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From the DNA Gyrase Inhibitor Cyclothialidine to a New Class of Antibacterial Agents

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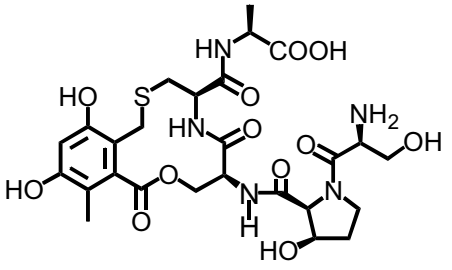
Abstract: Cyclothialidine (Ro 09-1437) is a potent DNA gyrase inhibitor that was isolated from *S. filipinensis* NR0484. In this account we describe the identification of potent antibacterials structurally derived from this virtually neat enzyme inhibitor.

Keywords: Antibacterials · Cyclothialidine · DNA gyrase inhibitor · Natural products

The identification of DNA gyrase [1] as the biological target of the quinolones (gyrase A inhibitors) [2], *e.g.* ciprofloxacin, and of the coumarin antibiotics (gyrase B inhibitors) [3], *e.g.* novobiocin, aroused a general interest in inhibitors of this enzyme as potential antibacterial drugs. By screening microbial broths for the *in vitro* inhibition of the supercoiling activity of DNA gyrase, cyclothialidine (**1**, Ro 09-1437) was discovered and isolated in low yield from the fermentation broth of *S. filipinensis* NR0484 [4]. The structure of **1** features a 12-membered lactone ring that is fused to a highly substituted benzene ring and partly incorporated in a pentapeptide chain. Cyclothialidine was found to be a potent inhibitor of DNA gyrase and its mode of action was shown to be inhibition of the ATPase activity conferred by the B subunit of DNA gyrase [5][6]. Although hardly exhibiting any growth-inhibitory activity against intact bacterial cells probably due to poor cellular permeation, **1** was considered to be a promising lead structure whose chemical modification might open up a route to a new class of antibacterials. In this account we describe how we developed out of the virtually neat enzyme inhibitor **1** congeners of potent antibacterial activity.



Cyclothialidine (**1**)

In order to explore and exploit the antibacterial potential of **1**, we have applied a strategy addressing the following major tasks.

1. Development of a Flexible Synthetic Scheme Allowing the Preparation of Many Analogues (Scheme 1)

Accordingly, a cysteine derivative **IV** is benzylated either by using a bromide **II** in the presence of base in an alkylative thiolation reaction, *viz. e.g.* the total synthesis of cyclothialidine [7], or alternatively by coupling with a benzaldehyde **III** in a reductive thiolation with triethyl silane/trifluoroacetic acid [8]. The latter reaction represents a key step in the preparation of derivatives of the most potent 12-methoxy series (*e.g.* compounds **3**-**12**). The amine **V** is then coupled with a carboxylic acid **VI** and the formation of the bicyclic lactone is finally accomplished by lactonization of an ω-hydroxy acid under Mitsunobu conditions.

2. Investigation of the Structural Requirements for Gyrase Inhibitory Activity (Scheme 2)

With the lactone **2** we had identified a first active analogue, indicating that the peptidic side-chains of **1** were not important for activity. By individually disconnecting the five substituents of **2**, we learned that only the 14-hydroxy group, *i.e.* the partial structure **VIII**, was a prerequisite for the biological activity. The fact that the 14-hydroxy substituent could not be replaced by either an amino or a methoxy group was explained later on by X-ray structures [9a,b] displaying the exact binding mode of **1** to the subunit B of DNA gyrase (Fig.).

The variation of the lactone ring size revealed that a gyrase inhibitory activity can be found for 11- to 16-membered lactones and that the 14-membered lactone **6** is the most potent one. Not surprisingly, we eventually also identified *seco*-compounds, *e.g.* **9**, that inhibited DNA gyrase [11b]. As a consequence, the 'minimal structure' **IX** served as structural basis of the chemical programme [10][11a,b].

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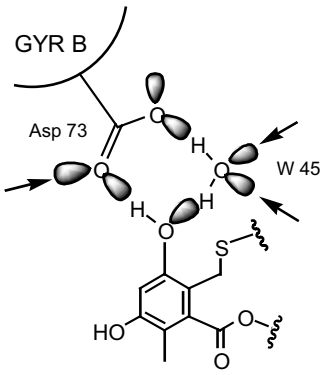

Fig. Binding mode of cyclothialidine at the DNA gyrase B protein

3. Optimization for *in vitro* Activity (Scheme 3, Table)

Whereas the truncated lead structure **2** exhibited only borderline activity, systematic variation of its substituents and combination of the best groups – in particular the 12-methoxy group, the thiolactam function and the oxadiazole entity – led to compounds of remarkable potency and the improved enzyme inhibitory property of the 14-membered lactones augmented the antibacterial activity even more. Thus, congeners of **1**, such as **10**, do not inhibit typical Gram-negative strains – they cover, however, broadly the spectrum of Gram-positive bacteria and their potency exceeds that of novobiocin and vancomycin.

4. Optimization for *in vivo* Efficacy (Scheme 3, Table)

A first series of analogues displaying considerable *in vitro* activity turned out to be inefficacious in a septicaemia model in mice due to unfavorable pharmacokinetics. Their rapidly decreasing plasma concentrations were attributed to glucuronidation of the phenolic parent compounds as well as to their distinct lipophilic properties. The introduction of hydrophilic groups, *e.g.* a hydroxymethyl entity at the 7β-position, or an amino function at the methyl-oxadiazole, of **10**, indeed afforded *in vivo* active compounds (**11**, **12**) showing ED_{50}s of 8–12 mg/kg.

Thus, we have demonstrated that the gyrase inhibitory principle contained in cyclothialidine can be considered as the basis for a new class of antibacterial agents. It remains, however, to be shown if this class can provide clinically useful drugs.

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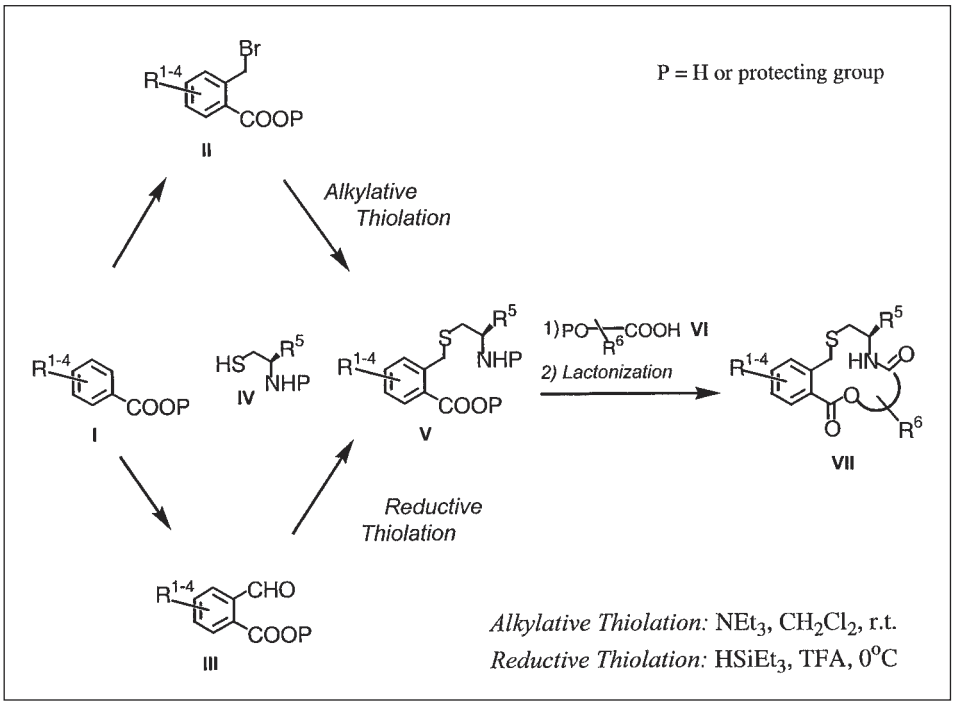
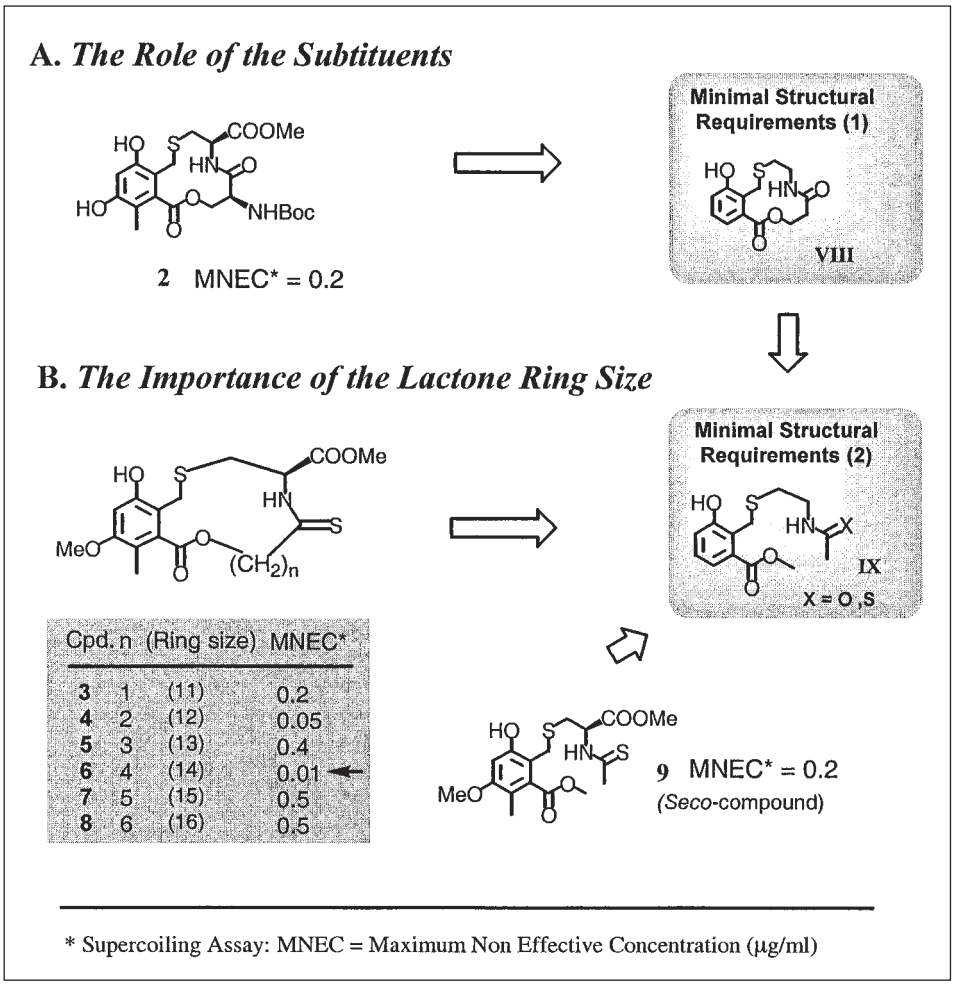
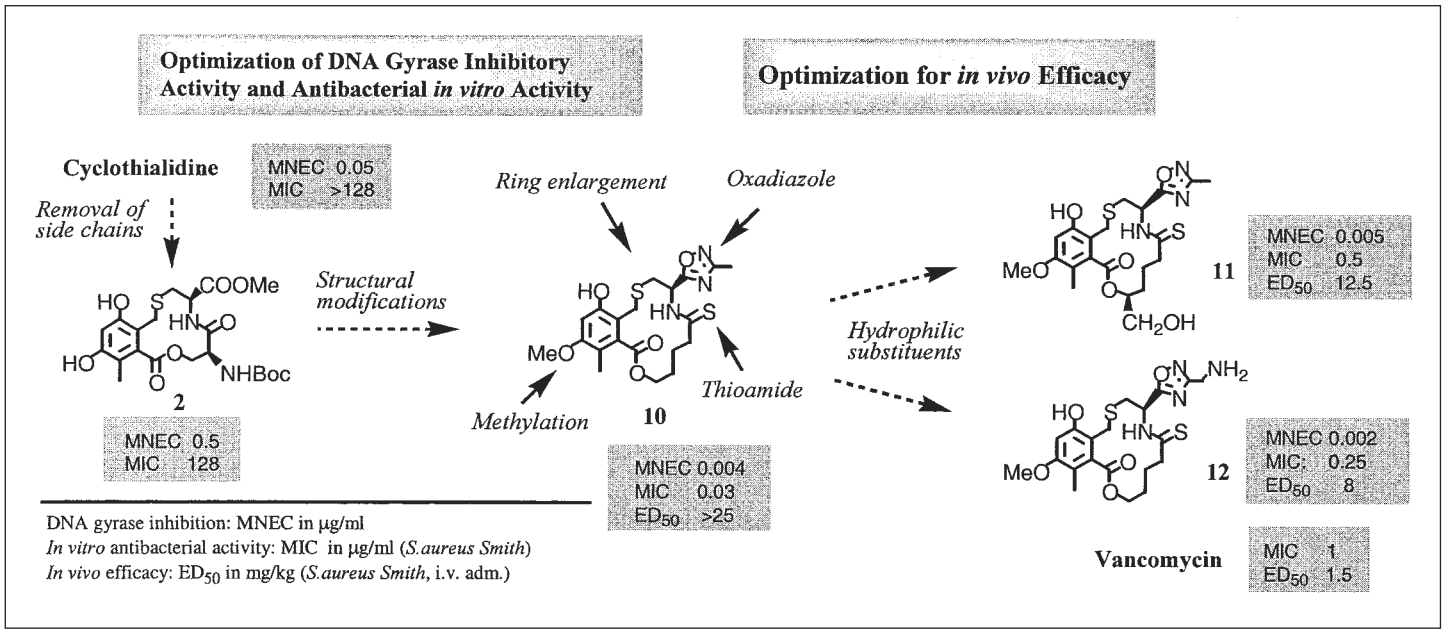


Scheme 1. Synthetic routes to cyclothialidine analogues



Scheme 2. Structural requirements for DNA gyrase inhibition



Scheme 3. Major development steps of the cyclothialidine programme

Table. Antibacterial activity of cyclothialidine congeners

	10	**11**	**12**	**Novobiocin**	**Vancomycin**
DNA Gyrase Inhibition: MNEC (µg/ml)[1)]	0.004	0.005	0.002	0.1	-
Antibacterial Activity *in Vitro*: MIC (µg/ml)[2)]					
Escherichia coli ATCC 25922	> 64	> 64	64	> 64	> 64
Xanthomonas maltophilia IAC 739	0.5	4	4	> 64	> 64
Staphylococcus aureus 25923	0.12	0.5	0.5	0.25	1
Staphylococcus aureus Smith	0.03	0.5	0.25	0.12	1
Staphylococcus epidermidis ATCC 14990	0.016	0.12	0.12	0.25	2
Enterococcus faecalis 6	0.25	0.25	0.5	8	2
Streptococcus pyogenes b15	0.25	0.5	0.5	2	0.5
***In Vivo* Efficacy : ED_{50} (mg/kg)[3)]**	> 25	12.5	8	3	1.5

1) Supercoiling assay. MNEC = Maximum non effective concentration. 2) Agar dilution (Mueller-Hinton medium). Inoculum 10^4 CFU/spot. MIC = Minimum inhibitory concentration. 3) Septicaemia in mice, *S. aureus* Smith, i.v.

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