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Design, Synthesis and Biological Evaluation of Oxazolidinone–Quinolone Hybrids

Christian Hubschwerlen,* Jean-Luc Specklin, Christine Sigwalt, Susanne Schroeder and Hans H. Locher

Morphochem AG, Schwarzwaldallee 215, CH-4058 Basel, Switzerland

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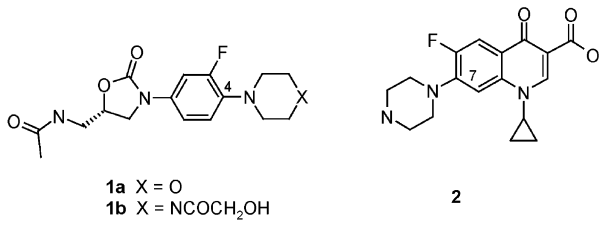
Abstract—Oxazolidinone–quinolone hybrids that combine the pharmacophores of a quinolone and an oxazolidinone were synthesised and shown to be active against a variety of resistant and susceptible Gram-positive and fastidious Gram-negative organisms. The best compounds in this series overcome all types of resistance in relevant clinical Gram-positive pathogens. The nature of the spacer greatly influences the antibacterial activity. The dual mode of action could be demonstrated for compounds having a piperazinyl spacer. Antibacterial activity was higher at acidic pH.

Introduction

The growing incidence of bacterial resistance to antibiotics represents a serious medical and socio-economical problem. The situation is getting particularly critical in hospital settings where Gram-positive pathogens like staphyloccocci and enteroccoci are becoming multiresistant to β-lactam, quinolone and glycopeptide antibiotics.[1] Resistance to linezolid **1a** (LZD; Chart 1), the recently marketed oxazolidinone antibiotic, has already been observed in clinical isolates of *Staphylococcus aureus* and *Enterococcus* sp.[2,3] Therefore, there is an urgent need for new classes of antibiotics that are more potent and less prone to resistance development than the currently marketed antibacterials. Besides the exploitation of new targets there is an other approach consisting of combining two pharmacophores in one molecule. These two pharmacophores, by addressing the active site of two different targets, offer the possibility to overcome the current resistance and in addition to reduce the appearance of new resistant strains. A similar strategy was already applied to sulfanilyl–fluoroquinolone and trimethoprime–fluoroquinolone hybrids.[4,5] In the first class, this principle could not be demonstrated since sulfonamide activity was not retained.[6] In contrast, the second class clearly addressed the two targets DNA gyrase and dihydrofolate reductase and both resistances could be overcome in vitro to some extent.

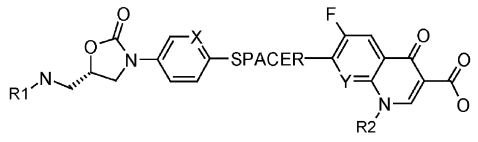
Several SAR studies on the oxazolidinones and quinolones have demonstrated a high tolerance for structural variation at the 4- respectively 7-positions of the phenyl ring.[7,8]

As eperezolid **1b** and ciprofloxacin **2** (CIP) possess both a piperazine substituent, it was appealing to merge these two molecules through this common substituent. The present paper describes the synthesis and the biological evaluation of such oxazolidinone–quinolone hybrids **3** where the two pharmacophores were linked through a spacer found either in quinolones or oxazolidinones.



1a X = O
1b X = NCOCH$_2$OH

2



3

Chart 1.

*Corresponding author. Tel.: +41-61695-2103; fax: +41-61695-2122; e-mail: christian.hubschwerlen@morphochem.ch

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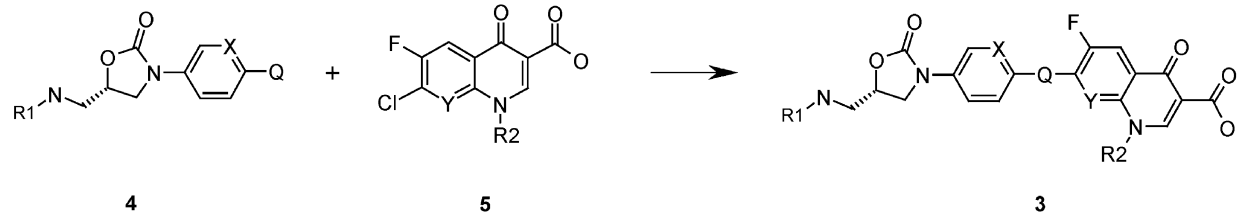
Chemistry

The synthesis of the oxazolidinone–quinolone hybrids **3** is described in Scheme 1. The appropriate oxazolidinone intermediates **4** containing a terminal basic nitrogen in the Q substituent at the 4-position of the aromatic ring were prepared following the synthetic strategy adopted for the synthesis of eperezolid, starting from 3,4-difluoronitrobenzene or 2-chloro-5-nitropyridine respectively (Scheme 2).[9]

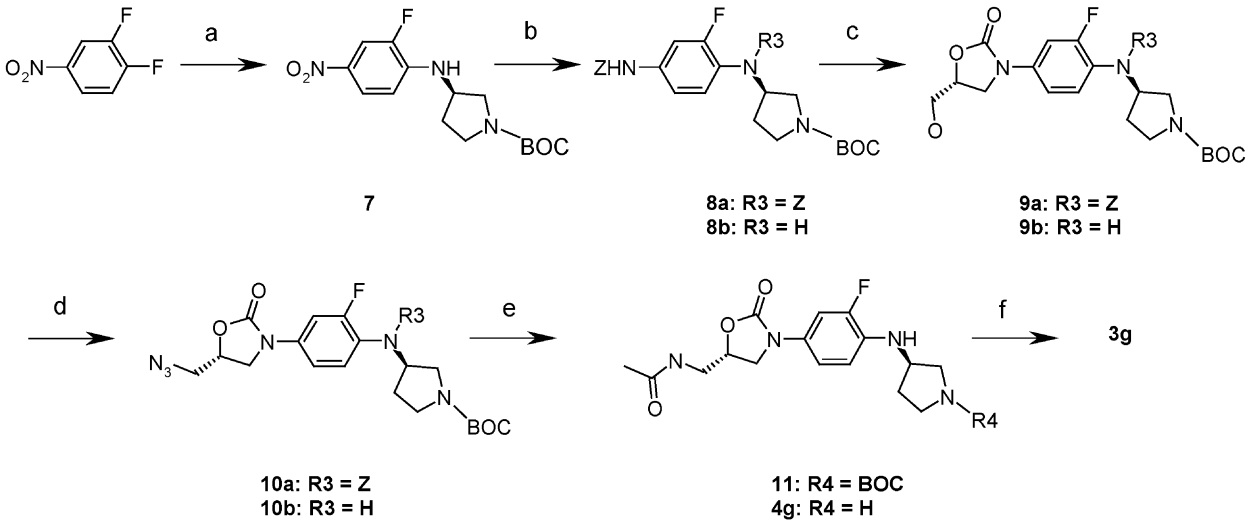
The 7-chloro or -fluoroquinolone building blocks **5**, when not commercially available, were synthesised using published procedures.[10] The key coupling reaction was performed either under thermal condition or under microwave irradiation in the presence of an organic base like DABCO, *N*,*N*-dimethyl-*p*-toluidine or triethylamine in a polar solvent like DMSO or 1-methyl-2-pyrrolidinone. This reaction was found to be sluggish with quinolones **5** (Y = CH) and required the activation of the quinolone moiety by the prior formation of a boron complex.[11] In the case of the naphthyridones **5** (Y = N), the boron activation was found to be troublesome at the stage of the hydrolysis. Alternatively, the naphthyridone intermediates **5** were treated with triethylchlorosilane in presence of triethylamine at 100 °C. In general the yields were higher in the naphthyridone series (Table 1).

Results and Discussion

The oxazolidinone–quinolones hybrids **3** were tested for in vitro antibacterial activity against a panel of well-characterized susceptible and resistant Gram-positive and -negative bacteria. Data for selected strains are



Scheme 1. Strategy for the assembly of the oxazolidinone-quinolone hybrids **3**.



Scheme 2. (a) (1) 3(*R*)-1-Allyloxycarbonyl-3-amino-pyrrolidine, Et_3N; (2) $PdCl_2(PPh_3)_2$, AcOH, Bu_3SnH; (3) BOC_2O; (b) (1) H_2, Pd/C; (2) ZCl, $NaHCO_3$; (c) *n*-BuLi, *R*-(−)-glycidyl butyrate; (d) (1) MsCl, TEA; (2) NaN_3; (e) (1) H_2, Pd/C; (2) Ac_2O, AcOH; (3) Et_3SiH, TFA; (f) **5a** (Y = CH; R_2 = cyclopropyl), DABCO.

Table 1. Yield of preparation of the oxazolidinone–quinolone hybrids **3**

	R1	X	Q	Y	R2	Yield (%)
3a	COMe	CF	Piperazinyl	CH	Cyclopropyl	26
3b	COMe	CF	Piperazinyl	N	Cyclopropyl	77
3c	COMe	N	Piperazinyl	N	Cyclopropyl	47
3d	C(=S)OMe	CF	Piperazinyl	N	Cyclopropyl	23
3e	COMe	CF	Piperazinyl		C–CH_2–N(Me)	21
3f	COMe	CF	3-Amino-azetidinyl	N	Cyclopropyl	30
3g	COMe	CF	3(*R*)-Amino-pyrrolidinyl	CH	Cyclopropyl	5
3h	COMe	CF	3(*R*)-Amino-pyrrolidinyl	N	Cyclopropyl	5

Table 2. In vitro antibacterial activity of oxazolidinone-quinolone hybrids **3** against selected bacteria (MICs in μg/mL)

Compd	Bacterial strain[a]										
	Sau1	Sau2 QRSA	Sau3 LZD-res.	Efs1	Efs2 LZD-res.	Efm1 VRE	Spn1	Spn2 QR	Hin	Mca	Eco
1a (LZD)	2	1	64	2	64	2	0.5	0.5	8	4	>64
2 (CIP)	0.5	32	0.5	1	1	>32	0.5	32	<0.03	<0.03	<0.03
3a	0.25	0.25	4	0.125	4	0.5	0.25	0.25	4	0.5	32
3b	0.125	0.125	8	0.125	4	0.25	0.125	0.25	1	0.25	32
3c	4	1	>32	2	>32	>32	0.125	0.25	8	64	>64
3d	0.25	0.125	>32	0.25	16	0.25	0.125	0.25	16	0.25	>64
3e	0.5	0.125	8	0.125	8	0.5	0.125	0.25	8	1	>64
3f	0.06	0.25	0.06	0.5	0.5	8	0.06	0.25	0.06	0.125	2
3g	<0.03	1	<0.03	0.06	0.125	16	0.5	2	0.06	0.06	0.5
3h	<0.03	1	0.06	0.125	0.06	8	0.06	<0.06	<0.03	<0.03	0.5

[a]Sau1, *S. aureus* ATCC 29213; Sau2, *S. aureus* MRSA, quinolone-resistant; Sau3, *S. aureus* linezolid-resistant; Efs1, *Enterococcus faecalis* ATCC 29212; Efs2, *E. faecalis* linezolid-resistant; Efm1, *E. faecium* vancomycin- and quinolone-resistant; Spn1, *Streptococcus pneumoniae* ATCC 49619; Spn2, *S. pneumoniae* quinolone-resistant; Hin, *Haemophilus influenzae* 11; Mca, *Moraxella catarrhalis* ATCC 8176; Eco, *Escherichi coli* ATCC 25922.

reported as minimum inhibitory concentration (MIC) expressed in μg/mL (Table 2). For comparison, CIP (**2**) and LZD (**1a**) were employed as reference drugs. As can be deduced from these data, all of the synthesised compounds exhibited potent antibacterial activity. This activity can be modulated through the nature of the spacer and the two pharmacophores.

Compounds **3a–e** which possess a piperazinyl linker displayed an antibacterial spectrum similar to linezolid by overcoming quinolone resistance in *S. aureus* (strain Sau2), *Streptococcus pneumoniae* (strain Spn2) and enteroccocci (strain Efm1). On the other hand, activity against linezolid-resistant strains was somewhat lower than towards wildtype strains, but nevertheless much better than linezolid. The nature of the oxazolidinone pharmacophore also plays an important role and the same general trend was observed as in the oxazolidinone series (i.e. **3d** ≥ **3b** > **3c**).

In contrast, compounds **3f–h** which contain an amino pyrrolidinyl or amino azetidinyl spacer have a more pronounced quinolone type antibacterial spectrum (i.e., potent activity against Gram-negative bacteria and reduced activity against quinolone-resistant bacteria, especially enteroccoccal strains like Efm1).

Table 3. Activity of oxazolidinone–quinolone hybrids **3** against DNA gyrase, topoisomerase IV, and in an in vitro transcription/translation assay

Compd	DNA gyrase[a]	Topo IV[b]	Inhib. of protein synthesis[c]
	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
3a	20	100	2.8
3b	50	10	2.8
3g	0.2	1	>20
LZD	NT	NT	4.1
CIP	0.5	5	>20

NT, not tested.
[a]Supercoiling assay with *E. coli* gyrase.
[b]Topoisomerase IV relaxation assay.
[c]In vitro transcription/translation assay with *E. coli* S30 Extract System (Promega).

Despite this general trend, representatives of both new classes are superior to their respective comparators and even to the ad hoc combination of both (see below).

Selected compounds were tested against 20 strains each of recent clinical isolates of methicillin- and quinolone-resistant *S. aureus* (MRSA, QRSA), vancomycin-resistant enterococci (VRE), and penicillin-resistant *S. pneumoniae* (PRSP). The MIC90% (MIC that inhibits ≥90% of all strains) for MRSA were 0.5, 0.5, 2, 2, >32 and 2 μg/mL for compounds **3a**, **3b**, **3g**, LZD, CIP, and the 1:1 combination of LZD and CIP, respectively. For VRE the MIC90% values were 0.125, 0.25, 8, 2, >32, and 4 μg/mL respectively, and for PRSP strains 0.125, 0.25, 0.25, 1, 4, and 1 μg/mL respectively. This data illustrates the superior activity of **3a** and **3b** against these multi-resistant organisms.

In order to confirm this dual mode of action, we measured for compounds **3a–b**, **g** both the inhibition of the protein synthesis in an in vitro transcription/translation assay and the inhibition of the enzymes that are targeted by the quinolones, that is DNA gyrase and topoisomerase IV (Table 3). The enzyme data confirmed the observed antibacterial activity with compounds **3a,b** showing a strong protein synthesis inhibition and compound **3g** a strong DNA gyrase and topoisomerase IV inhibition. Moreover, compounds **3a**,**b** also displayed activity on topoisomerase IV and to a lesser extent on the DNA gyrase indicating the dual mode of action of these molecules. This difference of behaviour between **3a**,**b** and **3g** can best be explained by the different molecular shape induced by the nature of the different spacers. This may result in a preferred binding of the

Table 4. pH-dependent antibacterial activity (*S. aureus* Sau2)

Compd	MIC (μg/mL) at pH			
	6.4	6.8	7.4	7.8
LZD	0.5	0.5	1	1
CIP	32	32	16	16
3a	0.03	0.125	0.25	0.5
3b	0.03	0.06	0.25	0.5
3g	0.125	0.25	0.5	1

molecules in one or the other active site of the two targets. Compounds **3a**,**b** seem to be able to address multiple targets.

In contrast to LZD and CIP, the activity of compounds **3a**,**b** is dependent on extracellular pH, that is MICs were lower at acidic pH (Table 4). This could be explained by an increased intrabacterial drug concentration when the outside pH is lower than the intracellular pH ('alkaline trapping').[6] This property may contribute to potent antibacterial activity and may be beneficial for the treatment of infections involving acidic environments as, for example, found in abscesses or inflamed tissues.[12]

Conclusion

A series of oxazolidinone-quinolone hybrid antibacterials has been discovered. Their in vitro antibacterial activity encompasses a large variety of clinically relevant susceptible as well as resistant (i.e. MRSA, QRSA, VRE and PRSP) Gram-positive and fastidious Gram-negative bacteria. Various spacers are tolerated and through their 3-D orientation influence the nature of the mode of inhibition and the resulting antibacterial spectrum. The most active compound **3b** was 4–16 fold more active than LZD and overcomes all types of resistance in relevant clinical Gram-positive pathogens.[13]

Experimental

General

Proton (^{1}H NMR) magnetic resonance spectra were recorded in DMSO-d_6 on a Bruker dpx 300 spectrometer. Chemical shifts (δ) are given in ppm and refer to TMS as internal standard and coupling constants are given in Hz. Mass spectra were recorded on a HP1100 LC/API150 EX with an ESI source and a single quad analyser. Microwave experiments were performed using Coherent Synthesis™ technology on a SmithCreator™ workstation (Personal Chemistry AB, Sweden). Flash column chromatography was performed with Silica 32-63 60 Å silica gel (Brunschwig). The term 'worked up' refers to the following sequence of operations: the aq layer was extracted twice with AcOEt, the resulting organic layer was then washed with H_2O and brine, dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. 7-Chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-[1,8]naphthyridine-3-carboxylic acid and 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid were purchased from commercial sources. Ciprofloxacin and linezolid were synthesised in our laboratories.

Synthesis of the oxazolidinone building blocks 4

(3*R*)-3-(2-Fluoro-4-nitro-phenylamino)-pyrrolidine-1-carboxylic acid allyl ester (6). A solution of 3,4-difluoronitrobenzene (5.01 g; 31.5 mmol), (3*R*)-1-allyloxycarbonyl-3-amino-pyrrolidine[14] (5.1 g; 30 mmol) and Et_3N (6.27 mL; 31.5 mmol) in AcOEt (100 mL) was stirred at reflux for 20 h. The reaction mixture was then worked up. The residue was crystallized from Et_2O–hexane to yield **6** (5.76 g; 18.6 mmol; 59%) as a yellow powder. NMR: 1.09–2.24 (m, 2H), 3.29–3.72 (m, 4H), 4.21–4.28 (m, 1H), 4.52 (d, $J=1.5$, 2H), 5.15–5.32 (m, 2H), 5.87–5.99 (m, 1H), 6.94 (t, $J=9$, 1H), 7.19 (d, $J=6$, 1H), 7.9–7.99 (m, 2H).

(3*R*)-3-(2-Fluoro-4-nitro-phenylamino)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (7). To a solution of **6** (5.76 g; 18.6 mmol) in THF (60 mL) were added $PdCl_2(PPh_3)_2$ (0.13 g; 0.186 mmol), AcOH (12.12 mL; 37.2 mmol) and tributyltin hydride (49.87 mL; 37.2 mmol). The reaction was stirred at rt for 1 h. The suspension was then diluted with Et_2O (100 mL) and the solid, collected by filtration, was washed with Et_2O and hexane and then dried. The solid was resuspended in THF (10 mL) and treated with Boc_2O (4.87g; 30 mmol). After stirring at rt for 3 h, the reaction mixture was worked up and the residue was crystallized from Et_2O–hexane to afford **7** (4.15 g; 12.6 mmol; 68%) as a yellow powder. NMR: 1.25 (s, 9H), 1.75–2.07 (m, 2H), 3.07–3.5 (m, 4H), 4.05–4.1 (m, 1H), 6.77–6.83 (t, $J=9$, 1H), 7.01 (d, $J=5.7$, 1H), 7.77–7.85 (m, 2H).

(3*R*)-3-[Benzyloxycarbonyl-(4-benzyloxycarbonylamino-2-fluoro-phenyl)-amino]-pyrrolidine-1-carboxylic acid *tert*-butyl ester and (3*R*)-3-[(4-benzyloxycarbonylamino-2-fluoro-phenyl)-amino]-pyrrolidine-1-carboxylic acid *tert*-butyl ester (8a and 8b). A solution of **7** (4 g; 12.29 mmol) in AcOEt (100 mL) and MeOH (50 mL) was hydrogenated over Pd/C 10% (1 g). At the end of the reaction, the catalyst was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in AcMe (100 mL) and treated at 0 °C with benzylchloroformate (3.63 mL; 25.8 mmol) in presence of a satd. solution of $NaHCO_3$ (25 mL). The reaction was stirred overnight at rt. The solvent was evaporated and the aq layer was extracted twice with AcOEt and worked up. The residue was chromatographed (AcOEt–hexane 1:1) to give an unseparable equimolar mixture of **8a** and **8b** (6.03 g; 10.8 mmol; 99%) as a reddish oil. MS: 562.4 $(M-H)^-$, 428.4 $(M-H)^-$. Method ESI$^-$. This material was carried on without further characterization.

(3*R*)-3-{Benzyloxycarbonyl-[2-fluoro-4-{(5*R*)-5-hydroxymethyl-2-oxo-oxazolidin-3-yl}-phenyl]-amino}-pyrrolidine-1-carboxylic acid *tert*-butyl ester and (3*R*)-3-{[2-fluoro-4-{(5*R*)-5-hydroxymethyl-2-oxo-oxazolidin-3-yl}-phenyl]-amino}-pyrrolidine-1-carboxylic acid *tert*-butyl ester (9a and 9b). *n*-BuLi (1.6 M solution in *n*-hexane; 7.62 mL; 12.2 mmol) was added dropwise at −78 °C to a crude solution of **8a** and **8b** (6.02 g; 10.8 mmol) in THF (40 mL). The mixture was stirred at −78 °C for 10 min and warmed up to reach 0 °C. *R*-(−)-Glycidyl butyrate (2.11 g; 14.6 mmol) was added dropwise and the reaction was allowed to reach rt. After stirring overnight, the reaction was worked up. The residue was crystallized from AcOEt-hexane to provide an unseparable equimolar mixture of **9a** and **9b** (3.36 g; 6.48 mmol; 60%) of off-white solid. MS: 530.3 $(M+H)^+$, 396.1 $(M+H)^+$,

Method ESI$^+$. This material was carried on without further characterization.

(3*R*)-3-{[4-{(5*R*)-5-Azidomethyl-2-oxo-oxazolidin-3-yl}-2-fluoro-phenyl]-benzyloxycarbonyl-amino}-pyrrolidine-1-carboxylic acid *tert*-butyl ester and (3*R*)-3-{[4-{(5*R*)-5-Azidomethyl-2-oxo-oxazolidin-3-yl}-2-fluoro-phenyl]-amino}-pyrrolidine-1-carboxylic acid *tert*-butyl ester (10a and 10b). Methanesulfonylchloride (0.805 mL; 10.8 mmol) was added dropwise at 0 °C to a crude solution of **9a** and **9b** (3.36 g; 10.8 mmol) and Et_3N (2.05 mL; 10.8 mmol) in CH_2Cl_2 (40 mL). The reaction was stirred at rt for 1 h. The reaction was diluted with H_2O and worked up. The residue was dissolved in DMF (10 mL), and NaN_3 (1.38 g; 10.8 mmol) was added. After stirring at 80 °C for 20 h, the reaction mixture was concentrated to dryness and the residue was worked up to afford an unseparable equimolar mixture of **10a** and **10b** (4.07 g; 10.7 mmol; 99%) as an amorphous off-white material. MS: 555.5 $(M+H)^+$, 421.3 $(M+H)^+$, Method ESI$^+$. The latter was carried on without further characterization.

(3*R*)3-{4-[(5*S*)-5-(Acetylaminomethyl)-2-oxo-oxazolidin-3-yl]-2-fluoro-phenylamino}-pyrrolidine-1-carboxylic acid *tert*-butyl ester (11). A solution of **10a** and **10b** (4.2 g; 7.3 mmol) in AcOEt (50 mL) was hydrogenated over Pd/C 10% (400 mg). At the end of the reaction, the catalyst was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in AcOH (5 mL) and Ac_2O (2 mL) was added. After stirring at rt for 2 h, the solvents were evaporated and the residue was worked up to yield **11** (3.1 g; 7.3 mmol) as a crude amorphous off-white material. MS: 437.5 $(M+H)^+$, Method ESI$^+$. This mixture was carried on without further characterization.

***N*-{(5*S*)-3-[3-Fluoro-4-{(3*R*)-pyrrolidin-3-ylamino}-phenyl]-2-oxo-oxazolidin-5-ylmethyl]-acetamide (4g; R1 = Ac, X = CF, Q = 3(*R*)-aminopyrrolidinyl).** A solution of triethylsilane (0.93 mL; 7.3 mmol) and **11** (3.1g, 7.3 mmol) in CH_2Cl_2 (20 mL) was treated with TFA (20mL). After stirring at rt for 20 h, the volatiles were evaporated and the residue was dissolved in H_2O and neutralized with a satd $NaHCO_3$ solution. After evaporation in vacuo, the residue was taken up in a CH_2Cl_2–MeOH (1:1) mixture. This suspension was treated with Fuller's earth, filtered and the filtrate evaporated to yield **4g** (2.1 g; 6.2 mmol; 85%) as an off-white amorphous solid. NMR: 1.87 (s, 3H), 2.21 (m, 1H), 3.16 (dd, $J=12$ and 2, 1H), 3.36 (m, 6H), 3.70 (dd, $J=6$ and 9, 1H), 4.18 (m, 1H), 4.71 (m, 1H), 5.64 (d, $J=5$, 1H), 6.83 (t, $J=9$, 1H), 7.12 (d, $J=7$, 1H), 7.42 (dd, $J=14$ and 2, 1H), 8.29 (t, $J=6$, 1H), 9.00 (s, 1H). MS: 337.6 $(M+H)^+$, Method ESI$^+$.

***N*-[(5*S*)-2-Oxo-3-(6-piperazin-1-yl-pyridin-3-yl)-oxazolidin-5ylmethyl]-acetamide (4c; R1 = Ac, X = N, Q = piperazinyl).** This compound was prepared according to the procedure reported for **4g** starting with 4-(5-amino-pyridin-2-yl)-piperazine-1-carboxylic acid *tert*-butyl ester in 10% overall yield.[15] NMR: 1.80 (s, 3H), 2.81 (m, 3H), 3.46 (m, 5H), 3.62 (dd, $J=6$ and 10, 1H), 4.09 (t, $J=9$, 1H), 4.71 (d, $J=9$, 1H), 7.8 (dd, $J=9$ and 3, 1H), 8.21 (d, $J=3$, 1H), 8.31 (m, 1H). MS: 320.1 $(M+H)^+$, Method ESI$^+$.

Thioacetamide {[(5*S*)-3-[3-fluoro-(1-piperazinyl) phenyl]-2-oxo-5-oxalidinyl]methyl}-carbamothioic acid methyl ester (4d; R1 = C(=S)OMe, X = CF, Q = piperazinyl). This compound was prepared in 99% from 4[4-[(5*S*)-5-aminomethyl-2-oxo-oxazolidin-3-yl]-2-fluoro-phenyl]-piperazine-1-carboxylic acid *tert* butyl ester[17] according to ref 18. NMR: 3.66 (s, 8H), 3.37 (m, 1H), 3.69–3.77 (m, 3H), 3.88 (s, 3H), 4.16 (t, $J=9$, 1H), 4.90 (m, 1H), 7.12 (t, $J=9$, 1H), 7.23 (dd, $J=9.5$ and 2.5, 1H), 7.51 (dd, $J=15$ and 2.5, 1H), 9.57 (t, $J=4.8$, 1H). MS: 369.1 $(M+H)^+$, Method ESI$^+$.

***N*-{(5*S*)-3-[4-(azetidin-3-ylamino)-3-fluoro-phenyl]-2-oxo-oxazolidin-5-ylmethyl}-acetamide (4f; R1 = Ac, X = CF, Q = 3-amino-azetidinyl).** This compound was prepared according to the procedure reported for **4g** starting from 3,4-difluoronitrobenzene and 1-diphenylmethyl-3-azetidinamine in an 14% overall yield.[16] NMR: 1.87 (s, 3H), 3.2–3.5 (m, 6H), 3.64–3.71 (m, 2H), 3.92–4.4 (m, 2H), 4.17 (m, 1H), 5.74–5.78 (m, 1H), 6.57–6.7 (m, 1H), 7.0 (d, $J=9$, 1H), 7.1–7.5 (m, 1H), 8.31 (m, 1H). MS: 322.34 $(M+H)^+$, Method ESI$^+$.

Preparation of the oxazolidinone-quinolone hybrids 3

7-(4-{4-[(5*S*)-5-(Acetylamino-methyl)-2-oxo-oxazolidin-3-yl]-2-fluoro-phenyl}-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (3a). A mixture of 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylate boron diacetate[11] **5a** (0.103 g; 0.25 mmol), *N*-[{(5S)-3[3-fluoro-4-(1-piperazinyl)phenyl]-2-oxo-5-oxazolidinyl}methyl]-acetamide[9] **4a** (0.1 g; 0.3 mmol) and *N*,*N*-dimethyl-*p*-toluidine (0.054 mL; 0.375 mmol) in 1-methyl-2-pyrrolidinone (0.5 mL) was stirred at 120 °C for 12 h. The reaction mixture was poured into water and the resulting crystals were collected by filtration and purified by chromatography on silica (CH_2Cl_2–MeOH 19:1) to afford **3a** (0.38 g; 0.06 mmol; 26%) as a beige solid. Mp 315–320 °C (dec). NMR: 1.32 (m, 2H), 1.44 (m, 2H), 1.95 (s, 3H), 3.34 (m, 4H), 3.52 (t, $J=5.8$, 3H), 3.60 (m, 4H), 3.83 (dd, $J=7$ and 8, 1H), 3.96 (m, 1H), 4.21 (t, $J=8$, 1H), 7.24–7.34 (m, 2H), 7.61–7.67 (dd, $J=5$ and 2, 1H), 7.75–7.77 (d, $J=8$, 1H), 8.06 (d, $J=15$, 1H), 8.35 (t, $J=6$, 1H), 8.79 (s, 1H), 15.3 (s, 1H). MS: 582.4 $(M+H)^+$; 580.4 $(M-H)^-$. Anal. calcd for $C_{29}H_{29}F_2N_5O_6$ (0.45 H_2O): C, 59.89; H, 5.03; N, 12.04; H_2O, 1.37. Found: C, 59.49; H, 5.04; N, 11.84, H_2O, 1.38.

7-(4-{[(5*S*)-5-(Acetylamino-methyl)-2-oxo-oxazolidin-3-yl]-2-fluoro-phenyl}-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (3b). A suspension of 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-[1,8]naphthyridine-3-carboxylic acid **5b** (0.1 g; 0.35 mmol), **4a** (0.13 g; 0.39 mmol), Et_3N (0.119 g; 1.17 mmol) and $ClSi(Me)_3$ (0.085 g; 0.78 mmol) in DMSO (2 mL) was heated to 150 °C in a microwave oven for 10 min. After evaporation under reduced pressure, the residue was taken up in H_2O, fil-

tered and the solid was chromatographed (CH_2Cl_2–MeOH 9:1). The fractions were collected and evaporated. The residue was crystallized from MeCN to afford **3b** (0.154 g; 0.27 mmol; 77%) as a pale yellow solid. NMR: 1.11 (m, 2H), 1.21 (m, 2H), 1.83 (s, 3H), 3.17 (m, 4H), 3.41 (t, $J=5.8$, 3H), 3.71 (m, 2H), 4.01–4.11 (m, 5H), 4.71 (m, 1H), 7.11 (t, $J=8$, 1H), 7.18 (dd, $J=7$ and 2, 1H), 7.51 (dd, $J=8$ and 2, 1H), 8.05 (d, $J=13$, 1H), 8.24 (t, $J=6$, 1H), 8.59 (s, 1H), 15.1 (s, 1H). MS: 583.3 $(M+H)^+$, 581.6 $(M+H)^-$ Method ESI^+, ESI^-. Anal. calcd for $C_{28}H_{28}F_2N_6O_6$: C, 56.94; H, 4.93; N, 14.23. Found: C, 57.01; H, 4.97; N, 14.04, H_2O, 1.38.

7-(4-{5-[(5*S*)-5-(Acetylamino-methyl)-2-oxo-oxazolidin-3-yl]-pyridin-2-yl}-1-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (3c). This compound was prepared according to the procedure reported for **3b** starting from **4c** and **5b**. NMR: 1.2-1.4 (m, 4H), 1.85 (s, 3H), 3.45 (m, 6H), 3.61 (m, 6H), 3.71 (m, 1H), 4.1 (t, $J=8$, 1H), 4.67 (m, 1H), 7.00 (d, $J=9$, 1H), 7.63 (d, $J=8$, 1H), 7.83 (d, $J=9$, 1H), 7.93 (d, $J=14$, 1H), 8.25 (s, 2H), 8.67 (s, 1H), 15.1 (s, 1H). MS: 565.8 $(M+H)^+$ Method ESI^+. Anal. calcd for $C_{28}H_{28}F_2N_6O_6$ (0.5 H_2O): C, 56.44; H, 5.09; N, 17.06; H_2O, 1.56. Found: C, 56.59; H, 5.08; N, 16.89, H_2O, 1.56.

1-Cyclopropyl-6-fluoro-7-(4-{2-fluoro-4-[(5*S*)-5-(methoxythiocarbonylamino-methyl)-2-oxo-oxazolidin-3-yl]-phenyl}-piperazin-1-yl)-4-oxo-1,4-dihydro-[1,8]-naphthyridine-3-carboxylic acid (3d). This compound was prepared according to the procedure reported for **3b** starting from **4d** and **5b**. NMR: 1.11–1.27 (m, 5H), 3.22 (m, 4H), 3.80 (m, 4H), 3.91 (s, 3H), 4.1 (m, 4H), 4.95 (m, 1H), 7.13–7.24 (m, 2H), 7.56 (dd, $J=15$ and 3, 1H), 8.12 (d, $J=15$, 1H), 8.66 (s, 1H), 9.54 (t, $J=6$, 1H), 15.23 (s, 1H). MS: 615.2 $(M+H)^+$ Method ESI^+. Anal. calcd for $C_{28}H_{28}F_2N_6O_6S$: C, 54.72; H, 4.59; N, 13.67; S, 5.22. Found: C, 54.41; H, 4.76; N, 13.33; S, 5.0.4.

9-(4-{4-[(5*S*)-5-(Acetylamino-methyl)-2-oxo-oxazolidin-3-yl]-2-fluoro-phenyl}-piperazin-1-yl)-8-fluoro-3-methyl-6-oxo-2,3-dihydro-6H-1-oxa-3,3a-diaza-phenalene-5-carboxylic acid ethyl ester (12). A solution of 8,9-difluoro-3-methyl-6-oxo-2,3-dihydro-6H-1-oxa-3,3a-diaza-phenalene-5-carboxylic acid ethyl ester[10] (0.1 g; 0.32 mmol) and **4a** (0.216 g; 0.64 mmol) in a mixture of pyridine (1 mL) and DMSO (1 mL) was heated to 120 °C for 20 h. The solvents were evaporated under reduced pressure and the residue was taken up in H_2O. The solid was collected by filtration and purified by chromatography (CH_2Cl_2–MeOH 9:1) to yield **12** (0.044 g; 0.07 mmol; 22%) as an off-white solid. NMR: 1.4 (t, $J=8$, 3H), 2.0 (s, 3H), 3.15 (s, 3H), 326 (m, 4H), 3.30 (d, $J=6$, 2H), 3.61 (m, 5H), 3.74 (dd, $J=8$ and 9, 1H), 5.2 (m, 2H), 5.38 (m, 2H), 5.65 (m, 1H), 7.23–7.40 (m, 2H), 7.61 (d, $J=15$, 1H), 7.65 (d, $J=15$, 1H), 8.4 (t, $J=6$, 1H), 8.62 (s, 1H). MS: 627.7 $(M+H)^+$, Method ESI^+.

9-(4-{4-[(5*S*)-5-(Acetylamino-methyl)-2-oxo-oxazolidin-3-yl]-2-fluoro-phenyl}-piperazin-1-yl)-8-fluoro-3-methyl-6-oxo-2,3-dihydro-6H-1-oxa-3,3a-diaza-phenalene-5-carboxylic acid (3e). A solution of **12** (0.044 g; 0.32 mmol) in a mixture of 37% aq. HCl (1mL) and AcOH (1 mL) was heated to 80 °C for 20 h. The solvents were evaporated. The residue was dissolved in a MeOH–CH_2Cl_2 (1:1; 5 mL) mixture and treated with Et_3N (0.5 mL). After evaporation to dryness, the residue was taken up in AcOH (1 mL) and treated with Ac_2O (1 mL). The reaction was left to proceed for 20 h, and the solvents were evaporated in vacuo. The residue was purified by preparative HPLC (H_2O–MeCN gradient) to provide **3e** (0.009 g; 0.06 mmol; 21%) as an off-white solid. NMR: 1.84 (s, 3H), 3.02 (s, 3H), 3.11 (m, 4H), 3.50 (m, 4H), 3.7 (dd, $J=8$ and 8.6, 1H), 4.09 (t, $J=8$, 1H), 4.71 (m, 1H), 5.3 (b, 1H), 7.13 (t, $J=8$, 1H), 7.20 (dd, $J=7$ and 2, 1H), 7.51 (dd, $J=8$ and 2, 1H), 7.63 (d, $J=13$, 1H), 8.25 (t, $J=6$, 1H), 8.78 (s, 1H), 15.1 (s, 1H). MS: 599.2 $(M+H)^+$, 597.7 $(M-H)^-$, Method ESI^+, ESI^-.

7-(3-{4-[5(*S*)-5-(Acetylamino-methyl)-2-oxo-oxazolidin-3-yl]-2-fluoro-phenylamino}-azetidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (3f). This compound was prepared according to the procedure reported for **3b** starting from **4f** and **5b**. NMR: 1.11–1.24 (m, 4H), 1.87 (s, 3H), 3.44 (t, $J=5$, 2H), 3.68–3.76 (m, 2H), 4.09 (t, $J=8$, 1H), 4.32 (b, 2H), 4.51–4.57 (m, 1H), 4.67–4.77 (m, 3H), 6.24 (d, $J=7$, 1H), 6.74 (t, $J=9$, 1H), 7.14 (d, $J=9$, 1H), 7.50 (dd, $J=14$ and 2, 1H), 8.04 (d, $J=11$, 1H), 8.27 (t, $J=6$, 1H), 8.60 (s, 1H), 15.41 (s, 1H). MS: 569.5 $(M+H)^+$, Method ESI^+.

7-[(3*R*)-3-{4-[(5*S*)-5-(Acetylamino-methyl)-2-oxo-oxazolidin-3-yl]-2-fluoro-phenylamino}-pyrrolidin-1-yl]-1cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-1-carboxylic acid (3g). A solution of **5a** (0.204 g; 0.5 mmol), **4g** (0.052 g; 0.75 mmol) and DABCO© (0.112 g; 1 mmol) in DMSO (5 mL) was stirred for 50 h at 120 °C. After evaporation under reduced pressure, the residue was resuspended in EtOH (10 mL), treated with Et_3N (0.1 mL) and further stirred at rt for 20 h. The mixture was diluted with H_2O (20 mL). The solid was collected by filtration and crystallized in MeOH–EtOH–CH_2Cl_2 to afford **3g** (0.024 g; 0.027 mmol; 5.5%) as a beige powder. NMR: 0.9 (t, $J=8$, 1H), 1.00 (m, 2H), 1.15 (d, $J=4$, 1H), 1.6 (s, 3H), 1.96 (m, 1H), 2.15 (m, 1H), 3.15 (m, 1H), 3.22–3.68 (m, 6H), 3.9 (m, 1H), 4.15 (m, 1H), 4.5 (m, 1H), 5.3 (d, $J=7$, 1H), 6.73 (t, $J=7$, 1H), 6.95 (m, 2H), 7.15 (dd, $J=15$ and 2, 1H), 7.68 (d, $J=15$, 1H), 8.08 (t, $J=6$, 1 h), 8.42 (s, 1H). MS: 582.4 $(M+H)^+$, Method ESI^+.

7-[(3*R*)-3-{4-[(5*S*)-5-(Acetylamino-methyl)-2-oxo-oxazolidin-3-yl]-2-fluoro-phenylamino}-pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-[1,8]-naphthyridine-3-carboxylic acid (3h). This compound was prepared according to the procedure reported for **3g** starting from **4g** and **5b**. NMR: 1.0–1.3 (m, 7H), 1.84 (s, 3H), 2.07 (m, 1H), 3.4 (t, $J=5$, 1H), 3.70 (m, 1H), 3.8–4.0 (m, 2H), 4.07 (t, $J=9$, 1H), 4.30–4.5 (m, 3H), 4.66 (m, 1H), 5.17 (d, $J=6$, 1H), 6.87 (t, $J=12$, 1H), 7.07 (d, $J=8$, 1H), 7.40 (dd, $J=14$ and 2, 1H), 8.00 (d, $J=14$, 1H), 8.02 (t,

$J=6$, 1 h), 8.58 (s, 1H), 15.4 (s, 1H). MS: 583.2 $(M+H)^+$, Method ESI^+.

Microbiological methods

Minimal inhibitory concentrations (MICs) were determined by a microdilution method following NCCLS guidelines, except that IsoSensitest broth (Oxoid; Basingstoke, UK) was used.[19] The medium was supplemented with 3% lysed horse blood for fastidious organisms. Serial dilutions in 96-well plates were prepared with the help of a Biomek 2000 robot. The pH of the test medium was 7.2–7.3, unless otherwise stated. The bacterial strains used were from the Morphochem collection. Clinical isolates were originally obtained from the Kantonspital Basel, Switzerland, and from other European and US hospitals.

In vitro transcription/translation assay

Inhibition of cell free protein synthesis was determined with a coupled transcription/translation assay (*Escherichia coli* S30 Extract System, Promega, Madison, WI, USA) using the plasmid pBestLuc as DNA template.[20] After pre-incubation for 10 min, the reactions were initiated by adding 0.1 μg of plasmid DNA and incubated at 37 °C for 35 min. Then, the reaction was stopped by cooling in ice, 15 μL was added to 15 μL luciferase substrate (Bright Glo, Promega, Madison, WI, USA), and luminescence was quantified in a Tecan Spectrafluor Plus plate reader.

Topoisomerase assays

Inhibition studies on *E. coli* DNA gyrase and topoisomerase IV were performed by published procedures.[21,22] Compounds were tested initially at a wide range of concentrations, which were then fine-tuned to determine exact IC_{50}s and confirm the results. Compounds were serially diluted in water, re-heating gently where required, to solubilize them, and added to the reaction before the addition of the enzyme. Briefly, DNA gyrase activity was measured in a supercoiling assay using relaxed pBR322 DNA and supercoiled pBR322 DNA was used for the topoisomerase IV relaxation assay. The IC_{50} for gyrase supercoiling was visually assessed as the concentration of compound, which led to a 50% reduction of the supercoiled band and a spread of topoisomers above. The IC_{50} for relaxation was determined visually, as being the compound concentration at which the relaxed band was reduced by 50% and a supercoiled band with topoisomers appeared.

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