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Discovery of pyrazolthiazoles as novel and potent inhibitors of bacterial gyrase

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ABSTRACT

Bacterial DNA gyrase is an attractive target for the investigation of new antibacterial agents. Inhibitors of the GyrB subunit, which contains the ATP-binding site, are described in this communication. Novel, substituted 5-(1*H*-pyrazol-3-yl)thiazole compounds were identified as inhibitors of bacterial gyrase. Structure-guided optimization led to greater enzymatic potency and moderate antibacterial potency. Data are presented for the demonstration of selective enzyme inhibition of *Escherichia coli* GyrB over *Staphlococcus aureus* GyrB.

Due to the emergence of bacteria resistant to current therapeutic agents the investigation of new antibiotics has become a necessary endeavor.[1] Bacterial DNA gyrase is a well-established target with commercial success, exemplified by quinolones such as ciprofloxacin (**1a**).[2] However, resistance is now a problem for this class of antibacterials in addition to most other classes.[3] Gyrase consists of two heterodimeric subunits, GyrA and GyrB. The quinolone class of molecules inhibits GyrA and induces cell death by trapping the gyrase–DNA complex, inducing oxidative damage, and preventing DNA replication.[4] Compounds like novobiocin (**1b**) inhibit GyrB, which blocks ATPase activity, thus depriving the source of energy needed for DNA replication.[5] GyrB as a target offers an opportunity such as a lack of cross-resistance to the quinolones. Some other known GyrB inhibitors are the cyclothialidines (represented by **1c**) and pyrrolamides (**1d**) from Astra-Zeneca.[6,7] We have previously reported the aminobenzimidazoles[8] (**1e**) and here we describe the pyrazolthiazole class of GyrB inhibitors (**1**) (Fig. 1).

In our efforts to identify novel starting points to discover inhibitors of the Gyrase B (GyrB) subunit, a high-throughput screen designed to identify compounds inhibiting the ATP-ase activity of the GyrB subunit was conducted. Compound **1** was identified and was shown to be a moderately potent inhibitor of GyrB (*E. coli* K_i = 2.2 μM). An X-ray crystal structure of compound **1** bound to *Staphlococcus aureus* GyrB was obtained, and showed a hydrogen bond network between the pyrazole moiety of **1**, Asp81 (Asp73 in *E. coli* numbering) and a highly conserved structural water molecule (Fig. 2). These hydrogen bonds correspond with those of the terminal carbamate of novobiocin in the published *E. coli*/novobiocin structure.[8] Initial investigation of the 5-position of the pyrazole demonstrated that an ethyl ester was equipotent to the methylthiol. Direct alkyl analogs such as the ethyl (**1g**), were less potent (*E. coli* K_i = 4.8 μM) (Table 1).

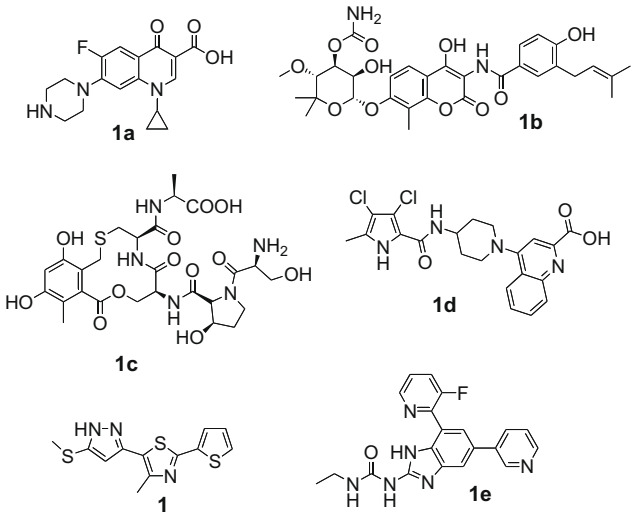

Fig. 1. Inhibitors of bacterial DNA gyrase

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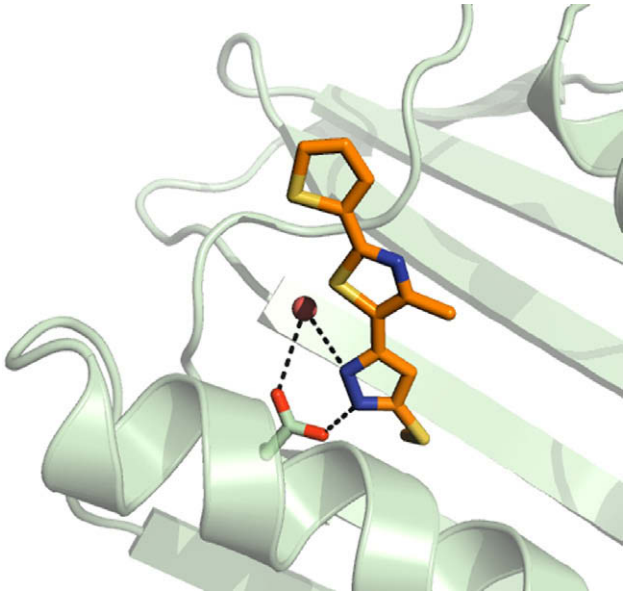
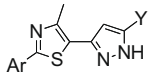


Fig. 2. Crystallographic data showing the pyrazole moiety of compound **1** binding interaction with Asp81 and a conserved water molecule in *S. aureus* GyrB. PDE code 3G75.

Table 1
Escherichia coli enzyme activity



Compound	Ar	Y	*E. coli* K_i (μM)
1	Thiophene	SMe	2.9
1f	Thiophene	COOEt	2.6
1g	Thiophene	CH_2CH_3	4.8
1h	Phenyl	SMe	3.5
1i	Phenyl	COOEt	1.7

To optimize the substituent at the 2-position of the thiazole, examination of the structure suggested that targeting Arg 136 (*E. coli* numbering) as an additional residue for hydrogen bond formation could lead to additional potency. To that effect, the 2-(pyridin-3-yl)thiazole pyrazole compound **2e** was prepared. Compound **2e** shows a 5–10-fold improvement in potency over compounds **1f** and **1i** with a K_i of 0.33 μM The hydrogen of the pyridyl nitrogen makes a hydrogen bond with Arg 136 (data not shown), which mimics the hydrogen bond observed with the exocyclic carbonyl oxygen of the coumarin ring in the published novobiocin structure.[8] From the X-ray structure of compound **1**, it was also determined that substituents bigger than methyl at the 4-position of the thiazole ring should be tolerated (compounds **2a–2d**, **2f–2k**). It was found that this position of the scaffold preferred lipophilic substituents, such as cyclohexyl **2i** (K_i of 0.12 μM) which was more potent than cyclopropyl **2j** (K_i of 0.24 μM), and 2.5-fold more potent than methyl **2e** (K_i of 0.33 μM). The 4-position of the thiazole could also tolerate the introduction of heteroatoms (**2c**, **2d**, **2f–h**). This resulted in compounds that maintained potency but had a better solubility profile. However, antibacterial activity was not observed against wild type strains of *E. coli*, *S. aureus*, or *S. pneumoniae* at an MIC concentration of 64 μg/mL, although compounds **2j** and **2k** did have moderate activity against the hypersensitive *S. aureus* Smith strain (MIC = 4 g/mL and 1.3 g/mL, respectively).

Analysis of the X-ray structures of **1** and additional analogs above suggested that further exploration of the 5-position of the pyrazole was warranted. Since it appeared that substituents of the size of carboethoxy could be tolerated, amides and carbamates were explored. The ethyl amides at the 5-position of the pyrazole shown in Table 3 were observed to be approximately twice as potent GyrB inhibitors as the corresponding ethyl ester analogs (compounds **3c** vs **2k** and **3g** vs **2i**). Some of these analogs exhibited moderate antibacterial activity. The nitrogen linked piperidine substituted at the 4-thiazole position, **3b**, was potent against the enzyme and showed that it could penetrate the bacteria, demonstrating weak MIC against the *E. coli tol C* pump knockout strain (MIC = 32 μ/mL). However, the *N*-linked piperidine compounds (**3b** and other substituted *N*-linked piperidines not shown) were also found to possess chemical stability issues limiting their usefulness. The (*S*)-2-piperidine **3d** demonstrated some *S. aureus* and *E. coli tol C* antibacterial potency with significant *S. pneumoniae* activity. The *R*-isomer **3e** was very weak showing only modest binding relative to the *S*-enantiomer. A more lipophilic substituent, such as the phenyl **3c**, showed greater antibacterial potency. These compounds however, lacked sufficient solubility to be investigated further. The compounds were tested intermittently for *S. aureus* enzyme inhibitory gyrase activity and generally were equivalent with the *E. coli* K_i values in the ester and amide series.

The carbamates shown in Table 4 were extremely potent in terms of enzymatic inhibition, due to formation of an additional hydrogen bond between the sidechain of Asn 46 and the carbamate carbonyl, and demonstrated the most potent *E. coli tol C* activity. These carbamates however, appeared to be effluxed from the bacteria since there was no observable antibacterial potency against wild type *E. coli* strains. These compounds showed moderate inhibition of *S. aureus* gyrase but antibacterial activity was not observed against the *S. aureus* ATCC29213 and *S. pneumoniae* ATCC10015 strains at MIC concentrations of 64 μ/mL. While the piperidine analogs (**4b** and **4c**) penetrated the bacteria, the cyclohexyl compound (**4e**) did not and was inactive, though potent against the enzyme. Interestingly, the *S. aureus* enzyme activity was significantly weaker. From the X-ray structure (Fig. 3**A** and **B**), it was observed that there was less space in this portion of the binding site of the *S. aureus* GyrB subunit relative to *E. coli*. The shape of the binding pocket formed by Ile-51, Leu-103, and Ile-175 in the observed *S. aureus* crystal structure, containing the methyl carbamate analog **4f**, is significantly different from the observed *E. coli* GyrB structure containing a propargyl carbamate analog **4b** (Fig. 3**A**). In the *E. coli* enzyme structure, the isoleucines are valines and the Leu-103 is a methionine (Fig. 3**A**). The propargyl carbamate when shown superimposed on the *S. aureus* GyrB surface revealed a lack of space for efficient binding (Fig. 3**B**), which

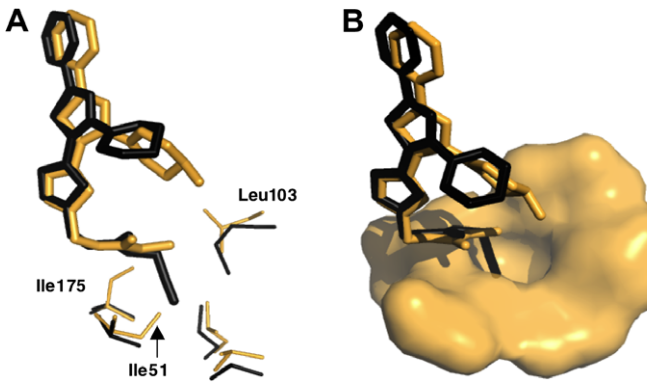
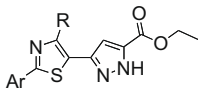


Fig. 3. (**A**) Superposition of X-ray crystal structures of compound **4f** (orange) bound to *S. aureus* GyrB. (PDE code 3G7B) and compound **4b** (black) bound to *E. coli* GyrB. (PDE code 3G7E). (**B**) Portion of the molecular surface of compound **4f** shown with compounds **4f** (orange) and **4b** (black).

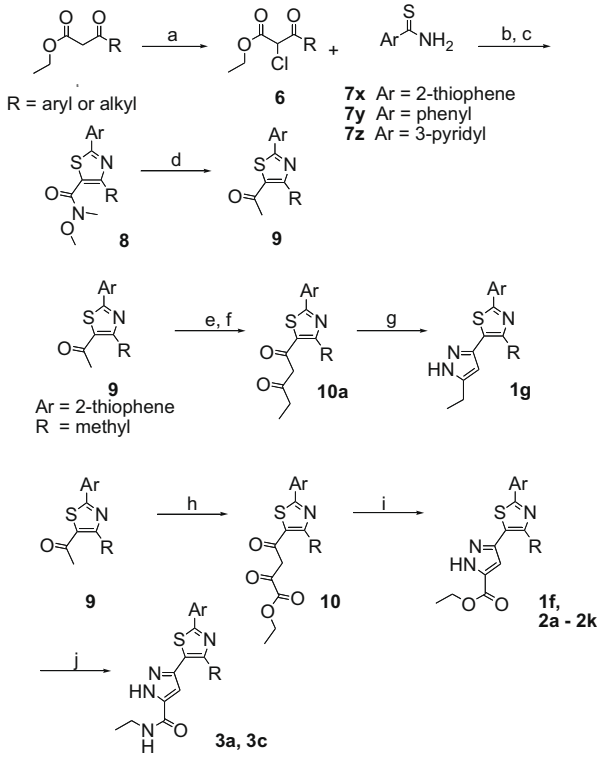
Table 2
Escherichia coli enzyme activity



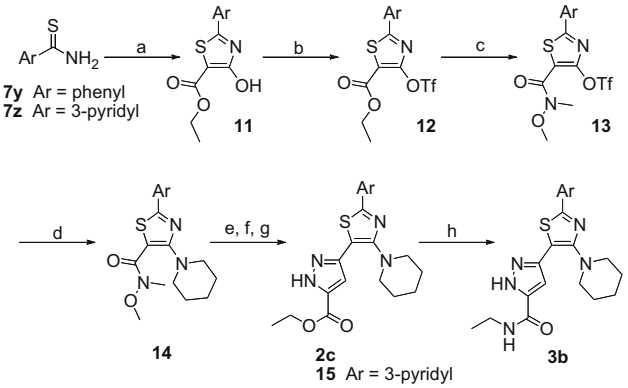
	Ar	R	*E. coli* K_i (μM)
2a	Phenyl	Propyl	0.8
2b	Phenyl	Phenyl	0.14
2c	Phenyl	1-Piperidine	0.21
2d	Phenyl	4-Morpholine	0.63
2e	3-Pyridyl	Methyl	0.33
2f	3-Pyridyl	4-Methyl-1-piperidine	1.05
2g	3-Pyridyl	Methoxymethyl	0.28
2h	3-Pyridyl	Hydroxymethyl	0.67
2i	3-Pyridyl	Cyclohexyl	0.12
2j	3-Pyridyl	Cyclopropyl	0.24
2k	3-Pyridyl	Phenyl	0.07

explains the 10 times increase in potency in *E. coli* over *S. aureus* for these longer carbamate analogs.

The synthesis of the ethyl ester compounds described in Tables 1 and 2 when the 4-position on the thiazole is alkyl or aryl (Scheme 1), started with the chlorination of the commercially available β-keto ester **5**. In instances when the β-keto ester was not available from commercial sources, it was prepared via the route described by Holmquist et al.[9] The chlorinated product **6** was condensed with the arylthioamide **7x–z** followed by direct conversion of the ethyl ester to the Weinreb amide **8** using dimethylaluminum chloride. A Grignard addition afforded the methyl ketone **9**, which was subjected to a Claisen condensation to yield the desired keto ester **10**. Subsequent condensation with hydrazine



Scheme 1. Reagents and conditions: (a) SO_2Cl_2, CH_2Cl_2, 0 °C to rt; (b) ethanol, reflux; (c) Me_2AlCl, *N,O*-dimethylhydroxy-amine hydrogen chloride, CH_2Cl_2, 0 °C; (d) MeMgBr, THF, 0 °C; (e) lithium bis(trimethylsilyl) amide, propionaldehyde, THF, −78 °C to −20 °C; (f) Dess–Martin periodinane, *t*-butyl alcohol, CH_2Cl_2, 0 °C; (g) hydrazine hydrate, ethanol; (h) KO*t*Bu, diethyl oxalate, THF, rt; (i) hydrazine, acetic acid, ethanol; (j) ethyl amine, methanol, reflux.

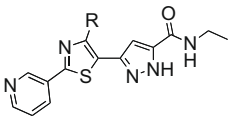


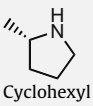
Scheme 2. Reagents and conditions: (a) diethylbromomalonate, pyridine, toluene reflux; (b) trifluoroacetic anhydride, 2,6-lutidine, CH_2Cl_2, 0 °C; (c) Me_2AlCl, *N,O*-dimethylhydroxyl-amine hydrogen chloride, CH_2Cl_2, 0 °C; (d) piperidine, toluene reflux; (e) MeMgBr, THF, 0 °C; (f) KO*t*Bu, diethyl oxalate, THF, rt; (g) hydrazine hydrate, acetic acid, ethanol; (h) ethyl amine, methanol reflux.

afforded the desired pyrazole esters. The lithium enolate of methyl ketone **9** was also condensed with propionaldehyde, and subsequent oxidation with Dess–Martin periodinane resulted in the desired β-diketone **10a**, which was converted to ethylpyrazole **1g**. The nitrogen-linked C-4 thiazole analogs (Scheme 2) were synthesized by first condensing diethylbromomalonate with the arylthioamide **7y** or **7z** to afford the 4-hydroxythiazole **11** as described by Kerdesky et al.[10] Treatment of **11** with trifluoroacetic anhydride and 2,6-lutidine afforded triflate **12**. The ethyl ester moiety in **12** was converted to the Weinreb amide **13**, and subsequent SnAr displacement of the triflate with piperidine afforded **14**. The synthesis was completed as described in Scheme 1.

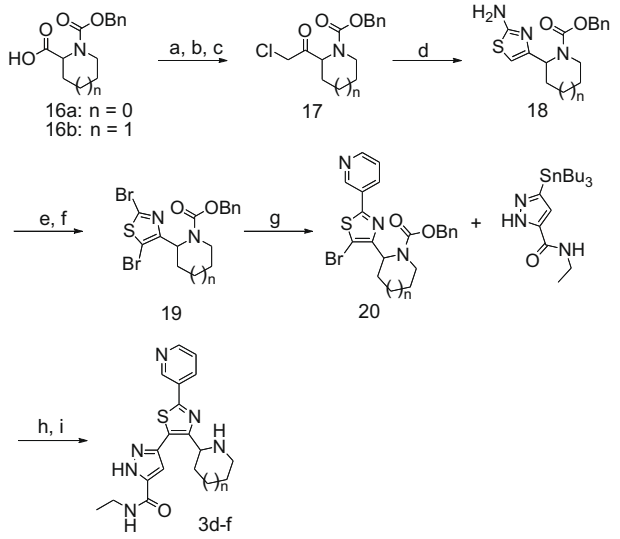
The pyrazole amides **3a–c**, **3g** described in Table 3 were prepared from the esters **15, 2e**, **2i**, **2k**, respectively, by heating with ethyl amine in methanol.

Table 3
Escherichia coli enzyme activity and antibacterial activity (MIC)

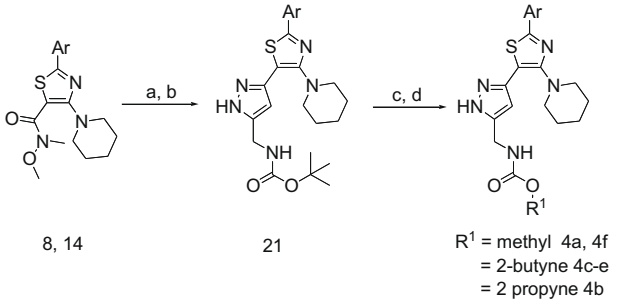


	R	*E. coli* K_i (μM)	MIC[a] (μg/mL)
3a	Methyl	0.35	All MIC > 64
3b	1-Piperidine	0.090	[d]*E. coli tol C* = 32
3c	Phenyl	0.045	[b]*S. aureus* = 8 [c]*S. pneumoniae* = 2 [d]*E. coli tol C* = 4
3d		0.040	[b]*S. aureus* = 32 [c]*S. pneumoniae* = 1.5 [d]*E. coli tol C* = 48
3e		3.4	All MIC >64
3f		0.082	[d]*E. coli tol C* = 32
3g	Cyclohexyl	0.062	[d]*E. coli tol C* = 16
3h	4-Cl-Phenyl	0.047	[b]*S. aureus* = 8 [c]*S. pneumoniae* = 4 [d]*E. coli tol C* = 8

[a] Observed antibacterial activities.
[b] *S. aureus* ATCC29213.
[c] *S. pneumoniae* ATCC10015.
[d] *E. coli tol C* strain CAG12184(CGSC)



Scheme 3. Reagents and conditions: (a) isobutyl chloroformate, TEA, ethyl acetate, 0 °C to rt; (b) CH_2N_2/Et_2O, ethyl acetate; (c) 6 N HCl, ethyl acetate; (d) thiourea, ethanol, reflux; (e) NBS, CH_3CN, 0 °C; (f) isoamyl nitrite, $CuBr_2$, PEG 200, CH_3CN; (g) 3-(1,3,2-dioxborinan-2-yl)pyridine, 2 N aq $NaHCO_3$, $Pd(PPh_3)_4$, DME, reflux; (h) $(Ph_3P)_2PdCl_2$, dioxane, reflux; (i) BBr_3, TFA, CH_2Cl_2, 0 °C.



Scheme 4. Reagents and conditions: (a) *tert*-butyl prop-2-ynylcarbamate, *n*-butyllithium, THF, −15 °C to −10 °C; (b) hydrazine hydrate, ethanol; (c) TFA, CH_2Cl_2; (d) but-2-yn-1-ol (**4c–e**), 1,1-carbonyldiimidazole, THF.

The synthesis of the 2-piperidine and 2-pyrrolidine analogs (Scheme 3) started with commercially available CBZ-protected 2-carboxylic acid **16**, which was activated by making the mixed anhydride. This was subjected to reaction with diazomethane, followed by treatment with 6 N HCl to afford chloromethyl ketone **17**. Condensation of **17** with thiourea afforded the desired 2-aminothiazole **18**, which was brominated at the 5-position on the thiazole ring with *N*-bromosuccinamide. The dibromo compound **19** was synthesized by aniline diazotization followed by treatment with copper bromide, using PEG 200 to maintain solubility. Suzuki coupling with 3-(1,3,2-dioxborinan-2-yl)pyridine proceeded selectively at the 2-position of thiazole **20**, and subsequent Stille coupling with the desired pyrazole stannane[11] afforded compounds **3d–f**.

The synthesis of the carbamate compounds in Table 4 started with alkynylation of the Weinreb amides (**8**, **14**) (Scheme 4). The amide was displaced by the dianion of BOC-propargyl amine. Subsequent treatment with hydrazine hydrate afforded pyrazole **21**. The BOC protecting group was cleaved with TFA, and the resulting amine was converted to the desired carbamates **4a**–**f**, by treatment with the desired alcohol in the presence of CDI.[12]

In conclusion, the pyrazolothiazole class evolved from a micromolar high throughput screen hit, to a class of GyrB inhibitors with potent enzyme and moderate antibacterial activity. They possess activity against *S. aureus* and *S. pneumoniae*, but appear to be actively effluxed from *E. coli* based on *E.coli tol C* mutant activity versus *E. coli* wild type activity. Perhaps the most interesting observation to arise from this study is the ability of the carbamate analogs to act as selective inhibitors of *E. coli* GyrB over *S. aureus* GyrB. Structural studies explain this selectivity by defining the differences of the binding site created by Ile-51, Leu-103, and Ile-175 in *S. aureus* GyrB compared to *E. coli* where the isoleucines are valines and Leu-103 is a methionine.

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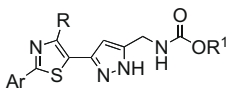
12. For full experimental details on similar compounds see: US Patent 0024030 A1, 2005.

Table 4
Staphlococcus aureus, *Escherichia coli* enzyme activity and antibacterial activity (MIC).



	Ar	R	R^1	*S. aureus* K_i(μM)	*E. coli* K_i(μM)	MIC (μg/mL) *E. coli tol C*
4a	3-Pyridyl	1-Piperidine	Methyl	0.300	0.056	>64
4b	3-Pyridyl	1-Piperidine	$CH_2C{\equiv}CH$	0.240	0.011	1.0
4c	3-Pyridyl	1-Piperidine	$CH_2C{\equiv}CCH_3$	0.140	<0.004	0.5
4d	3-Pyridyl	Methoxymethyl	$CH_2C{\equiv}CCH_3$	1.10	0.014	8.0
4e	3-Pyridyl	Cyclohexyl	$CH_2C{\equiv}CCH_3$	0.069	<0.004	>64
4f	Phenyl	4-Hydroxy-1-piperidine	Methyl	ND[a]	1.08	>64

[a] Not tested for *S. aureus* activity