Bioorganic & Medicinal Chemistry Letters 23 (2013) 6598–6603

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and biological evaluation of α-substituted isonipecotic acid benzothiazole analogues as potent bacterial type II topoisomerase inhibitors

Lorraine C. Axford [a,*], Piyush K. Agarwal [d], Kelly H. Anderson [a], Laura N. Andrau [a], John Atherall [c], Stephanie Barker [b], James M. Bennett [b], Michael Blair [a], Ian Collins [b], Lloyd G. Czaplewski [b], David T. Davies [b], Carlie T. Gannon [a], Dushyant Kumar [d], Paul Lancett [b], Alastair Logan [b], Christopher J. Lunniss [a], Dale R. Mitchell [c], Daniel A. Offermann [a], James T. Palmer [a], Nicholas Palmer [c], Gary R. W. Pitt [a], Stéphanie Pommier [b], Daniel Price [b], B. Narasinga Rao [d], Rashmi Saxena [d], Tarun Shukla [d], Amit K. Singh [d], Mahipal Singh [d], Anil Srivastava [d], Christopher Steele [c], Neil R. Stokes [b], Helena B. Thomaides-Brears [b], Edward M. Tyndall [a], David Watson [c], David J. Haydon [b]

[a] Biota Holdings Ltd, 10/585 Blackburn Road, Notting Hill, Victoria 3168, Australia
[b] Biota Europe Ltd, Begbroke Science Park, Begbroke, Oxfordshire OX5 1PF, United Kingdom
[c] BioFocus, Chesterford Research Park, Saffron Walden, Essex CB10 1XL, United Kingdom
[d] Jubilant Chemsys Ltd, B-34, Sector-58, Noida 201301, India

ARTICLE INFO

Article history:
Received 25 June 2013
Revised 26 October 2013
Accepted 28 October 2013
Available online 4 November 2013

Keywords:
DNA gyrase
Topoisomerase
Antibacterial

ABSTRACT

The discovery and optimisation of a new class of benzothiazole small molecules that inhibit bacterial DNA gyrase and topoisomerase IV are described. Antibacterial properties have been demonstrated by activity against DNA gyrase ATPase and potent activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Haemophilus influenzae*. Further refinements to the scaffold designed to enhance drug-likeness included analogues bearing an α-substituent to the carboxylic acid group, resulting in excellent solubility and favourable pharmacokinetic properties.

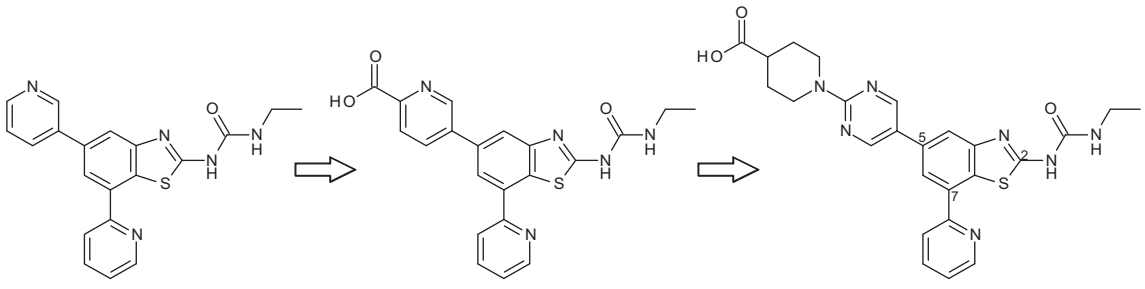
Drug resistance in pathogenic bacteria is increasing throughout the world, leading to higher mortality and increased healthcare costs.[1] There is a huge need to develop new classes of therapeutic agents to address this problem.

Type II topoisomerases catalyse the interconversion of DNA topoisomers by transporting one DNA segment through another.[2] Bacteria encode two type II topoisomerase enzymes, DNA gyrase and DNA topoisomerase IV.[3] DNA gyrase controls DNA supercoiling and relieves topological stress. Topoisomerase IV decatenates daughter chromosomes following replication and can also relax supercoiled DNA. Bacterial type II topoisomerases form a heterotetrameric complex composed of two subunits. DNA gyrase forms an A_2B_2 complex comprised of GyrA and GyrB, whereas topoisomerase IV forms a C_2E_2 complex comprised of ParC and ParE.[3] The bacterial type II topoisomerases are highly conserved enzymes allowing the design of broad-spectrum inhibitors. Furthermore, the GyrB and ParE subunits are functionally similar,[3] having an ATPase domain in the N-terminal domain and a C-terminal domain that interacts with the other subunit (GyrA and ParC respectively) and the DNA. The conservation between the DNA gyrase and topoisomerase IV active sites suggests that inhibitors of the sites should simultaneously target both type II topoisomerases.[3] Such 'dual-targeting' inhibitors are attractive because they have the potential to reduce the development of target-based resistance. Ideally, an antibiotic based on the inhibition of bacterial type II topoisomerases would be selective for the bacterial enzymes and relatively inactive against the eukaryotic type II topoisomerases.

The authors have previously described a class of benzothiazoles[4–7] which inhibits bacterial DNA gyrase activity. In this communication, a class of related compounds that include a carbocyclic or heterocyclic ring comprising of an α-substituted carboxylate, is described. Early hits such as **1** (Fig. 1)[4] showed antibacterial activity but had limited solubility. As solubility is important in clinical administration of antibacterial drugs targeting both intravenous (IV) and oral delivery, solubilising moieties were

* Corresponding author. Tel.: +61 3 9915 3700; fax: +61 3 9915 3701.
E-mail address: l.offermann@biota.com.au (L.C. Axford).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.10.058



	1	2	3
IC_{50}[a] (μg/mL)	0.014	0.001	0.010
E. fs MIC (μg/mL)	0.12	0.12	0.015
Solubility (μg/mL)	6.25	50	>800

Figure 1. Progression of early hits to isonipecotic acid containing analogues. [a]DNA gyrase ATPase (*S. aureus*).

explored that would not interfere with the promising antimicrobial profile. It was discovered that the addition of a carboxylic acid moiety appended to the C-5 aryl group, **2**, indeed improved the solubility[8] at physiological pH from 6.25 μg/mL to 50 μg/mL, whilst maintaining antibacterial activity against the target pathogens (minimal inhibitory concentration (MIC) data for *Enterococcus faecalis* shown as an example).

By linking the carboxylate to the C-5 aryl group by a cyclic amine, for example the isonipecotic acid appendage, **3**, both the antibacterial activity and the solubility increased. It was discovered that the addition of a substituent α to the carboxylic acid improved the PK profile of the series without loss of potency or solubility. Compounds of this class possessed enhanced solubilities at physiologically acceptable pH, enabling IV administration, along with excellent oral bioavailability, lower clearance, and higher exposure compared to compounds without an α substituent. The discovery and preparation of this class of compounds are now described.

During the exploration of C-5, a 2-pyridyl group was maintained at the C-7 position of the benzothiazole core. Boronic acid intermediate **4** was prepared as an advanced precursor, according to Scheme 1.

Synthesis commenced with bromination of 2-amino-5-nitrophenol **5** in acetonitrile followed by deamination with sodium nitrite and sulfuric acid in ethanol. Benzylation of phenol **6** and reduction of the nitro group afforded **7**. Addition of benzoyl isothiocyanate to **7** furnished *N*-benzoylthiourea **8**, which was readily cleaved to the thiourea **9** with sodium hydroxide. Bromine-mediated cyclisation to the benzothiazole core followed, with subsequent conversion to the ethyl urea **10**. Stille coupling installed the C-7 pyridyl group. Treatment of **11** with methane sulfonic acid yielded alcohol **12**, which upon conversion to triflate **13** and a Miyaura borylation reaction yielded the key intermediate **4** for further derivatisation.

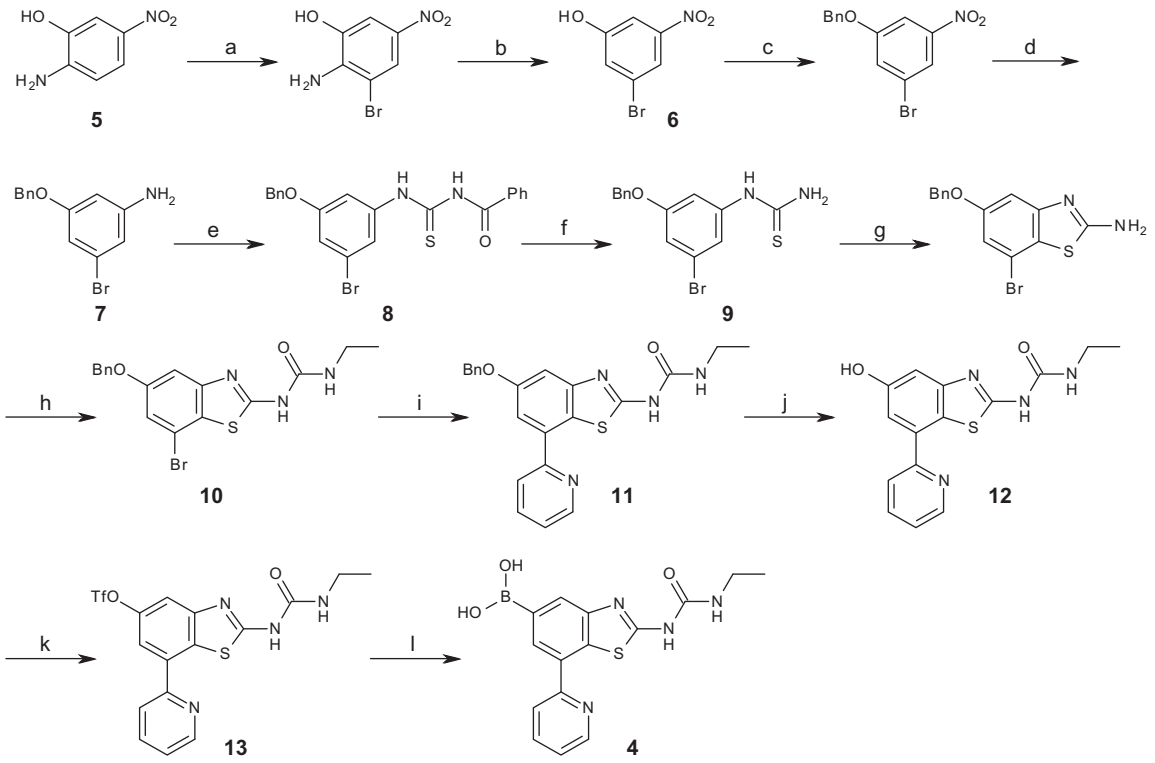
Initially, the introduction of an α substituent to the carboxylic acid was investigated. As shown in Scheme 2, commercially available ethyl *N*-Boc-piperidine-4-carboxylate **14** was treated with lithium di*iso*propylamide at −78 °C, quenched with an alkyl halide, then warmed to room temperature to furnish **15**. Removal of the Boc group with hydrogen chloride in 1,4-dioxane gave crude **16** which was then used in an aromatic nucleophilic substitution reaction with 5-bromo-2-chloropyrimidine to yield bromide **17**. Suzuki cross-coupling with boronic acid **4**, followed by saponification, yielded the target products.

Alternative α-substituents were prepared by quenching the lithiated species (step a) with alternative electrophiles that is, S-(trifluoromethyl)dibenzothiophenium trifluoromethanesulfonate (CF_3) or methanesulfonyl chloride (SO_2Me). Some analogues were prepared via a modified synthetic route or from alternative starting materials as described elsewhere.[9]

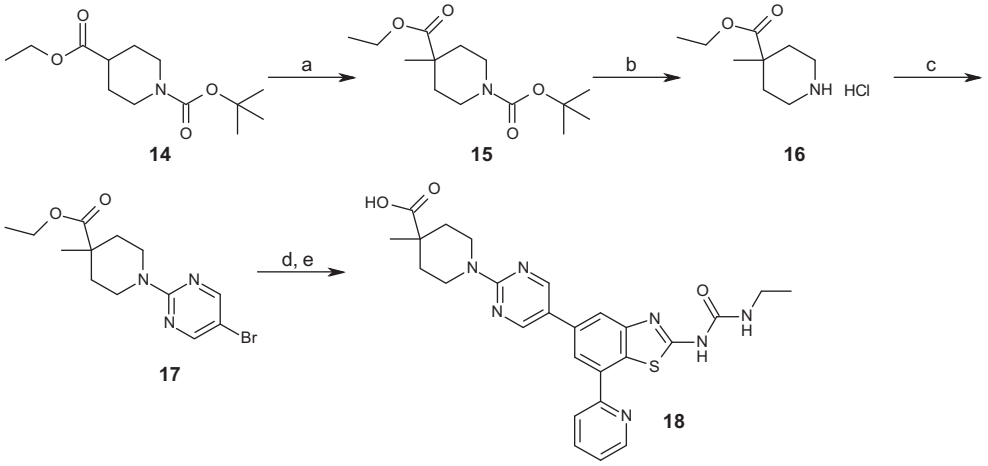
Compounds were tested in a DNA gyrase ATPase activity assay.[10] The antimicrobial activity of the compounds was measured by susceptibility testing using the broth microdilution method[11] and MICs were determined against *Staphylococcus aureus* (ATCC 29213), *E. faecalis* (ATCC 29212), *Streptococcus pyogenes* (ATCC 51339) and *Haemophilus influenzae* (ATCC 49247).

Table 1 indicates the structure activity relationship (SAR) within the series. All analogues tested displayed potent activity in the DNA gyrase ATPase assay. Compounds **3** and **18** have IC_{50} values for *S. aureus* topoisomerase IV (0.012 and 0.008 μg/mL respectively)[10] comparable to their *S. aureus* DNA gyrase ATPase IC_{50} values. Compounds **3** and **18** also showed specificity for bacterial topoisomerases, with no inhibition of human topoisomerase II being observed.[7] A dual targeting intracellular inhibitor of GyrB and ParE would be expected to show a low frequency of resistance (FoR).[13] The FoR to compound **3** and **18** in *S. aureus* (ATCC 29213) was determined.[7] At concentrations equivalent to four- and eight-fold the MICs no spontaneous mutants were isolated, giving measured FoRs of $\leqslant 10^{-10}$. Taken together this suggests the compounds are potent and specific inhibitors of both DNA gyrase and topoisomerase IV. Small alkyl groups gave the best antibacterial profile as well as excellent solubility and stability in mouse microsomes (intrinsic clearance, CL_{int}). For example, methyl **18** and ethyl **19** groups were shown to have the most favourable activity across the panel. Aryl containing groups such as phenyl **20** lost activity against *S. pyogenes* (*S. py*) and *H. influenzae* (*H. in*) and demonstrated poor solubility. Addition of a fluorine-containing group such as CF_3 **21** led to a marked decrease in activity, particularly against *S. aureus* (*S. au*). Other electron withdrawing groups such as SO_2Me **22** led to a further decrease in activity across the species tested. Electron rich groups such as OH **23** and NH_2 **24** generally showed poor activity, particularly against *S. aureus*, *E. faecalis* (*E. fs*) and *H. influenzae*. The nature of the α-substituent coupled with the carboxylate was key to fine tuning the ideal profile of the series. With this information in mind, subsequent analogues were prepared with an α-methyl substituent in order to obtain further SAR.

Next, the effect of the heterocycle directly attached to the C-5 position of the core was investigated. A set of analogues was prepared[9] using the α-methyl isonipecotic acid group as the optimal group from the earlier exploration. Selected compounds are described in Table 2. The 3,5-pyrimidyl group **18** (data in Table 1) gave the best overall profile, followed by the 3-pyridyl **25**. The 3,5-pyrimidyl and 3-pyridyl groups showed an improvement in



Scheme 1. Reagents and conditions: (a) bromine, MeCN, 30 °C, 1 h; 85%; (b) sulfuric acid (conc.), EtOH, −10 to 50 °C then $NaNO_2$, reflux, 3 h; 84%; (c) benzyl bromide, K_2CO_3, acetone, rt, 15 min then reflux, 3 h; 51%; (d) $SnCl_2{\cdot}2H_2O$, THF, reflux, 3 h; 97%; (e) benzoyl isothiocyanate, acetone, rt, 45 min; 85%; (f) NaOH, H_2O, 70 °C, overnight; 78%; (g) bromine, MeCN, 0 °C, 30 min then rt, 1 h; 59%; (h) ethyl isocyanate, 1,4-dioxane, 80 °C, overnight; 75%; (i) 2-tributylstannyl pyridine, $Pd(PPh_3)_4$, DMF, 100 °C, 16 h; 70%; (j) methane sulfonic acid, dichloromethane, rt, 3 h; 97%; (k) *N*-phenylbis(trifluoromethane sulfonamide), DIPEA, DMF, rt, 3 h; 68%; (l) bis(neopentylglycolato) diboron, $Pd(dppf)Cl_2{\cdot}DCM$, KOAc, DMSO, 80 °C, 1.5 h; 88%.

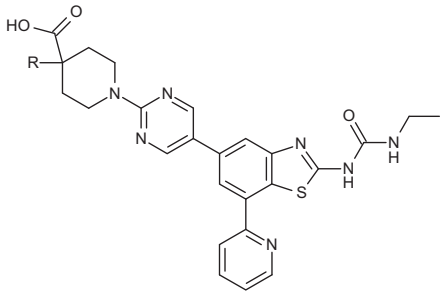


Scheme 2. Reagents and conditions: (a) LDA, THF, −78 °C, 45 min, then methyl iodide, −78 °C to rt, 6 h; 65%; (b) 4 M HCl in 1,4-dioxane, rt, 30 min; (c) 5-bromo-2-chloropyrimidine, DIPEA, EtOH, 70 °C, 1 h; 75% (over two steps); (d) **4**, $Pd(PPh_3)_4$, Na_2CO_3 (2 M, aq), EtOH, 80 °C; 2 h; 22%; (e) KOtBu, DMSO, rt, 2 h; 65%.

activity against *S. aureus* and *H. influenzae* compared to other analogues. Switching to a pyrazine analogue **26** resulted in a fourfold drop in activity against *S. aureus* and *H. influenzae* relative to 3,5-pyrimidyl. Regiochemical substitution also had an effect on antimicrobial activity. *Meta* substitution **27** was less favourable than *para* (relative to the benzothiazole core) particularly illustrated by comparing MICs against *S. aureus*. Switching to a five-membered ring such as 1,2,4-thiadiazole **28** led to poor activity against all pathogens as well as a minor drop-off in enzyme activity. In all cases, data suggest that solubility and microsomal stability were relatively unchanged by these modifications. 3,5-Pyrimidine was selected as the optimal heterocycle for the preparation of further analogues.

Modifications of the isonipecotic acid ring were then explored (Table 3). Five-, six- and seven-membered rings were synthesised.[9] Again, all analogues showed good enzyme data. Pyrrolidine compound **29** showed good activity against *S. pyogenes* and *H. influenzae*, however the alternative substitution pattern **30** displayed more modest activity against all pathogens tested. The piperidine analogue **18** (data in Table 1) showed improved activity, with the

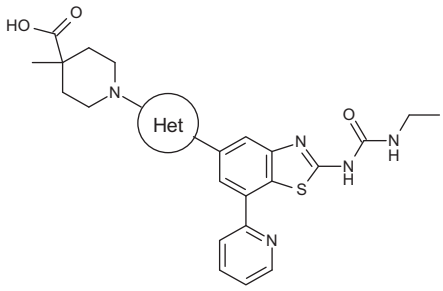
Table 1
Effect of various α-substituents on antibacterial activity

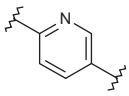
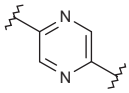
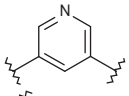
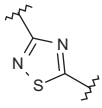


#	R	IC_{50}[a] (μg/mL)	MIC (μg/mL)				Solubility[8] (μg/mL)	CL_{int}[12] (mL/min/kg)
			S. au	*S. py*	*E. fs*	*H. in*		
3	H	0.010	0.12	0.03	0.015	2	>800	<5.8
18	Me	0.008	0.12	0.06	0.015	2	>800	<5.8
19	Et	0.012	0.06	0.06	0.015	4	>800	<5.8
20	Ph	0.013[b]	0.5	0.5	0.06	>16	12.5	<5.8
21	CF_3	0.016	2	0.25	0.25	16	>800	N.D.
22	SO_2Me	0.015[b]	>16	4	4	>16	>800	N.D.
23	OH	0.003[b]	8	0.12	0.5	8	100	N.D.
24	NH_2	0.025[b]	>16	4	>16	>16	25	N.D.

N.D. Not determined.
[a] DNA gyrase ATPase (*S. aureus*).
[b] DNA gyrase ATPase (*Escherichia coli*).

Table 2
Effect of the heterocycle on antibacterial activity



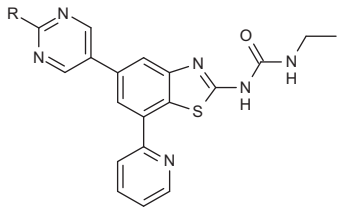
#	Het	IC_{50}[a] (μg/mL)	MIC (μg/mL)				Solubility[8] (μg/mL)	CL_{int}[12] (mL/min/kg)
			S. au	*S. py*	*E. fs*	*H. in*		
25		0.012	0.12	0.12	0.03	2	>800	<5.8
26		0.02	0.5	0.12	0.03	8	>800	<5.8
27		0.015	1	0.06	0.06	4	>800	N.D.
28		0.056	1	1	N.D.	>16	N.D.	N.D.

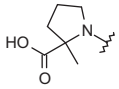
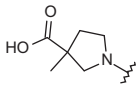
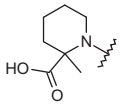
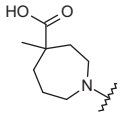
N.D. Not determined.
[a] DNA gyrase ATPase (*S. aureus*).

alternate regiochemisty **31** being disfavoured as well as exhibiting poor solubility. The azepane analogue **32** showed comparable potency, solubility and microsomal stability to **18**, apart from a decrease in *H. influenzae* activity. All analogues were prepared racemically apart from **18** which offered a fortuitous synthetic advantage due to its achiral nature.

Suitable compounds were selected and their PK profile determined in mouse and/or rat.[14] The maximum concentration following oral administration (C_{max}), area under the curve (AUC_{IV}), bioavailability (*F*) and clearance following IV administration (*CL*) were calculated from the measured plasma concentrations. Selected results are shown in Table 4.

Table 3
Effect of the acid attachment ring



#	R	IC_{50}[a] (μg/mL)	MIC (μg/mL)				Solubility[8] (μg/mL)	CL_{int}[12] (mL/min/kg)
			S. au	*S. py*	*E. fs*	*H. in*		
29		0.005	0.5	0.06	0.25	1	>800	N.D.
30		0.04	2	0.5	N.D.	4	>800	N.D.
31		0.007[b]	0.5	0.5	0.5	8	6.25	N.D.
32		0.006	0.12	0.12	N.D.	8	>800	<5.8

N.D. Not determined.
[a] DNA gyrase ATPase (*S. aureus*).
[b] DNA gyrase ATPase (*E. coli*).

Table 4
Calculated PK parameters following administration at 3 mg/kg

#	Species	C_{max} (μg/mL)	AUC_{IV} (μg hr/mL)	*F* (%)	*CL* (mL/min/kg)
3	Mouse	0.07	0.9	8	55
18	Rat	1.8	17.4	48	2.8
25	Mouse	1.7	7.6	33	6.5
25	Rat	0.6	6.6	31	7.6
19	Mouse	6.1	14.3	87	3.5
19	Rat	3.5	27.7	80	1.5

The results clearly demonstrate that the addition of a substituent α to the carboxylic acid significantly improved the PK profile of this class of compound when compared to compound **3**. Pleasingly, the maximum oral concentration, AUC and bioavailability all showed much greater values and decreased clearance rates were observed. For example, the addition of an ethyl group at the α position, compared to a hydrogen substituent (compounds **3** vs **19** in mouse), gave an 87-fold improvement in maximum concentration, a 16-fold improvement in AUC_{IV} and a 10-fold increase in bioavailability. Clearance was reduced by more than 15-fold. This trend was shown to be consistent over all compounds tested. Furthermore, the rat plasma protein binding remains the same for both compounds **3** and **18** (99.3% in each case)[7] giving weight to improvements in the pharmacology by the addition of an α substituent, rather than merely variation in protein binding. Other compounds have similar values.

In summary, the design, synthesis and antibacterial activity of a new class of compound have been reported. The compounds display outstanding antibacterial activity with inhibition of both DNA gyrase and topoisomerase IV, promising PK profiles, good microsomal stability and excellent solubility. Further investigation of these compounds as potential antibacterial agents is underway and will be reported elsewhere in due course.

References and notes

1. Magiorakos, A.-P.; Srinivasan, A.; Carey, R. B.; Carmeli, Y.; Falagas, M. E.; Giske, C. G.; Harbarth, S.; Hindler, J. F.; Kahlmeter, G.; Olsson-Liljequist, B.; Paterson, D. L.; Rice, L. B.; Stelling, J.; Struelens, M. J.; Vatopoulos, A.; Weber, J. T.; Monnet, D. L. *Clin. Microbiol. Infect.* **2012**, *18*, 268.
2. Levine, C.; Hiasa, H.; Marians, K. J. *Biochim. Biophys. Acta, Gene Struct. Expr.* **1998**, *1400*, 29.
3. East, S. P.; zaplewski, L. G.; Haydon, D. J. In *Designing Multi-Target Drugs*; Morphy, J. R., Harris, C. J., Eds.; RSC Publishing, 2012; pp 335–352. Chapter 20.
4. Haydon, D.J.; Czaplewski, L.G.; Palmer, N.J.; Mitchell, D.R.; Atherall, J.F.; Steele, C.R.; Ladduwahetty, T.; *PCT Int. Appl.* WO2007/148093.
5. Haydon, D.J.; Czaplewski, L.G.; *PCT Int. Appl.* WO2009/074810.
6. Haydon, D.J.; Czaplewski, L.G.; *PCT Int. Appl.* WO2009/074812.
7. Stokes, N. R.; Thomaides-Brears, H. B.; Barker, S.; Bennett, J. M.; Berry, J.; Collins, I.; Czaplewski, L. G.; Gamble, V.; Lancett, P.; Logan, A.; Lunniss, C.; Peasley, H.; Pommier, S.; Price, D.; Smee, C.; Haydon, D. J. *Antimicrob. Agents Chemother.* **2013**, in press. http://dx.doi.org/10.1128/AAC.00719-13
8. The solubility of the compounds was measured using a turbidimetric method. A series of doubling dilutions of compounds were prepared in neat dimethyl sulfoxide (DMSO). 5 μL samples were diluted 20-fold into 95 μL volumes of 100 mM phosphate buffered saline (PBS, pH 7.4) in microtitre assay plates and allowed to reach equilibrium at room temperature for 24 h. The absorbance within each well of the plate was read spectrophotometrically at a wavelength of 620 nm. A precipitate forms when the maximum aqueous solubility level is reached. The value quoted is the highest concentration where the compound was in solution that is, where no measurable precipitate was visible.
9. For experimental details on these and similar compounds see: Palmer, J.T.; Lunniss, C.J.; Offermann, D.A.; Axford, L.C.; Blair, M.; Mitchell, D.; Palmer, N.; Steele, C.; Atherall, J.; Watson, D.; Haydon, D.; Czaplewski, L.; Davies, D.; Collins, I.; Tyndall, E.M.; Andrau, L.; Pitt, G.R. W.; *PCT Int. Appl.* WO2012/045124.
10. *ATPase assay:* Enzymes were purchased from Inspiralis Ltd (Norwich, United Kingdom). For the *S. aureus* DNA gyrase ATPase assay, the final assay composition was 10 nM DNA gyrase enzyme (A_2B_2 complex), 0.08 mg/mL DNA, 40 mM HEPES pH 7.6, 10 mM magnesium acetate, 0.5 M potassium glutamate, 0.01 mg/mL BSA and 2 mM DTT, 1 mM ATP and 5% DMSO solution containing the compounds. For the *E. coli* DNA gyrase ATPase assay, the final assay composition was 10 nM DNA gyrase enzyme (A_2B_2 complex), 0.08 mg/mL DNA, 35 mM Tris pH 7.5, 24 mM KCl, 2 mM $MgCl_2$, 6.5% glycerol, 0.1 mg/mL BSA and 2 mM DTT, 1 mM ATP and 5% DMSO solution containing the compounds. For the *S. aureus* topoisomerase IV ATPase assay, the final assay composition was 25 nM topoisomerase IV enzyme (C_2E_2 complex), 0.01 mg/mL DNA, 50 mM Tris–HCl pH 7.5, 5 mM magnesium chloride, 0.35 M potassium

glutamate, 0.05 mg/mL BSA and 5 mM DTT, 1 mM ATP and 1% DMSO solution containing the compounds. The reactions, in a volume of 25 μL, were started by the addition of the ATP and allowed to incubate at 30 °C for 60 min. Reactions were stopped by adding 0.2 mL malachite green solution (0.034% malachite green, 10 mM ammonium molybdate, 1 M HCl, 3.4% ethanol, 0.01% tween 20). Colour was allowed to develop for 5 min and the absorbance at 600 nm was measured spectrophotometrically. The half-maximum inhibitory concentration (IC_{50}) values were determined from the absorbance readings using no compound and no enzyme controls.

11. Clinical and Laboratory Standards Institute, Approved Standard—Seventh Edition; **2006**, Vol. 26, No. 2, Document M7–A7.
12. *Mouse microsome stability assay:* Compounds were tested at 10 μM in duplicate with a final concentration of 0.1% v/v DMSO. Mouse liver microsomes (Life Technologies) were added to a final protein concentration of 1 mg/mL and reactions were started by the addition of NADPH to a concentration of 1 mM. Reactions were stopped at 0, 10, 30 and 60 min by the addition of DMSO (100 μL). Samples were extracted using acetonitrile and centrifugation, and the supernatants were analysed by LCMS. $T_{1/2}$ values were determined from the slope of the peak areas over time, which was then used to calculate the intrinsic clearance.
13. O'Neil, A. J.; Chopra, I. *Expert Opin. Investig. Drugs* **2004**, *13*, 1045.
14. The compound concentration in plasma was measured by LC/MS/MS following a single IV or oral administration of the compounds at a dose of 3 mg/kg. IV dose formation was administered as a single bolus dose through the tail vein, whilst oral dose formulation was administered by an oral gavage needle. In both cases, the dose volume was 5 mL/kg. Blood was collected from rats using a jugular vein catheter and from anesthetised mice through a capillary guided into the retro-orbital plexus. The collected blood was centrifuged to obtain plasma and the compounds extracted into methanol prior to determining the compound concentration by LC/MS/MS.