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Design and structure–activity relationships of potent and selective inhibitors of undecaprenyl pyrophosphate synthase (UPPS): Tetramic, tetronic acids and dihydropyridin-2-ones

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Abstract—Based on a pharmacophore hypothesis substituted tetramic and tetronic acid 3-carboxamides as well as dihydropyridin-2-one-3-carboxamides were investigated as inhibitors of undecaprenyl pyrophosphate synthase (UPPS) for use as novel antimicrobial agents. Synthesis and structure–activity relationship patterns for this class of compounds are discussed. Selectivity data and antibacterial activities for selected compounds are provided.

Undecaprenyl pyrophosphate synthase (UPPS) is an enzyme essential for bacterial viability. It catalyzes *cis*-double bond formation during sequential condensation of eight isopentenyl pyrophosphate (IPP) molecules with farnesyl pyrophosphate (FPP) to form C_{55} undecaprenyl pyrophosphate, which is the lipid carrier for the precursors of various cell wall structures, such as peptidoglycan, teichoic acids, and *O*-antigens.[1] This critical biological function as well as an active site amenable to small molecule inhibition, as revealed by crystallographic structures, make UPPS an attractive target for the discovery of novel antibacterial agents.[2]

In the present study, we describe the first potent and selective inhibitors of UPPS.[3] The work profited from the recently published co-crystal structure of UPPS with the natural substrate farnesyl pyrophosphate in the active site of the enzyme.[2b] Based on the crystal structure, the pyrophosphate head of FPP is bound to the backbone NHs of Gly29 and Arg30 as well as the side chains of Asn28, Arg30, and Arg39 through hydrogen bonding (Fig. 1). The hydrocarbon moiety of FPP interacts with the side chains of hydrophobic amino acids. The micromolar inhibitor of UPPS, tetramic-acid derivative **1a**, was identified in a high throughput screening and can be docked into the active site occupying the FPP binding site (Fig. 1).

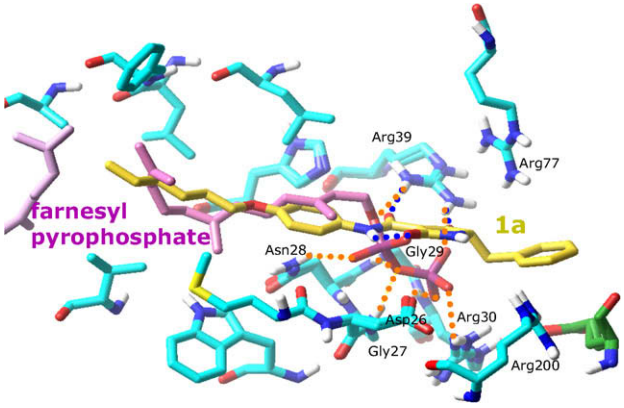

Figure 1. Structure of undecaprenyl pyrophosphate synthase (UPPS) from *E. coli* in complex with farnesyl pyrophosphate (in pink color; H-bonds in orange). Tetramic-acid derivative **1a** (yellow color) is docked into the binding site occupied by FPP, showing two H-bonds to Arg39 and an internal H-bond (in blue).

The analysis of this binding mode resulted in the hypothesis that two hydrogen acceptors and a hydro-

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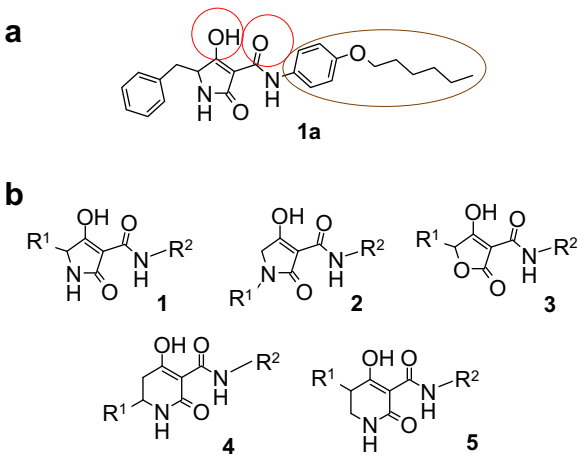
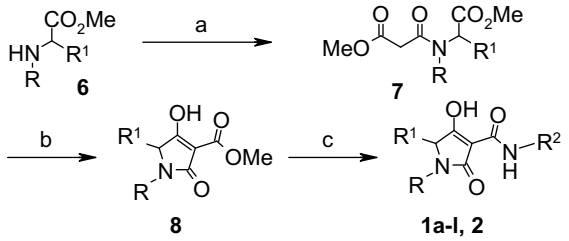


Figure 2. (a) Chemical structure of **1a** with the 4-hydroxy and amide bond oxygen atoms as hydrogen acceptors and the 4-hexyloxyphenyl group as hydrophobic contact. (b) Classes of potent UPPS inhibitors identified using the pharmacophore hypothesis.

phobic group serve as pharmacophoric points (Fig. 2a) whereas the ring carbonyl oxygen together with the exocyclic amide NH forms a hydrogen bond to stabilize the conformation of the molecule.

This hypothesis proved fruitful in our endeavour to design and synthesize novel UPPS inhibitors. Along with tetramic acids **1**, *N*-alkylated tetramic acid **2**, tetronic acids **3** and the two regioisomeric dihydropyridin-2-ones **4** and **5** match this pharmacophore and demonstrate potent inhibition of UPPS (Fig. 2b).

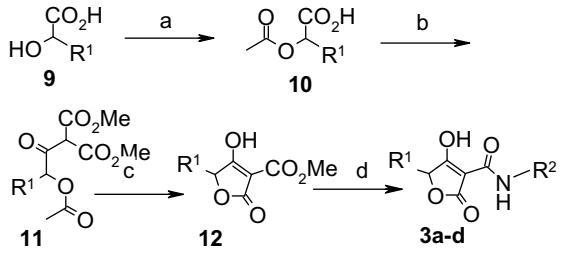
Synthesis: Tetramic acids (**1** and **2**) were prepared in three steps from commercially available racemic α-amino acid methyl esters **6**. Acylation with methyl malonyl chloride provided the intermediates **7** which were subjected to Lacey–Dieckmann cyclization conditions to yield 3-methoxycarbonyl tetramic acids **8** as building blocks in good yields.[4] Formation of 3-carboxamides was best achieved with microwave heating at temperatures between 100 and 120 °C in THF or ethanol as solvent. Higher temperatures caused significant decarboxylation and formation of vinylogous amides.[5] In the case of **1g** (R^1 = 2-imidazolylmethyl) removal of the trityl-protecting group with trifluoroacetic acid completed the synthesis (Scheme 1).



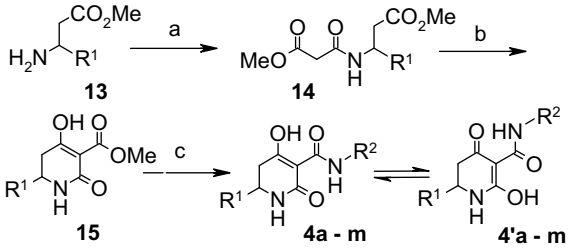
Scheme 1. Reagents and conditions: (a) methyl malonyl chloride, CH_2Cl_2, Et_3N, rt, 2 h, 72–98%; (b) NaOMe, MeOH, reflux, 2 h, 76–99%; (c) R^2-NH_2, THF or EtOH, microwave synthesizer, 100–120 °C, 5–8 min, 40–80%, for **1g**: then TFA, rt, 2 h.

Enantioselective synthesis of 3-acyl tetramic acids **1** starting from enantiomerically pure amino acids was not pursued because of reported partial racemization under the cyclization conditions.[6] Instead, racemates could be separated into the pure enantiomers (ee > 98%) by chiral chromatography on a Chiralpak AD column. Sodium salts of **1** were isolated by precipitation after the addition of 0.95 equiv aqueous 1 M NaOH to a solution of the acid in methanol.

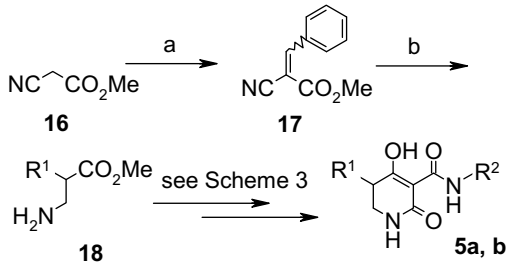
The synthesis of 3-acyl tetronic acids **3** employed the methodology by Igglessi-Markopoulou.[7] α-Hydroxy acids **9** were *O*-acylated with acetic anhydride followed by *C*-acylation between dimethyl malonate and the *N*-hydroxybenzotriazole ester of the *O*-protected α-hydroxy acid **10**. Cyclization to compounds **12** was achieved by treatment with 20% HCl in dioxane for 16 h. Amide bond formation with amines in toluene under reflux



Scheme 2. Reagents and conditions: (a) acetic anhydride, pyridine, 16 h, 100%; (b) 1—HOBt, DCC, THF, 0 °C, 16 h; 2—NaH, dimethyl malonate, 0 °C to rt, 3 h; (c) dioxane, 20% HCl, 16 h, 60% (b + c); (d) R^2-NH_2, toluene, reflux, 1 h, 40–60%.

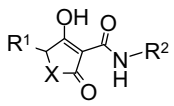
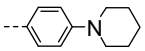
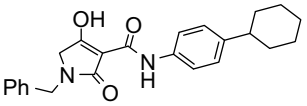


Scheme 3. Reagents and conditions: (a) methyl malonyl chloride, CH_2Cl_2, Et_3N, rt, 2 h, 76–100%; (b) NaOMe, MeOH, reflux, 2 h, 12–77%; (c) R^2-NH_2, THF, microwave synthesizer, 120–150 °C, 5–8 min, 40–80%.



Scheme 4. Reagents and conditions: (a) piperidine, toluene, benzaldehyde, reflux, 4 h, 74%; (b) for R^1 = $PhCH_2$: Pearlman's cat., EtOH, concd HCl, 50 psi H_2, rt, 3 d, 91%; for R^1 = cyclohexyl-CH_2: PtO_2, EtOH, concd HCl, 50 psi H_2, rt, 3 d, 77%.

Table 1. In vitro activity against *S. pneumoniae* (sp) UPPS, human (hu) FPPS (IC_{50}s, μM) and minimal inhibitory concentration (MIC, μg/mL)[9]

	X	R^1	R^2	spUPPS	huFPPS	*Enterococcus faecalis*	*Staphylococcus aureus*	*S. pneumoniae*
1a	NH	$PhCH_2$	4-Hexyloxyphenyl	19				
1b	NH	$PhCH_2$	4-Cyclohexylphenyl	1.8	>100	8	0.5	1
1c	NH	H	4-Cyclohexylphenyl	58				
1d	NH	Ph	4-Cyclohexylphenyl	3.5	>100	4	2	2
1e	NH	$PhCH_2CH_2$	4-Cyclohexylphenyl	0.16	>100	4	4	1
1f	NH	Isobutyl	4-Cyclohexylphenyl	1.3	>50	2	0.5	1
1g	NH	2-Imidazolyl-methyl	4-Cyclohexylphenyl	4.2		16	16	8
1h	NH	Methoxymethyl	4-Cyclohexylphenyl	>50				
1i	NH	$PhCH_2CH_2$	4-Biphenyl	0.12	>100	16	4	2
1j	NH	$PhCH_2CH_2$		0.80	>50	8	4	4
1k	NH	$PhCH_2CH_2$	4-Fluorophenyl	2.5		64	16	16
1l	NH	$PhCH_2CH_2$	Cyclohexyl	35		32	32	64
2				0.3	8	1	0.5	
3a	O	Ph	4-Chlorophenyl	24	>50	>64	>64	64
3b	O	Ph	3,5-Dichlorophenyl	2.0	>50	32	32	4
3c	O	$PhCH_2$	4-Cyclohexylphenyl	0.5	>50	32	64	1
3d	O	$PhCH_2$	4-Biphenyl	1.1		32	>128	4

conditions furnished the tetronic-acid derivates **3** (Scheme 2).

The less common carboxamides of dihydropyridin-2-one-3-carboxamides **4** were synthesized in a similar fashion to the tetramic acids **1** starting from β-amino acid esters **13** (Scheme 3). Reaction with methyl malonyl chloride provided the malonyl amides **14** which were condensed to the esters **15**.[8] The amides **4** were formed by reaction with amines in tetrahydrofuran in varying yields under microwave conditions at higher temperatures (120–150 °C) compared to the tetramic acids **1**. Enantiomerically pure compounds **4** were obtained starting from commercially available (*S*)- or (*R*)-β-amino acid esters **13**.

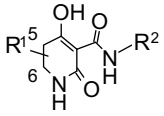
Compounds **4** exist in DMSO-d_6 as a ~1:1 mixture of the two tautomers **4** and **4′** with different orientation of the amide substituent R^2.[8] In addition, these compounds are weak acids with a pK_a of ~6.5–7 compared to the tetramic and tetronic acids **1**, **2** and **3** with pK_a's of 3–3.5.

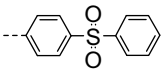
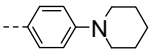
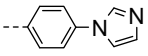
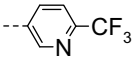
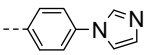
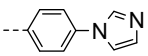
Regioisomeric compounds **5** were synthesized in the same way from the corresponding β-amino acid esters **18** (Scheme 4). The two amino acid esters **18** (R^1 = benzyl and cyclohexylmethyl) were easily prepared from the Knoevenagel product **17** by hydrogenation with either Pearlman's catalyst or PtO_2 under otherwise identical conditions.

Results and discussion: Table 1 shows the structure–activity relationship (SAR) for a number of substituents R^1 and R^2 within the tetramic (X=NH) and tetronic acid series (X=O). The potency of the initial hit **1a** could be improved by changing the 4-hexyloxyphenyl substituent to the conformationally more restricted 4-cyclohexylphenyl group (**1b**). Hydrophobic groups at the R^1 position proved beneficial (**1d–f**), whereas the unsubstituted compound **1c** showed significantly reduced enzyme inhibition. Polar R^1 substituents (**1g**, **h**) are less tolerated and resulted in higher IC_{50} values. Interestingly, the enantiomers of the active compound **1e** had almost identical enzyme activity (data not shown).

Sodium salts of compounds **1** provided similar potency as the free acids. Optimization of R^2 resulted in arylphenyl groups (e.g., **1i**) or heterocycloalkyl-phenyl groups (e.g., **1j**). Activity decreased with smaller aromatic substituents (**1k**) and dropped further with aliphatic groups (**1l**) at this position. The substituent R^1 could be moved to the ring nitrogen (**2**) without loss

Table 2. In vitro activity against *S. pneumoniae* (sp) UPPS, human (hu) FPPS (IC_{50}s, μM) and minimal inhibitory concentration (MIC, μg/mL)[9]



	R^1	R^2	spUPPSs	huFPPS	*E. faecalis*	*S. aureus*	*S. pneumoniae*
4a	6-Ph	4-Cyclohexylphenyl	0.2	>50	8	4	4
4b	(*S*)-6-Ph	4-Cyclohexylphenyl	0.07	>100	64	4	4
4c	(*R*)-6-Ph	4-Cyclohexylphenyl	0.18	>50	64	4	4
4d	6-$PhCH_2$	4-Cyclohexylphenyl	0.04	>50	64	32	8
4e	6-Cyclohexyl	4-Cyclohexylphenyl	0.06		32	>32	>32
4f	6-Isobutyl	4-Cyclohexylphenyl	0.2		64	>64	4
4g	6-$PhCH_2$	4-Phenoxyphenyl	0.11	>50	64	16	32
4h	6-$PhCH_2$	4-Anilinophenyl	1.1				
4i	6-$PhCH_2$		8.5		16	32	16
4j	6-$PhCH_2$		0.11	>50	16	64	64
4k	6-$PhCH_2$		0.63		32	32	32
4l	6-$PhCH_2$		0.14	>50	16	16	8
4m	6-$PhCH_2$	Cyclohexyl	0.07	>50	64	64	64
5a	5-$PhCH_2$		8.8		4	64	32
5b	5-Cyclohexylmethyl		2.1		4	16	16

of activity demonstrating that the ring NH is probably not a key element for interaction with the binding site. Tetronic acids (**3a**–**d**) showed similar enzyme activities and the 4-cyclohexylphenyl substituent proved to be one of the best R^2 groups here, too.

The SAR of the dihydropyridin-2-one carboxamides **4** and **5** is summarized in Table 2. The SAR shows similarity with the tetramic acids **1** but the IC_{50} values are typically lower. At the R^1 position, hydrophobic groups are preferred with the benzyl and cyclohexyl substituent being the most potent groups (**4a**, **d**–**f**). As observed before, the difference in activity for the two enantiomers (**4b**, **c**) is very small with a slight preference for the *S*-enantiomer **4b**.

The 4-cyclohexylphenyl group in R^2 position was again one of the best substituents (**4d**) but a number of other substituents, as demonstrated with compounds **4g**, **j**, **l**, including the aliphatic cyclohexyl group (**4m**), showed excellent inhibitory activity. 4-*N*-Imidazolyl-phenyl as a polar residue was tolerated with a slight loss in activity (**4k**). A comparison between the three compounds **4g**, **h** and **i** shows that the conformation of the R^2 substituents impacts activity: the compound with the strongly non linear 4-benzenesulfonylphenyl substituent (**4i**) shows less activity than the compounds with the more linear 4-anilino- or 4-phenoxy-phenyl group (**4g**, **h**). Shifting the R^1 substituent from the 6-position to the 5-position is tolerated (**5a**, **b**) but a direct comparison of compound **5a** versus **4k** indicates a ~10-fold loss in activity. All compounds tested showed good selectivity towards human farnesyl pyrophosphate synthase (FPPS), a *trans*-prenyltransferase which is the target of bisphosphonate drugs.[10]

Most tetramic- and tetronic acids **1**–**3** and dihydropyridiones **4**–**5** exhibited Gram-positive antibacterial activity in the range of 0.5–64 μg/mL (Tables 1 and 2). A comparison of the three compound classes shows better antibacterial activity for the tetramic acids **1** and reasonable correlation between enzyme inhibition and MIC effectiveness in *Streptococcus pneumoniae*, whereas the more potent UPPS inhibitors **4** are typically weaker antibacterial agents. This might be attributed to differences in physical–chemical properties and cell permeability between the two compound series.

In conclusion, new UPPS inhibitors were designed inspired by the binding mode of the natural substrate farnesyl pyrophosphate and subsequently synthesized. This design principle may find use in the development of new antibacterial compounds. Some of these compounds (e.g., **1i**, **j**, **4a**) show sub-micromolar spUPPS enzyme inhibition and antibacterial activity against Gram-positive bacteria. However, in the presence of blood serum a large shift to higher MICs in the tested Gram-positive bacteria is observed. This finding will require further optimization of compound properties to develop the UPPS inhibitors shown here into new antibacterial agents for in vivo use.

References and notes

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3. Several UPPS inhibitors were identified by a high throughput screening but no structures were disclosed (a) Li, H.; Huang, J.; Jiang, X.; Seefeld, M.; McQueney, M.; Macarron, R. *J. Biomol. Screen.* **2003**, *8*, 712; Less selective bisphosphonates as UPPS inhibitors are described in (b) Guo, R.-T.; Cao, R.; Liang, P.-H.; Ko, T.-P.; Chang, T.-H.; Hudock, M. P.; Jeng, W.-Y.; Chen, C. K.-M.; Zhang, Y.; Song, Y.; Kuo, C.-J.; Yin, F.; Oldfield, E.; Wang, A. H.-J. *PNAS* **2007**, *104*, 10022.
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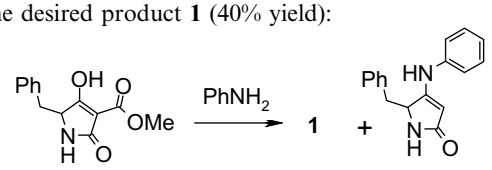
5. Reaction of 5-benzyl-3-methoxycarbonyl tetramic acid with aniline at 200 °C for 10 min provided 5-benzyl-4-phenylamino-1,5-dihydro-pyrrol-2-one in 34% yield next to the desired product **1** (40% yield):



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8. Weiss, R.; Presser, A.; Seebacher, W. *Monatsh. Chem.* **2003**, *134*, 1129.
9. *spUPPS enzyme biochemical assay: S. pneumoniae* UPPS was expressed and purified as an N-terminal His_6 fusion. The purified enzyme was then mixed with liposome made from *Escherichia coli* total lipid extract (Avanti Polar Lipids, Inc., Alabaster, AL). The enzyme reaction was carried out in 100 mM Tris–HCl, pH 7.3, 50 mM KCl, 1 mM $MgCl_2$, 0.01% Triton X-100, 20 μg/mL BSA, 3 μM FPP, 16 μM IPP, and 2 U/mL *E. coli* inorganic phosphatase. The inorganic phosphate generated in the reaction was then quantified with Biomol Green reagent (Biomol International, Plymouth Meeting, PA). Human FPPS reaction was carried out under the same condition as the one for UPPS using 8 μM DMAPP and 16 μM IPP. *MICs:* The quantitative in vitro determination of minimum inhibitory concentration (MICs) of novel compounds against Gram-positive aerobic bacteria was performed by microdilution broth method of testing as recommended by the standards approved by the Clinical and Laboratory Standards Institute (formerly, NCCLS), *Methods for Dilution Antibacterial Susceptibility Tests for Bacteria that Grow Aerobically*—7th edition. Approved Standard Document M7-A7, Vol. 20. No. 2 CLSI, Wayne, PA.
10. Rondeau, J.-M.; Bitsch, F.; Bourgier, E.; Geiser, M.; Hemmig, R.; Kroemer, M.; Lehmann, S.; Ramage, P.; Rieffel, S.; Strauss, A.; Green, J. R.; Jahnke, W. *ChemMedChem* **2006**, *1*, 267.