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Potent DNA gyrase inhibitors; novel 5-vinylpyrazole analogues with Gram-positive antibacterial activity

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Abstract—In this study, we designed and synthesized novel 5-vinylpyrazole analogues by decreasing the lipophilicity of the parent compounds **1a**,**b**; 3-[(3-methoxycarbonyl)cyclohexylaminomethyl]indazoles while keeping the van der Waals interaction with the lipophilic area of DNA gyrase B. The selected compound **8bb** exhibited good antibacterial activity against staphylococci and enterococci, including multi-drug resistant strains.

The increasing use of antibacterial agents such as β-lactams, macrolides, vancomycin or quinolones has resulted in the emergence of multi-drug resistant pathogens, especially Gram-positive bacteria.[1,2] Over the past decade, DNA gyrase has drawn much attention as selected target for finding potent antibacterial agents against multi-drug resistant strains.[3]

We have previously found compounds **1a**,**b** (Fig. 1) as potent and selective inhibitors of DNA gyrase with moderate antibacterial activity against multi-drug resistant strains as well as susceptible strains (Table 1).[4] Docking studies of **1a** with a 43 kDa fragment of DNA gyrase subunit B from *Escherichia* (*E.*) *coli* revealed that (1) the indazole scaffold forms postulated H-bond network with Asp73 and H_2O, (2) the methyl ester moiety is H-bonded to Arg136, (3) the benzyloxy side chain interacts—van der Waals interaction—with the lipophilic area around Ile94, (4) an additional H-bond is formed between Gly77 and the nitrogen atom on the secondary amine of **1a** (Fig. 2).

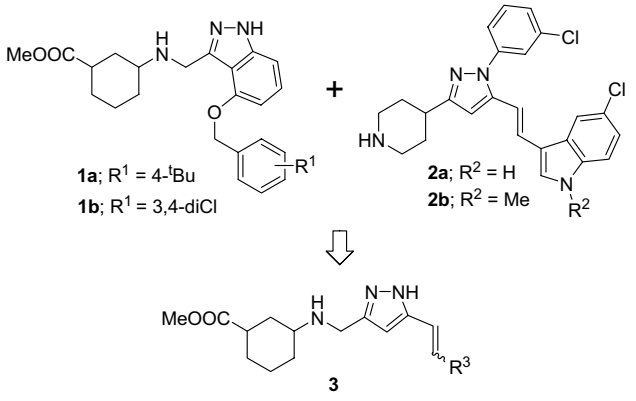

Figure 1.

In addition to our studies on compounds **1a**,**b**, we have previously reported a new class of antibacterial agents with potent inhibitory activity against bacterial type II topoisomerase using a new screening system for specific inhibitors of chromosome partitioning in *E. coli*.[5,6] Among these compounds, the pyrazole analogues **2a**,**b** (Fig. 1) showed potent Gram-positive antibacterial activity (Table 1).[7]

Although compounds **1a**,**b** and **2a**,**b** were active in vitro, they had apparently no in vivo antibacterial activity and

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Table 1. Inhibitory activity against *E. coli* DNA gyrase and topoisomerase IV (IC_{50}) and minimum inhibitory concentration (MIC) of compounds **1a**,**b** and **2a**,**b**

No.	IC_{50} (μg/mL)			MIC (μg/mL)			
	Gyrase[a]	Topo IV[b]	Topo II[c]	*S. aureus*		*E. faecalis*	
				FDA 209P[d]	KMP9[e]	ATCC 29212[d]	KU 1777[f]
1a	4	>128	>400	4	4	8	4
1b	1	>128	>400	4	4	4	4
2a	14	8.0	>400	1	1	2	2
2b	14	8.0	>400	1	1	2	2

(CAM: clarithromycin, ABPC: ampicillin, SPFX: sparfloxacin, VCM: vancomycin).
[a] DNA gyrase supercoiling activity.
[b] Topoisomerase IV decatenation activity.
[c] Human topoisomerase II relaxation activity.
[d] Susceptible strain.
[e] SPFX-, CAM-, ABPC-resistant strain.
[f] SPFX-, VCM-resistant strain.

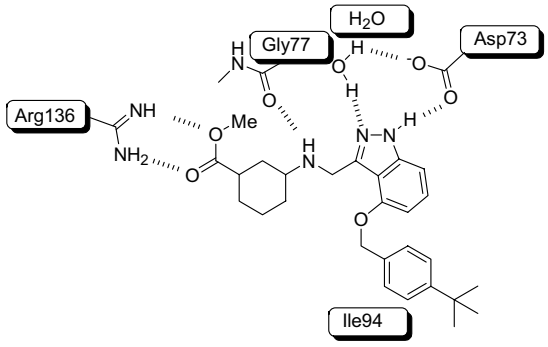


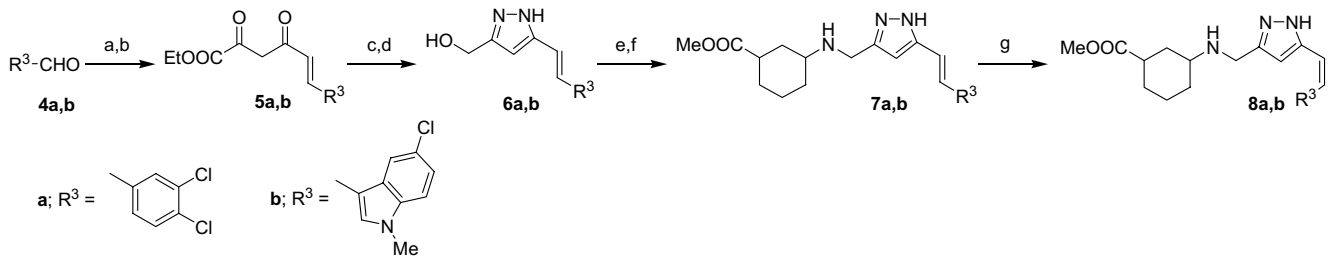
Figure 2. Schematic representation of the indazole analogue **1a** bound to the 43 kDa fragment of DNA gyrase B from *E. coli*.

showed weak antibacterial activity in the presence of 10% horse blood, indicating that these compounds, due to their lipophilicity, bind to serum proteins.

From the findings above and in order to improve the bioavailability and antibacterial activity of **1a**,**b**, we generated the novel 5-vinylpyrazoles **3** from the 3-[(3-methoxycarbonyl)cyclohexylaminomethyl]indazole derivatives **1a**,**b** and the cited 5-vinylpyrazole analogues **2a**,**b** (Fig. 1). The idea behind our strategy was to decrease the lipophilicity of compounds **1a**,**b** while keeping the van der Waals interaction with the lipophilic area around Ile94 of the DNA gyrase B.

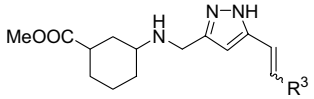
The novel 5-vinylpyrazole analogues were prepared as shown in Scheme 1. Condensation of the aldehydes **4a**,**b** with acetone in the presence of aq NaOH in EtOH,[8] followed by condensation reaction with $(COOEt)_2$ and NaOEt gave the 1,3-diketones **5a**,**b**. Cyclization of **5a**,**b** with NH_2NH_2 in EtOH, and reduction with $LiAlH_4$ afforded the 3-hydroxymethylpyrazoles **6a**,**b**. Chlorination of **6a**,**b** with $(COCl)_2$, followed by reaction with excess 3-(methoxycarbonyl)cyclohexylamine gave the desired products **7a**,**b**. Next, separation of the diastereomeric mixture of **7b** using CHP-20P (reverse phase column) furnished the less polar diastereoisomer **7ba** and the more polar diastereoisomer **7bb**, respectively.[9] Moreover, we found out that the (*E*)-forms **7a**, **7ba** and **7bb** were easily isomerized to the (*Z*)-forms **8a**, **8ba** and **8bb**, respectively, by irradiation with visible light in highly diluted MeOH. The stereochemistry of each isomer of **7** and **8** was determined on the basis of NOE experiments. For example, in compound **8a**, an enhancement was observed between the two protons of the vinyl moiety. On the other hand, in compound **7a**, no enhancement was observed between the two protons of the vinyl moiety.

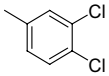
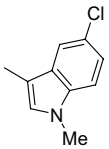
Next, compounds **7a**, **7ba**, **7bb**, **8a**, **8ba** and **8bb** were tested for inhibition of DNA gyrase and topoisomerase IV,[10,11] and their MIC values against *Staphylococcus* (*S.*) *aureus* and *Enterococcus* (*E.*) *faecalis* including susceptible and multi-drug resistant strains were determined by a microdilution method (Table 2).



Scheme 1. Synthesis of the novel 5-vinylpyrazole analogues. Reagents and conditions: (a) acetone, aq NaOH, EtOH; (b) $(COOEt)_2$, NaOEt, Et_2O; (c) NH_2NH_2, EtOH; (d) $LiAlH_4$, THF (e) $(COCl)_2$, CH_2Cl_2; (f) 3-methoxycarbonyl-cyclohexylamine (mixture of two stereoisomers), $CHCl_3$–MeOH; (g) *hν*, MeOH.

Table 2. Inhibitory activity against *E. coli* DNA gyrase (IC_{50}) and minimum inhibitory concentration (MIC) of the novel 5-vinylpyrazole analogues



No.		R^3	IC_{50} (μg/mL) Gyrase	MIC (μg/mL)			
				S. aureus		*E. faecalis*	
				FDA 209P	KMP9	ATCC 29212	KU 1777
7a	*E*		16	4	8	16	16
8a	*Z*		16	4	8	16	16
7ba	*E*		1	8	8	32	32
7bb	*E*		1	16	16	64	32
8ba	*Z*		1	4	8	8	8
8bb	*Z*		0.5	8	16	64	32

Compounds **7a** and **8a** showed less potent DNA gyrase inhibitory activity and slightly less potent antibacterial activity against four Gram-positive strains than the parent compound **1b** (Table 2). These results suggest that the 2-[3,4-(dichlorophenyl)]vinyl moiety renders good permeability through bacterial cell membrane. Since **7a** and **8a** inhibited DNA gyrase with the same IC_{50} value, it was assumed that both compounds bind to DNA gyrase in a similar manner. In addition, both compounds **7a** and **8a** showed the same MIC value against four Gram-positive strains.

Compounds **7ba**, **7bb**, **8ba** and **8bb** exhibited the same or 2-fold more potent inhibitory activity against DNA gyrase compared with the parent compound **1b**, although their antibacterial activity was less potent than that of **1b**. Among these compounds, **8bb** showed the most potent inhibitory activity against DNA gyrase. In addition, **8ba** and **8bb** showed slightly more potent antibacterial activity against four Gram-positive strains than **7ba** and **7bb**, respectively. Finally, the less polar diastereoisomers **7ba** and **8ba** exhibited slightly more potent antibacterial activity than the more polar diastereoisomers **7bb** and **8bb**, respectively (Table 2).

In this study, all the novel 5-vinylpyrazole analogues had moderate to strong inhibitory activity against DNA gyrase, but not against topoisomerase IV[11] (IC_{50}s > 128 μg/mL) and human topoisomerase II[12] inhibitory activity (IC_{50}s > 400 μg/mL). In addition, all compounds exhibited antibacterial activity against both susceptible and multi-drug resistant strains.

Compound **8bb** exhibited 8-fold more potent inhibitory activity against DNA gyrase than **1a**. On the other hand, we previously reported that the 3-[(3-methoxycarbonyl)cyclohexylaminomethyl]indazole portion of **1a** has three H-bond interactions with the 43 kDa fragment of DNA gyrase B on ATP binding site (Fig. 2).[4] Taken together, these results suggest that **8bb** interacts with the 43 kDa fragment of DNA gyrase B on ATP binding site as does **1a**.

In summary, we have designed and synthesized novel 5-vinylpyrazole analogues by decreasing the lipophilicity of the parent compounds **1a**,**b** while keeping the van der Waals interaction with the lipophilic area around Ile94 of the DNA gyrase B. The selected compound **8bb** (clogP = 4.630) was less lipophilic than **1a** (clogP = 6.445) or **1b** (clogP = 5.925),[13] and showed more potent inhibitory activity against DNA gyrase than the parent compounds. Although **8bb** showed weaker antibacterial activity against four Gram-positive strains than the parent compounds **1a**,**b**, it exhibited relatively good antibacterial activity against staphylococci and enterococci, including multi-drug resistant strains. These results provide a good strategy for the development of Gram-positive antibacterial agents targeting DNA gyrase.

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References and notes

1. Witte, W. J. *Antimicrob. Agents Chemother.* **1999**, *14*, 1.
2. Marchese, A.; Schito, G. C.; Debbia, E. A. *J. Chemother.* **2000**, *12*, 12.
3. Ferrero, L.; Cameron, B.; Manse, B.; Lagneaux, D.; Crouzet, J.; Famechon, A.; Blanche, F. *Mol. Microbiol.* **1994**, *13*, 641.
4. Tanitame, A.; Oyamada, Y.; Ofuji, K.; Kyoya, Y.; Suzuki, K.; Ito, H.; Kawasaki, M.; Nagai, K.; Wachi, M.; Yamagishi, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, preceding paper in this issue. doi:10.1016/j.bmcl.2004.03.044.
5. Wachi, M.; Iwai, N.; Kunihisa, A.; Nagai, K. *Biochimie.* **1999**, *81*, 909.
6. Hiraga, S.; Niki, H.; Ogura, T.; Ichinose, C.; Mori, H.; Ezaki, B.; Jaffe, A. *J. Bacteriol.* **1989**, *171*, 1496.
7. *J. Med. Chem.*, in press.

8. Edwards, M. L.; Ritter, H. W.; Stemerich, D. M.; Stewart, K. T. *J. Med. Chem.* **1983**, *26*, 431.
9. Compounds **7a** and **8a** were an inseparable mixture of diastereomers.
10. Sato, K.; Inoue, Y.; Fujii, T.; Aoyama, H.; Inoue, M.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* **1986**, *30*, 777.
11. Peng, H.; Marians, K. J. *J. Biol. Chem.* **1993**, *268*, 24481.
12. Spitzner, J. R.; Chung, I. K.; Muller, M. T. *Nucleic Acids Res.* **1990**, *18*, 1.
13. The clogPs of these compounds were calculated by cLogP for Windows Version 4.0 (Biobyte Corp., Claremont, CA, U.S.A).