl]pyrazole **18**. Although most of the synthesized pyrazole derivatives revealed potent antibacterial activity against not only *S. aureus* FDA 209P but also *S. aureus* KMP9, **16** exhibited the most potent antibacterial activity among all the compounds synthesized in this study.

To examine whether the pyrazole derivatives are effective against multidrug-resistant Gram-positive bacteria, MIC values of the selected pyrazole derivatives **1**, **12**, and **16** were determined against quinolone-resistant clinical isolates and coumarin-resistant laboratory isolates of Gram-positive organisms and were compared with those of sparfloxacin and novobiocin.[9] As shown in Table 2, MIC values of sparfloxacin against 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 32 μg/mL, respectively. On the other hand, **1**, **12**, and **16** revealed the same antibacterial activity against sensitive and multidrug resistant Gram-positive bacteria. 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, **1**, **12**, and **16** showed the same antibacterial activity against sensitive and coumarin-resistant *S. aureus*. Compound **16** demonstrated the most potent antibacterial activity against sensitive and resistant Gram-positive bacteria with across-the-board MIC values of 1−2 μg/mL. Thus, it is suggested that the pyrazole derivatives have potent antibacterial activity against susceptible and quinolone- and coumarin-resistant Gram-positive bacteria.

Table 3. Inhibitory Effects of Selected Pyrazole Derivatives against DNA Gyrase and Topoisomerase IV

	IC_{50} (μg/mL)				
	1	**12**	**16**	SPFX	NB
S. aureus DNA gyrase[a]	128	32	14	14	0.25
S. aureus DNA Topo IV[b]	128	32	14	14	28
E. coli DNA gyrase[a]	>128	32	14	0.25	0.25
E. coli DNA Topo IV[b]	128	32	8.0	6.9	3.5
human DNA Topo II[c]	200	50	>400	>400	>400

[a] DNA gyrase supercoiling activity. [b] Topoisomerase IV decatenation activity. [c] Topoisomerase II relaxation activity.

Next, the inhibitory activity of the selected compounds **1**, **12**, and **16** against DNA gyrase and topoisomerase IV was examined. As shown in Table 3, **12** and **16** having potent antibacterial activity strongly inhibited DNA gyrase and topoisomerase IV isolated from *S. aureus* and *E. coli*. The SAR for this inhibition was almost parallel to that for the antibacterial activity mentioned above. In addition, MIC values for both compounds correlated well with IC_{50} values especially in Gram-positive microoraganisms (Tables 2 and 3), indicating that inhibition of topoisomerases by pyrazole derivatives causes bacterial cell growth inhibition. Moreover, **16** did not inhibit human topoisomerase II even at 400 μg/mL. This result indicates that **16**, like sparfloxacin and novobiocin, selectively inhibits bacterial topoisomerase.

In summary, we have described the synthesis and structure−activity relationships of new pyrazole analogues. The 5-[(*E*)-2-(5-chloroindol-3-yl)vinyl]pyrazole **16**, one of the most active compounds, showed potent antibacterial activity against not only susceptible strains but also multidrug-resistant strains. In addition, **16** showed a more potent antibacterial activity against clinically isolated quinolone- and coumarin-resistant Gram-positive bacteria than sparfloxacin and novobiocin, respectively. We are pursuing further modifications of this novel pyrazole scaffold that can potently inhibit DNA gyrase and topoisomerase IV.

Experimental Section

General Procedures for Synthesis of the 3- and 5-(4-Piperidyl)pyrazole Derivatives. Methods A and B. (a) To an EtOH (30 mL) solution of 1,3-diketones **3** (10 mmol) was added hydrazine hydrate (20 mmol) or monosubstituted hydrazine (20 mmol), and the reaction mixture was refluxed for 12 h. To this reaction mixture was added 10% citric acid (30 mL), and the resulting solution was taken up in AcOEt (100 mL), washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The residue was purified by column chromatography (silica gel, $CHCl_3$−MeOH) to give 1-unsubstituted 3-[4-(1-*tert*-butyloxycarbonyl)piperidyl]pyrazoles **4** or a mixture of 1-substituted 3-[4-(1-*tert*-butyloxycarbonyl)piperidyl]pyrazoles **5** and 1-substituted 5-[4-(1-*tert*-butyloxycarbonyl)piperidyl]pyrazoles **6**.

(b) A solution of **4** or a mixture of **5** and **6** (10 mmol) in CH_2Cl_2 (30 mL) containing CF_3COOH (0.5 mL) was stirred for 4 h at room temperature. The reaction mixture was evaporated, and the resulting residue was separated using CHP-20P (reverse phase) column chromatography and/or recrystalliza-

tion from suitable solvents to give the 3-(4-piperidyl)pyrazoles or the 5-(4-piperidyl)pyrazoles, respectively.

Acknowledgment. We are grateful to Drs. K. Chiba, Y. Furutani, and Y. Nishikawa for their encouragement throughout this work. Thanks are also due to members of the pharmacology section for their pharmacological support and to members of the analytical chemistry section for their elements analysis and spectral measurements. We also thank Keith Poole, Department of Microbiology and Immunology, Queen's University, for supplying *E. coli* K901.

Supporting Information Available: Experimental procedures and characterization data of novel synthetic compounds, combustion analysis data, a table of antibacterial activity of selected pyrazole derivatives against different strains including Gram-negative bacteria, and procedures of biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ferrero, L.; Cameron, B.; Manse, B.; Lagneaux, D.; Crouzet, J.; Famechon, A.; Blanche, F. Cloning and Primary Structure of *Staphylococcus aureus* DNA Topoisomerase IV: A Primary Target of Fluoroquinolones. *Mol. Microbiol.* **1994**, *13*, 641−653.

(2) Miyamoto, T.; Matsumoto, J.; Chiba, K.; Egawa, H.; Shibamori, K.; Minamida, A.; Nishimura, Y.; Okada, H.; Kataoka, M.; Fujita, M.; Hirose, T.; Nakano, J. Synthesis and Structure−Activity Relationships of 5-Substituted 6,8-Difluoroquinolones, Including Sparfloxacin, a New Quinolone Antibacterial Agent with Improved Potency. *J. Med. Chem.* **1990**, *33*, 1645−1656.

(3) Kim, O. K.; Ohemeng, K. A. Patents on DNA gyrase inhibitors: January 1995 to March 1998. *Expert Opin. Ther. Pat.* **1998**, *8*, 959−969.

(4) Maxwell, A. The Interaction between Coumarin Drugs and DNA Gyrase. *Mol. Microbiol.* **1993**, *9*, 681−686.

(5) Boehm, H.; Boehringer, M.; Bur, D.; Gmuender, H.; Hunber, W.; Klaus, W.; Kostrewa, D.; Kuehne, H.; Luebbers, T.; Meunier-Keller, N.; Mueller, F. Novel Inhibitors of DNA Gyrase: 3D Structure Based Biased Needle Screening, Hit Validation by Biophysical Methods, and 3D Guided Optimization. A Promising Alternative to Random Screening. *J. Med. Chem.* **2000**, *43*, 2664−2674.

(6) Wachi, M.; Iwai, N.; Kunihisa, A.; Nagai, K. Irregular Nuclear Localization and Anucleate Cell Production in *Escherichia coli* Induced by a Ca^{2+} Chelator, EGTA. *Biochimie* **1999**, *81*, 909−913.

(7) Rowley, M.; Broughton, H. B.; Collins, I.; Baker, R.; Emms, F.; Marwood, R.; Patel, S.; Patel, S.; Ragan, C. I.; Freedman, S. B.; Leeson, P. D. 5-(4-Chlorophenyl)-4-methyl-3-(1-(2-phenylethyl)-piperidin-4-yl)isoxazole: A Potent, Selective Antagonist at Human Cloned Dopamine D4 Receptors. *J. Med. Chem.* **1996**, *39*, 1943−1945.

(8) Lam, P. Y. S.; Clark, C. G.; Saubern, S.; Adams, J.; Winters, M. D.; Chan, D. M. T.; Combs, A. New Aryl/Heteroaryl C−N Bond Cross-Coupling Reactions via Arylboronic Acid/Cupric Acetate Arylation. *Tetrahedron Lett.* **1998**, *39*, 2941−2944.

(9) Stieger, M.; Angehrn, P.; Wohlgensinger, B.; Gmunder, H. GyrB Mutations in *Staphylococcus aureus* Strains Resistant to Cyclothialidine, Coumermycin, and Novobiocin. *Antimicrob. Agents Chemother.* **1996**, *40*, 1060−1062.

JM030394F