CHEMMEDCHEM

DOI: 10.1002/cmdc.200800117

New DNA Polymerase IIIC Inhibitors: 3-Subtituted Anilinouracils with Potent Antibacterial Activity in vitro and in vivo

Niels Svenstrup,* Kerstin Ehlert, Christoph Ladel, Alexander Kuhl, and Dieter Häbich[a]

The development of resistance has rendered several antibiotics clinically ineffective, and there is an urgent medical need for potent and safe antibacterials with a novel and valid mode of action. To avoid cross-resistance, they should preferably inhibit targets that are not addressed by established antibiotics. In this respect, 6-anilinouracils represent a promising lead structure. They target the Gram-positive DNA polymerase IIIC, a target that is associated with a bactericidal mode of action. Moreover, they have no cross-resistance to marketed antibiotics. This paper describes the synthesis and biological characterization of structurally novel anilinouracils, some of which display potent in vivo efficacy in murine models of bacterial septicemia.

Introduction

Efficient antibiotics[1] are one of the major achievements of pharmaceutical research and development, assessed on the basis of the contributions of antibiotic medicines to human health and quality of life.[2] Antibiotics have saved millions of lives and eased patients' suffering. Often they have been perceived as "wonder-drugs", and until recently public opinion tended to regard mortality and morbidity caused by infectious diseases as things gone by.[3]

Bacteria, however, are able to overcome antibiotic pressure by a variety of smart mechanisms that facilitate the selection of resistant organisms.[4] Antibiotic-resistant Gram-positive bacteria represent a growing challenge: the efficacy of once powerful drugs such as the β-lactams and the macrolides is continually decreasing due to the emergence of multi-resistant bacteria.[5] These pathogens carry resistance genes that spread in the bacterial population across geographic and interspecies boundaries.[6]

Nonetheless, more and more big pharmaceutical companies are exiting the antibacterial field and the pipeline for new antibiotics is running dry.[7] Only a few new antibiotics have been introduced to the market in recent years: the oxazolidinone linezolid,[8] the ketolide telithromycin,[9] the lipopeptide daptomycin,[10] and the minocycline derivative tigecycline.[11] Except daptomycin, they all target bacterial protein biosynthesis.

Development of resistance is the inevitable result of antibiotic use, and it limits the efficacy and life span of every antibiotic. In fact, the first clinical cases of resistance development against linezolid were reported soon after its introduction.[12] Correct antibiotic use will slow down resistance, but cannot prevent it. Only the persistent discovery and development of new antibiotics will guarantee future therapy.[13] Established antibiotics use only a limited set of mechanisms,[14] therefore new structural classes of antibiotics that address novel and valid targets are urgently needed.[15]

At present, 6-anilinouracils represent the most promising lead structure for targeting the Gram-positive DNA polymerase IIIC.[16–18] This compound class evolved from aryldiazouracils and their corresponding arylhydrazine uracils **1** (Figure 1). Several cycles of medicinal chemistry optimization have led to compounds such as **2** and **3** with an improved physicochemical and biological profile. The valine ester **3**, a prodrug of the corresponding alcohol, was designed to improve aqueous solubility and to facilitate intravenous application.[19]

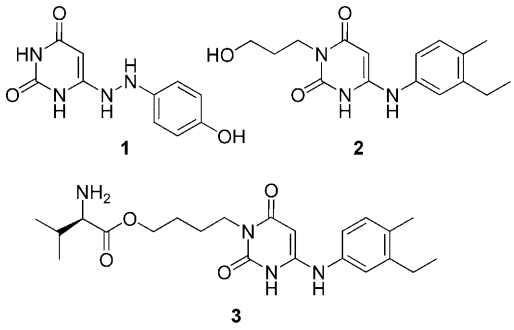


Figure 1. Published DNA Pol IIIC inhibitors of the anilinouracil class.

DNA polymerase IIIC (DNA Pol IIIC) is the polymerase that is required for the replication of chromosomal DNA in Gram-positive bacteria with low guanidine and cytosine content,[20,23] and thus represents a highly interesting target for the therapy of some of the microorganisms that are most commonly associated with nosocomial infections today.

DNA Pol IIIC has little homology with the mammalian DNA polymerase α or the Gram-negative DNA polymerase III, and consequently DNA Pol IIIC inhibitors are expected to specifical-

[a] *Dr. N. Svenstrup, Dr. K. Ehlert, Dr. C. Ladel, Dr. A. Kuhl, Dr. D. Häbich*
Global Drug Discovery, Bayer Schering Pharma
Bayer HealthCare AG, 42096 Wuppertal (Germany)
Fax: (+49) 202 364061
E-mail: Niels.Svenstrup@BayerHealthcare.com

ly kill Gram-positive bacteria with no unwanted interactions with the mammalian cellular replication machinery.[21] Anilinouracils show a bactericidal mode of action and address a novel target that is not covered by any antibiotic on the market or in clinical development. Herein we detail our progress in the novel class of anilinouracils, inhibitors of the Gram-positive DNA Pol IIIC.[22]

Results and Discussion

Synthetic chemistry

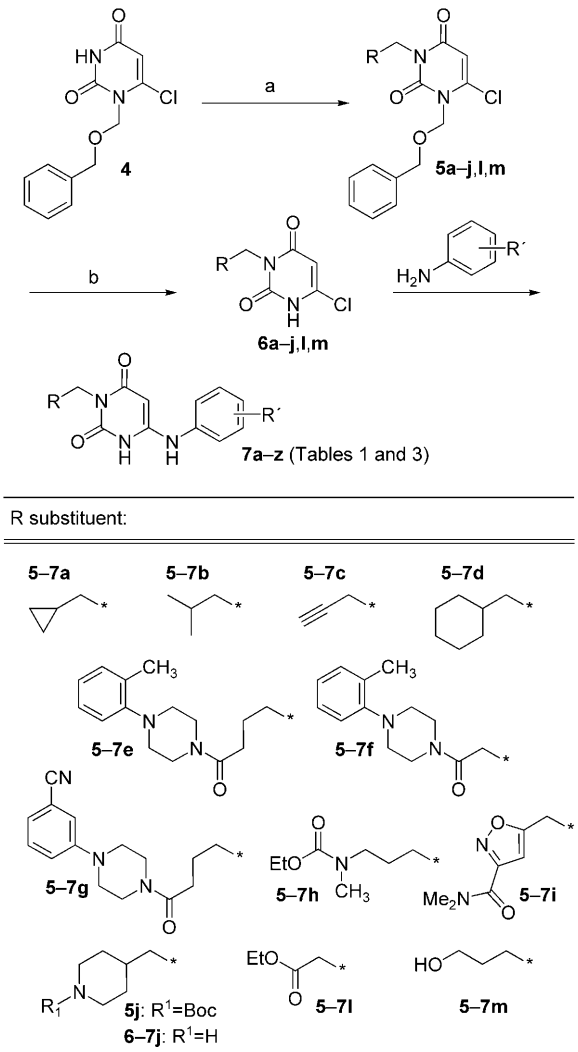
We were particularly interested in exploring the structure–activity relationship (SAR) of the 3-substituent and in re-investigating the SAR of the aryl part of the aniline moiety. The chemistry for the incorporation of substituted anilines was well established.[20,23] The available methods for the synthesis of 3-substituted anilinouracils, however, were limited and cumbersome; this prompted us to develop a new synthetic methodology.[25]

Synthesis by protecting group strategy

In general, the selective N3 alkylation of uracils is not possible directly, because the N1 position tends to be more reactive.[24] To overcome this problem, we recently developed a protecting group strategy for the synthesis of 3-alkyl-substituted anilinouracils.[25] This strategy is considerably more convenient than the reported four-step synthesis,[26] which involves the conversion of an amine via an urea into a barbituric acid by heterocyclization with diethyl malonate. Subsequent chlorination with phosphorous oxychloride followed by nucleophilic replacement with an aniline produces the desired anilinouracil.

Wright et al. addressed some of these shortcomings in a much improved two-step synthesis via alkylation of 2-methoxy-6-amino-4-pyrimidone and following one-pot arylamination and demethylation.[27] The yield and regioselectivity in the first step is generally poor however, and a demanding chromatographic separation is usually involved, which makes this method less useful for medium-throughput medicinal chemistry. Our approach, based on the alkylation of a 1-benzyloxymethyl-protected derivative of 6-chlorouracil, proved to be general, and could be employed for the preparation of anilinouracils that bear a broad variety of functional groups (Scheme 1).

1-Benzyloxymethyl-6-chlorouracil **4**[28] was alkylated with alkyl halides in *N*,*N*-dimethylformamide (DMF) by using cesium carbonate as the base in good yields. Alternatively, a Mitsunobu protocol could be used by employing neutral conditions that were compatible with base-sensitive functional groups.[29] Deprotection of derivatives **5** was achieved with anhydrous trifluoroacetic acid (TFA) under conditions that selectively hydrolyze the benzyloxymethyl group without affecting esters and other hydrolytically sensitive groups (although acetals are cleaved in most cases). The resulting 3-substituted 6-chlorouracils **6** could conveniently be isolated by crystallization.

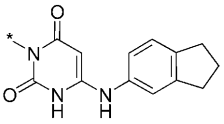
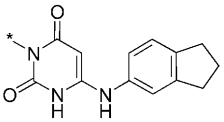
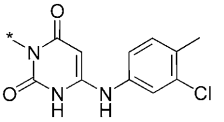
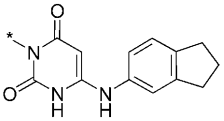
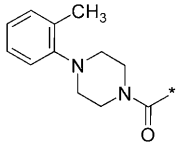
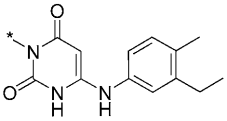
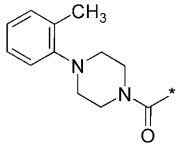
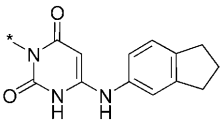
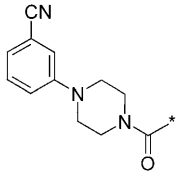
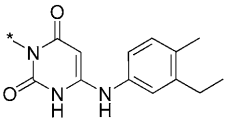
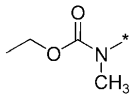
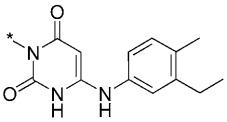
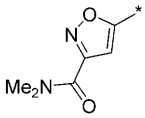
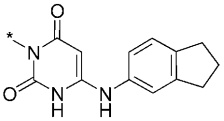
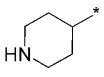
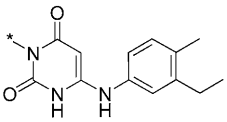
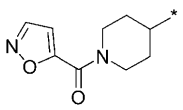
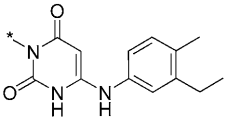
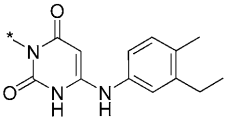


Scheme 1. Novel and improved synthesis of antibacterial anilinouracils. Reagents and conditions: a) RCH_2X (X = Cl, Br, I, or OTs), Cs_2CO_3, DMF; b) TFA, Δ.

In a final step, the aniline moiety was introduced by nucleophilic substitution (Scheme 1) as described in the reported synthesis.[25] By this synthetic methodology, a large number of structurally diverse anilinouracils **7a–z** was prepared, many of which were previously synthetically inaccessible (Table 1). In addition, compound **2** (Table 1), a known DNA Pol IIIC inhibitor,[17] could readily be synthesized for the purpose of comparison, whereas its reported synthesis involved five synthetic steps.

Optimal yields were achieved with activated alkylating agents such as α-bromo esters and ketones, or with halomethyl heterocycles (compound **7i**, Table 1), but the scope is not limited to such substrates. Nonactivated alkylating agents also reacted well, as demonstrated by the congeners **7b**, **7d**, and **7h**, as well as by the synthesis of 3-hydroxypropyl-6-chlorouracil **6m**, the precursor of **2**, (obtained in 67% overall yield for the alkylation and deprotection steps). Longer reaction times were usually required here. The general synthetic sequence (Scheme 1), as illustrated by the synthesis of 3-(cyclo-

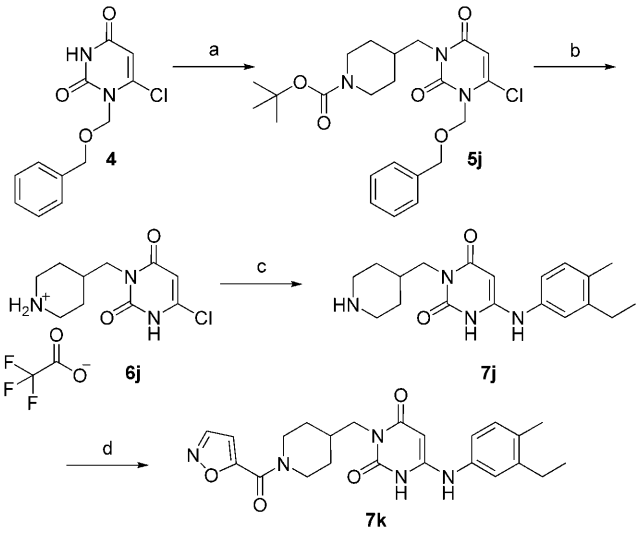
Table 1. Effect of 3-substitution on antibacterial potency.[a]

Compound	Function	Spacer	Core Moiety	IC_{50} [nM][b]	MIC [μg mL^{-1}][c]
7a		–CH_2–		300	4/4/16
7b		–CH_2–		1000	64/16/64
7c		–CH_2–		800	16/16/64
7d		–CH_2–		2500	64/32/64
7e		–$(CH_2)_3$–		300	8/64/4
7f		–CH_2–		13000	>256/64/>256
7g		–$(CH_2)_3$–		100	4/32/4
7h		–$(CH_2)_3$–		5	8/16/8
7i		–CH_2–		1200	32/16/32
7j		–CH_2–		900	32/128/256
7k		–CH_2–		100	16/8/8
2		–$(CH_2)_3$–		300	8/8/16

[a] Structures **7a–k** according to Figure 3. [b] DNA Pol IIIC activity was assayed by using DNA Pol IIIC from *S. aureus*. [c] Values reported for *S. aureus* / *S. pneumoniae* / *E. faecalis*; reference antibiotic: linezolid (MIC *S. aureus* 1 μg mL^{-1}, MIC *S. pneumoniae* 2 μg mL^{-1}, MIC *E. faecalis* 2 μg mL^{-1}).

propylmethyl)-6-(2,3-dihydro-1*H*-inden-5-ylamino)uracil **7a**, via **5a** and **6a**, is described in the Experimental Section.

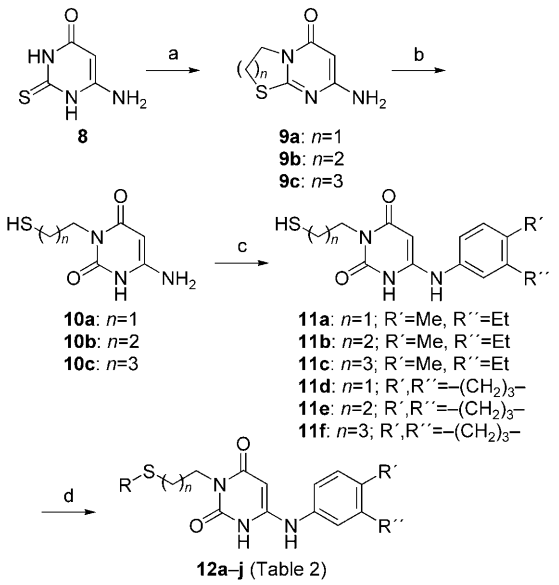
The Mitsunobu alkylation generally proceeded more slowly but in good yields. Reaction times of 24 h or more were often required (Scheme 2). The same synthetic method was used to prepare building blocks for further functionalization such as the piperidine **7j**, which could be acylated under standard conditions by using 1-benzotriazolyloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) in tetrahydrofuran (THF) (Scheme 2).[29]



Scheme 2. Alkylation of **4** by the Mitsunobu protocol. Reagents and conditions: a) DEAD, PPh_3, 72 h, RT, THF; b) TFA, Δ; c) 3-ethyl-4-methylaniline·HCl, iPr$_2$EtN, 150 °C, 2 h; d) isoxazole-5-carboxylic acid, PyBOP, iPr$_2$EtN, THF, RT, 24 h.

Thioethers

For probing distal bonding interactions around the central binding pocket of DNA Pol IIIC, we were interested in a straight-forward synthesis of 6-anilinouracils that bear aromatic and heterocyclic rings, and various functional groups that were connected to the uracil core by flexible linkers such as alkyl chains. For this purpose, we could not apply our benzyloxymethyl (BOM) protecting group strategy because the requisite (hetero)arylalkyl halides or (hetero)arylalkyl alcohols with linkers longer than two carbon atoms were not readily available. Instead, we decided to explore the SAR of distal substituents that were linked to the uracil 3-position by means of a thioether tool (Scheme 3), because this would be advantageous in several ways: a) by allowing us to use halomethyl derivatives of heterocycles and aromatic compounds as the commercially available starting materials; b) by providing an operationally simple and high-yielding synthesis that was suitable for a parallel chemistry setting (thiol alkylation); and c) by allowing access to a broad variety of chain lengths. In medicinal chemistry, thioethers are generally considered to be problematic due to their susceptibility to oxidative metabolism and the resulting high clearance in vivo. Therefore, the use of a thioether



Scheme 3. Synthesis of anilinouracils with thioether-linked 3-substituents. Reagents and conditions: a) $Br(CH_2)_{n+1}Br$, Cs_2CO_3, DMF; b) $NaOH_{(aq)}$, Δ; c) 3,4-disubstituted aniline·HCl (2 equiv), iPr$_2$EtN (1 equiv), 160 °C, 6 h; d) Cs_2CO_3 or DBU, alkyl halide, DMF, RT, 1 h.

function was intended only as a tool for rapid optimization of DNA polymerase inhibition in vitro. Potent derivatives could then be resynthesized with the corresponding metabolically stable alkyl linkers. Incidentally though, derivatives such as **12b** exhibited low-to-medium clearance in vivo, which is similar to the pharmacokinetic behavior of the corresponding amide or alkyl derivatives.

The 6-amino-3-thioalkyluracils **10a–c** were prepared by an adaptation of a published synthesis.[30] Commercially available 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **8** was treated with a dibromoalkane in DMF using cesium carbonate as the base to produce a heterobicyclic compounds **9a–c**, which were hydrolyzed with sodium hydroxide to give the thiols **10a–c** after acidification in high yield (Scheme 3). The aniline moiety was introduced by treatment with a mixture of the aniline and its corresponding hydrochloride salt at 160 °C for 6 h in a very clean reaction; alternatively, two equivalents of the aniline hydrochloride and one equivalent of *N*,*N*-diisopropylethylamine could be used. The resulting thioalkylanilinouracil **11a–f** could be further functionalized by alkylation with a variety of alkylating agents in good-to-excellent yields. Owing to the pronounced reactivity of the highly nucleophilic thiol, less-active alkylating agents such as epoxides and secondary alkyl halides could also be used, and even a number of aromatic nucleophilic substitutions were possible (e.g., with 1,3,4-thiadiazole-2-halides, 1,3-thiazole-2-bromide or pyridine-2-halides). Cesium carbonate worked well as a base, but for library syntheses using parallel chemistry we preferred the use of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) because it allowed precise dosing as a solution in DMF by using an automated liquid handler.

This synthetic route provides access to compounds **11a** and **11d** with ethylene, **11b** and **11e** with propylene and **11c** and

11 f with butylene spacers, respectively. The yields of the butylene-linked compounds were generally low however, apparently due to the formation on an unfavored seven-membered ring in the intermediate **9 c**. The C5-linked congeners were not prepared.

Aryl variations

Published synthetic efforts toward anilinouracils have focused on three distinct substituted aniline motifs: 3-ethyl-4-methylphenyl, 2,3-dihydro-1*H*-inden-5-yl, and 3-chloro-4-methylphenyl as shown in Figure 2. We were interested in re-investigating the reported SAR of this part of the molecule, and chose to prepare a structurally related array of 3,4-disubstituted anilines for incorporation into the anilinouracil. For comparability, we decided to employ the 3-(ethoxycarbonyl)methyl substituent for all compounds in this series.

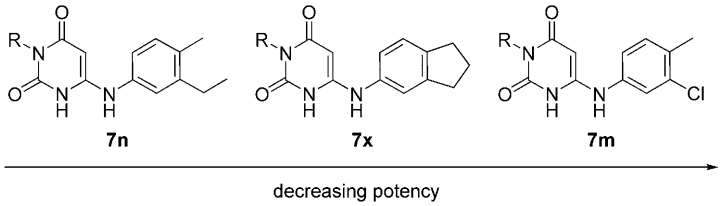
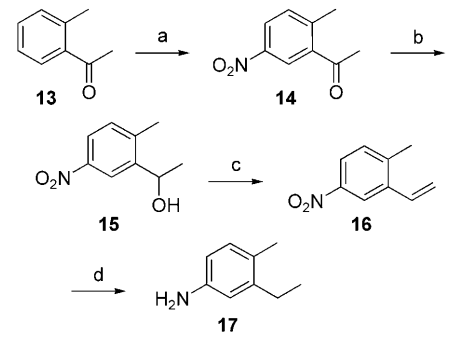


Figure 2. Commonly employed aniline moieties that were optimized for antibacterial potency; R = $EtOOCCH_2$.

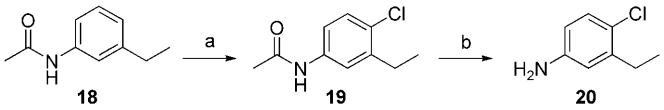
The anilines that were used for this purpose were in most cases prepared by reduction of the corresponding nitroarenes, which themselves were easily available by nitration of substituted benzene derivatives. Amongst the various other anilines that were synthesized, the following illustrate important synthetic techniques:

3-Ethyl-4-methylaniline **17** was previously prepared[31] by a Friedel–Crafts acetylation of *N*-acetyl 4-methylaniline, followed by Wolff–Kishner reduction of the keto group, and hydrolysis of the amide to give the free aniline. In our hands this synthesis generally produced poor yields. We chose to prepare **17** in a four-step sequence based on scalable chemistry (Scheme 4): commercially available 2′-methylacetophenone **13** was selectively nitrated in the 5-position to give 2′-methyl-5′-nitroacetophenone **14** in 36% yield. Reduction of the ketone with sodium borohydride was achieved in nearly quantitative yield to give 1-(2-methyl-5-nitrophenyl)ethanol **15**. The alcohol was treated with catalytic *p*-toluenesulfonic acid to give 2-methyl-5-nitrostyrene **16**, which yielded 3-ethyl-4-methylaniline **17** upon catalytic reduction over palladium on charcoal. The aniline is sensitive to oxidation, and is most conveniently isolated as the hydrochloride salt. This synthesis worked well on a 200 g scale with only minor modifications (by using distillation for the purification of steps a and c).

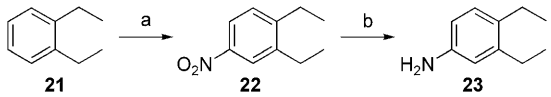
We prepared the known[32] 4-chloro-3-ethylaniline hydrochloride **20** in two steps from commercially available *N*-acetyl-3-ethylaniline **18** by selective 4-chlorination with HCl and *m*-chloroperbenzoic acid in DMF to give **19**, followed by hydrolytic removal of the acetyl protecting group in high yield (Scheme 5). The remaining anilines, when not commercially available, were prepared by a two-step nitration–reduction sequence as illustrated in Scheme 6 by using the synthesis of 3,4-diethylaniline **23** as an example. Frequently the nitrations proceeded with poor regioselectivity, in which case the isomeric nitrobenzenes had to be separated by column chromatography on silica gel. The substituted anilines **7l–z** were then incorporated into the anilinouracil by reaction with ethyl (4-chloro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)-acetate **6l**[33] at high temperature (Scheme 7).



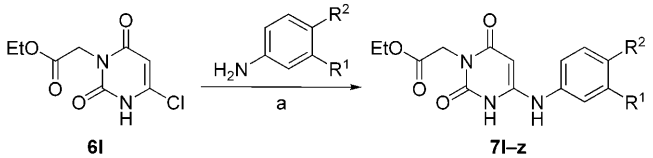
Scheme 4. Synthesis of 3-ethyl-4-methylaniline hydrochloride **17**. Reagents and conditions: a) HNO_3, H_2SO_4, 0 °C; b) $NaBH_4$, MeOH, 0 °C→RT; c) *p*-TsOH, toluene, reflux; d) 1) H_2, Pd/C, MeOH, RT, 2) HCl, Et_2O.



Scheme 5. Synthesis of 4-chloro-3-ethylaniline hydrochloride **20**. Reagents and conditions: a) HCl, *m*-CPBA, DMF; b) HCl, EtOH, reflux.



Scheme 6. Synthesis of 3,4-diethylaniline **23**. Reagents and conditions: a) HNO_3, AcOH, 10 °C; b) $HCOONH_4$, 10% Pd/C, MeOH, reflux.



Scheme 7. Synthesis of substituted aryl derivatives of anilinouracils. Reagents and conditions: a) 150–160 °C, 30–120 min.

Inhibition of DNA Pol IIIC and antibacterial properties

The uracil part of this compound class is thought to mimic the guanine moiety of dGTP, while the anilino moiety (aryl domain) presumably targets a hydrophobic pocket present only in the Gram-positive DNA polymerase III (Figure 2).[34] Binding of the phenylaminouracils most likely sequesters the enzyme into a nonproductive, reversible complex with the DNA leading to growth inhibition and cell death. Our experience has indeed shown that substitution in the base-pairing domain is detrimental to antibacterial activity, and also all tested substituents in the 5-position were inferior to hydrogen (Figure 3). Thus only the aryl ring of the aniline moiety and the 3-position of the uracil were considered for medicinal chemistry optimization.

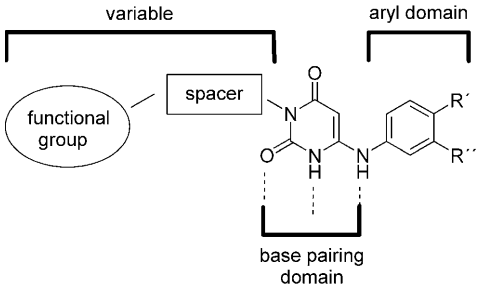


Figure 3. Basic pharmacophore model for anilinouracils.

3-Substitution

Protecting group strategy

Whereas the structure–activity relationship in the aniline moiety and the uracil part of the molecule was very narrow, a much wider array of substituents was allowed in the uracil 3-position. Small nonpolar substituents such as cyclopropylmethyl (compound **7a**), ethyl[35] or propargyl (compound **7c**) were well tolerated (Table 1), but appeared to have no substantial effect on the potency. More intricate substituents on the other hand did appear to participate in bonding interaction with the target as illustrated by compounds **7g** and **7k** which both gave IC_{50} values that approximated 100 nM. Minimum inhibitory concentrations (MIC) generally correlated with IC_{50} values, although compound **7h** appeared to be a special case, possibly an artifact: it was highly potent in the DNA Pol IIIC assay with an IC_{50} value of 5 nM, but its antibacterial activity was only slightly superior to reference compound **2**, which had an IC_{50} value of 300 nM under our assay conditions.

Irrespective of their good DNA Pol IIIC IC_{50} values, highly acidic and basic compounds such as **7j** exhibited no or decreased antibacterial activity; the same is the case for a related compound (not shown) that carried a $(CH_2)_3SO_3H$ group at N3, in spite of good target activity. Still, the polarity of the inhibitors could be fine-tuned within a certain polarity window by varying the 3-substituent without losing the antibacterial activity. Compared with **2**, isoxazole **7k** showed improved aqueous solubility and could be dosed intravenously in marked contrast to most reported DNA Pol IIIC inhibitors (except certain prodrugs specifically designed to enhance aqueous solubility).

Thioether linkage strategy

We used the thiol alkylation strategy for the preparation of a large number of 3-substituted anilinouracils; Table 2 below illustrates some of the findings. In the thioether series, it could be clearly demonstrated that certain larger 3-substituents had a higher binding affinity to DNA Pol IIIC and thus showed superior enzyme inhibition. For example, comparing compounds **12a** (3000 nM) and compound **12d** (50 nM) shows that the thiadiazole ring made a positive contribution (the N3-unsubstituted compound EMAU[37] has an $IC_{50} > 5000$ nM and MIC values of 16/64/32 under our assay conditions). Compound **12d** was about 60-fold more potent (IC_{50}), and about fourfold more active (MIC), a difference that is much greater than can be attributed to the differences in aryl substitution alone.

Turning the argument around, it is clear that the potency can be negatively influenced by the "wrong" 3-substituent. Compare compound **12b**, which is substituted with a 5-methyl-1,3,4-thiadiazol-2-yl group, with **12j**, which carries a 4-bromophenyl group, but is otherwise identical: although the two 3-substituents are sterically equivalent, **12b** is a potent compound (50 nM), but **12j** is practically inactive.

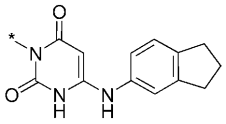
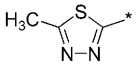
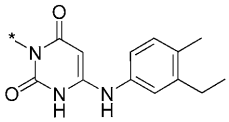
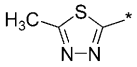
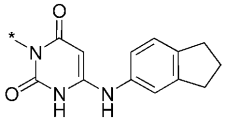
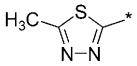
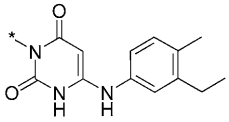
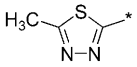
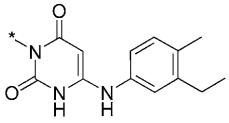
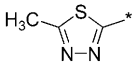
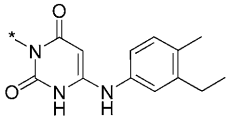
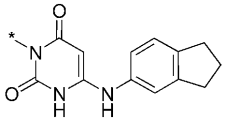
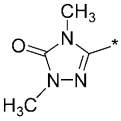
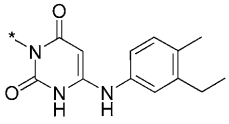
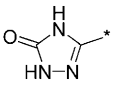
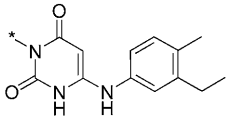
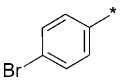
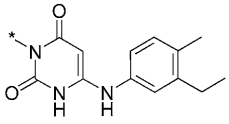
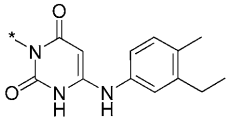
Clearly in this case the substituent is participating in an unfavorable interaction with the target, which results in an overall negative contribution to the binding energy. Thus, the 3-substituent definitely addresses a feature on the surface of the DNA polymerase III, although the nature of this feature seems less tangible than what is normally understood as a "binding pocket".

Comparing compounds **12h** and **12i** again makes it clear that the MIC value is clearly dependent on physicochemical parameters as well as the IC_{50} value; compound **12i**, although seemingly more potent in the enzyme assay, did not inhibit bacterial growth, probably because it does not penetrate well through the Gram-positive bacterial membrane. Overall, the 3-substituent plays a crucial role in determining the membrane-penetrating ability of the anilinouracils: **12b** was even active against intracellular bacteria such as *Listeria*, which grow within human cells in vitro, under conditions in which **2** and linezolid show no efficacy at all.

Aryl substituents

Although previous investigations[36] have already explored the role of aryl substitution in optimizing the antibacterial properties, we decided to look more closely at the structure–activity relationship of this moiety. The reported SAR was preferentially elucidated on the basis of MIC values, which inherently include the overlapping effects of membrane penetration and target affinity; because this can bias a compound in positive or negative ways and thus "contaminate" the SAR analysis, the inhibition of the DNA Pol IIIC from *B. subtilis*, a non-pathogenic organism, was also considered. To get an unbiased and thorough understanding of the SAR, we used our target assay to obtain

Table 2. Probing distal interactions with the 3-substituent by using a thioether building block approach.[a]

Compound	Function	Spacer	Core Moiety	IC_{50} [nM][b]	MIC [μg mL^{-1}][c]
12a		$-CH_2S(CH_2)_3-$		3000	64/64/64
12b		$-CH_2S(CH_2)_2-$		50	8/16/4
12c		$-CH_2S(CH_2)_2-$		250	32/8/16
12d		$-CH_2S(CH_2)_3-$		50	4/32/16
12e		$-CH_2S(CH_2)_4-$		13	4/8/4
12f		$-S(CH_2)_3-$		140	8/32/8
12g		$-S(CH_2)_2-$		600	64/32/64
12h		$-CH_2S(CH_2)_2-$		160	8/8/16
12i		$-CH_2S(CH_2)_2-$		70	256/128/256
12j		$-CH_2S(CH_2)_2-$		4220	>256/>256/>256
2		$-(CH_2)_3-$		300	8/8/16

[a] Structures **12a–j** according to Figure 3. [b] DNA Pol IIIC activity was assayed by using DNA Pol IIIC from *S. aureus*. [c] Values reported for *S. aureus* / *S. pneumoniae* / *E. faecalis*; reference antibiotic: linezolid (MIC *S. aureus* 1 μg mL^{-1}, MIC *S. pneumoniae* 2 μg mL^{-1}, MIC *E. faecalis* 2 μg mL^{-1}).

information regarding the direct interaction with the enzyme. In addition, we figured that because minor modifications in the aryl part of the molecule generally lead to major differences in activity, an improvement over the best aniline moiety so far (the 3-ethyl-4-methyl substitution pattern) could possibly result in significantly improved antibacterial potency. We chose to prepare this series with the 3-(ethoxycarbonyl)methyl substituent, a substituent that is associated with medium potency, to ensure that the aryl substituent would have the greatest possible effect on the overall potency within this series, thereby allowing a more precise read-out (Table 3):

The SAR in this part of the molecule was surprisingly narrow, and although we prepared a diverse number of anilines, only the 3,4-disubstituted derivatives were active. Even compound

Table 3. DNA Pol IIIC target activity and in vitro antibacterial activity against selected pathogens[a,c]

Compound	R^{1}[d]	R^{2}[d]	IC_{50} [μM][b]	MIC [μg mL^{-1}]		
				S. aureus	*S. pneumoniae*	*E. faecalis*
7l	$-CH_3$	$-CH_3$	4.7	16	32	32
7m	$-Cl$	$-CH_3$	7.0	16	8	32
7n	$-CH_2CH_3$	$-CH_3$	0.21	32	8	16
7o	$-CH(CH_3)_2$	$-CH_3$	28	>256	>256	>256
7p	$-CH_3$	$-CH_2CH_3$	30	256	>256	>256
7q	$-CH_2CH_3$	$-CH_2CH_3$	10	64	64	64
7r	$-CH_2CH_3$	$-Cl$	0.10	16	8	8
7s	$-CH_2CH_3$	$-H$	11	16	128	128
7t	$-SCH_3$	$-CH_3$	0.72	128	64	64
7u	$-CH_2CN$	$-CH_3$	14	128	64	128
7v	$-CH_2CF_3$	$-CH_3$	>100	>256	>256	>256
7w	$-(CH_2)_2-$		5.7	32	32	64
7x	$-(CH_2)_3-$		1.5	16	16	64
7y	$-(CH_2)_4-$		6.4	64	32	128
7z	$-CH_2OCH_2-$		18	>256	>256	>256

[a] Structures **7l–z** according to Scheme 7. [b] DNA Pol IIIC inhibition was assayed using DNA Pol IIIC from *S. aureus*. [c] Reference antibiotic: linezolid (MIC *S. aureus* 1 μg mL^{-1}, MIC *S. pneumoniae* 2 μg mL^{-1}, MIC *E. faecalis* 2 μg mL^{-1}). [d] R1 and R2 refer to the structure in Scheme 7.

7s, in which the methyl group was replaced by a hydrogen, was much less active than **7n**. Reversing the methyl and ethyl substituents as in compound **7p** was not tolerated. However, incorporating the 3- and 4-substituents into a ring was allowed, as was already known.[37] Apparently, a 5-membered ring such as in compound **7x** was optimal, because compound **7w** (four-membered ring) and compound **7y** (six-membered ring) were both about fivefold less active in the target assay. The SAR did not tolerate the incorporation of polar substituents into the (compound **7z**).

Replacing the methyl group of **7n** by a chlorine substituent (compound **7r**) indeed produced a small improvement over **7n**, in both the IC_{50} as well as the MIC values. This pattern represented a viable alternative to the 3-ethyl-4-methylphenyl motif.

In vivo investigations

Achieving convincing in vivo efficacy has been a notorious problem in the class of anilinouracils even with compounds that display high in vitro potency.[18] A possible explanation could either be poor pharmacokinetics, poor pharmaceutical properties (especially low aqueous solubility) or a combination of both. Indeed, in in vivo studies that are described in the literature, intraperitoneal (i.p.) dosing has been the predominant application route. In general, this route is chosen only as a "rescue" strategy for characterization of tool compounds that exhibit insufficient solubility for intravenous application.

Our initial efforts to achieve in vivo efficacy were unsatisfactory even with highly potent compounds such as **7a** or **7e.** Although **7a** exhibited good antibacterial activity against *S. aureus* (MIC = 4 μg mL^{-1}), it showed no efficacy in vivo even at a dose of 100 mg kg^{-1} dosed i.p. in a mouse model of staphylococcal septicemia. Because the pharmacokinetic properties of **7a** were on par with those of most other compounds within this series (Table 4),[22] we speculated that its poor pharmaceutical properties were responsible for the absence of in vivo efficacy.

Indeed, this hypothesis was supported by experiments in which we used variations in the 3-substituent to improve the pharmaceutical properties. After optimization cycles that focused on the inhibitor's physicochemical properties, we were able to identify potent compounds that were suitable for intravenous dosing, such as **7k** or **12b**, which showed highly significant in vivo efficacy against *S. aureus* sepsis in mice at doses of only 10 mg kg^{-1} i.v. This result compared favorably with compound **2**, which, in the same infection model, showed efficacy only at doses of 100 mg kg^{-1} i.p. or above. The in vivo efficacy of the most potent compound **7k** was similar or superior to the known DNA Pol IIIC inhibitor **2**: in a murine model of enterococcal sepsis in the group that was treated with **7k** at a dose of 5 mg kg^{-1} i.p. all animals survived. Five out of six animals survived in the linezolid group at a dose of 10 mg kg^{-1} i.v.

Table 4. In vivo activity of selected anilinouracils compared with **2** and linezolid in rodent models of bacterial septicemia.

Compound	IC_{50} [nM]	MIC [μg mL^{-1}][a]	Aqueous Solubility [mg L^{-1}]	Survival	
				S. aureus at Dose	*E. faecalis* at Dose
7a	250	4/4/16	3.3	0/6 100 mg kg^{-1} i.p.	n.d.
7k	100	16/8/8	90	3/6 10 mg kg^{-1} i.v.	6/6 5 mg kg^{-1} i.v.
12b	50	8/16/4	30	3/6 10 mg kg^{-1} i.v.	2/6 10 mg kg^{-1} i.v.
12h	160	8/8/16	290	0/6 10 mg kg^{-1} i.v.	n.d.
2	300	8/8/16	n.d.	2/6[b] 100 mg kg^{-1} i.p.	5/6 5 mg kg^{-1} i.p.
placebo	–	–	–	0/6	0/6
linezolid	–	1/2/2	>1000	6/6 10 mg kg^{-1} i.v.	5/6 10 mg kg^{-1} i.v.

[a] Minimal inhibitory concentration against *S. aureus* 133 / *S. pneumoniae* 1707/4 / *E. faecalis* ICB 27159. [b] *S. aureus* Smith.

Conclusions

In summary, we have prepared a number of novel substituted anilinouracils, many of which were specific inhibitors of the Gram-positive DNA polymerase IIIC enzyme with potent antibacterial activity in vitro. Newly developed synthetic methods were employed for thoroughly elaborating structure–activity relationships within the class of anilinouracils. The overall physicochemical properties of the anilinouracils could be favorably influenced by an appropriate choice of 3-substituent without loss of antibacterial activity, within a certain polarity window. This enabled us to identify anilinouracils with good antibacterial efficacy in vivo; these compounds were characterized in various rodent models of bacterial septicemia in direct comparison with previously described DNA Pol IIIC inhibitors and the marketed antibiotic linezolid. Combining the lessons that were learned in the optimization of potency and physicochemical profile, we were able to identify potent compounds that were suitable for intravenous dosing, such as **7k** or **12b**, which showed a pronounced in vivo efficacy in mouse models of *S. aureus* and *E. faecalis* sepsis.

In the class of the anilinouracils, our work represents a step forward in the understanding of the role of peripheral substituents and their influence on the physicochemical profile as well as the antibacterial potency in vitro and in vivo. This understanding will hopefully be of help in selecting a compound for clinical development.

Experimental Section

All reagents used were obtained from commercial sources and all solvents were of analytical grade. Sample purity of all products was in each case verified by analytical HPLC and ^{1}H NMR spectroscopy, and was consistently better than 98%, except for compound **7h** which was estimated to be 95% pure by LC/MS and NMR spectroscopy. ^{1}H NMR spectra were obtained on a Bruker 200 MHz or a 300 MHz NMR spectrophotometer, respectively, in [D_6]DMSO or $CDCl_3$ as a solvent unless otherwise stated, by using tetramethylsilane (TMS) or residual nondeuterated solvent as an internal standard. Chemical shifts are reported in δ (ppm) and the following abbreviations are used: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; br, broad. Mass spectra were run on a Finnigan MAT 900. Analytical HPLC was performed on an Agilent Technologies HP 1100 series HPLC system by using UV and diode array detection. All experiments involving live animals were carried out in compliance with local rules and regulations and were approved by an independent ethical committee.

1-(Benzyloxy)methyl-6-chloro-3-cyclopropylmethyluracil 5a: 1-(Benzyloxy)methyl-6-chlorouracil **4** (1.70 g, 6.4 mmol) was dissolved in dry DMF (15 mL) under argon and Cs_2CO_3 (4.15 g, 12.7 mmol) was added. The resulting mixture was stirred for 15 min, whereupon bromomethylcyclopropane (680 μL, 7.0 mmol) was added. The mixture was stirred at room temperature for 3 h, the solvent was removed in vacuo, and the crude product was dissolved in CH_2Cl_2 (150 mL) and washed with brine (3×50 mL). The organic phase was dried (Na_2SO_4) and evaporated in vacuo to give the product **5a** as a solid; yield: 1.76 g (86%). ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 7.32 (s, 5H), 6.12 (s, 1H), 5.49 (s, 2H), 4.63 (s, 2H), 3.64 (d, 2H), 1.12 (m, 1H), 0.43 (m, 2H), 0.30 ppm (m, 2H); MS (ESIpos): m/z = 321 $[M+H]^+$.

6-Chloro-3-cyclopropylmethyluracil 6a: A suspension of 1-(benzyloxy)methyl-6-chloro-3-cyclopropylmethyluracil **5a** (600 mg, 1.9 mmol) was suspended in TFA (30 mL) and heated at reflux for 45 min. The mixture was cooled to room temperature and evaporated in vacuo, whereupon MeOH (10 mL) was added and subsequently removed in vacuo. EtOAc (5 mL) was added, and the sample was left overnight to crystallize. The solid was filtered off, washed with Et_2O and dried to give **6a** as colorless crystals; yield: 320 mg (85%). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 7.45 (br s, 1H), 5.43 (s, 1H), 3.61 (d, 2H), 1.10 (m, 1H), 0.41 (m, 2H), 0.29 ppm (m, 2H); MS (ESIpos): m/z = 201 $[M+H]^+$.

3-Cyclopropylmethyl-6-(2,3-dihydro-1*H*-inden-5-ylamino)uracil 7a: A mixture of 6-chloro-3-cyclopropylmethyluracil **6a** (120 mg, 0.6 mmol) and 5-aminoindane (320 mg, 2.4 mmol) was heated under argon at 160 °C for 30 min. The mixture was cooled to room temperature, 2-propanol (5 mL) was added, and the resulting precipitate was filtered off and dried to give a crude product, which was purified by reversed-phase preparative HPLC (eluent MeCN/H_2O, 1:9 to 9:1) to give the product **7a** as a solid; yield: 123 mg (68%). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 10.33 (s, 1H), 8.08 (s, 1H), 7.23 (d, 1H), 7.08 (s, 1H), 6.96 (d, 1H), 4.74 (s, 1H), 3.52 (m, 2H), 2.85 (m, 4H), 2.04 (m, 2H), 1.12 (m, 1H), 0.39 (m, 2H), 0.29 ppm (m, 2H); MS (ESIpos): m/z = 298 $[M+H]^+$.

6-(2,3-Dihydro-1*H*-inden-5-ylamino)-3-(2-sulfanylethyl)-2,4-(1*H*,3*H*)-pyrimidinedione 11d (n = 1): 6-Amino-3-(2-sulfanylethyl)-2,4(1*H*,3*H*)-pyrimidinedione **10a**[30] (3.0 g, 16 mmol), 2,3-dihydro-1*H*-inden-5-amine (3.2 g, 24 mmol) and 2,3-dihydro-1*H*-inden-5-amine hydrochloride (4.08 g, 24 mmol) were heated under argon for 6 h at 160 °C. The reaction was cooled to room temperature, and the crude product was suspended in 50% aq EtOH (20 mL) and subsequently filtered off with suction. The washing procedure was repeated with 50% aq EtOH, then with Et_2O (2×20 mL), and the residue was dried in vacuo to give the product **11a** as a solid; yield: 4.71 g (97%). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 10.43 (s, 1H), 8.17 (s, 1H), 7.22 (d, 1H), 7.06 (s, 1H), 6.95 (d, 1H), 4.71 (s, 1H), 3.83 (t, 2H), 2.84 (q, 4H), 2.59 (q, 2H), 2.02 ppm (m, 2H); MS (ESIpos): m/z = 304 $[M+H]^+$.

6-(3-Ethyl-4-methylphenyl)amino-3-(2-sulfanylethyl)-2,4(1*H*,3*H*)-pyrimidinedione 11a: 6-Amino-3-(2-sulfanylethyl)-2,4(1*H*,3*H*)-pyrimidinedione **10a** (3.0 g, 16 mmol), 3-ethyl-4-methylaniline hydrochloride **17** (5.78 g, 34 mmol) and *i*Pr_2EtN (2.93 mL, 17 mmol) were heated under argon for 6 h at 160 °C. The reaction was cooled to room temperature, and the crude product was suspended in 50% aq EtOH (20 mL), and subsequently filtered off with suction. The washing procedure was repeated with 50% aq EtOH, then with Et_2O (2×20 mL), and the residue was dried in vacuo to give the product **11a** as a solid; yield: 4.83 g (98%). ^{1}H NMR (200 MHz, [D_6]DMSO): δ = 10.41 (s, 1H), 8.10 (s, 1H), 7.14 (d, 1H), 6.98–6.91 (m, 2H), 4.71 (s, 1H), 3.83 (t, 2H), 2.63–2.53 (m, 4H), 2.24 (s, 3H), 1.14 ppm (t, 3H); MS (ESIpos): m/z = 306 $[M+H]^+$.

6-(3-Ethyl-4-methylphenyl)amino-3-(3-sulfanylpropyl)-2,4-(1*H*,3*H*)-pyrimidinedione 11b (n = 2): 6-Amino-3-(3-sulfanylpropyl)-2,4(1*H*,3*H*)-pyrimidinedione **10b**[30] (2.98 g, 15 mmol), 3-ethyl-4-methylaniline hydrochloride **17** (7.63 g, 44 mmol) and *i*Pr_2EtN (3.87 mL, 22 mmol) were heated under argon for 6 h at 160 °C. The reaction was cooled to room temperature, and the crude product was suspended in 50% aq EtOH (20 mL), and subsequently filtered off with suction. The washing procedure was repeated with 50% aq EtOH, then with Et_2O (2×20 mL), and the residue was dried in

vacuo to give the product **11b** as a solid; yield: 4.25 g (90%). ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 10.48 (s, 1H), 8.15 (s, 1H), 7.05 (dd, 2H), 6.97 (s, 1H), 4.72 (s, 1H), 3.77 (t, 2H), 2.23 (s, 3H), 1.83–1.70 (m, 2H), 1.13 ppm (t, 3H), 4H are obscured by the DMSO peak; MS (ESIpos): m/z = 320 [M+H]$^+$.

General procedure for the thiol alkylation: 3-(2-{[(1,4-Dimethyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]sulfanyl}ethyl)-6-[(3-ethyl-4-methylphenyl)amino]pyrimidine-2,4(1*H*,3*H*)-dione **12h**: 6-(3-Ethyl-4-methylphenyl)amino-3-(2-sulfanylethyl)-2,4(1*H*,3*H*)-pyrimidinedione **11b** (50 mg, 0.16 mmol), 5-(chloromethyl)-2,4-dimethyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (26 mg, 0.16 mmol) and Cs_2CO_3 (160 mg, 0.49 mmol) were placed in a septum-closed vial, the vial was purged with argon for 5 min and the argon needle was removed, whereupon dry MeCN (3 mL) was added, and the resulting suspension was stirred overnight. The mixture was diluted with CH_2Cl_2 (10 mL) filtered through a plug of Celite, rinsing with CH_2Cl_2 (10 mL). The sample was evaporated in vacuo to give the product 12 h as a hard yellow glass; yield: 50 mg (73%). ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 8.76 (s, 1H), 7.12–6.98 (m, 3H), 4.74 (s, 1H), 3.91 (t, 2H), 3.75 (s, 2H), 3.27 (s, 3H), 3.15 (s, 3H), 2.67 (t, 2H), 2.21 (s, 3H), 1.12 ppm (t, 3H), 2H signals are obscured by the DMSO peak; MS (ESIpos): m/z = 431 [M+H]$^+$.

6-[(3-Ethyl-4-methylphenyl)amino]-3-(3-{[(5-methyl-1,3,4-thiadiazol-2-yl)methyl]sulfanyl}ethyl)pyrimidine-2,4(1*H*,3*H*)-dione 12b: The title compound was prepared analogously to compound **12h** above by starting from 6-(3-ethyl-4-methylphenyl)amino-3-(2-sulfanylethyl)-2,4(1*H*,3*H*)-pyrimidinedione **11b** (100 mg, 0.33 mmol), 2-(chloromethyl)-5-methyl-1,3,4-thiadiazole[38] (54 mg, 0.36 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (100 μL, 0.66 mmol) to give the product **12b** after purification by reversed-phase preparative HPLC; yield: 102 mg (74%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.53 (br s, 1H), 8.19 (br s, 1H), 7.14 (d, 1H), 6.98 (d, 1H), 6.95 (dd, 1H), 4.73 (s, 1H), 4.21 (s, 2H), 3.95 (t, 2H), 2.70 (m, 5H), 2.57 (q, 2H), 2.25 (s, 3H), 1.14 ppm (t, 3H); MS (ESIpos): m/z = 418 [M+H]$^+$.

2′-Methyl-5-nitroacetophenone 14: 2′-Methylacetophenone **13** (13.00 g, 97 mmol) was added to concd H_2SO_4 (30 mL) (**exothermic!**) while the temperature was maintained under 5 °C, then a cold mixture of H_2SO_4 (concd, 15 mL) and HNO_3 (d = 1.55, 10 mL) was added dropwise (**very exothermic!**) over 45 min, whilst keeping the temperature under 5 °C by using an acetone/CO_2 cooling bath. The mixture was poured into ice, extracted with Et_2O (2× 200 mL), washed with brine, dried and evaporated. The resulting crude oil was separated chromatographically through a plug of silica (gradient eluent: cyclohexane-dichloromethane 4:1 to pure dichloromethane) and evaporated to give the product **14** as an oil; yield: 6.31 g (36%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.54 (d, 1H), 8.23 (dd, 1H), 7.44 (d, 1H), 2.69 (s, 3H), 2.65 ppm (s, 3H).

1-(2-Methyl-5-nitrophenyl)ethanol 15: 2′-Methyl-5-nitroacetophenone **14** (5.20 g, 29 mmol) was dissolved in MeOH (100 mL) at 0 °C, $NaBH_4$ (3.29 g, 89 mmol) was added in four portions over 30 min, and the mixture was then stirred 12 h whilst warming to room temperature. The reaction was quenched by the addition of 5 N HCl (25 mL), evaporated to 1/3 volume, extracted with CH_2Cl_2 (2× 150 mL), the combined organic phases were washed with brine (100 mL), dried (Na_2SO_4) and evaporated to give the product **15** as a solid; yield: 4.91 g (93%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45–7.20 (several discrete multiplets, 3H), 5.27–5.13 (m, 1H), 2.44 (s, 3H), 1.50 ppm (m, 3H).

2-Methyl-5-nitrostyrene 16: 1-(2-Methyl-5-nitrophenyl)ethanol **15** (5.00 g, 28 mmol), *p*-toluenesulfonic acid (475 mg, 0.28 mmol) and hydroquinone (150 mg, 1.4 mmol) were dissolved in toluene (100 mL) and held at reflux for 20 h. The resulting solution was cooled to room temperature and washed with sat. aq Na_2CO_3 (2× 100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated to give the product **16** as a brown oil; yield: 4.52 g (100%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.32 (d, 1H), 8.00 (dd, 1H), 7.30 (d, 1H), 6.93 (dd, 1H), 5.80 (d, 1H), 5.48 (d, 1H), 2.44 ppm (s, 3H).

3-Ethyl-4-methylaniline 17: 10% Pd/C (500 mg) was placed in a 500-mL hydrogenation flask, and a solution of 2-methyl-5-nitrostyrene **16** (4.50 g, 28 mmol) in MeOH (150 mL) was added under argon. The resulting solution was subjected to hydrogenation in a Parr shaker at 4 bar for 4 h. The resulting solution was filtered through Celite, evaporated, redissolved in Et_2O (200 mL), washed with 1 M NaOH (100 mL) and brine (100 mL), dried over sodium sulfate and evaporated. The crude product was purified by flash chromatography through silica gel (eluent cyclohexane to CH_2Cl_2 to give the product **17** as an oil; yield: 2.65 g (71%). 3-Ethyl-4-methylaniline is most conveniently stored as the hydrochloride salt, which can be made by dissolving the free base in ether and adding gaseous HCl. The resulting nonhygroscopic crystalline material can be filtered off and dried in vacuo. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.91 (d, 1H), 6.52 (d, 1H), 6.45 (dd, 1H), 3.50 (br s, 2H), 2.54 (q, 2H), 2.19 (s, 3H), 1.18 ppm (t, 3H); MS (ESIpos): m/z = 136 [M+H]$^+$.

***N*-(4-Chloro-3-ethylphenyl)acetamide**[32] **19**: *N*-(3-Ethylphenyl)-acetamide (5.00 g, 30.6 mmol) was dissolved in dry DMF (20 mL), HCl (4 M in dioxane, 9.2 mL, 36.8 mmol) was added, followed by solid *m*-chloroperbenzoic acid (5.82 g, 33.7 mmol), and the resulting mixture was stirred at room temperature for 30 min, then diluted with H_2O (100 mL) and extracted with EtOAc (2×100 mL). The combined organic extracts were washed with sat. aq Na_2SO_4 (100 mL), $NaHCO_3$ (100 mL) and brine (150 mL), and evaporated in vacuo. The resulting crude product was chromatographed on silica gel by eluting with EtOAc/cyclohexane (1:1). Evaporation left the product **19** as a clear oil, which crystallized upon standing at room temperature; yield: 3.50 g (57%). MS (ESIpos): m/z = 198 [M+H]$^+$

4-Chloro-3-ethylaniline hydrochloride[32] **20**: *N*-(4-Chloro-3-ethylphenyl)acetamide **19** (3.50 g, 17.7 mmol) was dissolved in EtOH (50 mL) and concd. aq HCl (25 mL) was added followed by H_2O (25 mL), and the resulting mixture was held at reflux for 3 h. The mixture was cooled to room temperature and the resulting precipitate was filtered off, washed with Et_2O and dried to give the product **20** as a white solid; yield: 2.78 g (97%). MS (ESIpos): m/z = 156 [M−HCl+H]$^+$

Ethyl {4-[(4-chloro-3-ethylphenyl)amino]-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl}acetate 7r: Ethyl (4-chloro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl)acetate[39] **6l** (100 mg, 0.43 mmol), 4-chloro-3-ethylaniline hydrochloride **20** (124 mg, 0.65 mmol) and *i*Pr$_2$EtN (190 μL, 1.08 mmol) were heated at 150 °C for 35 min under argon. The crude product was cooled to room temperature and diluted with CH_2Cl_2 (50 mL), and subsequently washed with 0.5 M HCl (3× 50 mL) and brine (50 mL), dried (Na_2SO_4) and evaporated. The residue was subjected to purification by preparative reverse-phase HPLC to give the product **7r** as a crystalline solid; yield: 60 mg (38%). ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 10.62 (s, 1H), 8.40 (s, 1H), 7.40 (d, 1H), 7.18 (d, 1H), 7.07 (dd, 1H), 4.76 (s, 1H), 4.45 (s, 2H), 4.13 (q, 2H), 2.69 (q, 2H), 1.20 ppm (m, 6H); MS (ESIpos): m/z = 352 [M+H]$^+$.

***tert*-Butyl 4-({3-[(benzyloxy)methyl]-4-chloro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl}methyl)piperidine-1-carboxylate 5j**: 1-Benzyloxymethyl-6-chlorouracil **4** (5.0 g, 23 mmol), *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate[29] (6.19 g, 23 mmol) and triphenylphosphine (6.7 g, 26 mmol) were dissolved in dry THF (150 mL)

under argon and cooled to 0 °C, whereupon diethyl azodicarboxylate (4.0 mL, 26 mmol) was added dropwise over a period of 10 min. The resulting solution was stirred for 72 h at room temperature and evaporated, and the crude product was purified by chromatography on silica gel; yield: 7.43 g (69%). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.34$ (s, 5H), 5.91 (s, 1H), 5.56 (s, 2H), 4.69 (s, 2H), 4.08 m, 2H), 3.79 (d, 2H), 2.65 (m, 2H), 1.90 (m, 1H), 1.56 (m, 3H), 1.47 (s, 9H), 1.24 ppm (m, 3H); MS (ESIpos): $m/z = 464$ $[M+H]^+$;

6-Chloro-3-(piperidin-4-ylmethyl)pyrimidine-2,4(1*H*,*3H*)-dione trifluoroacetate 6j: *tert*-Butyl 4-({3-[(benzyloxy)methyl]-4-chloro-2,6-dioxo-3,6-dihydropyrimidin-1(2*H*)-yl}methyl)piperidine-1-carboxylate **5j** (9.85 g, 21.2 mmol) was dissolved in neat TFA (160 mL) and heated at reflux for 2 h. The resulting solution was cooled to room temperature, evaporated in vacuo, redissolved in MeOH (100 mL) and evaporated to give a crude product, which was recrystallized from EtOAc to give the product **6j**; yield: 5.43 g (90%). MS (ESIpos): $m/z = 244$ $[M-\text{TFA}+H]^+$.

6-[(3-Ethyl-4-methylphenyl)amino]-3-(piperidin-4-ylmethyl)pyrimidine-2,4(1*H*,*3H*)-dione 7j: 6-Chloro-3-(piperidin-4-ylmethyl)pyrimidine-2,4(1*H*,3*H*)-dione trifluoroacetate **6j** (1.0 g, 4.1 mmol), 3-ethyl-4-methylaniline hydrochloride **17** (0.85 g, 4.9 mmol) and $i\text{Pr}_2\text{EtN}$ (1.57 mL, 9.0 mmol) were heated at 150 °C for 2 h, then cooled to room temperature and diluted with CH_2Cl_2 (10 mL) and MeOH (5 mL). The resulting solution was poured into Et_2O (50 mL) with stirring, and the resulting precipitate was filtered off and dried to give the pure product; yield: 0.50 g, 35%). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 10.66$ (br s, 1H), 8.74 (br s, 1H), 8.61 (m, 1H), 8.40 (br s, 1H), 8.15 (d, 1H), 6.97 (d, 1H), 6.93 (dd, 1H), 4.74 (s, 1H), 3.63 (d, 2H), 3.22 (br d, 2H), 2.80 (m, 2H), 2.58 (m, 2H), 2.24 (s, 3H), 1.98 (m, 1H), 1.69 (br d, 2H), 1.36 (m, 2H), 1.14 ppm (t, 3H); MS (ESIpos): $m/z = 343$ $[M+H]^+$.

6-[(3-Ethyl-4-methylphenyl)amino]-3-{[1-(isoxazol-5-ylcarbonyl)-piperidin-4-yl]methyl}pyrimidine-2,4(1*H*,*3H*)-dione 7k: 6-[(3-Ethyl-4-methylphenyl)amino]-3-(piperidin-4-ylmethyl)pyrimidine-2,4(1H,3H)-dione **7j** (134 mg, 0.40 mmol), isoxazole-5-carboxylic acid (45 mg, 0.4 mmol) and 1-benzotriazolyloxytris(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP, 225 mg, 0.43 mmol) were suspended in dry THF (1.5 mL) and $i\text{Pr}_2\text{EtN}$ (56 mg, 0.43 mmol) was added, and the resulting mixture was stirred for 24 h. The solvent was removed, H_2O (0.5 mL) was added, and after 15 min stirring the resulting mixture was filtered through Celite, eluting with CH_2Cl_2/MeOH, 1:2 (50 mL). The solvent was removed, the residue was redissolved in EtOAc (50 mL) and washed with sat. aq $NaHCO_3$ (2×30 mL), the organic phase was then dried (Na_2SO_4) and evaporated. The crude product was finally purified by preparative reversed-phase HPLC; yield: 65 mg (37%). ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 10.43$ (s, 1H), 8.74 (d, 1H), 8.13 (s, 1H), 7.15 (d, 1H), 6.99 (d, 1H), 6.95 (dd, 1H), 6.89 (d, 1H), 4.74 (d, 1H), 4.37 (br d, 1H), 3.72 (br d, 1H), 3.64 (d, 2H), 3.11 (br t, 1H), 3.02 (m, 1H), 2.83 (br t, 1H), 2.56 (q, 2H), 2.24 (s, 3H), 2.03 (m, 1H), 1.74 (m, 1H), 1.64 (br m, 2H), 1.25–1.12 ppm (m, 5H); MS (ESIpos): $m/z = 438$ $[M+H]^+$.

DNA Pol IIIC activity. DNA Pol IIIC activity was assayed by an enzymatic coupled assay containing activated (partially digested with DNase I) calf thymus DNA as the template–primer DNA and deoxynucleoside triphosphates (dNTPs) as substrates. The reaction mixture contained 5 μL (25.0 A260 U mL^{-1}) of activated calf thymus DNA (Amersham); dATP, dTTP, dGTP, and dCTP at 20 μM each; 20 μM adenosine 5′-phosphosulfate; and 60 μM luciferin in 50 μL of 50 mM Tris–HCl (pH 7.5), 5 mM dithiothreitol, 10 mM $MgCl_2$, 30 mM NaCl, 0.1 mg mL^{-1} bovine serum albumin, 10% glycerol. The reaction was started by the addition of the Pol IIIC enzyme preparation. The mixture was incubated for 30 min at 30 °C prior to the addition of 0.01 mg mL^{-1} ATP sulfurylase (20 μL; 7.2 U mg^{-1}) from baker's yeast (Sigma). The mixture was further incubated for 15 min at 30 °C. Upon addition of 0.3 μg mL^{-1} firefly luciferase (20 μL, Promega), luminescence can be measured for 1 min in a luminometer. For the determination of the anti-Pol IIIC activities of the described *N*-3-substituted anilinouracils, dGTP as the competitive dNTP was omitted from the enzymatic assay.[22] Test compounds were dissolved in DMSO to a final concentration of not more than 2%.

Bacterial strains and growth conditions. The bacterial strains used in this study were *S. aureus* 133, *S. pneumoniae* 1707/4, and *E. faecalis* ICB 27159. All strains were subcultured on agar plates containing 5% sheep blood. Liquid cultures were prepared in brain heart infusion (BHI) medium containing 10% bovine serum for *S. pneumoniae* and *E. faecalis* at 37 °C.

MIC determinations. MICs were determined by the broth microdilution method with an inoculum of 5×10^5 CFU mL^{-1} in BHI medium. Growth was read after 18 h of incubation at 37 °C. For *S. pneumoniae* and *E. faecalis*, 10% bovine serum was added to the medium and incubation was performed under microaerophilic conditions. The test compounds were dissolved in DMSO and diluted to a concentration not greater than 2.5% DMSO.

Murine sepsis models. CFW1 mice (weight, 20 g; six mice per group) were infected with a single intraperitoneal (i.p.) injection of *S. aureus* 133 (0.25 mL that contained 10% mucin per mouse; 10^6 CFU/mouse) or *E. faecalis* ICB 27159 (100 μL that contained 5% mucin per mouse, 5×10^8 CFU per mouse). At 30 min after infection the mice were treated intravenously (i.v.) with 0.1 mL of test compound dissolved in 2% DMSO/12% Solutol at a concentration that was sufficient to give a dose of 10 mg kg^{-1} of body weight. The mice were monitored over a 5-day period, and the results are expressed as the number of surviving mice.

Keywords: anilinouracils · antibiotics · DNA polymerase IIIC · drug resistance · Gram-positive bacteria

[1] C. Walsh, *Antibiotics, Actions, Origins, Resistance*, 1st ed., ASM press, Washington DC, **2003**.
[2] C. J. Thomson, E. Power, H. Ruebsamen-Waigmann, H. Labischinski, *Curr. Opin. Microbiol.* **2004**, *7*, 445–450.
[3] J. Travis, *Science* **1994**, *264*, 360–362.
[4] a) C. Walsh, *Nature* **2000**, *406*, 775–778; b) G. D. Wright, *Curr. Opin. Chem. Biol.* **2003**, *7*, 563–569; c) M. N. Alekshun, S. B. Levy, *Cell* **2007**, *128*, 1037–1050.
[5] a) A. Coates, Y. Hu, R. Bax, C. Page, *Nat. Rev. Drug Discovery* **2002**, *1*, 895–910; b) Infectious Disease Society of America (IDSA): http://www.idsociety.org (last access August 12, 2008).
[6] a) G. D. Wright, *Curr. Opin. Chem. Biol.* **2003**, *7*, 563–569; b) *EARSS Annual Report 2006*: http://www.rivm.nl.
[7] a) S. J. Projan, *Curr. Opin. Microbiol.* **2003**, *6*, 427–430; b) S. J. Projan, *Drug Discovery Today* **2008**, *13*, 279–280.
[8] a) M. R. Barbachyn, C. W. Ford, *Angew. Chem.* **2003**, *115*, 2056–2070; *Angew. Chem. Int. Ed.* **2003**, *42*, 2010–2023; b) S. J. Brickner, M. R. Barbachyn, D. K. Hutchinson, P. R. Manninen, *J. Med. Chem.* **2008**, *51*, 1981–1990.
[9] Y.-Q. Xiong, T. P. Le, *Drugs Today* **2001**, *37*, 617–628.
[10] A. Raja, J. LaBonte, J. Lebbos, P. Kirkpatrick, *Nat. Rev. Drug Discovery* **2003**, *2*, 943–944.
[11] W. E. Rose, M. J. Rybak, *Pharmacotherapy* **2006**, *26*, 1099–1110.
[12] R. D. Gonzales, P. C. Schreckenberger, M. B. Graham, S. Kelkar, J. P. Quinn, K. DenBesten, *Lancet* **2001**, *357*, 1179.

[13] a) S. J. Projan, *Curr. Opin. Pharmacol.* **2002**, *2*, 513–522; b) I. Chopra, J. Hodgson, B. Metcalf, G. Poste, *Antimicrob. Agents Chemother.* **1997**, *41*, 497–503.
[14] F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich, *Angew. Chem.* **2006**, *118*, 5194–5254; *Angew. Chem. Int. Ed.* **2006**, *45*, 5072–5129.
[15] D. Häbich, F. von Nussbaum, *ChemMedChem* **2006**, *1*, 951–954.
[16] A. Ali, G. E. Taylor, *Expert Opin. Ther. Pat.* **2005**, *15*, 947–953.
[17] P. M. Tarantino, Jr., C. Zhi, J. J. Gambino, G. E. Wright, N. C. Brown, *J. Med. Chem.* **1999**, *42*, 2035–2040.
[18] Y. Rose, S. Ciblat, R. Reddy, A. C. Belley, E. Dietrich, D. Lehoux, G. A. McKay, H. Poirier, A. R. Far, D. Delorme, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 891–896.
[19] J. F. Daly, T. J. Giehl, N. C. Brown, C. Zhi, G. E. Wright, R. T. Ellison III, *Antimicrob. Agents Chemother.* **2000**, *44*, 2217–2221.
[20] G. E. Wright, N. C. Brown, *Curr. Opin. Anti-Infect. Invest. Drugs* **1999**, *1*, 45–48.
[21] M. H. Barnes, C. J. Leo, N. C. Brown, *Biochemistry* **1998**, *37*, 15254–15260.
[22] A. Kuhl, N. Svenstrup, C. Ladel, M. Otteneder, A. Binas, G. Schiffer, M. Brands, T. Lampe, K. Ziegelbauer, D. Haebich, K. Ehlert, *Antimicrob. Agents Chemother.* **2005**, *49*, 987–995.
[23] W. B. Cowden, I. A. Clark, N. H. Hunt, *J. Med. Chem.* **1988**, *31*, 799–801.
[24] L. Arias, A. Guzmán, S. Jaime-Figueroa, F. J. Lopez, D. J. Morgans Jr. , F. Padilla, A. Pérez-Medrano, C. Quintero, M. Romero, L. Sandoval, *Synlett* **1997**, 1233–1234.
[25] N. Svenstrup, A. Kuhl, K. Ehlert, D. Häbich, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3215–3218.
[26] C. E. Mueller, D. Shi, M. Manning Jr. , J. W. Daly, *J. Med. Chem.* **1993**, *36*, 3341–3349.
[27] C. Zhi, Z. Long, A. Manikowski, N. C. Brown, P. M. Tarantino Jr. , K. Holk, E. J. Dix, G. E. Wright, K. A. Foster, M. M. Butler, W. A. LaMarr, D. J. Skow, I. Motorina, S. Lamothe, R. Storer, *J. Med. Chem.* **2005**, *48*, 7063.
[28] E. D. Edstrom, Y. Wei, *J. Org. Chem.* **1994**, *59*, 6902–6903.
[29] N. Svenstrup, A. Kuhl, D. Flubacher, M. Brands, K. Ehlert, C. Ladel, M. Otteneder, J. Keldenich, PCT Int. Appl. WO 2003072574, **2003**.
[30] P. Pecorari, M. Rinaldi, M. P. Costi, *J. Heterocycl. Chem.* **1989**, *26*, 1701–1705.
[31] G. E. Wright, J. J. Gambino, *J. Med. Chem.* **1984**, *27*, 181–185.
[32] E. R. Krajniak, E. Ritchie, W. C. Taylor, *Aust. J. Chem.* **1973**, *26*, 1337–1351.
[33] Prepared as described in Scheme 1; see also Ref. [25].
[34] M. M. M. Butler, L. W. Dudycz, N. N. Khan, G. E. Wright, N. E. Brown, *Nucleic Acids Res.* **1990**, *18*, 7381–7387.
[35] D. J. Trantolo, G. E. Wright, N. C. Brown, *J. Med. Chem.* **1986**, *29*, 676–681.
[36] a) G. E. Wright, N. C. Brown, *J. Med. Chem.* **1980**, *23*, 34–38; b) C. Zhi, Z.-Y. Long, J. Gambino, W.-C. Xu, N. C. Brown, M. Barnes, M. Butler, W. LaMarr, G. E. Wright, *J. Med. Chem.* **2003**, *46*, 2731–2739.
[37] P. M. Tarantino, Jr., C. Zhi, G. E. Wright, N. C. Brown, *Antimicrob. Agents Chemother.* **1999**, *43*, 1982–1987.
[38] A. I. Glushkov, O. P. Shitov, A. V. Mezhenin, V. A. Tartakovsky, *Russ. Chem. Bull.* **2002**, *51*, 1332–1334.
[39] P. A. Link, H. C. van der Plas, F. Müller, *J. Heterocycl. Chem.* **1985**, *22*, 873–878.

Received: April 15, 2008
Revised: July 9, 2008
Published online on September 12, 2008

