Bioorganic & Medicinal Chemistry Letters 22 (2012) 6705–6711

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Structure-guided design, synthesis and biological evaluation of novel DNA ligase inhibitors with in vitro and in vivo anti-staphylococcal activity

Jean-Philippe Surivet *, Roland Lange, Christian Hubschwerlen, Wolfgang Keck, Jean-Luc Specklin, Daniel Ritz, Daniel Bur, Hans Locher, Peter Seiler, Daniel Stefan Strasser, Lars Prade, Christopher Kohl, Christine Schmitt, Gaëlle Chapoux, Eser Ilhan, Nadia Ekambaram, Alcibiade Athanasiou, Andreja Knezevic, Daniela Sabato, Alain Chambovey, Mika Gaertner, Michel Enderlin, Maria Boehme, Virginie Sippel, Pierre Wyss

Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, CH-4123 Allschwil, Switzerland

ARTICLE INFO

Article history:
Received 18 July 2012
Revised 23 August 2012
Accepted 25 August 2012
Available online 4 September 2012

Keywords:
Antibacterials
DNA ligase
Selective inhibitors
2-amino-[1,8]-naphthyridine-3-carboxamides
In vivo active

ABSTRACT

A series of 2-amino-[1,8]-naphthyridine-3-carboxamides (ANCs) with potent inhibition of bacterial NAD^+-dependent DNA ligases (LigAs) evolved from a 2,4-diaminopteridine derivative discovered by HTS. The design was guided by several highly resolved X-ray structures of our inhibitors in complex with either *Streptococcus pneumoniae* or *Escherichia coli* LigA. The structure–activity-relationship based on the ANC scaffold is discussed. The in-depth characterization of 2-amino-6-bromo-7-(trifluoromethyl)-[1,8]-naphthyridine-3-carboxamide, which displayed promising in vitro (MIC *Staphylococcus aureus* 1 mg/L) and in vivo anti-staphylococcal activity, is presented.

Over the last seventy years, antibacterial therapies have revolutionized medical practice and have contributed to the increase of the average lifespan. Unfortunately, the emergence of multidrug resistant bacteria has come along with the use of antibacterial drugs. The prevalence of such resistant pathogens has reached such an alarming level that immediate and drastic actions are urgently needed to ensure that effective therapeutic options remain available.[1] Beyond any economical and strategic considerations,[2] the scarcity of novel validated biological targets amenable to antibacterial therapy and the lack of new chemical entities not subject to cross-resistance with established anti-infective drugs, make the discovery and the clinical development of a novel antibiotic acting through a novel mode of action, one of the most fascinating scientific challenges.[3]

DNA ligases are essential for the maintenance of genome integrity as they are indispensable for DNA replication, recombination and repair. During these vital processes, 5'-PO_4^{2-} 3′-OH polynucleotide nicks are formed in double stranded DNA and must be resealed by ligase. NAD^+-dependent bacterial DNA ligase (LigA; EC 6.5.1.2) is a validated antibacterial target[4] but has so far received little attention despite the potential to reach broad-spectrum activity.[5] LigA isoenzymes are encoded by essential genes that show a high degree of conservation across a relevant spectrum of Gram-positive and Gram-negative pathogens.[6] The ligation mechanism consists first in the adenylation of a lysine in the active site of LigA by NAD^+ with release of nicotinamide mononucleotide. Then, the adenosine monophosphate (AMP) is transferred from the LigA-Lys to the 5′-end of the 5′-PO_4^{2-}-terminated DNA strand. Finally, the phosphodiester bond is formed via the nucleophilic attack of the 3′-OH end onto the activated 5′-PO_4^{2-}, with release of AMP.[7]

Bacterial LigA isoenzymes utilize NAD^+ as a cofactor and belong to a distinct phylogenetic cluster strictly separated from the cluster of mammalian ligases consuming ATP.[8] The dissimilarity between the NAD^+-dependent bacterial and the ATP-dependent human DNA ligases increases the chance to design selective inhibitors of the bacterial enzyme thereby minimizing the risk of mechanism based toxicity owing to human ligase inhibition.[9]

When we initiated our investigations, only two classes of selective LigA inhibitors displaying target related antibacterial activity had been reported: one derived from adenosine such as compound **1**[10] and the other belonging to the pyridochromanone family, exemplified by compound **2**[11] (Fig. 1). Recently, 4-amino-pyr-

* Corresponding author.
E-mail address: jean-philippe.surivet@actelion.com (J.-P. Surivet).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.08.094

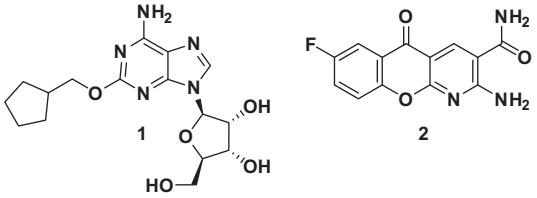


Figure 1. Reported LigA inhibitors **1** and **2**, targeting the ATP-binding site.

ido[2,3-*d*]pyrimidin-5(8*H*)-ones, displaying moderate Gram-positive antibacterial activities through selective LigA inhibition, have also been reported.[12]

Herein, we report the discovery of a novel class of DNA LigA inhibitors based on a 2-amino-[1,8]-naphthyridine-3-carboxamide (ANC) scaffold and our efforts to improve the biological activity leading to a compound displaying potent in vivo activity against *Staphylococcus aureus*.

A HTS campaign against the *Escherichia coli* LigA resulted in the identification of compounds **3** and **4** (Table 2). The two hits displayed IC50 in the low micromolar range against *S. aureus* LigA and in a higher range against the *E. coli* enzyme.[13] Neither the human DNA ligase 1[14] (huLig 1) nor the growth of wild-type bacteria[15] was inhibited by compounds **3** and **4**. However, LigA specific inhibitory activity could be shown with a panel of isogenic 'permeable' Δ*tolC* (efflux knock-out) Δ*rfaC* (truncated LPS) *E. coli* strains with low or high ligation activity. Thus, a particular ligase mutation (L15F amino acid exchange in *ligA*251)[16] rendered strains carrying only the *ligA*251 allele more sensitive toward specific LigA inhibitors resulting in a >20-fold reduced ligation activity.[17] The specificity of compound **3** for LigA was shown through the significant increase of MIC (minimal inhibitory concentration) when wild-type (wt) LigA (*pECLigA*) was over-produced in the isogenic *E. coli* strain. Compound **4** showed a similar activity pattern as compound **3**, however to a lower extent as a result of its weaker inhibitory potency against *E. coli* LigA (Table 1). The better biochemical potency and whole-cell activity of hit **3** over hit **4**, combined with a slightly better Ligand Efficiency (LE, 0.54 vs 0.4 Kcal/mol/non H-atom, calculated using *S. aureus* LigA inhibition data) and a higher amenability for structural modifications, prompted us to select the pteridine derivative **3** as a starting point for a medicinal chemistry program.[18]

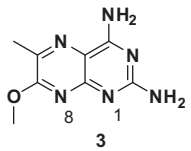
In parallel, the X-ray crystal structure of hit **3** (Fig. 2) bound within the AMP binding pocket of a truncated form of *Streptococcus pneumoniae* LigA was solved. The major binding interactions are a π-stacking interaction with the side chain of Tyr225 and a series of hydrogen bonds either with the backbone of the enzyme (Leu114) or amino-acid side chains (Glu113 and Lys290).[16]

A superposition of compounds **1** and **3** bound to LigA (Fig. 3) revealed that both compounds are covering the same space as the AMP unit of NAD^+, as found in the structure of NAD^+ bound to *Enterococcus faecalis* LigA (PDB code 1TAE).[19] The cyclopentyl unit of **1** and the 7-methoxy group of **3** are both occupying a part of a hydrophobic cave which we dubbed 'tunnel'. This cavity is not present in huLig 1 since it is blocked by the side chain of Trp742 (PDB code: 1X9N).[9,20] Replacement of the N-5 nitrogen of hit **3** by a carbon atom offered a new and promising exit vector in the direction of the ribose pocket. The 4-amino group of compound **3** interacted with the backbone of LigA (Lys115) through a water molecule (Fig. 2). Although compound **3** did not inhibit huDHFR, the removal of this 4-amino group, combined with the replacement of the N-3 nitrogen of **3** by a carbon atom was a preemptive measure to avoid undesired interactions with enzymes of the folate pathway, as experienced with related molecules.[21] We intended to combine features of compound **1** and a modified version of **3** (compound **5a**, Fig. 3) in order to reach simultaneously the ribose pocket as well as the hydrophobic 'tunnel'.

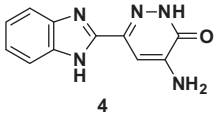
Compound **6** (Scheme 1) inhibited *E. coli* LigA (IC_{50} = 25 μM) and the growth of sensitized *E. coli* strain in the same range as compound **3**, but inhibited neither huLig 1 nor huDHFR at this concentration. We focused our efforts on exploring a suitable substitution pattern to fill the ribose site, initially defining R^3 as CF_3. Unfortunately, enzymatic inhibition measured for more than 100 compounds of formulae **8** and **9** (Scheme 1) on *E. coli* LigA remained in a range between 5 and 25 μM (IC50). The introduction of hydrophobic groups, such as aromatic or hetero-aromatic groups were usually not tolerated (compounds not shown), with the exception of phenyl groups substituted with polar functional groups such as in compound **8a** (Scheme 1). The introduction of aliphatic polar substitutions as in compound **9a** (Scheme 1), did not lead to improved enzymatic inhibitory activities either. The hydroxyl groups of the compound **9a** did not interact favorably with the existing H-bond network defined by water molecules and surrounding amino acid side chains in contrast to the hydroxyl groups of the ribose unit present in AMP.

The X-ray structure of the pyridochromanone **2** bound to truncated *E. faecalis* LigA (PDB code 3BA8)[22], revealed that an amide moiety strengthened the interaction with the enzyme backbone. This observation led us to establish the 2-amino-[1,8]- naphthyridine-3-carboxamide (ANC) series **5b** (Fig. 3), and further introduce a substitution on position 6 (R^2 substitution) to increase the interactions with the protein. During the course of our program, X-ray structures of two pyridopyrimidine derivatives bound to LigA have

Table 1
Biological activities of HTS validated hits **3** and **4** on LigAs and engineered *E. coli* strains



3

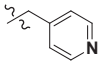
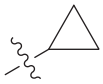
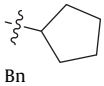
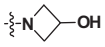
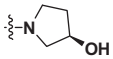
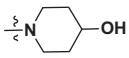
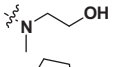
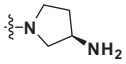


4

Compounds	IC_{50}[a] *S. aureus*	IC_{50}[a] *E. coli*	K_D[b]	MIC wt[c,f]	MIC Lig$^-$ [d,f]	MIC Lig$^+$ [e,f]
3	2.5	25	0.8	>16	2	>16
4	2.5	>25	20	>16	8	16

[a] IC_{50} on LigA (μM).
[b] Equilibrium dissociation constant measured on Biacore using *Streptococcus pneumoniae* LigA (μM).
[c] *E. coli* Δ*tolC*, Δ*rfaC*
[d] *E. coli* Δ*tolC*, Δ*rfaC*, *ligA*251.
[e] *E. coli* Δ*tolC*, Δ*rfaC*, ligA251, *pECLigA*.
[f] MICs expressed in mg/L.

Table 2
Enzymatic inhibitory and antibacterial activities[15] of ANC derivatives **5b** (see Fig. 3)

Compounds	R^1	R^2	R^3	IC_{50}[a] (μM)	MIC wt[b]	MIC Lig$^-$ [c,d]	K_D[e] (nM)	MIC[i]		
								S.a[f]	*S.p*[g]	*H.i*[h]
13a	H	H	$-CF_3$	0.5–0.6	16	0.125	101	8	>16	0.5
13b	Me	H	$-CF_3$	0.5	4	0.25	48	8	>16	0.5
13c	$-(CH_2)_2OMe$	H	$-CF_3$	10	>16	2	458	>16	>16	4
13d	$-(CH_2)_2OH$	H	$-CF_3$	10	>16	1	431	>16	>16	1
13e	$-CH_2NH_2$	H	$-CF_3$	2.5	>16	1	102	>16	>16	4
13f	Ph	H	$-CF_3$	2.5	32	0.5	237	>16	>16	8
17a	$-(CH_2)_2-$	–Cl		0.6	8	0.25	152	16	>16	8
17b	Me	Bn	–Cl	0.5	4	0.125	107	4	4	2
17c	Me		–Cl	0.8–1	8	0.125	116	8	>16	4
17d	Me		–Cl	1–2	>16	0.25	118	>16	8	2
20a	H	Br	CF_3	0.3	2	0.063	16	1	2	0.125
20b	H	Br	tBu	0.3–0.5	8	0.25	77	4	8	2
20c	H	Br		0.5	>16	4	184	>16	>16	>16
20d	H	Br		>5	>16	8	4312	>16	>16	>16
20e	H	Br		>5	>16	>16	2608	>16	>16	>16
22a	H	Me	CF_3	0.5	0.5	0.063	48	4	8	0.25
22b	H		CF_3	0.4	8	0.125	89	2	>16	1
22c	H	Et	CF_3	0.6	8	0.25	75	4	>16	2
22d	H		CF_3	0.8-1	>16	1	152	16	>16	8
22e	H		CF_3	0.4	2	0.125	83	1	16	0.5
22f	H		CF_3	2.5	>16	4	150	>16	16	16
22g	H	Bn	CF_3	0.8–1	16	0.125	141	>16	>16	4
22h	H	$-CH_2Bn$	CF_3	>10	>16	32	1216	>16	>16	>16
22i	H	$-CH_2OH$	CF_3	0.5	>16	2	389	>16	>16	2
30a		Br	CF_3	0.5	4	0.125	43	16	>16	4
30b		Br	CF_3	0.7	4	0.125	31	8	8	8
30c		Br	CF_3	0.7	8	0.25	39	>16	16	>16
30d		Br	CF_3	0.7	>16	0.5	57	8	8	4
30e		Br	CF_3	1–2	>16	1	86	>16	>16	8

[a] *E. coli* LigA.
[b] *E. coli* Δ*tolC*, Δ*rfaC*.
[c] *E .coli* Δ*tolC*, Δ*rfaC*, *ligA*251.
[d] All compounds showed MIC >16 on *E. coli* Δ*tolC*; Δ*rfaC, ligA*251, *pECLigA* except compound **20a** (MIC = 8).
[e] Equilibrium dissociation constant measured on Biacore using *S. pneumoniae* LigA (nM).
[f] *S. aureus* ATCC 29213.
[g] *S. pneumoniae* A-216.
[h] *Haemophilus influenzae* A921.
[i] MICs expressed in mg/L. None of the compounds reported in the table inhibited huLig1 or huDHFR.

been released.[23] These inhibitors share structural features with **5b**, but no biological data were published so far. A series of 5-substituted-7-trifluoro-ANC **13a–f** and 5,6-disubstituted-7-chloro-ANC **17a–d** were synthesized (Schemes 2 and 3) and evaluated (Table 2).

Sub-micromolar inhibitory activities against *E. coli* LigA were measured for compounds **13a** and **13b**, confirming the positive impact of the carboxamide that forms two H-bonds with the backbone of the protein (Fig. 4). Compounds **13a** and **13b** bearing either no or just a small substituent in position 5 displayed strong growth inhibitory activity on sensitized *E. coli* strains but lacked inhibitory activities against huDHFR and huLig1 respectively, confirming the excellent specificity and selectivity profile already observed for compound **6**. The same findings were observed with several 7-chloro-ANC (**17a–d**) featuring small R^1, which inhibited LigA as well as a sensitized *E. coli* strain. Derivative **17b** exhibited measurable antibacterial effects on wild type *S. aureus* and *Haemophilus influenzae* (Table 2). By contrast, increasing the size and/or the polarity of R^1 in position 5 caused a decrease of inhibition on *E. coli* LigA and sensitized *E. coli* strain (compounds **13c–f**).

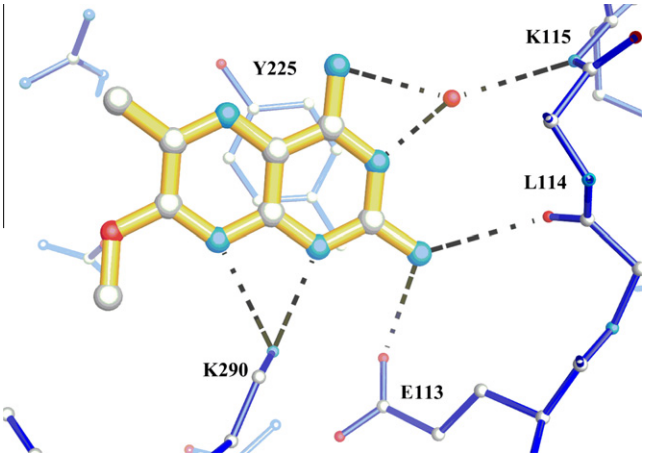


Figure 2. Co-crystal structure of HTS hit **3** with truncated *S. pneumoniae* LigA[16] (PDB code: 4GLW).

The two derivatives **20a** and **20b**, wherein R^3 is trifluoro or *tert*-butyl respectively, showed potent inhibition of *E. coli* LigA, low equilibrium dissociation constant on *S. pneumoniae* LigA enzyme, and strong growth inhibition of sensitized *E. coli* strains. Furthermore, compounds **20a** and **20b** showed significant antibacterial activity on wild-type *S. aureus*, *S. pneumoniae* and *H. influenzae*. Increasing either the size and/or the polarity of the R^3 (compounds **20c–e**) led to decreased activity.

The beneficial effect of the R^2 substitution on the antibacterial activities is obvious by comparing the activities of compounds **13a** and **20a**. Due to an easier chemical access to the ANC scaffold (Scheme 3), an extensive investigation around R^2 was performed keeping $R^3 = CF_3$. Various small alkyl groups were tolerated, providing compounds with significant antibacterial activity on wild-type *S. aureus* and *H. influenzae* (compounds **22a–e**, Table 2) while larger substituents such as a benzyl (compound **22g**) or a phenethyl (compound **22h**) displayed weaker or no measurable antibacterial activities. To further extend our exploration of the 6-bromo-7-trifluoromethyl-ANC scaffold, various N-5 substituted derivatives (**30a–e**) were synthesized (Scheme 4). While sub-micromolar inhibitory activities against *E. coli* LigA were recorded for hydroxyalkylamine compounds **30a–d**, only modest minimal inhibitory concentrations were obtained on wild-type *S. aureus* confirming again the detrimental impact of any large R^1 substitution filling the ribose pocket, on the antibacterial activities.

The SAR around the 2-amino[1,8]-naphthyridine scaffold was explored preparing two series of 7-trifluoromethyl-2-amino[1,8]-naphthyridines. Naphthyridines of formula **8** were obtained from the intermediate **6** (obtained by chlorination of the known derivative **7**[24]) via a Suzuki–Miyaura cross coupling reaction[25] using a variety of (hetero)aromatic boronic acids. Naphthyridines of formula **9** were prepared via a substitution reaction of compound **6** with secondary amines (Scheme 1). The synthesis of 5-substituted-7-trifluoromethyl-ANC **13** (Scheme 2) started with the condensation of ethyl diamino-acrylate **10** with β-substituted-β-methoxyvinyl trifluoromethyl ketones **11** affording the pyridines **12**. The reduction of the ester moiety with LAH and subsequent oxidation with MnO_2 led to the corresponding aromatic aldehydes that were further condensed with 2-cyanoacetamide in presence of TMG leading to derivatives **13a–f**. In case $R_1 = CH_2NHBoc$ or $(CH_2)_2$-OBn, a deprotection step was necessary using either HBr in AcOH or TFA. 5,6-Disubstituted-7-chloro-ANC **20a–e** were prepared *via* a condensation reaction of the α-substituted β-ketoesters **14** with ethyl diamino-acrylate **10**.[26] The resulting 6-amino-pyridin-2-ones **15** were refluxed in $POCl_3$ to afford the chloropyridines **16**, which were further converted to the ANC **17** using the usual three ultimate steps (as outlined in Scheme 2). A series of 2-amino-pyridines **19** and **21**, bearing various substitutions in position 6, were obtained by condensation of the sodio enolates **18** with ethyl diamino-acrylate **10** in presence of acetic acid. The regioselective bromination was performed with NBS, yielding the derivatives **19**. Palladium-catalyzed cross coupling reactions, carried out with various boronic acids, potassium trifluoroborate salts[27] or zinc reagents[28] provided derivatives **21**, bearing a variety of substitutions in position 6. If necessary, any remaining unsaturation was reduced by catalytic hydrogenation. The derivatives **20** were directly obtained from the intermediate **19** ($R = CF_3$) using a three steps sequence (reduction to the alcohol, oxidation to the aldehyde and condensation with 2-cyanoacetamide). The same sequence was applied to generate ANC derivatives **22a–i** from the esters **21** (Scheme 3). The synthesis of a library of 5-N-substituted ANC derivatives **30a–e** (Scheme 4) was achieved through nucleophilic substitution of the 5-chloro ANC **29** using various primary and secondary amines. The synthesis of the intermediate **29** was achieved from the known precursor **23**.[29] After chlorination in hot $POCl_3$, sequential displacement with the sodio anion of 4-methoxybenzylalcohol

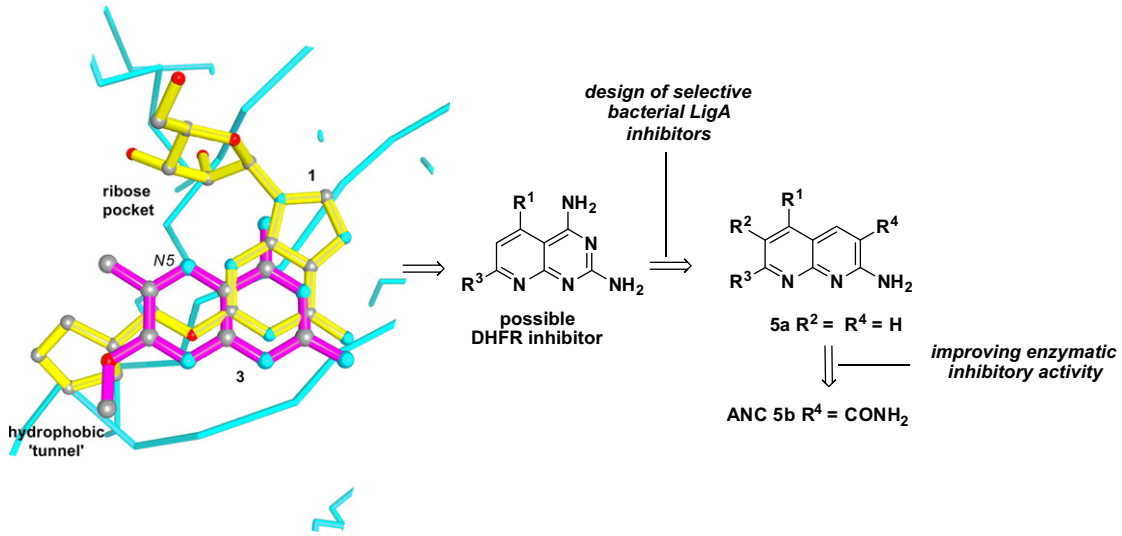
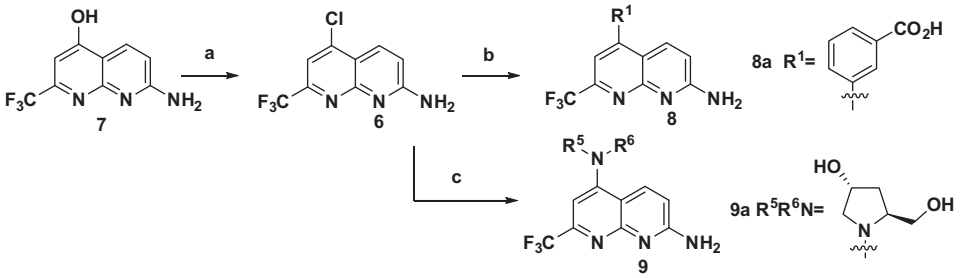
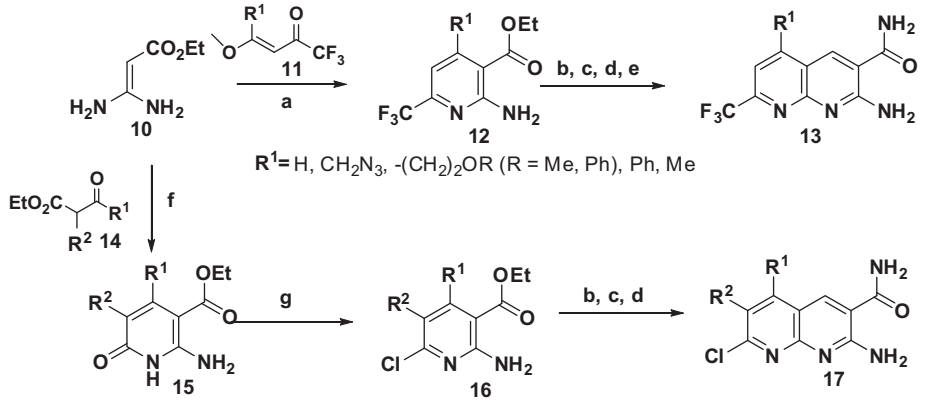


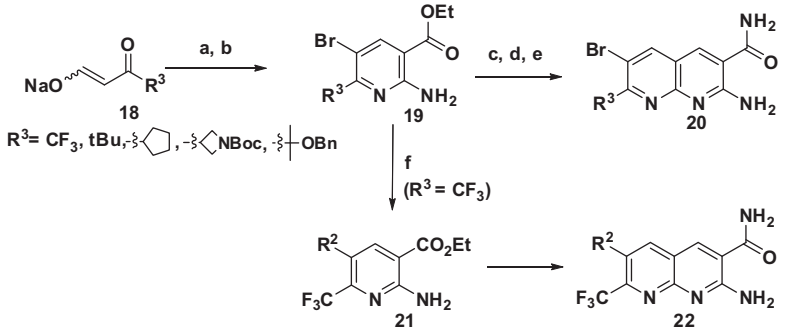
Figure 3. Structure guided design of novel ANCs starting from the superimposition of compounds **1** (yellow bonds) and **3** (magenta bonds).



Scheme 1. Reagents and conditions: Preparation of small librairies of 7-trifluoromethyl-2-amino[1,8]naphthyridines **8** and **9**: (a) $POCl_3$, reflux; (b) R^1-$B(OH)_2$, $(PPh_3)_4Pd$ (5 mol %), K_2CO_3, aq dioxane, reflux; (c) R^5R^6NH, NMP, 120 °C.



Scheme 2. Reagents and conditions: Synthesis of 5-substituted-7-trifluoromethyl-ANC **13** and 5,6-disubstituted-7-chloro- ANC **17**: (a) THF, 80 °C; (b) LAH, Et_2O, 0 °C, (when $R^1 = CH_2N_3$ then Boc_2O; (c) MnO_2, DCM, 40 °C; (d) 2-cyanoacetamide, TMG, EtOH, 60 °C; (e) when $R^1 = (CH_2)_2OBn$: HBr, AcOH, rt (to provide **13d**); when R^1 = NHBoc, TFA, rt (leading to **13e**); (f) pyridine, EtOH, Δ, (g) $POCl_3$, 90°C.



Scheme 3. Reagents and conditions: Synthesis of 6-substituted-7-trifluoromethyl-ANC **20** and **22**: (a) **10**, THF, 80 °C; (b) NBS, MeCN, rt; (c) LAH, Et_2O, 0 °C; (d) MnO_2, DCM, 40 °C; (e) 2-cyanoacetamide, TMG, EtOH, 60 °C, rt; (f) R^2 = Me: MeZnBr, cat. $Pd(PPh_3)_4$, dioxane, 80 °C; R^2 = cPr, Bn: $BnBF_3K$, cat. $Cl_2Pd(dppf)_2$, Cs_2CO_3, aq THF, Δ; R^2 = Et, cPent, $-(CH_2)_2Ph$: R'B$(OH)_2$, cat. $Pd(PPh_3)_4$, K_2CO_3, aq dioxane, Δ then H_2, cat. PtO_2, EA, rt.

and 3,4-dimethoxybenzylamine and cleavage of both protecting groups in TFA afforded the intermediate **27**. Subsequently, a treatment with $POCl_3$ followed by a bromination using NBS gave rise to the ester **28**. The transformation into naphthyridine **29** was then performed in three steps as described previously.

The physicochemical properties and metabolic stability profile of the most promising LigA inhibitor **20a** were determined in more detail. The aqueous solubility remained in the low single digit μg/mL range at all pHs.[30] Major human cytochrome P450 isoenzymes 2C9, 2D6 and 3A4 were not inhibited by compound **20a** (IC_{50} > 50 μM). It was stable in rat microsomes and hepatocytes with respective intrinsic clearances of 20 μL/(min∗mg) and 1.8 μL/(min∗10^6cells). Rat PK performed with compound **20a** revealed low clearance, moderate volume of distribution and high oral bioavailability (F = 80%) while subcutaneous administration (10 mg/kg) to mice resulted in high exposure (see Table 3). As significant free concentration was measured in mouse plasma for the compound **20a**, it was evaluated in a neutropenic murine thigh infection model[31] using a quinolone sensitive laboratory strain descendent of *S. aureus* DSM 11823 as infecting pathogen (**20a**; MIC = 0.5mg/L). When administered at a dose of 100 mg/kg (s.c.; 2h post-infection and once per experiment), **20a** provided a net static effect 24 h after infection (inoculum size 2×10^3 cfu i.m.; compound formulated in 5% DMSO/15% solutol HS15 in water). In vivo efficacy of a LigA inhibitor was already demonstrated on a similar aminal model with a metabolically labile adenosine analog.[10c]

Compound **20a** inhibited growth of various strains of *S. aureus* even in presence of human serum (Table 4), and displayed some

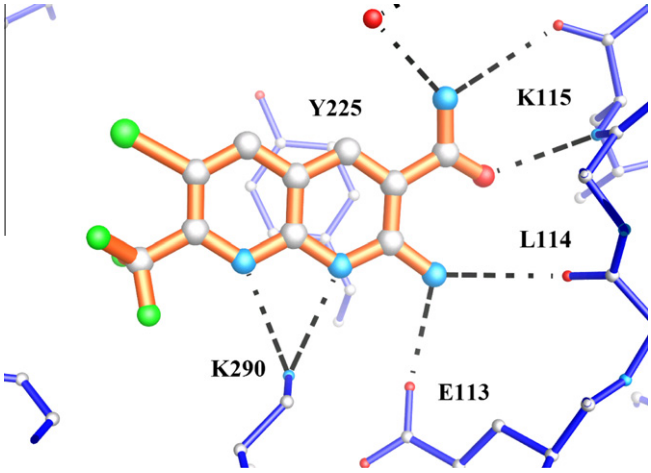


Figure 4. X-ray structure of ANC **20a** bound to *E. coli* LigA (PDB code: 4GLX).

antibacterial potency against other Gram-positive (*E. faecalis*) and RTI pathogens such as *S. pneumoniae* and *Moraxella catarrhalis* but it did not inhibit growth of *Enterococcus faecium*, and in general Gram-negative organisms. The IC_{50} against *S. pneumoniae* LigA was two to threefold higher than for *S. aureus* LigA. Not surprisingly for Gram-negative strains, the lack of activity can be attributed to poor permeability and high efflux (see permeable *E. coli* activity in Table 3). The complete absence of activity against *Saccharomyces cerevisiae* which has an ATP-dependent ligase, confirmed the excellent selectivity profile of our ANC inhibitors for the bacterial class of these enzymes.novel class of NAD^+-dependent LigA inhibitors, i.e. functionalized 2-amino-[1,8]-naphthyridine-3-carboxamides, has been obtained in a structure guided optimization approach starting from a HTS hit featuring a 2,4-diaminopteridine scaffold. The most

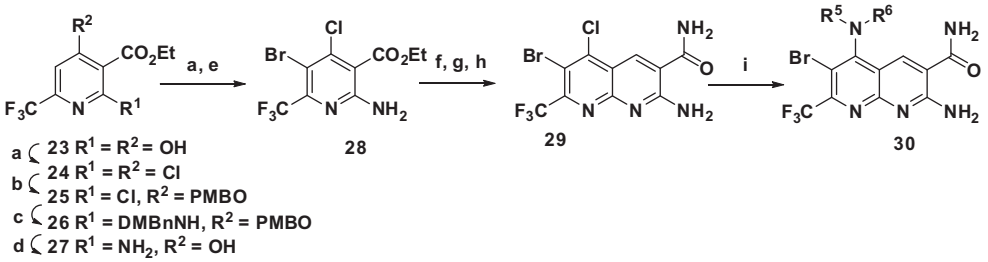
Table 4
Antibacterial activity[15] of compound **20a** against selected pathogens[32]

Gram-positive strains	MIC (mg/L)	Gram-negative strains	MIC (mg/L)
S. aureus A798	1	*H. influenzae* A921	0.125
S. aureus ATCC25923	1	*M. catarrhalis* A894	8
S. aureus ATCC25923[a]	2	*P. aeruginosa* ATCC27853	>32
E. faecium A949	>32	*K. pneumoniae* T6474	>32
E. faecalis ATCC29212	8	*A. baumannii* T6474	>32
S. pneumo.ATCC49619	16	*E. coli* ATCC25922	>32
C. difficile ATCCA1148	8	*S. cerevisiae* (yeast) A136	>32

[a] Measured in presence of 50% human serum.

potent inhibitors displayed in vitro antibacterial activities on Gram-positive *S. aureus* as well as fastidious Gram-negative *H. influenzae*. Target specificity of these inhibitors was established by comparing whole-cell assays using *E. coli* strains with low or high DNA LigA activity respectively. The establishment of the SAR required the development of synthetic pathways leading to highly functionalized 2-aminopyridines, a process not suited for high-throughput chemistry. Our investigations demonstrated 1) that substitution of position 5 did not lead to an increase of binding affinity and 2) that substituents in positions 6 and 7 have a strong influence on SAR.

In depth characterization of compound **20a** showed that its good metabolic stability (combined with an acceptable mouse plasma protein binding) led to sufficient free exposure to ensure in vivo efficacy in a murine infection model, using a *S. aureus* strain as an infecting pathogen. Complete characterization of biochemical and antibacterial properties of inhibitor **20a** will be discussed elsewhere. Further investigations of the ANC scaffold will focus on 1) improving the biochemical potency on various LigA enzymes to expand the antibacterial spectrum, 2) decreasing resistance frequen-



Scheme 4. Reagents and conditions: Synthesis of various 5-substituted 6-bromo- ANC **30**: (a) $POCl_3$, DMF, 90 °C; (b) 4-MeO-BnOH (PMB-OH), NaH, DMF, 0 °C; (c) 3,4-di-MeO-$BnNH_2$ (DMBNH_2), 100 °C; (d) TFA, 70 °C, 2 h; (e) NBS, MeCN, rt; (f) LAH, Et_2O, 0 °C; (g) MnO_2, DCM, 40 °C; (h) 2-cyanoacetamide, TMG, EtOH, 60 °C; (i) HNR^5R^6, Et_3N, NMP, 100 °C.

Table 3
PK parameters determined for compound **20a**

	CL[c]	V_{ss}[d]	$t_{1/2}$[e]	C_{max}[f]	AUC[g]	*f*AUC[g]
Rat (0.1 mg/kg, iv.[a] or 1 mg/kg po.[b])	11.0	0.71	2.3	0.41[h]	0.15 1.22[h]	0.021[i] 0.171[h,i]
Mouse (10 mg/kg, s.c.[b])	n/a	n/a	2.9	7.05	84.5	3.89[j]

[a] 5% DMSO/28.5% HPβCD in water.
[b] 5% DMSO/15% Solutol HS15 in water.
[c] Total plasma clearance (mL/min/kg).
[d] Volume of distribution at steady state (L/kg).
[e] Terminal half-life (h).
[f] Highest observed plasma concentration (μg/mL).
[g] Area under the concentration−time curve from time 0 to ∞ (iv.) or 24 h (po./sc., mg*h/L).
[h] Determined from the po. experiment.
[i] Unbound fraction *fu* = 0.14.
[j] Unbound fraction *fu* = 0.046. n/a: not applicable.

cies 3) optimizing the key physicochemical properties (solubility, plasma protein binding).

Acknowledgments

We would like to acknowledge Dr Naomi Tidten-Luksch for finalizing and depositing the protein X-ray structures, Markus Gude (High Throughput Medicinal Chemistry group), and the members of the the Preformulation and DMPK groups at Actelion Pharmaceuticals Ltd for the excellent support they provided.

References and notes

1. Boucher, H. W.; Talbot, G. H.; Bradley, J. S.; Edwards, J. E.; Gilbert, D.; Rice, L. B.; Scheld, M.; Spellberg, B.; Bartlett, J. *Clin. Infect. Dis.* **2009**, *48*, 1.
2. (a) Katz, M. L.; Mueller, L. V.; Polyakov, M.; Weinstock, S. F. *Nat. Biotechnol.* **2006**, *24*, 1529; (b) Kraus, C. N. *Curr. Opin. Microbiol.* **2008**, *11*, 434; (c) Projan, S. J. *Curr. Opin. Microbiol.* **2003**, *6*, 427.
3. (a) Silver, L. L. *Clin. Microbiol. Rev.* **2011**, *24*, 71; (b) Payne, D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L. *Nat. Rev. Drug Disc.* **2007**, *6*, 29.
4. (a) Olivera, B. M.; Lehman, I. R. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 1426; (b) Lehman, I. R. *Science* **1974**, *186*, 790; (c) Gellert, M. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 148; (d) Weiss, B.; Richardson, C. C. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *1021*, 57; (e) Gefter, M. L.; Becker, A.; Hurwitz, J. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 240.
5. (a) Swift, R. V.; Amaro, R. E. *Expert Opin. Drug Discov.* **2009**, *4*, 1281; (b) Dwivedi, N.; Dube, D.; Pandey, J.; Singh, B.; Kukshal, V.; Ramachandran, R.; Tripathi, R. P. *Med. Res. Rev.* **2008**, *28*, 545.
6. (a) Gottesman, M. M.; Hicks, M. L.; Gellert, M. *J. Mol. Biol.* **1973**, *77*, 531; (b) Kaczmarek, F. S.; Zaniewski, R. P.; Gootz, T. D.; Danley, D. E.; Mansour, M. N.; Griffor, M.; Kamath, A. V.; Cronan, M.; Mueller, J.; Sun, D.; Martin, P. K.; Benton, B.; McDowell, M.; Biek, D.; Schmid, M. B. *J. Bacteriol.* **2001**, *183*, 3016; (c) Gong, C.; Martins, A.; Bongiorno, P.; Glickman, M.; Shuman, S. *J. Biol. Chem.* **2004**, *279*, 20594; (d) Petit, M. A.; Ehrlich, S. D. *Nucleic Acids Res.* **2000**, *28*, 4642.
7. Tomkinson, A. E.; Vijayakumar, S.; Pascal, J. M.; Ellenberger, T. *Chem. Rev.* **2006**, *106*, 687.
8. (a) Pascal, J. M. *Curr. Opin. Struct. Biol.* **2008**, *18*, 96; (b) Wilkinson, A.; Day, J.; Bowater, R. *Mol. Microbiol.* **2001**, *40*, 1241.
9. Shuman, S. *J. Biol. Chem.* **2009**, *284*, 17365.
10. (a) Cavero-Tomas, M.; Gowravaram, M.; Huynh, H.; Ni, H.; Stokes, S. S. WO 2006-0405558.; (b) Stokes, S. S.; Huynh, H.; Gowravaram, M.; Albert, R.; Cavero-Tomas, M.; Chen, B.; Harang, J.; Loch, J. T., III; Lu, M.; Mullen, G. B.; Zhao, S.; Liu, C. F.; Mills, S. D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4556; (c) Mills, S. D.; Eakin, A. E.; Buurman, E. T.; Newman, J. V.; Gao, N.; Huynh, H.; Johnson, K. D.; Lahiri, S.; Shapiro, A. B.; Walkup, G. K.; Yang, W.; Stokes, S. S. *Antimicrob. Agents Chemother.* **2011**, *1088*, 55.
11. Broetz-Oesterhelt, H.; Knezevic, I.; Bartel, S.; Lampe, T.; Warnecke-Eberz, U.; Ziegelbauer, K.; Haebich, D.; Labischinski, H. *J. Biol. Chem.* **2003**, *278*, 39435.
12. (a) Gu, W.; Wang, T.; Maltais, F.; Ledford, B.; Kennedy, J.; Wei, Y.; Gross, C. H.; Parsons, J.; Duncan, L.; Arends, R.; Cameron, M.; Perola, E.; Green, J.; Charifson, P. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3693; (b) Wang, T.; Duncan, L.; Gu, W.; O'Dowd, H.; Wei, Y.; Perola, E.; Parsons, J.; Gross, C. H.; Moody, C. S.; Arends, S. J. R.; Charifson, P. S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3699.
13. IC_{50}s were measured and calculated as recommended in: Copeland, R. A. *Evaluation of Enzyme Inhibitors in Drug Discovery*; Wiley and Sons: New Jersey, 2005. Chapter 5.
14. Chafin, D. R.; Vitolo, J. M.; Henricksen, L. A.; Bambara, R. A.; Hayes, J. J. *EMBO J.* **2000**, *19*, 5492.
15. Clinical Laboratory and Standards Institute. Methods for Dilution Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard Seventh Edition, document. M7-A7, Clinical Laboratory and Standards Institute, Wayne, PA, USA, 2006.
16. For consistency, *E. coli* amino acid numbering is used throughout the publication.
17. (a) Lavesa-Curto, M.; Sayer, H.; Bullard, D.; MacDonald, A.; Wilkinson, A.; Smith, A.; Bowater, L.; Hemmings, A.; Bowater, R. P. *Microbiology* **2004**, *150*, 4171; (b) Dermody, J. J.; Robinson, G. T.; Sternglanz, R. *J. Bacteriol.* **1979**, *139*, 701.
18. Gajiwala, K. S.; Pinko, C. *Structure* **2004**, *12*, 1449.
19. Structurally related 2,4-diamino-7-dimethylamino-pyrimido[4,5-*d*]pyrimidine was also identified as a selective DNA LigA inhibitor at Eli Lilly & Co; see Meier, T. I.; Yan, D.; Peery, R. B.; McAllister, K. A.; Zook, C.; Peng, S.-B.; Zhao, G. *FEBS J.* **2008**, *275*, 5258.
20. Pascal, J. M.; O'Brien, P. J.; Tomkinson, A. E.; Ellenberger, T. *Nature* **2004**, *432*, 473.
21. Song, Y.; Hou, Y.; Gupta, S.; Reed, A.D.; Ghosh, D. US 2007-0082920. Some 2,4-diamino-pyrido[2,3-*d*]pyrimidines, claimed to act as NAD^+-dependent DNA Ligase inhibitors in this patent application, displayed in our hands, strong inhibition of huDHFR and potent antibacterial activity. The latter effect, readily antagonized by addition of thymidine in the growth medium, was likely due to bacterial DHFR inhibition.
22. Pinko, C.; Borchardt, A.; Nikulin, V.; Su, Y. available at http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=67865.
23. PDB IDs: 3BAA and 3BAB.
24. Ferrarini, P. L.; Mori, C.; Badawneh, M.; Calderone, V.; Calzolari, L.; Loffredo, T.; Martinotti, E.; Saccomanni, G. *Eur. J. Med. Chem.* **1998**, *33*, 383.
25. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
26. The condensation reaction between compounds **10** and **14** is sluggish, leading to ethyl 2-(4-R^1-5-R^2-6-oxo-1,6-dihydropyrimidin-2-yl)acetate as the major or sole product.
27. Molander, G. A.; Bernardi, C. R. *J. Org. Chem.* **2002**, *67*, 8424.
28. Negishi, E-i.; Hu, Q.; Huang, Z.; Qian, M.; Wang, G. *Aldrichim. Acta* **2005**, *38*, 71.
29. Adam, F. M.; Bish, G.; Calo, F.; Carr, C. L.; Castro, N.; Hay, D.; Hodgson, P. B.; Jones, P.; Knight, C. J.; Paradowski, M.; Parsons, G. C.; Proctor, K. J. W.; Pryde, D. C.; Rota, F.; Smith, M. C.; Smith, N.; Tran, T. D.; Hitchin, J.; Dixon, R. *Org. Process Res. Dev.* **2011**, *15*, 788.
30. Thermodynamic solubilities were measured after 24 h equilibration in the studied medium.
31. Andes, D.; Craig, W. A. *Int. J. Antimicrob. Agents* **2002**, *19*, 261.
32. *S. aureus*: *Staphylococcus aureus*; *E. faecium*: *Enterococcus faecium*; *E. faecalis*: *Enterococcus faecalis*; *S. pneumoniae* or *S. pneumo*: *Streptococcus pneumoniae*; *C. difficile*: *Clostridium difficile*; *K. pneumoniae*: *Klebsiella pneumoniae*; *M. catarrhalis*: *Moraxella catarrhalis*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *E. coli*: *Escherichia coli*; *A. baumannii*: *Acinetobacter Baumannii*; *S. cerevisiae*: *Saccharomyces cerevisiae*.