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Structure–activity relationships of tulipalines, tuliposides, and related compounds as inhibitors of MurA

Thomas Mendgen, Therese Scholz, Christian D. Klein *

Department of Medicinal Chemistry, Universität Heidelberg, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany

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ABSTRACT

The enzyme MurA performs an essential step in peptidoglycan biosynthesis and is therefore a target for the discovery of novel antibacterial compounds. We report here the inhibition of MurA by natural products from tulips (tulipalines and tuliposides), and the structure–activity relationships of various derivatives. The inhibition of MurA can be related to antibacterial activity, and MurA is probably one of the relevant molecular targets of the tulipaline derivatives. MurA inhibition by this class of compounds depends on the presence of the substrate UNAG, which indicates non-covalent suicide inhibition as observed previously for cnicin. With respect to selectivity, however, the reactivity against arbitrary sulfhydryl groups, such as in glutathione, could not yet be sufficiently separated from MurA inhibition in the present dataset.

Bacteria are surrounded by the peptidoglycan polymer, which is responsible for cell integrity. The first step in the biosynthesis of peptidoglycan is catalyzed by the enzyme UDP-*N*-acetylglucosamine 1-carboxyvinyltransferase (MurA). This step includes a nucleophilic attack of the 3′-hydroxyl group of UDP-*N*-acetylglucosamine (UNAG) to the C2 position of phosphoenolpyruvate (PEP) to form enolpyruvyl-UDP-*N*-acetylglucosamine (EP-UNAG) under release of phosphate (Fig. 1).[1,2]

The inhibition of an enzyme from the biosynthetic pathway of the bacterial cell wall induces cell lysis and is therefore a relevant approach for antibacterial drug discovery. In case of MurA the only inhibitor in clinical use is the epoxide fosfomycin, which covalently binds to Cys115 of MurA.[3]

Recently we reported a couple of potent inhibitors of MurA: These are natural products of the sesquiterpene lactone type with cnicin as its most potent representative.[4] We initially expected a covalent binding of the α,β-unsaturated carbonyl function to Cys115 of MurA, which would have been analogous to fosfomycin's binding mode. However, an irreversible binding mode could not be confirmed by bio-analytical methods such as mass spectrometry of proteolytic digests. Eventually, an X-ray structure of the cnicin–UNAG–MurA complex indicated an unexpected binding mode: The side chain of cnicin—closely resembling PEP, with the Michael acceptor and the diol group—is converted by MurA to a 'wrong' product, which remains bound to the active site.[5] This mode of action represents an auto-catalytic, non-covalent suicide inhibition. Attempts to generate larger amounts of the product or to detect it by mass spectrometry, remained ambiguous. In order to gain a further understanding of the underlying SAR and to increase the potency of the compounds, we decided to synthesize and evaluate a series of acrylic acid derivatives as mimics of the cnicin side chain.

Considering the preparation and modification of the side chain, we identified various constituents of tulips, tulipaline A and B, and their corresponding glycosides 1-tuliposide A and B (cf. Table 1).[6] The antibiotic activity of tulips was first described in 1943. It was also observed that certain fungi do not affect the pistil of tulips. The tulipalines and tuliposides, which are present in high concentrations in this part of the plants, are believed to be responsible for this effect.[6–10] While the present work was in progress, Ubukata and co-workers[11] described the antibiotic activity of tulipalines against a variety of pathogenic organisms and hypothesized that MurA was the responsible target protein.

In the first part of this study we investigated the inhibition of MurA by the natural products from tulips and their derivatives. **1-tuliposide A** and **1-tuliposide B** were extracted from freeze-dried tulips. The commercially available α-methylene-γ-butyrolactone **tulipaline A** was converted into **(±)-tulipaline B** with selenium dioxide. Ring opening was achieved with potassium hydroxide in methanol to obtain **1** and **2** in good yields (Scheme 1).

The IC_{50} values at the native *Escherichia coli* MurA and the mutant (C115D) enzyme were determined as described previously (Table 1).[4,12,13]Whereas **tulipaline A** has no inhibitory effect, its

Abbreviations: UNAG, UDP-*N* acetylglucosamine; EP-UNAG, enolpyruvyl-UDP-*N*-acetylglucosamine; DMP, Dess–Martin-periodinane; PEP, phosphoenolpyruvate; GSH, glutathione.

* Corresponding author. Tel.: +49 6221 544875; fax: +49 6221 546430.
E-mail address: c.klein@uni-heidelberg.de (C.D. Klein).

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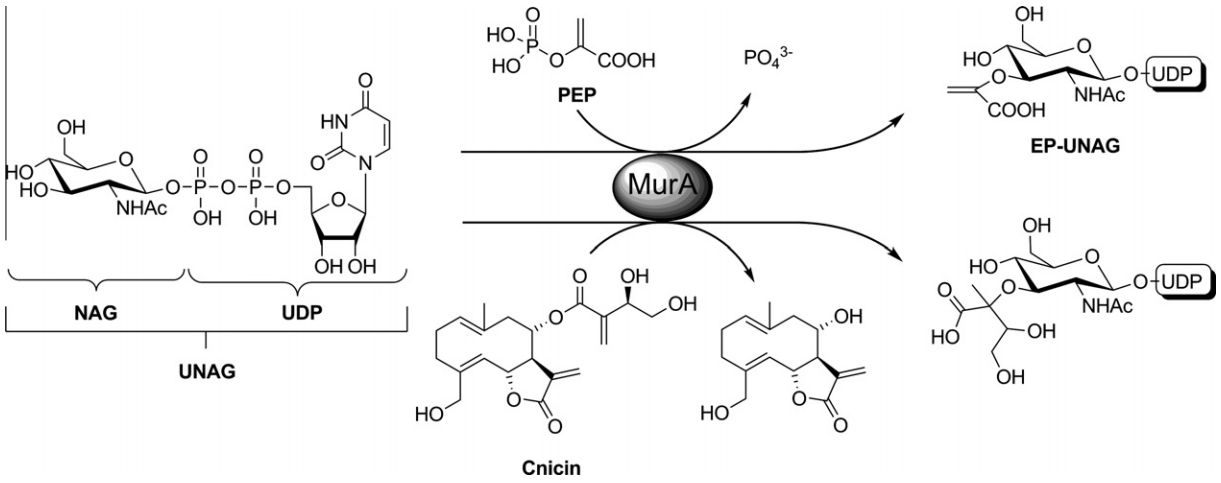

Figure 1. MurA catalyzes the formation of enolpyruvyl-UDP-*N*-acetylglucosamine (EP-UNAG) from UDP-*N*-acetylglucosamine (UNAG) and phosphoenolpyruvate (PEP). Cnicin acts as a substrate mimic for PEP, which leads to the formation of an UNAG–cnicin adduct that remains bound in the active site of MurA.

hydroxylated analog **(±)-tulipaline B**, which represents a lactonized form of the cnicin side chain, is a potent inhibitor of the native *E. coli* MurA. The glycoside **1-tuliposide B** with an IC_{50} value of 5 μM is highly active, whereas **1-tuliposide A** shows no inhibition of MurA. Since the only difference between the A- and B-series is the hydroxyl group, this moiety seems to be crucial. Both representatives of the B-series inhibit MurA in a time-dependent fashion, indicating either formation of a covalent enzyme-inhibitor complex or non-covalent suicide inhibition (cf. Fig. 2).

Stimulated by these encouraging results we synthesized various tulipaline derivatives to further modulate the steric and electronic properties of the compound series. Derivatives with shorter chain-length were synthesized according to Scheme 2. The ester **3** was formed by a Baylis–Hillman reaction, where the reaction time could be shortened from 3 days to 6 h by use of ultrasonic irradiation.[14,15] Subsequent hydrolysis afforded the carboxylic acid **4**.

Derivatives with longer chain-lengths were synthesized according to Scheme 3. Starting with DL-1,2-isopropylideneglycerol, the first step was an oxidation with Dess–Martin-periodinane (DMP) followed by a Baylis–Hillman reaction to form **5**.[16,17] Acetylation and deprotection of the dimethylacetal with *p*-TsOH lead to **6**. Deprotection under variable conditions afforded **7** and **8**.[6]

The **(±)-tulipaline B** analog **9** (Scheme 4) was synthesized from **5** using TFA/H_2O for deprotection and ring closure.[17] The following selective halogenation of the primary alcohol was problematic due to different side reactions of the double bond and the secondary alcohol. Finally, an Appel reaction with CBr_4 and PPh_3 furnished the bromo derivative **10**.

To form the dehydroxy-derivative of **9** a two step procedure was used. Starting with a Reformatzky reaction of bromomethylacrylate **11** and acetylethanal using activated zinc furnished intermediate **12**, which was deacetylated with sodium methanolate to give compound **13** (Scheme 5).[18]

Compound **15** was designed to mimic PEP more closely (cf. Scheme 6). Intermediate **11** was obtained by halogenation of **3** and converted into the phosphonic acid ester **14** using a Perkov reaction with triethyl phosphite.[19] Deprotection with concd HCl afforded **15** in quantitative yield.

The results of the enzymatic and antibacterial assays are given in Table 1 with fosfomycin and cnicin as reference compounds. From the top of Table 1, it becomes immediately clear that the A-series of tulipalines and related compounds is practically inactive, whereas the B-series is highly active in the enzymatic assay. As recently reported,[11] the side chain of tuliposide B is very important for a strong antibacterial activity and a small change in the structure leads to complete loss of activity. These results are consistent with our observations presented here, indicating that MurA is one of the major molecular targets of tulipalines and tuliposides. Compound **2**, the open-ring analog of **(±)-tulipaline B**, is the single carboxylic acid in the dataset that shows activity (cf. the inactive compds **1**, **4**, **8**, and **15**). It appears that the gem-diol moiety adjacent to the methylene group, as realized in compd **2** (and, in masked form, in **(±)-tulipaline B**) represents the optimum structure: The extension with a third hydroxy group (compds **6**, **7**, **8**) reduces activity, and 'contraction' (compds **3** and **4**) results in completely inactive compounds. The inactivity of compds **3** and **4** indicates that the relevant factor is not the inductive effect of the adjacent hydroxy function on the methylene group, but—in line with the observed binding mode of the UNAG–cnicin-adduct[5]—the bioisosteric similarity of the gem-diol moiety to PEP's phosphate group. This SAR can be extended to the lactonized compounds, which have a similar activity as the corresponding ring-opened methyl esters (**7** ↔ **9**). The essentiality of a hydroxy group adjacent to the methylene moiety becomes evident in the comparison of compounds **9** and **13**.

All active analogs except compound **2** are either esters or lactones. Apart from compound **2**, the carboxylic acids are inactive. It has been hypothesized by others[11] that lactonization is a prerequisite for antibacterial activity. Our data indicates that lactones and esters are both active in the MurA assay and the antibacterial screen. We therefore conclude that the relevant effect is not a steric (cyclic vs linear ester) but an electronic one (acid/anion vs neutral compound). The outlier, compound **2**, may either have a particularly strong propensity to form the lactone in situ, or the other structural features of the compound (which otherwise represents the optimum structure as discussed above) offset the detrimental electronic effect of the carboxylic acid group.

The bromo-analogs **10** and **11** were synthesized in an attempt to generate covalent UNAG-inhibitor adducts with increased electron density for protein-inhibitor co-crystallization experiments. Unfortunately, we were unable to obtain co-crystals of these compounds that were of sufficient quality for structure determination by X-ray diffraction. Of the phosphonic acid derivatives **14** and **15**, only the ester analog **14** showed some residual activity against the native MurA.

The C115D mutant MurA is practically resistant towards all tested compounds. Therefore, the cysteine residue must play a pivotal role in the formation of the UNAG-inhibitor adduct. It may be hypothesized that a covalent intermediate between inhibitor and the thiol group of Cys115 is generated before the formation of