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Synthesis and antibacterial activity of a novel series of DNA gyrase inhibitors: 5-[(*E*)-2-arylvinyl]pyrazoles

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Abstract—The 2-arylvinyl moiety in 1-(3-chlorophenyl)-3-(4-piperidyl)-5-[(*E*)-2-(5-chloro-1*H*-indol-3-yl)vinyl]pyrazole **2**, which has previously shown improved DNA gyrase inhibition and target-related antibacterial activity, was transformed to other groups and the in vitro antibacterial activity of the synthesized compounds was evaluated. Many of the 5-[(*E*)-2-arylvinyl]pyrazoles synthesized in this study exhibited potent antibacterial activity against quinolone-resistant clinical isolates of Gram-positive bacteria with minimal inhibitory concentration values equivalent to those against susceptible strains.

Antibacterial resistance to hospital-acquired Gram-positive bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant enterococci (VRE), has been increasing at an alarming rate. Furthermore, these drug-resistant bacteria commonly infect healthy people in large communities, thus creating a serious health problem around the globe. Consequently, the discovery of novel and potent bactericides is the best way to overcome bacterial resistance and develop effective therapies.

Bacterial DNA gyrase is a proven target for antibacterial chemotherapy.[1] Quinolones, such as sparfloxacin[2] (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 novobiocin (NB, Fig. 1), have also been known as antibacterial agents.[3] Although many efforts have been dedicated to finding potent antibacterial agents that can overcome bacterial resistance, promising new lead structures of DNA gyrase inhibitors have not been found.[4–9]

Using a new screening system for specific inhibitors of chromosome partitioning in *Escherichia* (*E.*) *coli*,[10,11] we have previously reported compounds **1**, **2** and **3** (Fig. 1) as moderate inhibitors of DNA gyrase and topoisomerase IV with potent antibacterial activity against multidrug resistant strains as well as susceptible

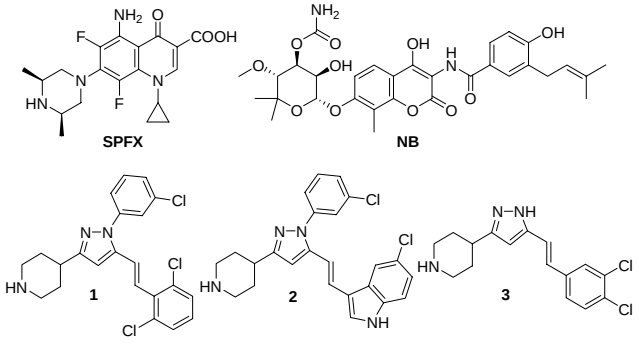

Figure 1.

Keywords: DNA gyrase inhibitor; 5-[(*E*)-2-Arylvinyl]pyrazoles; Multidrug resistant strains.
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strains.[12] In addition, we have published the synthesis and structure–activity relationships (SARs) of 3-substituted pyrazole derivatives.[13] In this study, we derived the 2-arylvinyl moieties of **1**, **2** and **3** to other 2-arylvinyl and 2-arylethyl substituents to find novel potent DNA gyrase inhibitors with antibacterial activity against MRSA, PRSP and VRE. Here, we report the synthesis and SARs of a series of 5-[(*E*)-2-arylvinyl]pyrazoles and 5-(2-arylethyl)pyrazoles.

The novel 5-[(*E*)-2-arylvinyl]pyrazoles **7a**–**d**, **8a**,**b** were prepared as shown in Scheme 1. Condensation of aldehydes **4** with acetone using aqueous NaOH gave the various 2-acetylvinyl derivatives **5**. The requisite intermediate 1,3-diketones **6** were synthesized by coupling reaction of the *N*-[*tert*-butoxycarbonyl (Boc)]-piperidine-4-carboxylic acid with **5** in THF using 1,1′-carbonyldi-1*H*-imidazole (CDI) and lithium hexamethyldisilazide (LHMDS).[14] Condensation of **6** with 3-chlorophenylhydrazine hydrochloride, followed by cleavage of the Boc group under acidic conditions and separation using CHP-20P (reverse phase) column chromatography and/or recrystallization gave one of the isomers, the 1-(3-chlorophenyl)-5-[(*E*)-2-arylvinyl]pyrazoles **7a**–**d** as acid salts. The position of 3-chlorophenyl groups in **7a**–**d** was determined on the basis of nuclear Overhauser effects (NOE) experiments. For example, in compound **7b**, a correlation of NOE was observed between the protons of the vinyl moiety and the *ortho* protons of the 3-chlorophenyl moiety.

The 1*H*-pyrazoles **8a**,**b** were synthesized by condensation of **6** with hydrazine hydrate and aqueous NaOH in a similar manner to that described above. 5-(2-Arylethyl)pyrazoles **10a**,**b** and **11** having 2-arylethyl groups at the 5-position on the pyrazole ring instead of the 2-arylvinyl of **1**, **2** and **8b** were prepared from the corresponding 1,3-diketones **9** (Scheme 1), which were prepared by hydrogenation of **6**, in a similar manner to that described above.

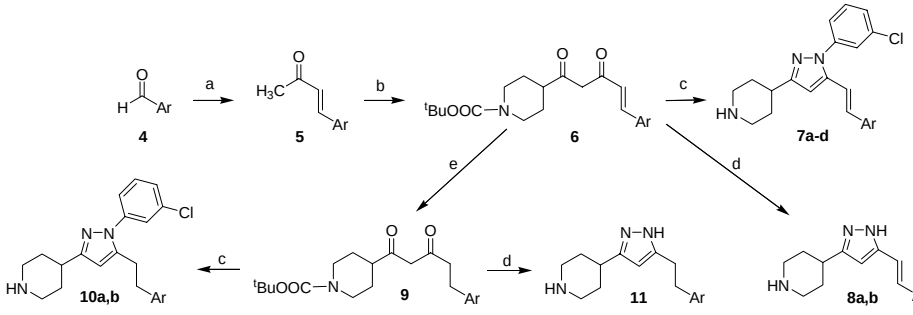
To investigate the effects of R groups on the pyrazole ring on the antibacterial activity, the vinyl moieties in **1**, **2** and **3** were transformed to other groups and the minimal inhibitory concentration (MIC) of compounds obtained was evaluated against two Gram-positive microdilution method (Table 1).

The (*E*)-2-[4-(3,4-dichlorobenzyloxy)phenyl]vinyl derivative **7a** and the (*E*)-2-(3,4-dichlorophenyl)vinyl derivative **7b** showed almost the same antibacterial activity against the four strains of bacteria compared to **1**. The (*E*)-2-(5-chloro-1-methylindol-3-yl)vinyl derivative **7c** and the (*E*)-2-(5-chloro-1*H*-indol-2-yl)vinyl derivative **7d** showed 2-fold less potent antibacterial activity against *Staphylococcus* (*S.*) *aureus* than **2**. Compound **7c**, which was the *N*-methyl derivative of **2**, did not show antibacterial activity against *E. coli* NIHJ JC-2. This result suggests that the 5-chloro-1*H*-indol-3-yl groups in **2** and **7d** are important for antibacterial activity against Gram-negative bacteria. The 2-(2,6-dichlorophenyl)ethyl derivative **10a** and the 2-(5-chloro-1*H*-indol-3-yl)ethyl derivative **10b** exhibited 4- to 16-fold less potent antibacterial activity than the corresponding 5-[(*E*)-2-arylvinyl]pyrazoles **1** and **2**, respectively. This result suggests that the vinyl moieties in the R groups appended on the pyrazoles are essential for antibacterial activity.

The 1*H*-pyrazoles **3** and **8a** showed almost the same antibacterial activity against two Gram-positive bacteria compared to 1-(3-chlorophenyl)pyrazoles **7a** and **7b**, respectively. Remarkably, compound **8a** showed 8-fold more potent antibacterial activity against *E. coli* NIHJ JC-2 than the corresponding 1-(3-chlorophenyl)pyrazole **7a**. Also, the 5-(2-arylethyl)pyrazole **11** showed almost the same antibacterial activity against two Gram-positive bacteria compared to the corresponding 1-(3-chlorophenyl)pyrazole **10a**. Compound **8b** resulted in less potent antibacterial activity compared to the 1-(3-chlorophenyl)pyrazole **1**. Thus, the substituents on the pyrazole ring of the isomers of the 5-[(*E*)-2-dichlorophenylvinyl]pyrazole derivatives show different effects on the antibacterial activity from each other.

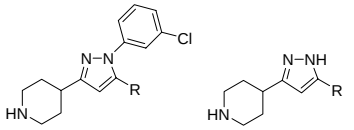
Since the 1*H*-pyrazoles **3** and **8a** showed nearly the same antibacterial activity against *E. coli* NIHJ JC-2 and W3110 Δ*acrA*, it is presumed that these derivatives are not affected by bacterial outer membrane pump. The pyrazole derivatives synthesized in this study showed almost same antibacterial activity against susceptible and resistant *S. aureus*.

To examine whether the novel 5-[(*E*)-2-arylvinyl]pyrazoles are effective against multidrug resistant Gram-positive bacteria, MIC values of selected compounds against

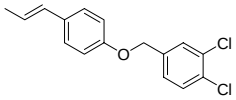
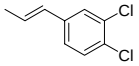
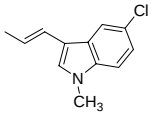
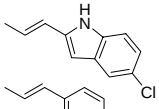
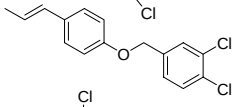
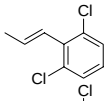
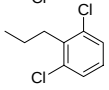
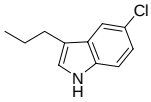
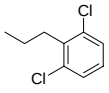
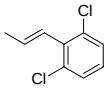
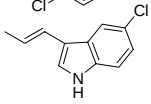
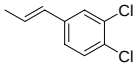


Scheme 1. General synthesis of the pyrazole derivatives. Reagents: (a) acetone, aqueous NaOH, EtOH; (b) (i) LHMDS, THF (ii) *N*-Boc-piperidine-4-carboxylic acid, CDI, THF; (c) (i) 3-chlorophenylhydrazine·HCl, aqueous NaOH, EtOH; (ii) TFA, CH_2Cl_2; (d) (i) $NH_2NH_2{\cdot}H_2O$, EtOH; (ii) TFA, CH_2Cl_2, (e) H_2, 5% Pd–C, EtOH.

Table 1. Antibacterial activity of the pyrazole derivatives



1, 2, 7a-d, 10a,b **3, 8a,b, 11**

No	R	MIC (μg/mL)			
		S. aureus		*E. coli*	
		FDA 209P[a]	KMP9[b]	NIHJ JC-2[a]	W3110 Δ*acrA*[c]
7a		2	4	32	4
7b		8	4	16	4
7c		2	2	>128	4
7d		2	4	16	4
8a		2	2	4	2
8b		128	>128	>128	>128
10a		32	32	>128	16
10b		16	16	64	16
11		32	64	128	64
1		4	4	64	4
2		1	1	8	4
3		4	4	8	4
SPFX	—	0.125	128	0.032	0.004
NB	—	0.25	0.25	64	0.5

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

quinolone-resistant clinical isolates of Gram-positive bacteria were determined and compared with those of **1**, **2** and sparfloxacin (Table 2). Although the ratio of MIC values of sparfloxacin against susceptible and resistant strains was more than 32, that of **7c**, **8a** and **10b** ranged between 1 and 2. Thus, the 5-[(*E*)-2-arylvinyl]pyrazoles were effective against quinolone-resistant Gram-positive bacteria. In particular, compounds **7c** and **8a** demonstrated the most potent antibacterial activity against susceptible and resistant Gram-positive bacteria with across-the-board MIC values of 1–2 μg/mL.

Table 2. Antibacterial activity of the selected 5-[(*E*)-2-arylvinyl]pyrazoles against susceptible and resistant Gram-positive bacteria

Organism[a]	MIC (μg/mL)					
	7c	**8a**	**10b**	**1**	**2**	SPFX
S. aureus FDA 209P (MSSA) [b]	2	2	16	4	1	0.125
S. aureus KMP9 (MRSA) [c]	2	2	16	4	1	128
S. pneumoniae ATCC49619 (PSSP) [b]	1	2	16	4	2	0.125
S. pneumoniae KT2524 (PRSP)[c]	1	1	8	4	2	4
E. faecium ATCC19434 (VSE)[b]	2	2	16	8	2	0.25
E. faecium KU1778 (VRE)[d]	1	1	16	8	2	64

[a] SPFX: sparfloxacin, CAM: clarithromycin, ABPC: ampicillin, VCM: vancomycin.
[b] Susceptible strain.
[c] SPFX-, CAM-, ABPC-resistant strain.
[d] SPFX-, CAM-, ABPC-, VCM-resistant strain.

To elucidate the mechanism by which the novel 5-[(*E*)-2-arylvinyl]pyrazoles induce their antibacterial activity, the inhibitory activity of selected compounds **7c** and **8a** against DNA gyrase and topoisomerase IV isolated from *E. coli* was examined (Table 3).[15,16] Compounds **7c** and **8a** showed moderate inhibition against the two enzymes (IC_{50} = 13.9–27.8 μg/mL). There was a good correlation between the MICs and the IC_{50}s of **2**, **7c** and **8a** (Tables 1 and 3), suggesting that inhibition of the DNA gyrase and topoisomerase IV by the 5-[(*E*)-2-arylvinyl]pyrazoles suppresses bacterial cell growth. While ratio of inhibition of DNA gyrase and topoisomerase IV of sparfloxacin was more than 20, that of **7c** and **8a** ranged between 1.2 and 2.0. Thus, **7c** and **8a** showed almost the same inhibitory activity against both enzymes as the dual inhibitors. These results suggest that resistance against the 5-[(*E*)-2-arylvinyl]pyrazoles would not be easily developed by bacteria.

In this study, we have described the synthesis and SARs of the novel pyrazole derivatives. Among the 1-(3-chlorophenyl)pyrazole derivatives, **7c** had comparatively strong antibacterial activity, whereas among the 1*H*-pyrazole derivatives, **8a** showed comparatively strong antibacterial activity. Compounds **7c** and **8a** exhibited potent antibacterial activity against not only susceptible strains but also multidrug resistant strains. We are pursuing further modifications of the 5-[(*E*)-2-arylvinyl]pyrazole scaffold to obtain more potent inhibitors of both DNA gyrase and topoisomerase IV.

Table 3. Inhibitory effects of the selected 5-[(*E*)-2-arylvinyl]pyrazoles against DNA gyrase and topoisomerase IV

Compound	IC_{50}(μg/mL)[a]	
	Gyrase[b]	Topo IV[c]
7c	13.9	16
8a	13.9	27.8
2	8.0	13.9
SPFX	0.25	6.9

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

Acknowledgments

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