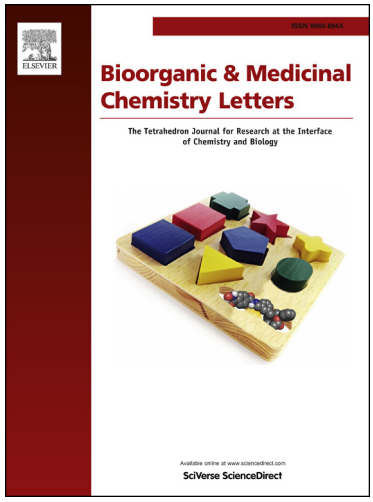

Novel hydroxyl tricyclics (*e.g.* GSK966587) as potent inhibitors of bacterial type IIA topoisomerases

Timothy J Miles, Alan J Hennessy, Ben Bax, Gerald Brooks, Barry S Brown, Pamela Brown, Nathalie Cailleau, Dongzhao Chen, Steven Dabbs, David T Davies, Joel M Esken, Ilaria Giordano, Jennifer L Hoover, Jianzhong Huang, Graham E Jones, Senthill K Kusalakumari Sukmar, Claus Spitzfaden, Roger E Markwell, Elisabeth A Minthorn, Steve Rittenhouse, Michael N Gwynn, Neil D Pearson

PII: S0960-894X(13)00847-0
DOI: http://dx.doi.org/10.1016/j.bmcl.2013.07.013
Reference: BMCL 20678

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 2 June 2013
Revised Date: 7 July 2013
Accepted Date: 9 July 2013

Please cite this article as: Miles, T.J., Hennessy, A.J., Bax, B., Brooks, G., Brown, B.S., Brown, P., Cailleau, N., Chen, D., Dabbs, S., Davies, D.T., Esken, J.M., Giordano, I., Hoover, J.L., Huang, J., Jones, G.E., Kusalakumari Sukmar, S.K., Spitzfaden, C., Markwell, R.E., Minthorn, E.A., Rittenhouse, S., Gwynn, M.N., Pearson, N.D., Novel hydroxyl tricyclics (*e.g.* GSK966587) as potent inhibitors of bacterial type IIA topoisomerases, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: http://dx.doi.org/10.1016/j.bmcl.2013.07.013

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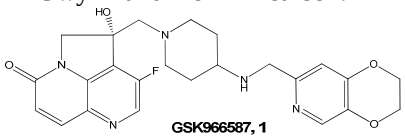

GSK966587, 1

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Novel hydroxyl tricyclics (*e.g.* GSK966587) as potent inhibitors of bacterial type IIA topoisomerases

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[a]*Diseases of the Developing World CEDD, GlaxoSmithKline, Calle Severo Ochoa, 2, 28760, Tres Cantos, Madrid, Spain*
[b]*Infectious Diseases CEDD, GlaxoSmithKline, Gunnels Wood Road, Stevenage, SG1 2NY, UK*
[c]*Computational and Structural Chemistry, GlaxoSmithKline, Gunnels Wood Road, Stevenage, SG1 2NY, UK*
[d]*Infectious Diseases CEDD, GlaxoSmithKline, Third Avenue, Harlow, CM19 5AW, UK*
[e]*Infectious Diseases CEDD, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*
[f]*Protein Dynamics DPU, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Antibacterials;
Bacterial type IIA topoisomerase;
Hydroxyl tricyclics;
Pharmacokinetic;
In vivo efficacy;

ABSTRACT

During the course of our research to find novel mode of action antibacterials, we discovered a series of hydroxyl tricyclic compounds that showed good potency against Gram-positive and Gram-negative pathogens. These compounds inhibit bacterial type IIA topoisomerases . Herein we will discuss structure activity relationships in this series and report advanced studies on compound 1 (GSK966587) which demonstrates good PK and *in vivo* efficacy properties. X-ray crystallographic studies were used to provide insight into the structural basis for the difference in antibacterial potency between enantiomers.

There is an unmet medical need for novel antibiotics[1] due to the increasing prevalence of resistant bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *enterococci* (VRE) and fluoroquinolone resistant pathogens.

Bacterial type IIA DNA topoisomerases are well validated targets as they are inhibited by both quinolones and novobiocin. However both these drug classes suffer from toxicity issues: novobiocin has very limited use and quinolones are used in adults only. Moreover resistance to fluoroquinolones is on the increase making the development of novel antibiotics against this well-validated target attractive.[2]

It has been established through mutational (Black *et al*)[3a] and X-ray crystallography studies (Bax *et al*)[3b] that compounds (such as GSK299423[3b]) with a similar mode of action to **1**[4] bind to the topoisomerases in a different manner than that of the quinolones.[3c] These compounds have a novel mechanism, which overcomes issues of target-mediated resistance that have developed in the clinic.[2] Compounds with a similar mechanism but of a distinct chemical series have also been discussed in the literature.[3d-h]

In the course of our efforts to exploit this mode of action to develop an antibiotic with a good safety profile, we discovered a series of hydroxyl tricyclic compounds (*e.g.* GSK966587, **1**[4]) which have potent antibacterial properties.

This paper will discuss the structure activity relationships (SAR) of the left hand side (LHS) and central unit portion of this series of hydroxyl tricyclic compounds (e.g. **1**[4]) as shown below in Figure 1.

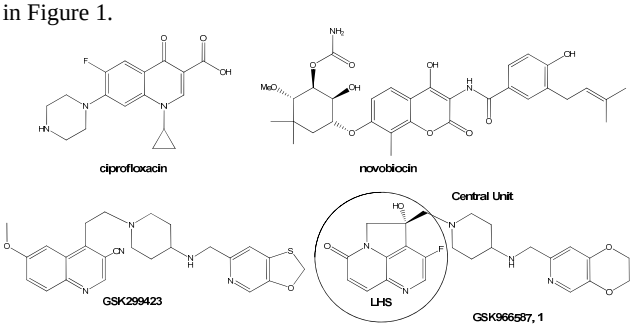
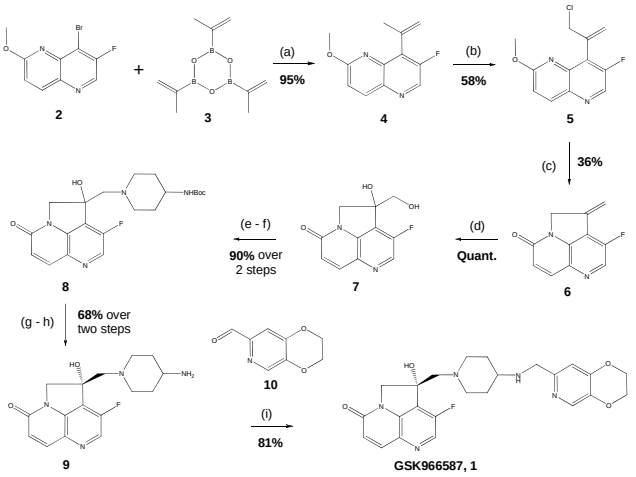


Figure 1. Bacterial Type IIA DNA topoisomerase inhibitors

The synthesis of hydroxyl tricyclic compound **1**[4] is described in Scheme 1. A Suzuki cross coupling of 8-bromo-7-fluoro-2-methoxy-1,5-napthridine **2**,[5] with pyridine-tris(1-methylethenyl)

* Corresponding author. Tel.: +34-650-349-771; fax: +34-918-070-550; e-mail: tim.j.miles@gsk.com

boroxin **3**[4, 6] then chlorination using cerium (III) chloride heptahydrate[7] afforded chloride **5** in an overall yield of 55%. Subsequent cyclisation using NaI then dihydroxylation afforded the diol **7** in 96% yield over two steps. Activation of the primary hydroxyl group as the tosyalte was achieved using dibutyltin oxide[8] and displacement with NBoc piperidine afforded the NBoc protected intermediate **8** in 90% yield. The free amine **9** was liberated by Boc deprotection and then separated into enantiomers by prepative chiral HPLC. The desired (4*S*)-enantiomer was placed under reductive alkylation conditions with aldehyde **10**[9] to give the hydroxylated tricyclic **1**[4] in 81% yield.



Scheme 1. Reagents and conditions: (a) **2**[5] (1 equiv), $Pd(P(Ph)_3)_4$ (5 mol%), dimethoxy ethane, RT, 30 min then **3**[4, 6] (0.4 equiv), K_2CO_3 (1 equiv), H_2O, reflux, 10 h; (b) $CeCl_3.7H_2O$ (1 equiv), NaClO (2 equiv), ^tBuOH, RT, 10 min; (c) NaI (10 equiv), acetone, reflux, 18 h; (d) AD-mix , ^tBuOH, H_2O, RT, 16 h; (e) TsCl (1 equiv), $^nBu_2SnO$ (5 mol%), Et_3N (1.5 equiv), DCM, THF, DMF, RT, 18 h; (f) 1,1-dimethylethyl 4-piperidinylcarbamate (1.33 equiv), $NaHCO_3$, DMF, EtOH, RT, 2.5 d; (g) HCl in 1,4-dioxane (4M), DCM, MeOH, RT, 1.5 h; (h) Chiral Separation using Chiralpak AD column; (i) **10**[9] (1 equiv), $Na(OAc)_3BH$ (2 equiv), DCM, MeOH, 0°C RT, 2.5 h.

From Table 1 it can be seen that this series of compounds has broad spectrum activity against Gram-positive (*Staphylococcus. aureus* WCUH 29, *Streptococcus pneumoniae* 1629) and Gram-negative (*Haemophilus influenzae* H128) bacteria. These are just representative strains and the potency was maintained against a more extensive panel of organisms.

We indirectly measured target potency by using a gyrase-dependent DNA replication assay reconfigured in an unpublished format at GSK using Scintillation Proximity Assay (SPA) technology.[10] For this series of compounds the IC_{50} in this assay tracked the whole cell antibacterial potency well.

Within these hydroxyl tricyclic examples and for the bacterial strains shown in Table 1 the following observations can be made: enantiomer **1**[4] is better tolerated for antibacterial potency than **11**[4a]. It is possible to alter the bridgehead 4-OH in **1**[4] with either F (**12**[4a]) or H (**13**[11]). Variations of alternative LHS subunits are tolerated; examples shown in Table 1 are naphthyridine (**1**[4]), quinoline (**14**[12]), quinoxaline (**15**[13]) and a further substituted naphthyridine (**16**[4a]). Finally, alternative central units such as **17**[4a] and **18**[4a] are tolerated, although not as potent as the parent compound **1**[4] within these bacterial strains. As described below, structural studies have shown that these central units would be largely solvent exposed, without target or DNA interaction.

The 2.6Å crystal structure of compound **1**[4] in complex with DNA and *S. aureus* DNA gyrase was determined using a similar technique as previously described in the literature.[3b, 14] The binding mode for compound **1**[4] was generally similar to that described for GSK299423[3b], apart from a novel hydrogen bonding interaction between the bridgehead 4-OH and an NH_2 from an adenine base of the DNA (O to N distance of 3.1A as shown in Figure 2).

Compounds **1**[4] and **11**[4a] are enantiomers that have very different whole cell and molecular target activity against *S. aureus* (Table 1). An analytical gel filtration assay[3b, 14] was used to identify a DNA sequence that gave a stable complex with compound 1 and to investigate the stability of complexes of compound **1**[4] or compound **11**[4a] with eight different 20-base-pair DNA duplexes and DNA gyrase. The results showed that with compound **1**[4] all eight DNA duplexes gave stable complexes (Figure 2), while for compound **11**[4a] the stability of the complexes was variable and four duplexes gave little or no stable complex. An unfavourable interaction between the chiral centre at the bridgehead of compound **11**[4a] and the surrounding DNA may partially account for this poor activity.

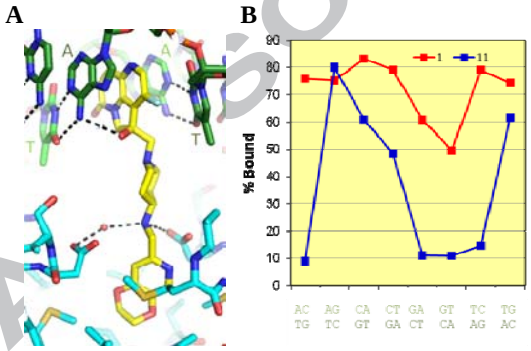


Figure 2. **A** The 2.6Å crystal structure[14] of compound 1, GSK966587 (yellow carbons) in a complex with DNA (green carbons) and DNA gyrase (cyan carbons). **B** An analytical gel filtration assay[3b] was used to assay the stability of complexes of compound **1**[4] (red) or it's enantiomer compound **11**[4a] (blue) with eight different DNA duplexes. The bases surrounding the compound binding site were varied, as indicated (top line 5'to 3'; bottom line complementary strand sequence is shown 3'to 5').

The hERG inhibition of the compounds described was generally related to their lipophilicity and basicity.[15a-c] Several compounds within this series were identified with hERG inhibition that was suitable for progression.

To further profile this series of compounds we decided to examine compound **1**[4] in PK and *in-vivo* efficacy studies. We will now discuss each of these studies in turn.

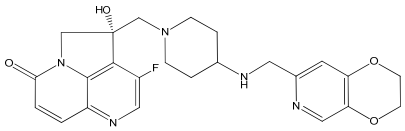
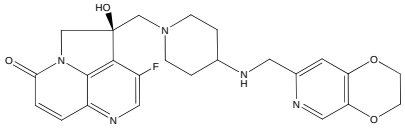
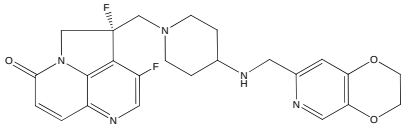
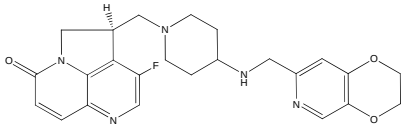
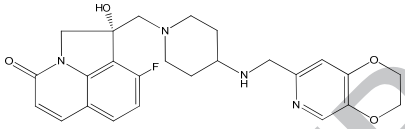
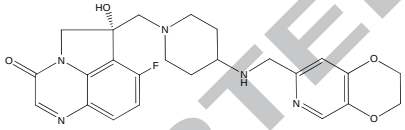
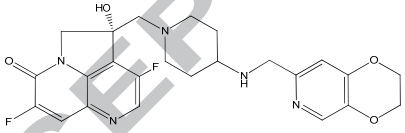
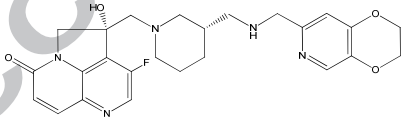
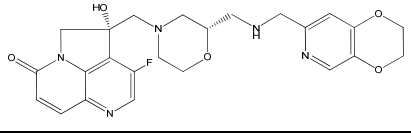
Table 2. Summary of *in vivo* pharmacokinetics and *in vitro* study results for compound **1**.[16]

Parameters (Units)	Rat	Dog	Monkey	Human
IV DMPK				
Dose (mg/kg)	3	3	3	nd
CL_b (mL/min/kg)	62.2	18.1	25.1	nd
Vss (L/kg)	8.6	6.4	3.4	nd
Half-life (h)	3.1	7.1	2.3	nd
Oral DMPK				
Dose (mg/kg)	5	5	5	nd
AUC (ug.h/mL)	0.46	2.82	1.92	nd
Oral Bioavailability (%)	34	63	55	nd
Plasma Protein Binding (% bound)	42.29 ± 9.60	17.74 ± 8.68	21.91 ± 7.35	22.25 ± 5.97
CL_i microsomes (mL/min/g liver)	<0.5	<0.5	1.6	<0.5

nd: not determined.

As can be seen in Table 2,[16] the blood clearance was high in the rat (~100% of hepatic blood flow) but moderate in dog and monkey (50-60% of hepatic blood flow). The renal clearance in dog and monkey was low to moderate (~27% and 15% of the total clearance in dog and monkey, respectively), and volume of distribution was high (greater than total body water). Reasonable oral bioavailability (FPO) was achieved in all species. Plasma protein binding and microsomal intrinsic clearance of **1**[4] were similar across species.

Table 1: Antibacterial activity of a set of hydroxyl tricyclic compounds[10, 15d-e, 17]

Compound	Structure	*H. influenzae.* DNA gyrase-dependent DNA replication IC_{50} (μg/ml)[10]	MIC (μg/ml) *H. influenzae* / *S. aureus* / *S. pneumoniae*[17]	hERG inhibition PatchXpress (IC_{50}, μM)[15d]	hERG inhibition whole cell patch (IC_{50}, μM)[15e]
1[4] (GSK966587)		0.035	1, 0.125, 0.125	239	310
11[4a] (enantiomer of GSK966587)		>1.324	>16, 8, 4	nd	nd
12[4a]		0.04	0.5, 0.03, 0.06	16	nd
13[11]		0.035	0.25, 0.03, 0.06	58	122
14[12]		0.071	1, 0.06, 0.125	nd	nd
15[13]		0.058	0.5, 0.125, 0.125	101	206
16[4a]		0.037	0.5, 0.25, 0.25	289	nd
17[4a]		1.213	16, 4, 4	nd	nd
18[4a]		0.361	4, >16, 4	nd	nd

Haemophilus influenzae H128, *Staphylococcus aureus* WCUH 29, *Streptococcus pneumoniae* 1629, nd: not determined.

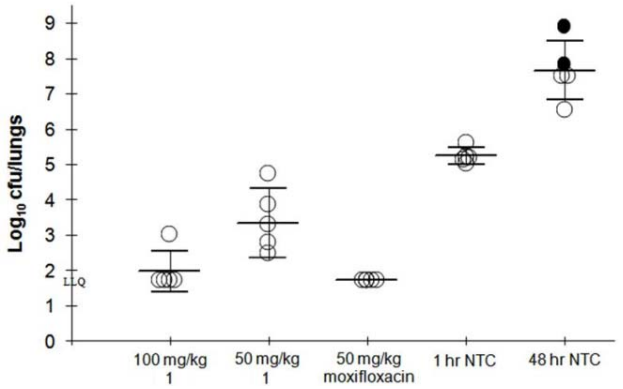


Figure 3. *In vivo* efficacy of compound **1** (GSK966587)[4] against *S. pneumoniae* 1629. Each circle represents bacterial counts recovered from the lungs of one animal. Filled circles represent animals sacrificed prior to the end of the study.

Compound **1**[4] (GSK966587) was progressed into *in vivo* efficacy studies, including a *S. pneumoniae* 1629 mouse respiratory tract infection model , Figure 3.[18] Non-neutropenic mice were infected in the lungs *via* intranasal inhalation and received either **1**[4] or a positive control (moxifloxacin) *via* oral gavage at 1, 7, 24 and 31 hours post infection. Pharmacokinetics were evaluated at these doses to determine the exposure in blood required for efficacy.

After 48 hours, bacterial numbers in non-treated control animals (NTC) increased by more than 2 $\log_{10}$ colony forming units (cfu) compared to those at the start of therapy (1 hour NTC), indicating a robust infection was achieved. Compound **1**[4] showed excellent efficacy in this model. At 50 mg/kg BID, a mean reduction in bacterial counts of approximately 2 log10 cfu was obtained compared with 1hour NTC. The higher dose reduced bacterial burden below the limit of detection (1.7 $\log_{10}$ cfu/lungs) in 4/5 animals.

The AUC measured for the 50 mg/kg BID dose was approximately 50 µg.h/ml per day with an associated C_{max} of approximately 8 µg/ml. This data demonstrates that excellent *in vivo* efficacy can be obtained for a compound from this class in a clinically relevant animal infection model.

In conclusion, we achieved our goal of demonstrating efficacy and promising PK attributes with a tool compound **1**[4]. Progression of compound **1**[4] towards the clinic was discontinued due to hepatic portal tract lesions observed in the 14-day dog GLP safety toxicology study across all dose levels. This type of toxicity has not been observed for other compounds within this class, therefore we continue to evaluate the class for clinical development.

Acknowledgment

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

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10. (a) Moses, R. E.; Richardson, C. C. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 674; (b) Winston, S.; Masushita, T. *J. Bacteriol*., **1975**, *123*, 921; (c) Bosworth, N.; Towers, P. *Nature*, **1989**, *341*, 167; The published toluenized cell DNA replication assay was configured to a microtiter-based Scintillation Proximity (SPA) format. *H. influenzae* was rendered permeable to nucleotide substrates and cofactors by treatment with 1% toluene for 10 min. DNA gyrase activity was determined by measuring gyrase-dependent DNA replication in the toluenized cells by the ATP-dependent incorporation of 33P-TTP. Final concentrations (per well): 20% toluenized H. influenzae cells, 0.05 µCi 33P-TTP, 33 µM dATP, 33 µM dGTP, 33 µM dCTP,13 µM dTTP, 2 mM ATP, 70 mM KPO_4, 5 mM DTT, 13 mM $MgSO_4$, 0.01% BSA, 0.01% CHAPS, pH 7.4. Reactions, incubated at 37 ^oC for approximately 30 min, were stopped with 10% TCA, and SPA beads added for scintillation counting.
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14. A complex of 1 (GSK966587) with S. aureus GyrB27-A56(GKdel/Tyr123Phe) (ref 3b) and a 20 base-pair DNA heteroduplex (sequence below) was purified by size exclusion chromatography in 100mM Na_2SO_4, 20mM HEPES, 5mM $MnCl_2$, pH 7.0. The purified complex, 6.4mg/ml in 33mM Na_2SO_4, 20mM HEPES, 13mM NaCl, 1.7mM $MnCl_2$, pH 7.0 was crystallised by the hanging drop method against 11% PEG 5000MME, 100mM BisTris pH6.2. A crystal was transferred into 15% glycerol + well solution and frozen in liquid nitrogen. A 2.6Å dataset was collected from a single crystal at the ESRF on beamline ID23-1 (cell P61 a=b=93.9Å, c=416.4Å). The structure was refined starting from the complex with GSK299423 (PDB code: 2XCS - cell P61 a=b=93.3Å, c=412.8Å – ref 3b), and coordinates have been

deposited in the PDB with accession code: 4bul. The 20bp DNA duplex used to determined the GSK299423 crystal structures (ref 3b) did not give stable complex with GSK966587. Analytical gel filtration studies (as described in online methods section and supplementary figure 6 of ref 3b) were used to identify a DNA sequence that gave stable complex with GSK966587; the sequence used for the structure was:
5' TGTGCGGTGTACCTACGGCT 3'
3' ACACGCCACATGGATGCCGA 5'
In further analytical gel filtration studies the central base-pairs (indicated by Xs below) in a DNA duplex of sequence:
5'-TGTGCGGTGXXCCTACGGCT – 3'
3'-ACACGCCACXXGGATGCCGA – 5'
were varied as described in the legend to figure 2.

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16. All animal experiment protocols were approved by the Animal Care and Use Committee at GlaxoSmithKline Pharmaceuticals (PA, US). The pharmacokinetics of compound **1** 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 LC/MS/MS analysis and the concentration-time data were analyzed by non-compartmental methods (WinNonlin, v4.1). 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.
17. *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 ~5.5 X 10^5 cfu/ml
18. *Infection model details*: *S. pneumoniae* 1629 was grown overnight on trypticase soy agar plates supplemented with 5% sheep blood. The inoculum was prepared by harvesting bacterial growth from the plates into phosphate-buffered saline. Mice were infected with *S. pneumoniae* 1629 by intranasal inhalation of a 50 µl inoculum, receiving approximately 7.6 $\log_{10}$ cfu/mouse. At 48 h post infection, the animals were euthanized and the lungs excised for the enumeration of viable bacteria. PK was performed separately using infected mice.

Graphical Abstract

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19.

Novel hydroxyl tricyclics (*e.g.* GSK966587) as potent inhibitors of bacterial type IIA topoisomerases

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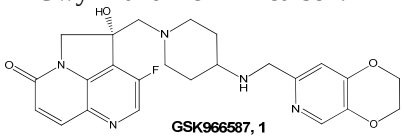

GSK966587, 1