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Novel cyclohexyl-amides as potent antibacterials targeting bacterial type IIA topoisomerases

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ABSTRACT

As part of our wider efforts to exploit novel mode of action antibacterials, we have discovered a series of cyclohexyl-amide compounds that has good Gram positive and Gram negative potency. The mechanism of action is via inhibition of bacterial topoisomerases II and IV. We have investigated various subunits in this series and report advanced studies on compound **7** which demonstrates good PK and in vivo efficacy properties.

The need for novel mechanism antibacterials to combat the global spread of diseases caused by resistant bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococci* (VRE) has never been greater.[1]

Bacterial DNA type IIA topoisomerases have been much studied targets in the field of antibacterial research. This has led to major successes such as the quinolones and novobiocin (Fig. 1). However, these drugs have also suffered from toxicity issues, which have greatly limited novobiocin use and have largely prevented quinolone use in children. Additionally, resistance to fluoroquinolone agents is on the rise and therefore there is a real need for a new antibiotic.[2]

In the course of our efforts to develop antibacterials with novel modes of action and good safety profile, we have synthesized a series of cyclohexyl-amide compounds which have potent antibacterial properties. They target both the GyrA subunit of DNA gyrase and the ParC subunit of topoisomerase IV.[3a] This dual targeting should confer resistance benefits in a clinical setting.

It has been established through mutational (Black et al.) and our X-ray crystallography studies (Bax et al.) that the mode of action and the binding to the topoisomerases of similar compounds is different to that of the quinolones.[3a,b] These compounds can be viewed as novel series of antibacterials which should address clinically validated targets without the issue of existing resistance problems in the clinic. Compounds with a similar mechanism but of a distinct chemical series have also been discussed in the literature.[3c–e]

Our initial medicinal chemistry efforts were involved in surveying the potency requirements of the Left Hand Side (LHS), central unit and Right Hand Side (RHS) portion of our molecule as shown below in Figure 1.

The general synthesis of these compounds is described in Scheme 1 for compound **7**. The synthesis of subunits of type **2** (aryl bromides or triflates) is well described in the patent literature. [4a] The palladium catalysed amidation reaction of subunits of type **2** and **3** gave us ready access to intermediate **4**. This reaction is quite general and is the subject of another publication.[4b] Standard deprotection and reductive alkylation gave us access to the target compounds. The syntheses of aldehydes of type **6** and hydroxyamide **3** have been reported previously.[4,4d]

The synthesis of central unit **13** is depicted in Scheme 2.[4a] Enantiopure carboxylic acid **8** is converted to bromolactone **9**,[4e] before being treated with aqueous ammonia to give amide **10**. Azide displacement establishes the desired *trans* 1,4 configuration and standard azide reduction and primary amine protection then gives **13**.

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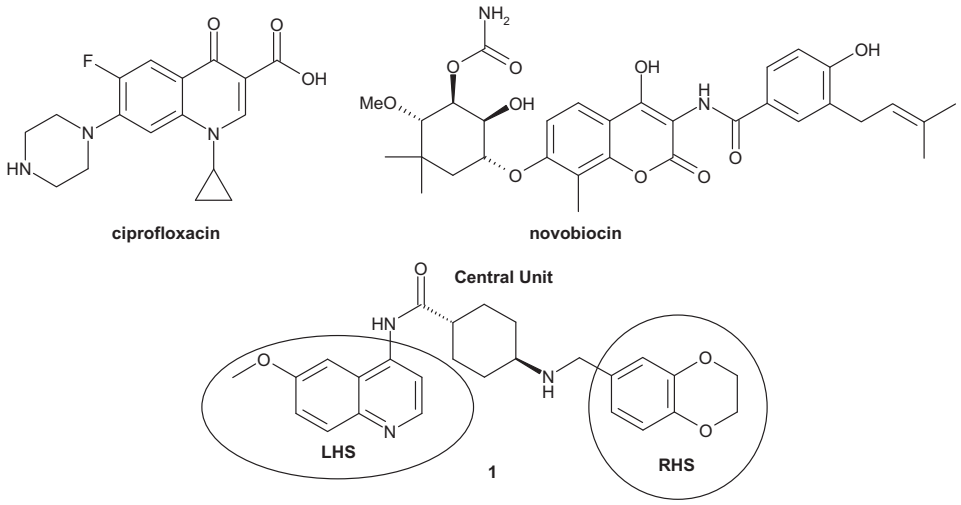
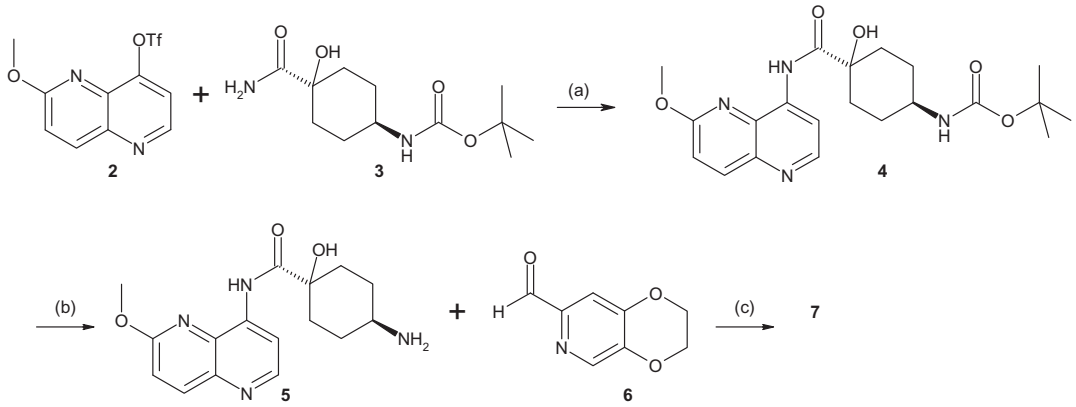
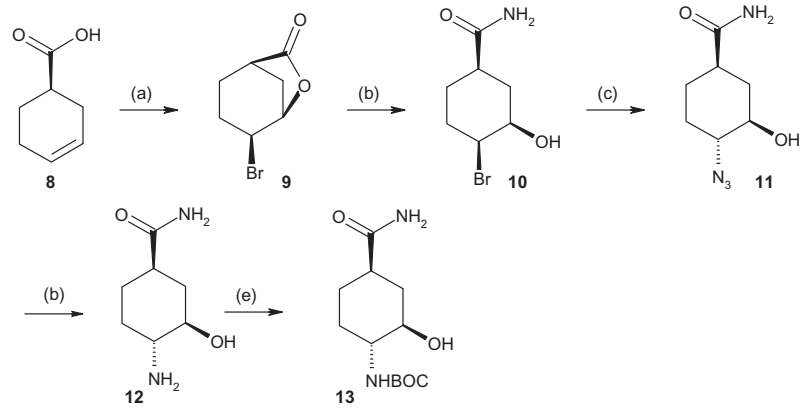


Figure 1. Type IIA bacterial DNA toposisomerase inhibitors.



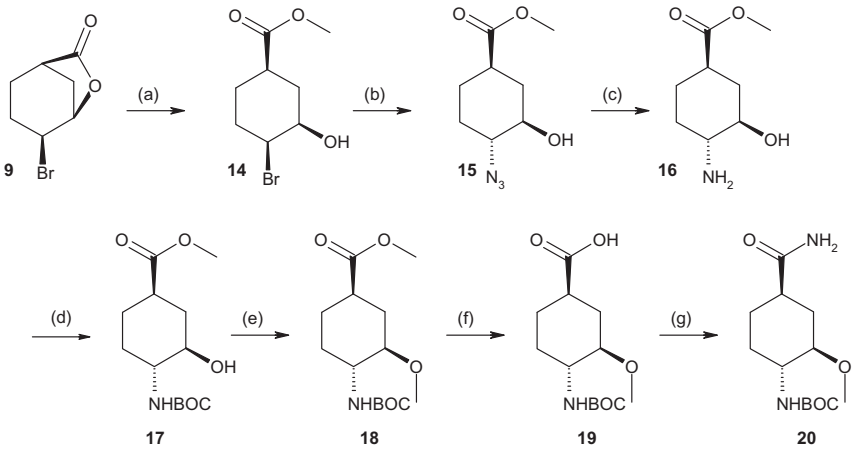
Scheme 1. Reagents and conditions: (a) aryl-triflate **2** (1 equiv), cyclohexyl-amide **3** (1.05 equiv), $Pd_2(dba)_3$ (3 mol %), (±)-BINAP (6 mol %), Cs_2CO_3 (2.5 equiv), 1,4-dioxane, 85 °C, 18 h; (b) TFA, DCM then basic extraction; (c) $NaBH(OAc)_3$, $CHCl_3$, MeOH, 3 Å molecular sieves.



Scheme 2. Reagents and conditions: (a) TMSBr, DIPEA, DMSO; (b) aq NH_3, rt, 24 h; (c) NaN_3, DMF, 60 °C, 15.5 h; (d) Pd/C, 1 atm H_2, MeOH rt, 22 h; (e) Boc_2O, DIPEA, MeOH, 1,4-dioxane, 16 h.

The synthesis of central unit **20** is shown in Scheme 3.[4a] Taking bromolactone **9** and opening the lactone ring under basic methanolic conditions gave ester **14**. Again, azide displacement resulted in the desired *trans* 1,4 configuration. Standard azide reduction and primary amine protection yielded **17**. A key chemoselective alkylation with methyl iodide and silver(I) oxide allowed access to methoxy intermediate **18**. The ester was then converted to the primary amide under standard conditions.

With all of the desired sub-units in hand we proceeded to synthesize a range of compounds, a subset of which is shown in Table 1.

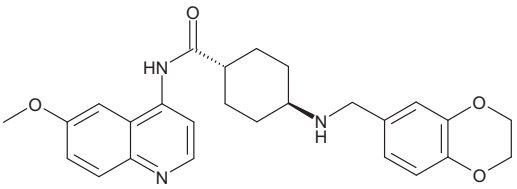
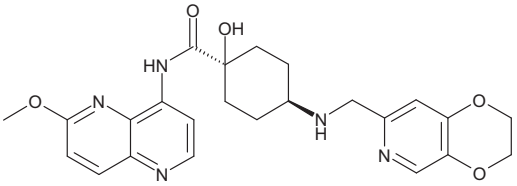
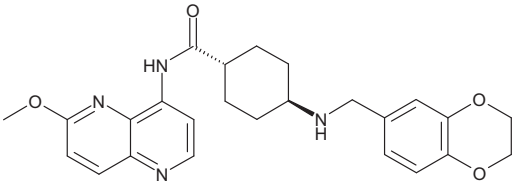
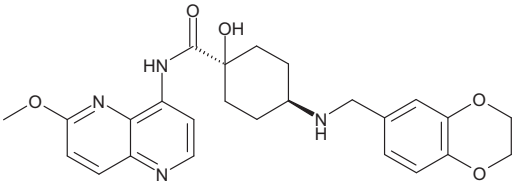
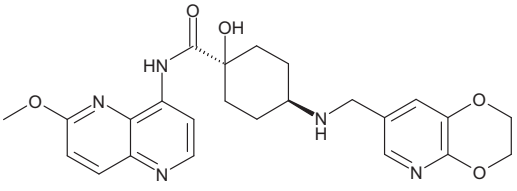


Scheme 3. Reagents and conditions: (a) $NaHCO_3$, MeOH, 18 h; (b) NaN_3, DMF, 60 °C, 8 h; (c) Pd/C, 1 atm H_2, MeOH, acetic acid rt, 18 h; (d) Boc_2O, DIPEA, THF, 50 °C, 18 h; (e) silver(I) oxide, methyl iodide, 3 Å molecular sieves, DCM, rt, 18 h; (f) LiOH, MeOH, H_2O, rt, 18 h; (g) HOAt, EDC-HCl, NH_4HCO_3, rt, 18 h.

As can be seen from Table 1, we kept the secondary amide linker constant. We then chose a variety of RHS subunits, LHS subunits, and cyclohexane ring substituents to explore the SAR of this series.

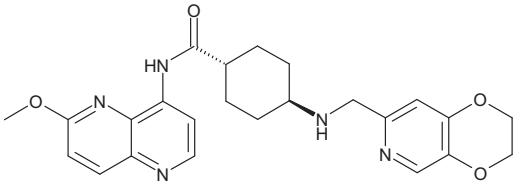
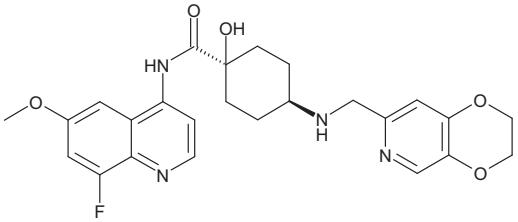
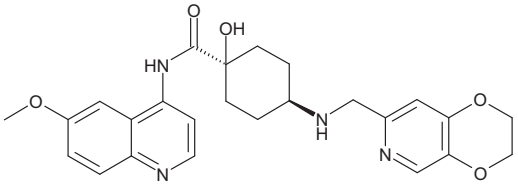
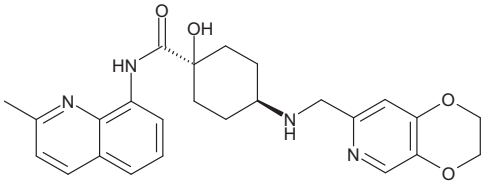
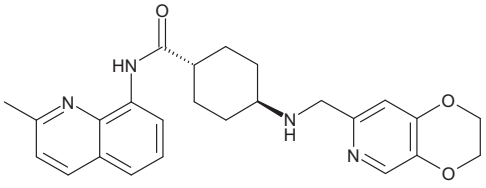
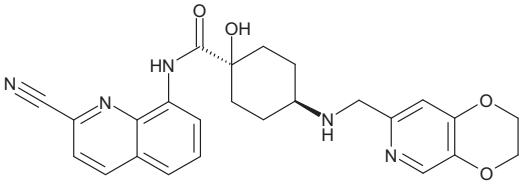
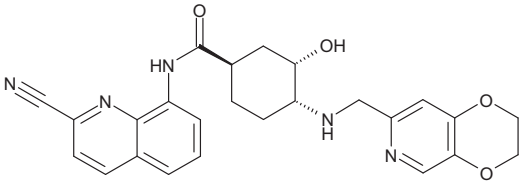
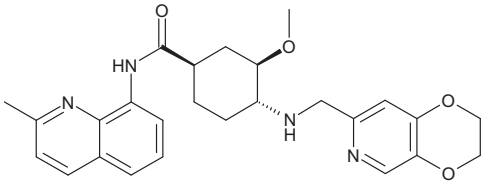
These compounds in Table 1 are well tolerated showing a broad spectrum activity against Gram-positive (*S. aureus* WCUH 29, *Streptococcus pneumoniae* 1629) and Gram-negative (*Haemophilus*

Table 1
In vitro activities of a set of cyclohexyl-amide compounds[5,7c,9]

Compound	Structure	*E. coli* DNA gyrase-dependent DNA replication IC_{50}[5] (μg/mL)	MIC (μg/mL) *H. influenzae*/ *S. aureus*/*S. pneumoniae*[9]	hERG inhibition whole cell patch clamp IC_{50}[7c] (μM)
1		0.017	2,[a] 0.03, 0.25	25
7		0.013	0.5, 0.01, 0.125	24
21		0.003	1, 0.03, 0.125	nd
22		0.004	0.5, 0.03, 0.03	25
23		0.010	2, 0.03, 0.06	52

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	*E. coli* DNA gyrase-dependent DNA replication IC_{50}[5] (μg/mL)	MIC (μg/mL) *H. influenzae*/ *S. aureus*/*S. pneumoniae*[9]	hERG inhibition whole cell patch clamp IC_{50}[7c] (μM)
24		0.008	0.25, 0.01, 0.03	29
25		0.009	1, 0.06, 0.125	113
26		0.010	1, 0.125, 0.125	200
27		0.050	2, 0.125, 0.125	2
28		0.069	2, 0.125, 0.25	nd
29		0.002	0.5, 0.03, 0.06	nd
30		0.001	0.5, 0.01, 0.03	nd
31		0.102	16, 0.5, 0.5	nd

Haemophilus influenzae H128, *Staphylococcus aureus* WCUH 29, *Streptococcus pneumoniae* 1629, nd: not determined.
[a] *Haemophilus influenzae* Q1 strain used.

influenzae H128) bacteria. These are representative strains but the potency was conserved in a more extensive panel of organisms. The apparent trend that this series of compounds is more potent towards Gram-positive than Gram-negative bacteria could be

attributed to reduced cell membrane penetration and/or efflux pump differences.

As can be seen in Table 1, it is possible to alter each subunit of compound **1**. From the benzodioxin RHS unit of **1**, nitrogens can be introduced to give pyridyl analogues such as **7** and **23**. Hydroxylation and methoxylation of the central unit is also possible, as in analogues **7**, **30** and **31**.

A variety of LHS alternatives to the quinoline LHS are possible, such as naphthyridines (**7**, **21–24**) and alternatively substituted quinolines (**25**, **27–31**).

We measured target potency using a gyrase-dependent DNA replication assay reconfigured in an unpublished format using Scintillation Proximity Assay (SPA) technology.[5] For this series of compounds this assay tracked well with whole cell antibacterial potency.

This data also tracks well with the good fit obtained by modelling studies, where **7** was docked into the X-ray crystal structure previously obtained.[3b,6]

As might be imagined from the basic nature of these compounds there was an issue of hERG inhibition. This was generally related to the lipophilicity of the compounds.[7a,b] Subtle structural changes may also play a part, for example compounds **7** and **26** have similar *c*Log*P* values (2.8 and 2.9, respectively) but their hERG IC_{50}s vary considerably (24 and 200 μM, respectively).

To assess the therapeutic window for our compounds given their hERG inhibition, we evaluated a tool compound **7** in a rat respiratory tract infection model, Figure 2.[8] In this model, rat lungs were infected with *S. pneumoniae* 1629 and animals were dosed orally with either **7** or a positive control (amoxicillin) (1, 7, 24 and 31 h post infection).

The non-treated control (NTC) rats showed an increase in *S. pneumoniae* 1629 colony forming units (cfu) after infection, 48 h NTC compared to 1 h NTC. Compound **7** had moderate activity at 25 mg/kg (approx. 2 $\log_{10}$ decrease in cfu/lungs compared to 48 h NTC) and caused full eradication of the infection at 50 mg/kg, Figure 2.

Based on multiple exposure studies, the AUC required for efficacy was approximately 12–24 μg h/mL per day and the C_{max} was approximately 3 μg/mL. This demonstrates excellent in vivo efficacy for a compound from this class in a relevant animal infection model.

We then decided to examine the full DMPK profile of compound **7**, Table 2.[10]

As can be seen in Table 2, the blood clearance was high in rat and monkey (73–76% of hepatic blood flow) but low in dog (28% of hepatic blood flow). The renal clearance in rat, dog and monkey was low (3–7% in each species). Excretion of unchanged compound may explain the high in vivo clearance but low in vitro liver microsomal clearance in the rat, however further studies would be necessary to confirm this. Microsomal metabolite ID studies identified a range of metabolites, of which mono-oxidation (hydroxy and/or N-oxide) products predominated across species. Reasonable oral bioavailability (F_{PO}) was achieved in the rat and the dog, greater than that in the monkey. Protein binding and intrinsic microsomal clearance of **7** was similar across species.

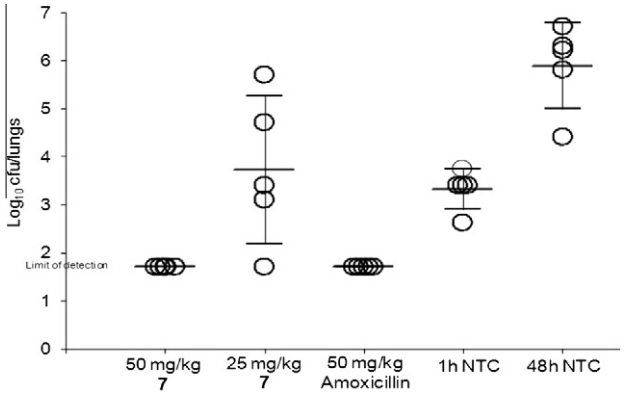


Figure 2. Oral efficacy of **7** against *S. pneumoniae* 1629 in a rat respiratory tract infection model.

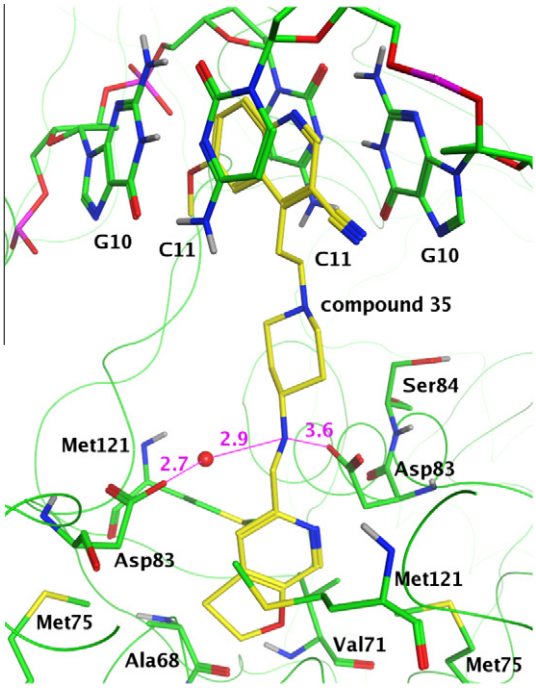


Figure 3. X-ray binding mode of GSK299423 in *S. aureus* gyrase (pdb code: 2XCS).[3b]

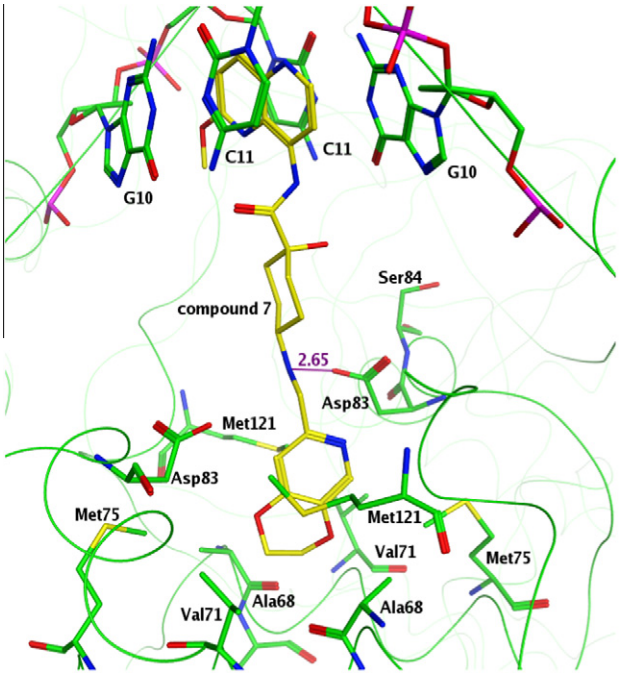


Figure 4. Compound **7** was manually placed in the binding site of GSK299423 in *S. aureus* gyrase and then optimised using Embrace (Maestro) with the OPLS2005 force field and GB/SA solvation model.

Table 2
Summary of in vivo pharmacokinetics and in vitro study results for compound **7**[10]

Parameters (units)	Rat	Dog	Monkey	Human
IV DMPK				
Dose (mg/kg)	2.9	2.6	2.7	–
CL_b (mL/min/kg)	41.1	8.4	34.2	nd
V_{ss} (L/kg)	2.41	3.7	4.1	nd
Half-life (h)	1.0	6.3	1.8	nd
Oral DMPK				
Dose (mg/kg)	4.6	4.5	4.4	nd
AUC (ug h/mL)	0.34	5.7	0.19	nd
MRT (h)	2.9	9.8	6.4	nd
Oral bioavailability (%)	18	63	9	nd
Plasma protein binding (% bound)	73.2	73.7	83.5	72.6
CL_i microsomes (mL/min/g liver)	2.9	2.2	2.6	0.8

nd = Not determined.

In conclusion, we achieved our goal of demonstrating efficacy and achieving promising PK attributes with a tool compound **7**. Further efforts are ongoing in this area to optimise all the required properties for clinical development.

Acknowledgment

Acknowledgment is given to all the chemists and biologists who have been involved in this work.

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6. Docking of **7** into X-ray crystal structure (Ref. 3b) in *S. aureus* gyrase can help to explain the antibacterial potency observed. Compound **7** binds very similarly to GSK299423 (Fig. 3). The key interactions are shown below, Figure 4.
7. (a) Hancox, J. C.; McPate, M. J.; El Harchi, A.; Zhang, Y. H. *Pharmacol. Ther.* **2008**, *119*, 118; (b) Recanatini, M.; Cavalli, A.; Masetti, M. *ChemMedChem* **2008**, *3*, 523; (c) Zhou, Z.; Gong, Q.; Ye, B.; Fan, Z.; Makielski, J. C.; Robertson, G. A.; January, C. T. *Biophys. J.* **1998**, *74*, 230; For hERG inhibition reviews: (d) Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishert, G. *J. Med. Chem.* **2006**, *49*, 4029.
8. Infection model details: *S. pneumoniae* 1629 was grown overnight on trypticase soy agar plates supplemented with 5% horse blood. The inoculum was prepared by harvesting bacterial growth from the plates into phosphate-buffered saline with a further 1/10 dilution into cooled molten nutrient agar (42 °C). Rats were infected with *S. pneumoniae* 1629 by intrabronchial instillation of 50 μL of the inoculum *via* non surgical intratracheal intubation. Animals received approximately 5.3 $\log_{10}$ cfu/rat. At 48 h post infection, the animals were euthanized and the lungs excised for the enumeration of viable bacteria.
9. *Minimum Inhibitory Concentrations*: MICs were determined by broth microdilution methods according to Clinical and Laboratory Standards Institute guidelines 7. The MIC was the lowest concentration of an antibacterial that showed no visible growth after incubation at 37 °C for 18–24 h, with a starting inoculum of $\sim 5.5 \times 10^5$ CFU/mL.
10. All animal experiment protocols were approved by the Animal Care and Use Committee at GlaxoSmithKline Pharmaceuticals (PA, USA). The pharmacokinetics of compound **7** were studied in male Sprague–Dawley rats, male Beagle dogs and male cynomolgus monkeys following single intravenous and oral administration. Absolute oral bioavailability was estimated using a cross-over study design ($n = 3$). Blood samples were assayed using protein precipitation followed by LCMS/MS analysis and the concentration–time data were analyzed by non-compartmental methods (WinNonlin, v3.2). The in vitro plasma protein binding and microsomal intrinsic clearance were determined as described in Xiang, H.; McSurdy-Freed, J.; Moorthy, G. S.; Hugger, E.; Bambal, R.; Han, C.; Ferrer, S.; Gargallo, D.; Davis, C. B. *J. Pharm. Sci.* **2006**, *95*, 2657.