Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 4229–4233

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure–Activity Relationship in the Oxazolidinone–Quinolone Hybrid Series: Influence of the Central Spacer on the Antibacterial Activity and the Mode of Action

Christian Hubschwerlen,* Jean-Luc Specklin, Daniel K. Baeschlin, Yves Borer, Sascha Haefeli, Christine Sigwalt, Susanne Schroeder and Hans H. Locher

Morphochem AG, Schwarzwaldallee 215, CH 4058 Basel, Switzerland

Received 8 May 2003; accepted 14 July 2003

Abstract—Oxazolidinone–quinolone hybrids, which combine the pharmacophores of a quinolone and an oxazolidinone, were synthesised and shown to be active against a variety of susceptible and resistant Gram-positive and Gram-negative bacteria. The nature of the spacer greatly influences the antibacterial activity by directing the mode of action, that is quinolone- and/or oxazolidinone-like activity. The best compounds in this series have a balanced dual mode of action and overcome all types of resistance, including resistance to quinolones and linezolid, in clinically relevant Gram-positive pathogens.

The increasing emergence and spread of multi-drug resistant Gram-positive bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA), penicillin- and macrolide-resistant *Streptococcus pneumoniae* and vancomycin-resistant enterococci represent a major threat in hospital settings.[1] Resistance to quinolones [e.g., ciprofloxacin **1** (CIP); Chart 1] is common and strains resistant to the newly introduced oxazolidinone linezolid **2** (LZD) have already been observed in clinical isolates of *S. 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.

The oxazolidinone–quinolone hybrids **3** which simultaneously act on two different cellular functions, DNA replication (DNA gyrase and topoisomerase IV) and protein synthesis, offer such an opportunity.[4] Initial representatives in this series have shown that modification of the two pharmacophores followed the overall general trend observed in the quinolone and oxazolidinone series. Notably, compounds **3a** and **3b** (Table 1), which differ only in the nature of the central spacer, displayed markedly different antibacterial activities. The present contribution describes our efforts to understand the influence of the spacer on the antibacterial activity as well as the mode of action and our attempts to obtain active compounds with a balanced dual mode of action.

Initial modifications of the spacer led to compounds with either poor activities or to the loss of one of the two modes of action (data not shown). However, a cluster of amino alcohol spacers gave compounds with interesting biological activities and was extensively

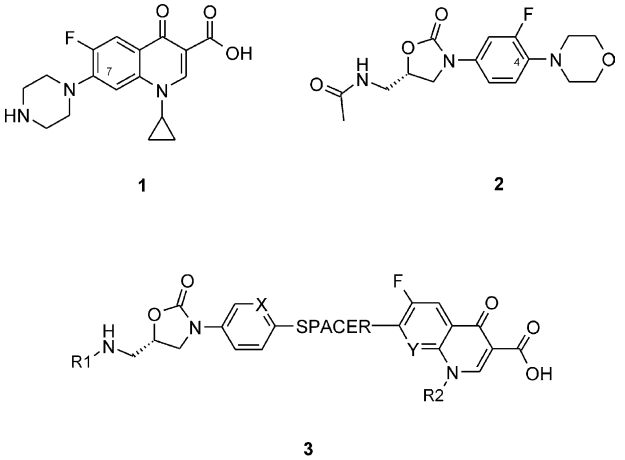


Chart 1.

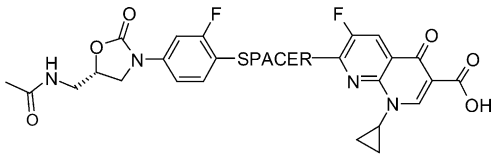
*Corresponding author. Tel.: +41-61695-2103; fax: +41-6195-2122; e-mail: christian.hubschwerlen@morphochem.ch

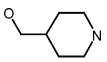
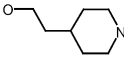
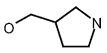
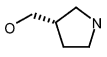
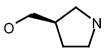
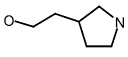
0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.07.028

studied. The oxazolidinone–quinolone hybrids **3c**–**n** (Table 1) were synthesised as described in Scheme 1 following a reported procedure.[4] 3,4-Difluoronitrobenzene (**4**) was reacted with the appropriate *N*-benzyloxycarbonyl protected amino alcohols. Zinc-mediated reduction of the nitro group and protection of the resulting amine with benzyl chloroformate led to compounds **5c**–**n**. The oxazolidinone ring was constructed as described for the synthesis of linezolid.[5] Finally, removal of the remaining protecting group and subsequent reaction with the commercially available 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-[1,8]-naphthyridine-3-carboxylic acid gave the corresponding target compounds **3c**–**n**. All the chemical structures of the compounds obtained were confirmed by ^{1}H NMR and mass spectra and the purity was demonstrated by HPLC analysis. The required benzyloxycarbonyl-protected amino alcohols were either commercially available or prepared according to published procedures.[6–13]

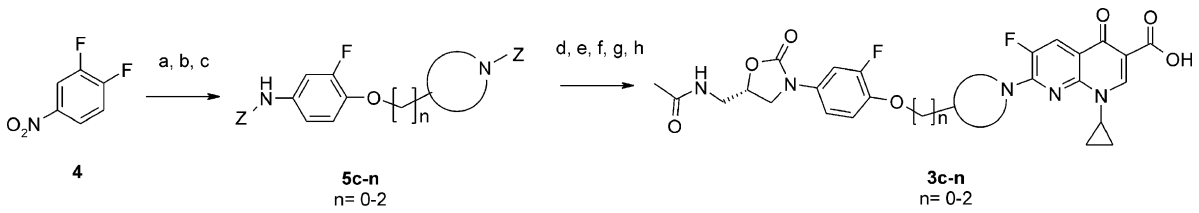
The oxazolidinone–quinolone hybrids **3a**–**n** were tested for in vitro antibacterial activity against a panel of well

Table 1. Antibacterial activities of oxazolidinon–equinolone hybrids against selected strains (MICs in μg/mL)



Compd	Spacer	Bacterial strain[a]							
		Sau1	Sau2 CIP^r	Sau3 LZD^r	Efs	Efm CIP^r	Hin	Eco1	Eco2 perm
1 (CIP)		0.5	>32	0.5	1	>32	≤0.03	≤0.03	≤0.03
2 (LZD)		2	1	64	2	2	8	>64	8
3a		0.25	0.25	8	0.125	0.25	1	32	0.06
3b		≤0.06	1	≤0.06	≤0.06	8	≤0.06	0.5	≤0.06
3c		0.25	0.5	≤0.06	0.5	2	0.125	1	≤0.06
3d		1	0.5	0.5	0.5	2	0.5	8	≤0.06
3e		≤0.06	0.25	≤0.06	0.125	1	≤0.06	1	≤0.06
3f		0.25	0.25	1	0.125	0.25	1	32	0.25
3g		32	16	16	16	>32	2	>32	8
3h		0.5	0.25	0.5	0.25	0.5	1	8	≤0.06
3i		0.125	0.125	1	≤0.06	0.125	0.5	>32	0.125
3j		0.25	0.125	1	0.125	0.125	2	>32	≤0.06
3k		0.125	0.125	≤0.06	0.125	0.25	0.125	1	≤0.06
3l		0.125	0.125	0.125	0.06	0.125	0.25	1	≤0.06
3m		≤0.06	0.25	≤0.06	0.125	0.5	0.125	1	≤0.06
3n		0.125	0.125	0.125	0.06	0.125	0.5	2	≤0.06

[a]Sau1, *S. aureus* ATCC 29213 (CIP and LZD susceptible); Sau2, MRSA, CIP resistant (gyrA: Ser-84-Leu, grlA: Ser-80-Phe); Sau3, *S. aureus* LZD resistant (23S rRNA: G2447T); Efs, *Enterococcus faecalis* ATCC 29212; Efm, *E. faecium* vancomycin and CIP resistant; Hin, *Haemophilus influenzae* 11; Eco1, *Escherichia coli* ATCC 25922; Eco2, *Escherichia coli* AS19, permeable mutant.



Scheme 1. Synthesis of compounds **3c–n**. Reagents and conditions: (a) corresponding *N*(*Z*)-protected alcohols, NaH, THF; (b) Zn, HCl, MeOH; (c) ZCl, $NaHCO_3$, acetone; (d) *n*-BuLi, *R*-(−)-glycidyl butyrate, THF, −78 °C; (e) (1) MsCl, TEA, CH_2Cl_2, 0 °C; (2) NaN_3, DMF, 80 °C; (f) (1) PPh_3, H_2O, THF, 80 °C; (2) Ac_2O, AcOH; (g) H_2, Pd/C, MeOH–AcOEt; (h) 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-[1,8]naphthyridine-3-carboxylic acid, TEA, $ClSi(Me)_3$, NMP, 120 °C.

characterized susceptible and resistant Gram-positive and Gram-negative bacterial strains (Table 1).[14,15] This panel included *S. aureus* resistant to CIP (containing the most frequently found mutations in gyrA and grlA) as well as an in vitro selected LZD resistant strain with a commonly observed mutation in the 23S rRNA (G2447U, *Escherichia coli* numbering).[2] These strains helped us to study the influence of the spacer on the antibacterial activity and the mode of action. This panel was completed with a strain each of *Haemophilus influenzae* and *E. coli*. Additionally, an outer membrane permeable mutant of *E. coli* was included to address penetration related issues.[16] For comparison, CIP and LZD were employed as reference drugs. In order to further study the mode of action of this new class of dual action antibacterials, we measured, for selected compounds, the inhibition of protein synthesis in an in vitro transcription/translation assay and the inhibition of the enzymes that are targeted by quinolones, that is DNA gyrase and topoisomerase IV (Table 2).[17,19]

Compound **3a**, bearing a piperazinyl spacer, displayed a strong oxazolidinone character although retaining some quinolone-like activity; it retained good activity against the CIP-resistant strains while it was less active against the LZD-resistant *S. aureus* strain. The enzyme assays confirmed the observed antibacterial activity pattern in that a strong protein synthesis inhibition (superior to LZD) was observed whilst the activity against the topoisomerases was weak.

By contrast, compound **3b** containing a 3-amino-pyrrolidinyl spacer, behaved more like a quinolone: the LZD-resistant *S. aureus* and the Gram-negative strains were highly susceptible, whilst the CIP-resistant strains had higher MICs.

Table 2. Activities of selected oxazolidinone–quinolone hybrids against topoisomerases and as inhibitors of protein synthesis

Compd	DNA gyrase[a] IC_{50} (μM)	Topo IV[b] IC_{50} (μM)	Protein synthesis[c] IC_{50} (μM)
3a	50	10	2.8
3f	50	5	5.2
3e	10	3	6.7
3k	2	2	1.5
LZD	NT	NT	4.1
CIP	0.5	2.5	>20

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

Compounds **3c–e**, containing a 3-hydroxy-azetidinyl or a 3-hydroxy-pyrrolidinyl spacer displayed a balanced dual mode of action with good activity against both CIP- and LZD-resistant strains. The quinolone character seemed to be slightly dominating, since the activity against the LZD-resistant strain was higher than against the CIP-resistant strains. The stereochemistry at position 3 on the pyrrolidine had an impact by modifying the contribution of the quinolone pharmacophore. Comparing the two diastereoisomers **3d** and **3e**, the (*S*) configuration (**3e**) enhanced notably the quinolone character (increased activity against LZD-resistant *S. aureus* and Gram-negative bacteria). Compound **3e** showed good activity in the topoisomerase IV assay whilst retaining activity as protein synthesis inhibitor.

Compounds **3f** and **3h**, containing a 4-hydroxy-piperidinyl or a 4-hydroxy-azepanyl spacer exhibited a very balanced type of activity against both LZD- and CIP-resistant strains with MICs between 0.25 and 1 μg/mL. However, compared to **3d** and **3e**, activities against *E. coli* and the LZD-resistant *S. aureus* were weaker, suggesting a more oxazolidinone-like character. In agreement with these data, compound **3f** was shown to act on both protein synthesis and the topoisomerases, although activities against the latter were less than those observed for compound **3e**. The direction of the connecting vector on the piperidine was very important, since the activity could be fully abolished by using a 3-hydroxy-piperidinyl spacer (**3g**).

In a further study we explored the influence of the length of the spacer. In the piperidine series, we compared compounds **3f** with **3i** and **3j** containing a 4-hydroxymethyl- and a 4-hydroxyethyl-piperidinyl spacer, respectively. All these compounds had potent activities and differences were not significant. The same was also observed in the pyrrolidine series (**3k**,**n**). The impact of the stereochemistry at position 3 on the pyrrolidine, although less marked than with the 3-hydroxy-pyrrolidine spacer, was still noticeable. The (*R*) configuration (compound **3m**) slightly enhanced the quinolone character (increased activity against LZD-resistant *S. aureus*) while the (*S*) configuration (**3l**) enhanced the oxazolidinone character (slightly better activity against the CIP-resistant strains). The antibacterial activity of the mixture of diastereomers **3k** was, as expected, very similar to the two pure diastereoisomers **3l** and **3m**. It was more than 2-fold more potent than LZD in the in vitro protein synthesis assay and almost equivalent to CIP in the gyrase and topoisomerase IV assays.

In the absence of structural information on the complexes of quinolones with DNA gyrase and oxazolidinones with the bacterial ribosome it is difficult to rationalise these results at the molecular level. However, some observations can be made. The general shape of the molecule, though very important (e.g., **3g**), is not the only determinant factor for the mode of action since compounds **3b** and **3d** behave very differently. The weak oxazolidinone activity in **3b** can be best explained by the presence of a free NH in the spacer that abolishes the antibacterial activity [all the intermediates in the synthesis containing the oxazolidinone linked to the spacers were devoid of antibacterial activity (data not shown)]. Although quinolones bearing some of the spacers described above as substituents at position 7 are known to be active, oxazolidinones being substituted at position 4 of the fluorophenyl ring through an oxygen atom are weaker than LZD.[22] This might suggest an additional contribution of the quinolone in the binding to the ribosome. In addition to the target enzymes other factors such as bacterial penetration and efflux systems may play an important role in defining the SAR, especially for Gram-negative bacteria such as *E. coli*. Although some oxazolidinone-quinolone hybrids were reasonably active against wild type *E. coli*, they were much more active against an outer membrane permeable mutant. This indicates a restricted penetration and/or an active efflux as is observed for LZD.

In summary, a new class of oxazolidinone-quinolone hybrids with potent antibacterial activity has been identified. The antibacterial spectrum and mode of action is highly dependent on the nature of the spacer. Overall the most active derivative was **3k** (and its pure diastereoisomers **3l** and **3m)** which contains a 3-hydroxyzmethyl-pyrrolidinyl spacer. These compounds were approximately 4-fold more active than CIP, and 8- to 16-fold more active than LZD against Gram-positive bacteria. Furthermore, due to the balanced dual mode of action the best representatives overcome all types of resistance in clinically important Gram-positive pathogens, including resistance to quinolones and LZD. In addition, compared to LZD, the antibacterial spectrum is extended to Gram-negatives. Hence, this new series, which also exhibits a strong bactericidal effect and a low propensity to resistance development (data not shown), has the potential for a promising alternative treatment of severe nosocomial Gram-positive infections.

Acknowledgements

Inhibition studies on DNA gyrase and topoisomerase IV were kindly performed by A. Maxwell and A. Howells, John Innes Enterprises, Ltd, Norwich, UK.

References and Notes

1. Fridkin, S. K.; Hill, H. A.; Volkova, N. V.; Edwards, J. R.; Lawton, R. M.; Gaynes, R. P.; McGowan, J. E., Jr. *Emerg. Infect. Dis.* **2002**, *8*, 697.
2. Tsiodras, S.; Gold, H. S.; Sakoulas, G.; Eliopoulos, G. M.; Wennersten, C.; Venkataraman, L.; Moellering, R. C. *Lancet* **2001**, *358*, 207.
3. Bassetti, M.; Farrel, P. A.; Allan, D. A.; Dembry, L. M.; Opal, J. E. *Abstracts of Papers*, 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, USA, Sept. 22–25, 2001; American Society for Microbiology: Washington, DC, 2001; Poster K-1193.
4. Hubschwerlen, C.; Specklin, J.-L.; Sigwalt, C.; Schroeder, S.; Locher, H. *Bioorg. Med. Chem.* **2003**, *11*, 2310.
5. Brickner, S. J.; Hutchinson, D. K.; Barbachyn, M. R.; Manninen, P. R.; Ulanowicz, D. A.; Garmon, S. A.; Grega, K. C.; Hendges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. *J. Med. Chem.* **1996**, *39*, 673.
6. Preparation of (*S*)-3-amino-1-pyrrolidinecarboxylic acid phenylmethyl ester and (*S* and *R*)-3-hydroxy-1-pyrrolidinecarboxylic acid phenylmethyl ester Sanchez, Joseph P.; Domagala, J. M.; Heifetz, C. L.; Priebe, S. R.; Sesnie, J. A.; Trehan, A. K. *J. Med. Chem.* **1992**, *35*, 1764.
7. Preparation of 3-hydroxy-1-azetidinecarboxylic acid phenylmethyl ester: Rosenberg, S. H.; Spina, K. P.; Condon, S. L.; Polakowski, J.; Yao, Z. *J. Med. Chem.* **1993**, *36*, 460.
8. Preparation of 4-hydroxy-1-piperidinecarboxylic acid phenylmethyl ester: Cooper, C. S.; Klock, P. L.; Chu, D. T. W.; Hardy, D. J.; Swanson, R. N.; Plattner, J. J. *J. Med. Chem.* **1992**, *35*, 8 1393.
9. Preparation of 3-hydroxy-1-piperidinecarboxylic acid phenylmethyl ester: Apostolopoulos, C. D.; Haroutounian, S. A. *J. Heterocycl. Chem.* **1995**, *32*, 1843.
10. Preparation of 4-hydroxymethyl-1-piperidinecarboxylic acid phenylmethyl ester: Yoneda, Y.; Kawajiri, S.; Sugimura, M.; Osanai, K.; Kito, F.; Ota, E.; Mimura, T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2663.
11. Preparation of 4-hydroxyethyl-1-piperidinecarboxylic acid phenylmethyl ester: Mazzini, C.; Lebreton, J.; Alphand, V.; Furstoss, R. *J. Org. Chem.* **1997**, *62*, 5215.
12. Preparation of 4-hydroxypropyl-1-piperidinecarboxylic acid phenylmethyl ester: Grumel, V.; Merour, J.-Y.; Lesur, B.; Giboulot, T.; Frydman, A.; Guillaumet, G. *Eur. J. Med. Chem. Chim. Ther.* **2002**, *37*, 45.
13. 4-Hydroxy-1-azepinecarboxylic acid phenylmethyl ester was prepared from 4-perhydroazepinone by treatment with benzylchloroformate and subsequent $NaBH_4$ reduction Roglans, A.; Marquet, J.; Moreno-Manas, M. *Synth. Com* **1992**, *22*, 1249.
14. Minimal inhibitory concentrations (MICs) were determined in cation-adjusted Mueller–Hinton Broth (BBL) by a microdilution method following NCCLS guidelines.[15] The pH of the test medium was 7.2–7.3. 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.
15. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 4th ed.; Approved standard: NCCLS Document M7-A4; National Committee for Clinical Laboratory Standards: Villanova, PA, USA, 1997.
16. Zorzopoulos, J.; de Long, S.; Chapman, V.; Kozloff, L. M. *FEMS Microbiol. Lett.* **1989**, *52*, 23.
17. Inhibition of cell free protein synthesis was determined with a coupled transcription/translation assay (*E. coli* S30 Extract System, Promega, Madison, WI, USA) using the plasmid pBestLuc as DNA template.[18] 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.

18. Kung, P.-P.; Casper, M. D.; Cook, K. L.; Wilson-Lingardo, L.; Risen, L.; Vickers, T. A.; Ranken, R.; Blyn, L. B.; Wyatt, J. R.; Cook, P. D.; Ecker, D. J. *J. Med. Chem.* **1999**, *42*, 4705.
19. Inhibition studies on *E. coli* DNA gyrase and topoisomerase IV were performed by published procedures.[20,21] 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.
20. Reece, R. J.; Maxwell, A. *J. Biol. Chem.* **1989**, *264*, 19648.
21. Peng, H.; Marians, K. J. *J. Biol. Chem.* **1993**, *268*, 24481.
22. Wedner-Wells, M. A.; Boggs, C. M.; Foleno, B. D.; Wira, E.; Bush, K.; Goldschmidt, R. M.; Hlasta, D. *J. Bioorg. Med. Chem. Lett.* **2001**, *11*, 1829.