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Novel pyrazole derivatives as potent inhibitors of type II topoisomerases. Part 1: Synthesis and preliminary SAR analysis

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Abstract—In an attempt to search for a new class of antibacterial agents, we have discovered a series of pyrazole analogs that possess good antibacterial activity for Gram-positive and Gram-negative organisms via inhibition of type II bacterial topoisomerases. We have investigated the structure–activity relationships of this series, with an emphasis on the length and conformation of the linker. This work led to the identification of tetrahydroindazole analogs, such as compound **1**, as the most potent class of compounds.

Over the past decade, type II topoisomerases have drawn much attention as selected targets for the discovery of potent antibacterial agents. Among the type II topoisomerases are DNA gyrase and topoisomerase IV. DNA gyrase is an essential prokaryotic type II topoisomerase with no direct mammalian counterpart.[1] It is involved in the vital processes of DNA replication, transcription, and recombination. DNA gyrase catalyzes the ATP-dependent introduction of negative supercoils into bacterial DNA as well as the decatenation and unknotting of DNA. Topoisomerase IV has a primary role in decatenation of daughter chromosomes following DNA replications. Those attributes make DNA gyrase an attractive target for drug discovery. DNA gyrase is mainly inhibited by quinolones and coumarins. Numerous quinolone antibacterial agents have been developed and are now widely used for the treatment of bacterial infectious diseases (e.g., ciprofloxacin, Fig. 1).[2,3] This class of antibacterial agents inhibits the DNA breakage-reunion cycle by binding to the A subunit and stabilizing the gyrase–DNA complex. Unfortunately, resistance to quinolones has emerged.[4,5] In addition to some quinolones, naturally occurring bacterial DNA gyrase inhibitors such as the coumarin (e.g., novobiocin, Fig. 1) have also shown some antibacterial activities.[6,7] The coumarins inhibit ATPase activity of DNA gyrase by competing with ATP for binding to the B subunit of the enzyme.[8,9] However, the coumarins suffer from a developing resistance profile and toxicity concerns.[3,10] Recently, multidrug-resistant Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSA), and vancomycin-resistant enterococci (VRE), have become a serious medical problem. To overcome the limitations of the known DNA gyrase inhibitors, it is important to identify new classes of compounds.

As part of our antibacterial program, we have identified a series of pyrazole derivatives as potent inhibitors of bacterial growth.[11] These compounds target the *parC* subunit of topoisomerase IV in *S. pneumoniae*. A series of resistant mutants have been identified which map to the *parC* subunit (unpublished results). We report herein the racemic synthesis and the preliminary structure–activity relationship of this series. Our initial efforts were aimed at evaluating the influence of the nature of the pyrazole on the antibacterial activity.

The synthesis of the initial pyrazole analogs is described in Scheme 1. Treatment of CBz-protected 4-piperidinone with *tert*-butoxybis(dimethylamino) methane in THF followed by hydrolysis (1 N HCl) of the corresponding enamine afforded the desired intermediate **3** in quantitative yield. Pyrazole ring formation was accomplished with (6-methoxy-quinolin-4-yl)-hydrazine

Keywords: Gyrase; Topoisomerase; Pyrazoles; SAR; Tetrahydroindazole.
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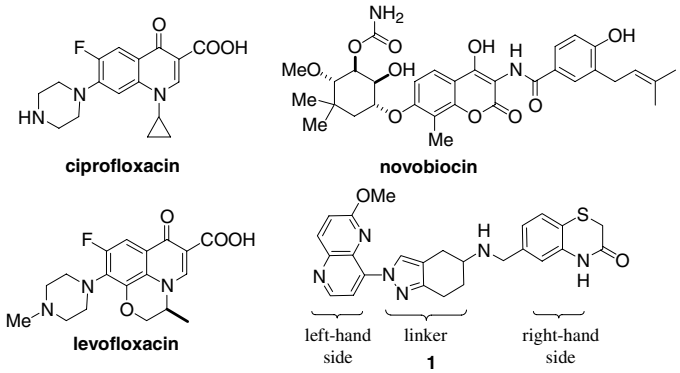
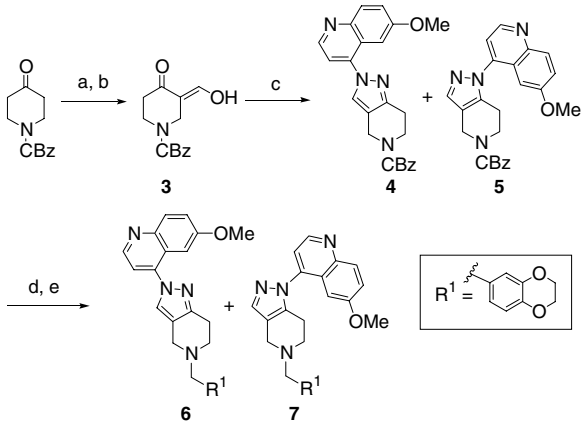


Figure 1. Selected inhibitors of type II topoisomerases.

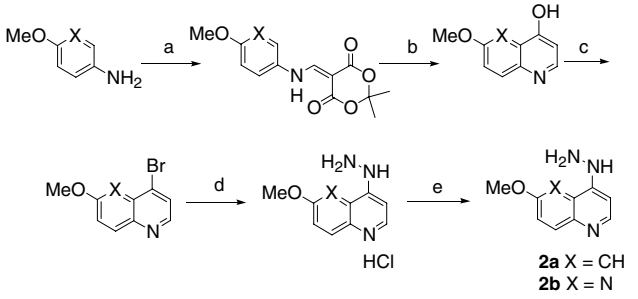


Scheme 1. Reagents and conditions: (a) $[(CH_3)_2N]_2CH(OtBu)$, THF, 65 °C; (b) 1 N HCl; (c) hydrazine **2a**, THF then *p*-TsOH; (d) 10% Pd/C, HCO_2NH_4, EtOH; (e) R^1CHO, $NaBH_3CN$, CH_3CN.

in THF. Complete dehydration of the intermediate is accomplished with addition of *p*-TsOH to afford the desired products, **4** and **5**, as a 3:2 mixture of regioisomers in 85% yield. A subsequent hydrogenolysis of the CBz protective group with 10% Pd/C and ammonium formate provided the unprotected secondary amine in quantitative yield. A final derivatization step was accomplished using various aldehydes in the presence of sodium cyanoborohydride in CH_3CN. Fortunately, the regioisomers, **6** and **7**, were readily separated by reversed phase chromatography. The structure was fully assigned using 2D NMR spectroscopy and NOE studies.

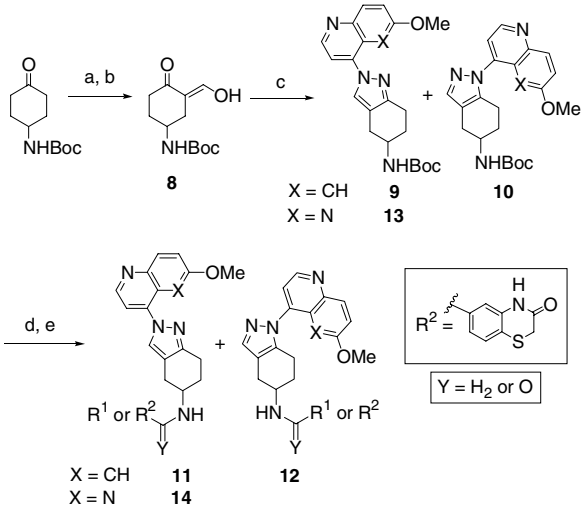
The synthesis of both quinoline and naphthyridine hydrazines is described in Scheme 2.[12] Treatment of substituted aniline with Meldrum's acid and triethyl orthoformate afforded the corresponding enamines in 89% (X = N) and 85% (X = CH) yield, respectively. The ring formation was accomplished in Dowtherm A at 260 °C to afford the desired products in 86% (X = N) and 78% (X = CH) yield, respectively. Subsequent bromination reaction using PBr_3 in DMF followed by hydrazine formation afforded the desired products **2a** and **2b** in 52% and 62% overall yields.

Analogs bearing an exocyclic nitrogen were obtained via a synthesis similar to the one described above (Scheme 3).



Scheme 2. Reagents and conditions: (a) Meldrum's acid, $CH(OEt)_3$; (b) Dowtherm A, 260 °C; (c) PBr_3, DMF; (d) NH_2NH_2, NMP, 140 °C; (e) basic extraction.

It is worth mentioning that the regiochemical outcome of the pyrazole ring formation resulted in a greater selectivity (ratio 8:1, after optimization) favoring isomer **9** when the intermediate 1,3-dicarbonyl **8** was used shortly after preparation. ^1H NMR analysis of this material showed various isomerizations over time.



Scheme 3. Reagents and condition: (a) $[(CH_3)_2N]_2CH(OtBu)$, THF, 65 °C; (b) 1 N HCl; (c) hydrazine **2a** or **2b**, THF then *p*-TsOH; (d) 4M HCl, dioxane; (e) R^1CHO or R^2CHO, $NaBH_3CN$, CH_3CN or R^2COOH, EDC, HOBt, DMF.

Interestingly, the same reaction was found to be regiospecific when (6-methoxy-[1,5]naphthyridin-4-yl)-hydrazine **2b** was used in place of (6-methoxy-quinolin-4-yl)-hydrazine **2a**. Indeed, the pyrazole ring formation led exclusively to isomer **13** (Scheme 3).

In addition, 2D NMR spectroscopy and NOE studies of both compounds **9** and **13** revealed a different orientation of the bicyclic system with respect to the pyrazole ring (Fig. 2). No additional correlations (H_{1a}–H_{3a} or H_{1b}–H_{2b}) were detected by the NOE study suggesting that the structures depicted in Figure 2 are energetically favored conformations.

Investigation into the importance of the ring size was conducted using a route similar to the one described above. Condensation of *N*-Boc-hexahydro-1*H*-azepin-4-one with *tert*-butoxybis(dimethylamino)methane yielded a 3:1 mixture of the two regioisomers **15** and **16** in quantitative yield. Subsequent condensation with hydrazine **2b** led to two single regioisomers in 65% yield, which were further derivatized to afford compounds **17** and **18** using the conditions as shown in Scheme 4.

The antibacterial activity of the compounds was evaluated in vitro against Gram-negative (*Escherichia coli*) and Gram-positive (*S. aureus and S. pneumoniae*) organisms and the results are summarized in Table 1. Exploration of the linker length revealed some interesting trends. Analogs bearing a nitrogen at the endocyclic position, compounds **19** and **20**, did not show any antibacterial activity. Extending the position of the nitrogen to an exocyclic position resulted in a potent inhibitor against Gram-positive organisms as seen with compound **21**. The corresponding regioisomer **22** was found to be about 32- and 64-fold less potent against *S. aureus* and *S. pneumoniae*, respectively. Interestingly, the lack of antibacterial activity of compound **21** against Gram-negative organisms was overcome by modifying the right-hand side. Analog **23**, bearing 4*H*-benzo[1,4]thiazin-3-one moiety, showed a 32-fold increase in potency against *E. coli* and similar activity against *S. aureus* and *S. pneumoniae*. In addition, compound **25**, bearing an amide functionality, displayed comparable antibacterial activity to its amine counterpart **23**. However, the regioisomeric compound **24** was found to have no antibacterial activity against both Gram-positive and Gram-negative organisms. Replacing the quinoline moiety on the pyrazole with naphthyridine ring did not affect the antibacterial activity; since both compounds **23** and **1** have similar potency against all three strains of bacteria. This result is interesting since we have shown that these two compounds display different orientations of the bicyclic system with respect to the pyrazole core structure. This would suggest that only the relative orientation of both the right and left-hand side of the molecule affects the antibacterial activity of the compounds and not the interaction of the linker itself (i.e., pyrazole) with the enzyme. An overlay of analogs **23** and **1** is depicted in Figure 3.

This was further demonstrated with compound **27**, where the exocyclic nitrogen was attached at the adjacent carbon. This compound showed similar inhibitory activity against topoisomerase IV (IC_{50} = 0.5 μM) with the corresponding regioisomer **25** (IC_{50} = 0.25 μM). Finally, expanding the size of the ring attached to the pyrazole as in analog **28** resulted in a compound with similar antibacterial activity to the parent analog **25**.

In summary, we have described the synthesis and structure–activity relationships of a series of racemic pyrazole analogs as potent antibacterial agents. The present investigation suggests the importance of the linker length and the orientation of both right and left-hand side moieties in the antibacterial activity. This work led to the identification of compound **1**, a tetrahydroindazole core structure, as one of the most active compounds, with potent antibacterial activity against both Gram-positive and Gram-negative organisms. Further development of the SAR on this compound will be presented in future publications.

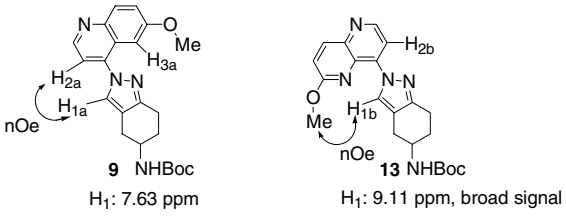
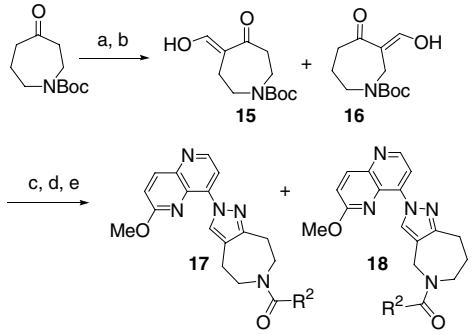


Figure 2. NMR studies and assignment of conformations.



Scheme 4. Reagents and condition: (a) $[(CH_3)_2N]_2CH(OtBu)$, THF, 65 °C; (b) 1 N HCl; (c) hydrazine **2b**, THF then *p*-TsOH; (d) 4 M HCl, dioxane; (e) R_2COOH, EDC, HOBt, DMF.

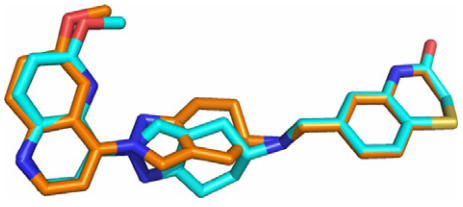
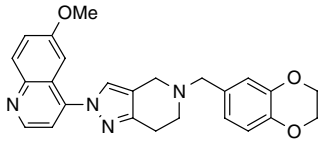
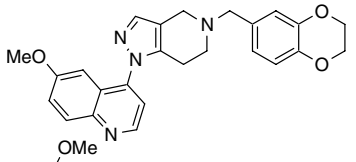
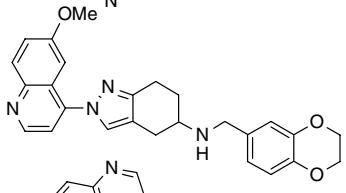
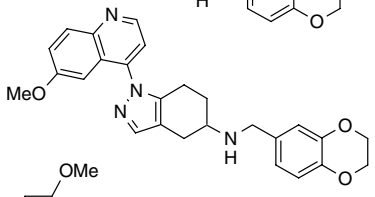
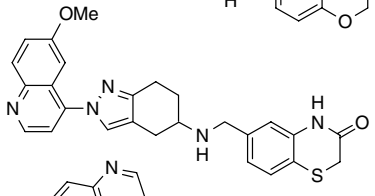
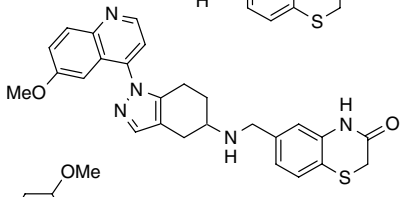
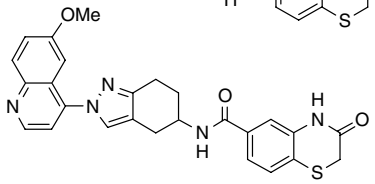
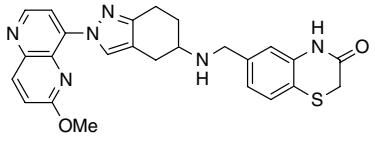
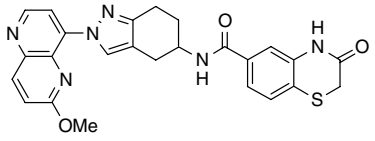
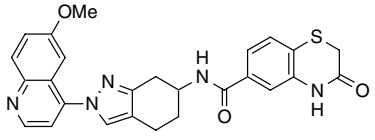
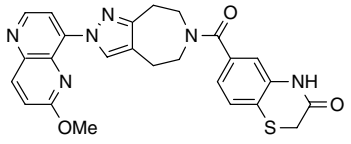


Figure 3. Superposition of **23** (orange) and **1** (blue).

Table 1. Antibacterial activity of selected set of pyrazole analogs

Compound	Structure	DNA gyrase/Topo IV IC_{50} (μg/mL)	E.c./S.a./S.p MIC (μg/mL)
19		>8/ND	>128/>128/>128
20		>8/ND	>128/>128/>128
21		2/0.06	64/0.5/0.5
22		>8/ND	>128/16/32
23		0.25/0.03	2/0.25/0.5
24		2/ND	>128/>128/>128
25		1/0.125	4/4/2
1		0.25/0.016	1/0.25/2
26		0.06/0.25	128/0.125/4
27[13]		>8/0.5	8/8/4
28		0.25/ND	2/1/2

E.c, *Escherichia coli* KL-16 (GSC strain 4245); S.a., *Staphylococcus aureus* ATCC13709; S.p, *Streptococcus pneumoniae* ATCC49619; ND, not determined.

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12. (a) The syntheses were adapted from procedures published in the literature. Davies, D.T.; Jones, G.E.; Markwell, R.E.; Pearson, N.D. WO 2003010138; (b) Hubschwerlen, C.; Surivet, J.-P.; Zumbrunn Acklin, C. WO 2006032466; (c) Cassis, R.; Tapia, R.; Valderrama, J. A. *Synthetic Commun.* **1985**, *15*, 125; (d) Grethe, G.; Uskokovic, Milan R.; U.S. Patent 3917612, 1975; (e) Raheem, I. T.; Goodman, S. N.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2004**, *126*, 706.
13. Compound **27** was synthesized following the procedure depicted in Scheme 3 starting with (3-Oxo-cyclohexyl)-carbamic acid *tert*-butyl ester.