Functionalized and lipophilic Prodrugs of novel Inhibitors of the bacterial RNAP

Alexandra Ruthenbeck[1], Walid AM Elgaher[2], Rolf Hartmann[2] and Chris Meier[1]

[1]*Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany.*
[2]*Helmholtz Institut für Pharmazeutische Forschung Saarland, Department of Drug Design and Optimization, Campus E8.1, 66123 Saarbrücken, Germany.*

Universität Hamburg
DER FORSCHUNG | DER LEHRE | DER BILDUNG
FAKULTÄT FÜR MATHEMATIK, INFORMATIK UND NATURWISSENSCHAFTEN

Development of **Prodrugs**

Antibacterial activity-determination (MIC_{95})
good against G+ strains*
no activity against G- bacteria**

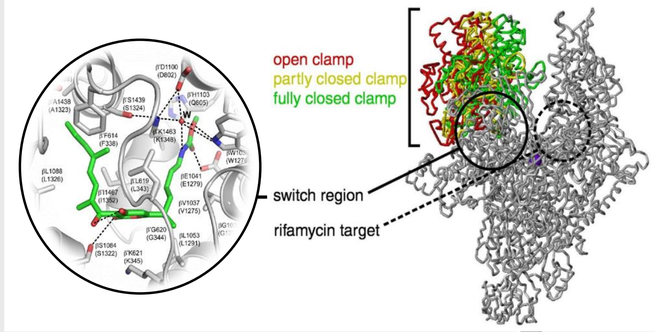
II
IC_{50} = 22 µM
E. coli RNAP

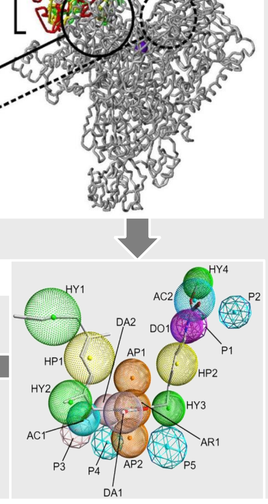
III
IC_{50} = 22 µM
E. coli RNAP

Discovery, optimization and expansion of a novel class of inhibitors

I
IC_{50} = 75 µM
E. coli RNAP

Identification of the "switch region" as a new druggable binding site
Co-crystal with bacRNAP inhibitor Myxopyronin B (from *Myxococcus fulvus)*





* B. subtilis, S. aureus, and E. coli TolC (efflux mutant) (MIC_{95} [µg/mL] = 8 – 14)
** E. coli, P. aeruginosa (MIC_{95} [µg/mL] >25 / MIC determination was limited due to insufficient solubility of the test compound)

Background

- bacterial RNA-Polymerase (RNAP) is an eligible target in antibacterial therapy, e.g. for rifamycins
- resistance development against the representative and in clinical therapy applied Rifampin illustrates the need for novel effective drugs
- the RNAP "switch region" constitutes an important domain mediating the conformational changes during RNA polymerization
- being approved as druggable, it emerges as a new basis for drug development

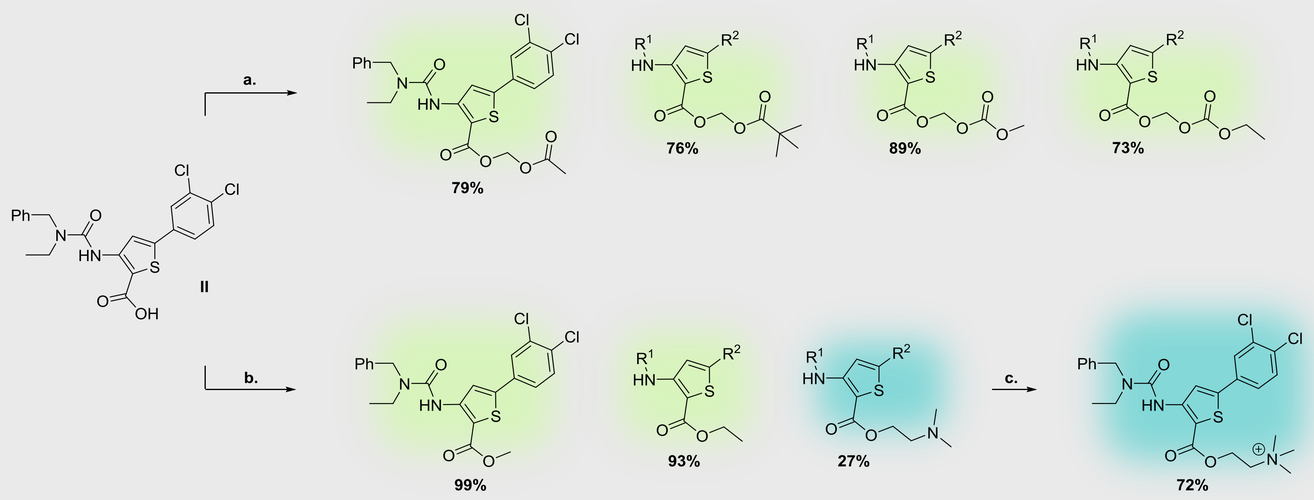
Novel inhibitors targeting the RNAP "switch region" were developed via a pharmacophore based virtual screening approach (**I**). Detailed binding mode characterization and *in vitro* testing confirmed high activity by blocking the "switch region", and structural optimization further improved inhibition (**II**, **III**). Antibacterial activities proved good effects on Gram-positive bacteria (S. aureus, B. subtilis) whereas against Gram-negative strains (E. coli K12, P. aeruginosa) compounds were inactive.[1,2] Correlating pharmacokinetic deficiencies in e.g. bio-availability were consequently approached by the development of prodrugs.

Objectives

To enhance the antibacterial efficiency of the parent compounds **II** and **III**, a bio-reversible modification of the carboxylic acid residue conveying differential functionalization constituted the aim of this study.

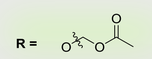
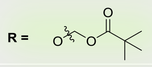
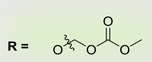
- drug efflux and reduced membrane-diffusion are presumably the major drawbacks
- prodrugs characteristics range broadly, from enabling passive diffusion to addressing an active uptake
- lipophilicity increase as established approach to relativize high potential distribution at carboxylic acid function eventually contributing to drug efflux
- active uptake induction via conjugation with moieties appealing a distinctive receptor interaction and subsequent transport
- synthesis of alkyl esters, choline-esters and linker-bridged siderophore-conjugates

Synthesis of Acyloxymethyl and Carboxyloxymethyl esters and Choline(analogue) esters

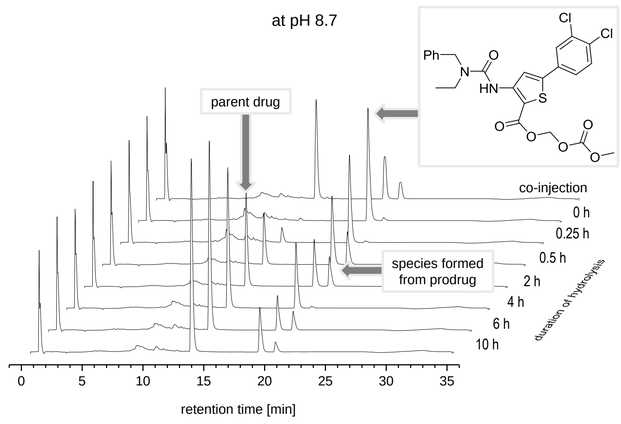


a. 1.75 eq. TEA, 1.85 eq. hal reagent*, DMF, rt, 18 h // **b.** 2 eq. DIAD, 2 eq. TPP, 1.5 eq. - 2 eq. ROH, DMF, rt, 4 h - 18 h // **c.** 1 eq. MeI, THF, rt, 20 h.
hal reagent*: bromomethyl acetate & chloromethyl pivaloate commercially purchased // chloromethyl(methyl)carbonate & chloromethyl(ethyl)carbonate synthesized from: 1 eq. chloromethyl chloroformiate, 2 eq. - 6 eq. ROH, 2.5 eq. pyridine, CH_2Cl_2, 0 °C → rt, 5 h, quant.

Determination of the hydrolytic Stability and enzymatic Activation

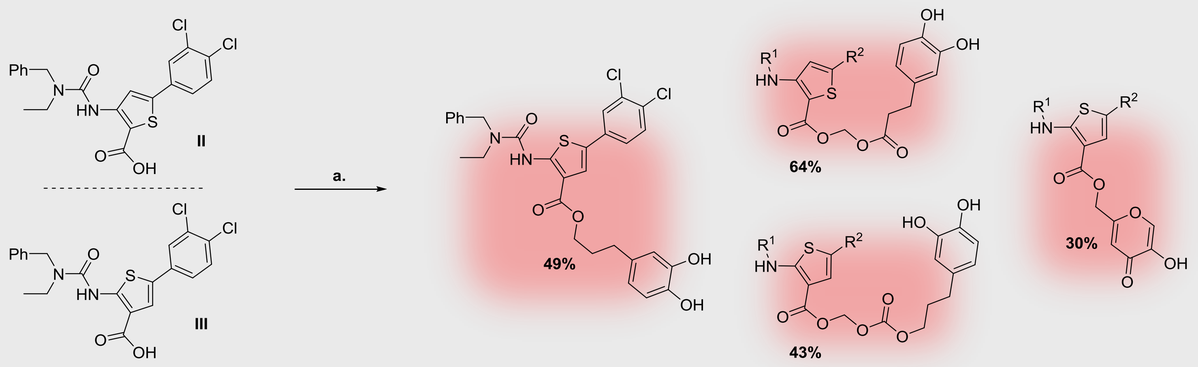
Prodrugs of II	Half-lives $t_{1/2}$ [h] for the release of the parent drug		
	enzymatic activation at pH 8.7	chemical hydrolysis at pH 7.3	(and pH 8.7)
R = 	2.6	124	3.2
R = 	2.1	14	-
R = 	2.5	70	9.6
R =	4.5	no significant release after 7d of incubation	

enzymatic hydrolyses of the carboxymethyl(oxymethyl)ester at pH 8.7

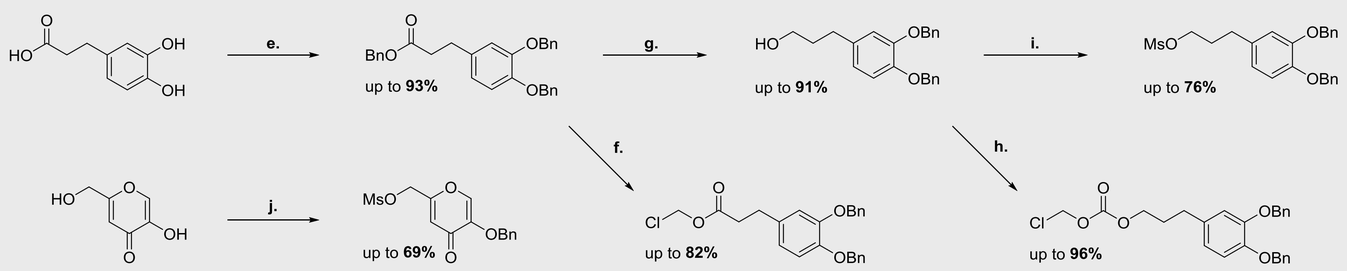


Hydrolysis protocols: *Determination of the chemical stability in phosphate buffered solution (PBS):* Prodrugs (DMSO sol., 0.05 mM) were incubated with PBS buffer (aq., 50 mM, pH 7.3 / pH 8.7) at 37 °C, and samples for analysis taken constantly at increasing times. *Investigation of the enzymatic activation w/ pig liver esterase (PLE):* Prodrugs (DMSO sol., 5 mM) were incubated with PLE (20 units/mL in PBS) 1:4 at 37 °C. After defined times samples were quenched w/ methanol, filtrated and deep-frozen until analysis. *Analysis of hydrolysis samples:* The course of the drug release was monitored via RP-HPLC (C18 column, TBAA buffer (10 mM, pH 7.3) w/ acetonitrile gradient from 5 %-100 % in 20 min, parent compound release confirmed via co-injection). Consumption of prodrug, formation of drug and resp. half-lives were evaluated plotting the area integral of chromatogram signals against the time, determining exponential growth/decay functions and therefrom calculating $t_{1/2}$.

Synthesis of Siderophore-Conjugates

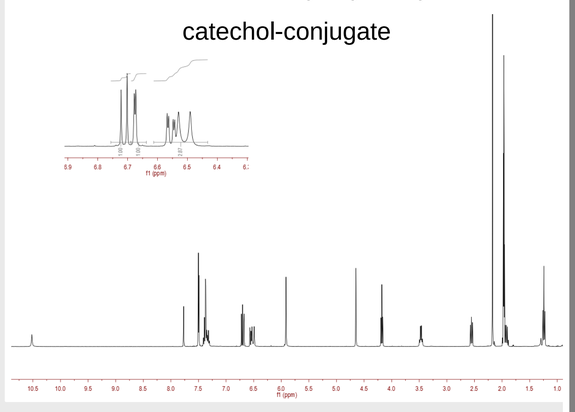


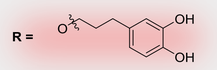
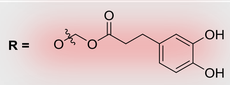
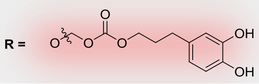
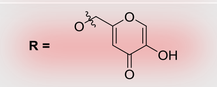
** syntheses of hal/OMs reagent



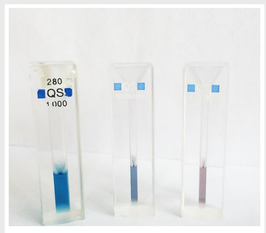
d.l. 1.75 eq. TEA, 1.85 eq. hal/OMs reagent**, (0.5 eq. TBAI), DMF, rt, 18 h, **II.** 5 eq. TMSI (1 M CH_2Cl_2), CH_2Cl_2, -25 °C→ rt, 3 h - 18 h.
hal/OMs reagent** synthesized from: **e.** 6 eq. BnBr, 4.5 eq. K_2CO_3, MeCN + 4% H_2O, 95 °C, 18 h; **f.I.** 2 eq. KOH, THF/H_2O 1:1, 50 °C, 18 h, **II.** 1.1 eq. chloromethyl chlorosulfate, 4 eq. $NaHCO_3$, 0.1 eq. nBu_4NHSO_4, CH_2Cl_2/H_2O 1:1, rt, 30 min; **g.** 4.5 eq. BH_3 (1 M THF), THF, 0 °C, 1 h → rt, 18 h; **h.** 1.25 eq. MsCl, 1.25 eq. TEA, CH_2Cl_2, 0° C, 1 h → rt, 2 h; **i.** 1.1 eq. chloromethyl chloroformiat, 2.5 eq. pydridine, CH_2Cl_2, 0 °C, 1 h → rt, 18 h; **j.I.** 3 eq. BnBr, 1.1 eq. NaOH (1 M aq. sol.), MeOH, 100 °C, 18 h, **II.** 1.25 eq. MsCl,1.25 eq. TEA, CH_2Cl_2, 0 °C → rt, 4 h.

^{1}H-NMR of the carboxyloxymethyl-linked catechol-conjugate



Prodrugs of II and III	CAS-assay
R = 	- 0.51
R = 	- 0.63
R = 	-0.54
R = 	-0.34
Vanchrobactin[4]	-0.63

Fe(III)-affinity of Siderophore-conjugates



- highest reactivity in CAS-assay shown by the acyloxymethyl- and carboxyl-oxymethyl-linked catechol-conjugates
- Fe(III) affinity comparable with the natural siderophore Vanchrobactin

Assay protocol: CAS solutions were prepared as in [3] and mixed 0.85:1 w/ prodrugs (DMSO/H_2O 1:3, 150 nM). Course of Fe(III) exchange was followed via spectrophotometric analysis (90 min / 24 h) as an absorbance decrease at 630 nm. Resp. intensity decay values were calculated against a blank.

Conclusion

- Selection of prodrugs with differential characteristics successfully synthesized
- Stability and activation studies confirmed the prodrug approach in principle
- Siderophore-conjugates showed good iron(III)-affinities in a competitive assay

In antibacterial activity tests with the prodrugs, the Choline ester showed interesting effects on Gram negative bacteria only.

References: [1] JH Sahner, M Groh, M Negri, J Haupenthal, RW Hartmann, *Eur. J. Med. Chem.*, **2013**, *65*, 223. [2] WAM Elgaher, M Fruth, M Groh, J Haupenthal, RW Hartmann, *RSC Adv.*, **2014**, *4*, 2177. [3] B Schwyn, JB Neilands, *Anal. Biochem.*, **1987**, *160*, 47-56. [4] A Souto, MA Montaos, M Balado, CR Osorio, J Rodríguez, ML Lemos, C Jiménez, *Bioorg. Med. Chem.*, **2013**, *21*, 295-302.

Acknowledgement: We are grateful to the University of Hamburg for the financial support.