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Design, synthesis and structure–activity relationship studies of novel indazole analogues as DNA gyrase inhibitors with Gram-positive antibacterial activity

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Abstract—In this study, we report the design, synthesis and structure–activity relationships of novel indazole derivatives as DNA gyrase inhibitors with Gram-positive antibacterial activity. Our results show that selected compounds from this series exhibit potent antibacterial activity against Gram-positive bacteria including multi-drug resistant strains that is methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE).

In recent years, the emergence and spread of multi-drug resistant microorganisms have become a serious problem in the treatment of infectious diseases.[1] To overcome this problem, new and effective antibacterial agents are needed.

Over the past decade, bacterial DNA gyrase has drawn much attention as a selected target for finding potent antibacterial agents.[2] Accordingly, a number of synthetic quinolone antibacterial agents have been developed and are now widely used for treatment of bacterial infectious diseases.[3] Quinolones inhibit DNA gyrase and topoisomerase IV, and cause bacterial cell death.[2] Besides the quinolones, naturally occurring antibacterial agents, such as novobiocin (NB; Fig. 1) have also been identified as bacterial DNA gyrase inhibitors.[4,5] Novobiocin inhibits ATPase activity of DNA gyrase by competing with ATP for binding to the gyrase subunit B. Using detailed 3D structural information of ATP binding site located on the subunit B of DNA gyrase, Hoffmann–La Roche's group has recently developed a new lead DNA gyrase inhibitor (**1**; Fig. 1).[6,7] X-ray studies of indazole analogues with a 24 kDa fragment of DNA gyrase B from *Staphylococcus* (*S.*) *aureus* revealed that (1) the indazole scaffold forms postulated H-bond network with Asp73 and H_2O, (2) the 'X' moiety stacks on the Glu50-Arg76 salt bridge and the 'Y' moiety is H-bonded to Arg136 and (3) the benzyloxy side chain interacts—van der Waals interaction—with the lipophilic area around Ile94 (Fig. 2).[6] Although compound **1**

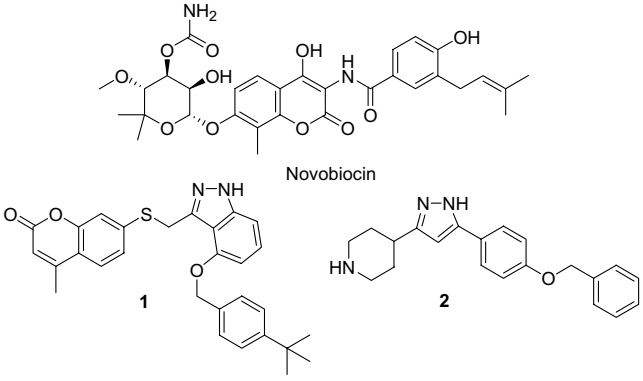

Figure 1.

Keywords: DNA gyrase inhibitor; Indazole analogues; Multi-drug resistant strains; ATP binding site.
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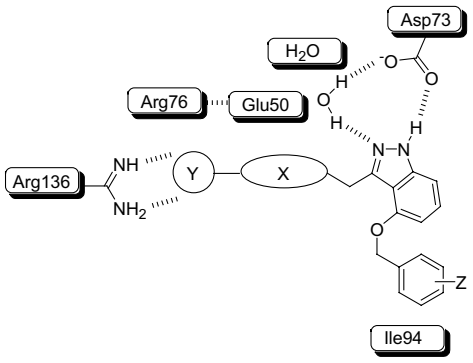


Figure 2. Schematic representation of an indazole analogue bound to the 24 kDa fragment of DNA gyrase B.

has strong inhibitory activity against DNA gyrase, its antibacterial activity is weak, suggesting that **1** cannot penetrate bacterial cells (Table 1).

Independently, we have previously discovered a new class of bacterial DNA gyrase inhibitors that have potent activity against *S. aureus* and *Enterococcus* (*E.*) *faecalis*.[8] Among these inhibitors, compound **2** having a piperidine ring was identified as a hit compound using a new screening system for specific inhibitors of chromosome partitioning in *Escherichia* (*E.*) *coli*.[9,10] As compound **2** exhibited both weak inhibitory activity against bacterial type II topoisomerase and moderate antibacterial activity against Gram-positive pathogens (Table 1), it was assumed that this compound could penetrate bacterial cells.

Using the results obtained with compound **2** to generate more potent DNA gyrase inhibitors with strong antibacterial activity, we attempted in this study to improve compound **1** ability to penetrate bacterial cells by replacing its coumarin moiety with a variety of basic amines such as the 4-piperidyl moiety of compound **2**.

The six compounds **6**, **8**, **10a**,**b** and **11a**,**b** were prepared as the first set of analogues (Scheme 1). Reaction of the 3-(bromomethyl)indazole **4**[6] with an excess or equivalent amount of the appropriate amine (**12a** or **12b**, etc.[11]), followed by protection of the amino function, if necessary, and deprotection of the *tert*-butyldiphenylsilyl (TBDPS) group afforded the intermediates **5**, **7** and **9a**,**b**. Benzylation of these phenols with 4-*tert*-butylbenzyl bromide and NaH in DMF, followed by cleavage of the protecting groups gave the desired products **6**, **8** and **10a**,**b**, respectively. Reduction of **10a**,**b** with $NaBH_4$ afforded the corresponding hydroxymethyl derivatives **11a**,**b**.

Compounds **6**, **8**, **10a**,**b** and **11a**,**b** were then tested for inhibition of DNA gyrase and topoisomerase IV,[12,13] and their MIC values against *S. aureus* and *E. faecalis* including susceptible and multi-drug resistant strains were determined by a microdilution method (Table 2). While all compounds showed some antibacterial activity, compounds **6**, **8** and **11a**,**b** exhibited only weak DNA gyrase inhibitory activity and no inhibition of topoisomerase IV (IC_{50}s > 128 μg/mL). On the other hand, the methyl ester derivatives **10a**,**b** showed strong DNA gyrase inhibitory activity with the same IC_{50} value (8 μg/mL). From these findings, we assumed that the methyl ester group of **10a**,**b** could form a specific H-bond with Arg136 of ATP biding site in the DNA gyrase B (Fig. 2).

With the results of **10a**,**b** in hand, we attempted to modify the position of 'Y' moiety, which forms a H-bond with Arg136 of the DNA gyrase B while keeping 'X' moiety as an amine group (Fig. 2). Compounds **13**–**19** with an ester group were synthesized using the corresponding amines instead of the methylamine or *N*-Boc piperazine in a similar manner to that described in Scheme 1.

Although compound **16** had strong inhibitory activity against DNA gyrase, it did not show any antibacterial activity, presumably because of low permeability through bacterial membrane. Compounds **13**, **14** and **15** had potent DNA gyrase inhibitory activity and showed moderate antibacterial activity (Table 2).

When the secondary amine derivatives **17**–**19** were tested, **17** and **18** had moderate inhibitory activity against DNA gyrase with IC_{50} values of 16 and 32 μg/mL,

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

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]
1	0.25	>128	>400	>128	>128	>128	>128
2	128	128	200	64	64	64	64
NB	0.25	25	>400	0.25	0.25	2	2

(CAM: clarithromycin, ABPC: ampicillin, SPFX: sparfloxacin, VCM: vancomycin)

[a] DNA gyrase supercoiling activity.

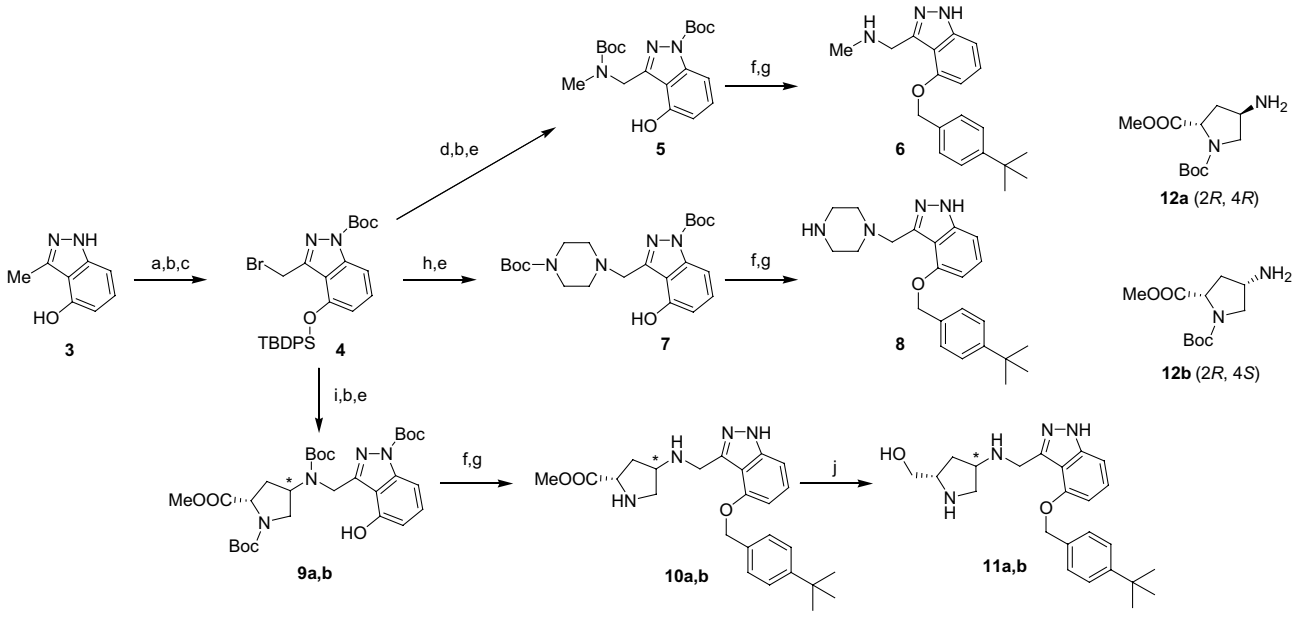
[b] Topoisomerase IV decatenation activity.

[c] Human topoisomerase II relaxation activity.

[d] Susceptible strain.

[e] SPFX-, CAM-, ABPC-resistant strain.

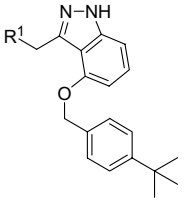
[f] SPFX-, VCM-resistant strain.

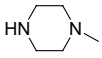
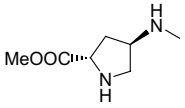
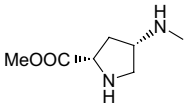
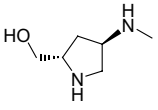


Scheme 1. Synthesis of **6**, **8**, **10a**,**b** and **11a**,**b**. Reagents and conditions: (a) TBDPSCl, imidazole, DMF; (b) Boc_2O, Et_3N, DMAP, CH_3CN; (c) NBS, $(PhCO_2)_2$, CCl_4; (d) aq CH_3NH_2 (excess), THF; (e) TBAF, THF; (f) *tert*-butylbenzylbromide, NaH, DMF; (g) TFA, CH_2Cl_2; (h) *N*-Boc-piperazine, Et_3N, THF; (i) **12a** or **12b** (excess), $CHCl_3$–CH_3OH; (j) $NaBH_4$, CH_3OH.

respectively. However, the carboxylic acid derivative **18** did not show any antibacterial activity, presumably because of low permeability. Compound **19**, on the other hand, had potent inhibitory activity against DNA

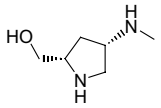
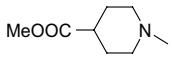
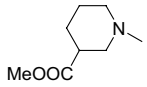
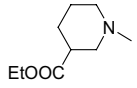
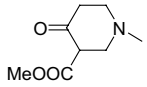
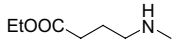
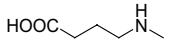
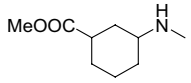
Table 2. Inhibitory activity against *E. coli* DNA gyrase (IC_{50}) and minimum inhibitory concentration (MIC) of the synthesized indazole analogues



No.	R^1	IC_{50} (μg/mL) Gyrase	MIC (μg/mL)			
			S. aureus		*E. faecalis*	
			FDA 209P	KMP9	ATCC 29212	KU 1777
6		64	32	64	32	16
8		64	16	32	32	16
10a		8	64	128	128	64
10b		8	64	64	64	64
11a		32	32	64	64	32

(*continued on next page*)

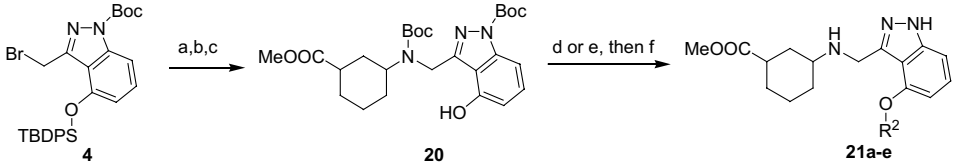
Table 2 (*continued*)

No.	R^1	IC_{50} (μg/mL) Gyrase	MIC (μg/mL)			
			S. aureus		*E. faecalis*	
			FDA 209P	KMP9	ATCC 29212	KU 1777
11b		64	64	64	64	64
13		16	32	32	32	32
14		16	32	64	64	32
15		8	16	32	16	16
16		4	>128	>128	>128	>128
17		16	16	16	16	16
18		32	>128	>128	>128	>128
19		4	4	4	8	4

gyrase ($IC_{50} = 4$ μg/mL) and good antibacterial activity against Gram-positive bacteria with MIC values of 4–8 μg/mL (Table 2).[14] Compound **19** revealed 16-fold less potent inhibitory activity against DNA gyrase and >32-fold more potent antibacterial activity against *S. aureus* than compound **1**. These results suggests that the 3-(methoxycarbonyl)cyclohexylamine moiety allows good permeability through bacterial membrane compared with other moieties.

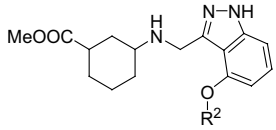
Next, transformation of the 4-*tert*-butylbenzyl moiety of **19** was examined. The substituted benzyloxy derivatives were prepared as shown in Scheme 2. Reaction of the 3-(bromomethyl)indazole **4** with an excess of 3-(methoxycarbonyl)cyclohexylamine, followed by protection of the amine function and deprotection of TBDPS group afforded the intermediate **20**. Benzylation of **20** with R^2X and NaH in DMF or R^2OH and Mitsunobu reagents, followed by cleavage of the protecting groups gave the desired products **21a**–**e**.[14]

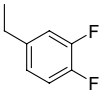
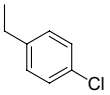
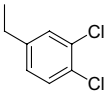
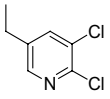
Among the non-, mono- or di-halogenated benzyl derivatives **21a**–**d**, the 3,4-dichlorobenzyl derivative **21d** revealed more potent DNA gyrase inhibitory activity than the parent compound **19** and moderate antibacterial activity against four Gram-positive strains (Table 3). As the lipophilicity of R^2 group increased, DNA gyrase inhibitory activity and antibacterial activity of the indazole analogues became stronger. However, the 3-pyridylmethyl derivative **21e** ended in less potent



Scheme 2. Synthesis of **21a**–**e**. Reagents and conditions: (a) 3-(methoxycarbonyl)cyclohexylamine (excess), THF; (b) Boc_2O, Et_3N, DMAP, CH_3CN; (c) TBAF, THF; (d) R^2X, NaH, DMF; (e) R^2OH, DIAD, Ph_3P, DMF; (f) TFA, CH_2Cl_2.

Table 3. Inhibitory activity against *E. coli* DNA gyrase (IC_{50}) and minimum inhibitory concentration (MIC) of 3-[(3-methoxycarbonyl)cyclohexylaminomethyl]indazoles



No.	R^2	IC_{50} (μg/mL) Gyrase	MIC (μg/mL)			
			S. aureus		*E. faecalis*	
			FDA 209P	KMP9	ATCC 29212	KU 1777
21a		8	64	64	64	64
21b		16	128	128	128	128
21c		16	32	32	64	32
21d		1	4	4	4	4
21e		64	>128	>128	>128	>128

DNA gyrase inhibitory activity and less antibacterial activity (Table 3).

All compounds tested in this study had selective DNA gyrase inhibitory activity, but had no topoisomerase IV[13] (IC_{50}s > 128 μg/mL) and no human topoisomerase II[15] inhibitory activity (IC_{50}s > 400 μg/mL). Compounds **19** and **21d** showed good antibacterial activity against not only susceptible but also multi-drug resistant strains.

Finally, docking studies of compound **19** with a 43 kDa fragment of the DNA gyrase B from *E. coli* (Fig. 3) revealed the following interactions: (1) specific H-bonds with both Asp73 and a water molecule, (2) ionic interaction with Arg136 and (3) lipophilic interaction with Ile94. Moreover, another H-bond could be formed between Gly77 and the nitrogen atom on the secondary amine of **19**.

In summary, we designed and synthesized a series of novel indazole derivatives as DNA gyrase inhibitors. Among these compounds, we have identified the 3-[(3-methoxycarbonyl)cyclohexylaminomethyl]indazole derivative **21d** as having potent inhibitory activity against DNA gyrase with exceptionally good antibacterial activity against staphylococci and enterococci, including multi-drug resistant strains.

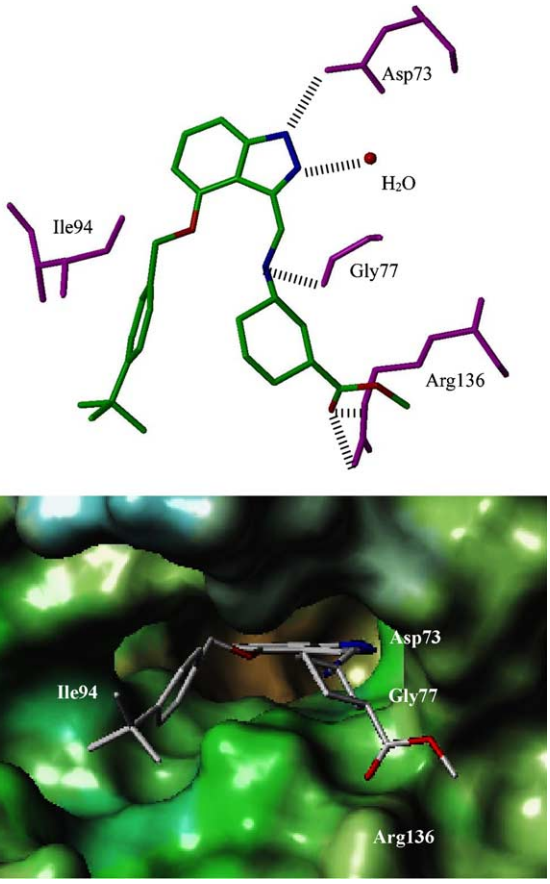


Figure 3. Docking study of the 43 kDa fragment of DNA gyrase B from *E. coli* with compound **19** (1*S*,3*R*). Docking studies of the four isomers of **19** and the crystal structure of DNA gyrase B (PDB code; 1EI1) were performed using the docking software FlexX (Tripos, Inc., St. Louis MO, U.S.A.).[16] The models of **19** (1*S*,3*R*) and (1*S*,3*S*) isomers had the same binding form as that shown in Figure 2. Molecular dynamic (MD) simulations were carried out on these two models for 1 ns at 298 K using the molecular modelling software InsightII/Discover95.0 (Accerlys, Inc., San Diego, CA, U.S.A.) with cvff force field[17] with each structure along the trajectory path being energy-minimized.

Acknowledgements

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