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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY EVALUATION OF A DUAL-DRUG BASED ON THE ANTIBACTERIAL PEPTIDE LUGDUNIN FUNCTIONALIZED WITH THE CARBONIC ANHYDRASE SULFONAMIDE INHIBITOR ACETAZOLAMIDE.

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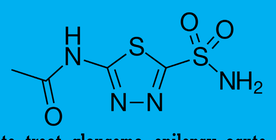
Acetazolamide (AAZ)



AAZ is a therapeutic used to treat glaucoma, epilepsy, acute mountain sickness, periodic paralysis, idiopathic intracranial hypertension, heart failure and to alkalinize urine. Moreover, acetazolamide is a first-generation carbonic anhydrase inhibitor

Potent inhibitor

Carbonic anhydrase (CA)

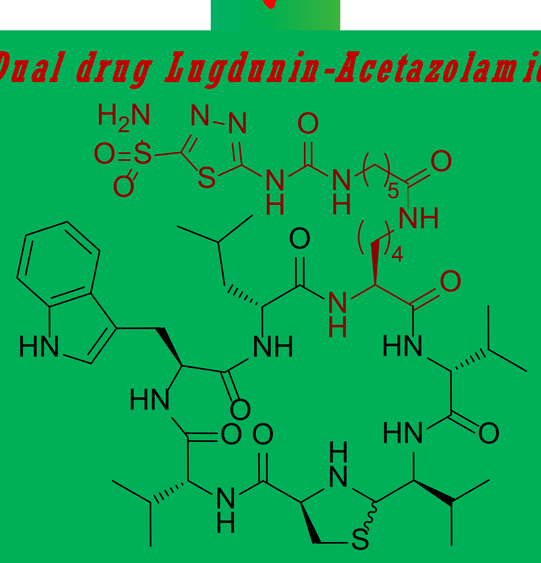
CA is a zinc-containing enzyme that plays a crucial role in various physiological processes by catalyzing the reversible hydration of carbon dioxide (CO_2) into carbonic acid (H_2CO_3). This family of metal enzymes is a promising target for the development of antibacterial compounds because it plays a key role for the survival of the microorganism.

Three major classes

α → Primarily found in animals but also present in some bacteria (Gram negative) and green algae. These are carbonic anhydrases present in humans and sulfonamides are powerful inhibitors.
β → Widely found in microorganisms, algae, plants, and invertebrates.
γ → Primarily found in bacteria [1]

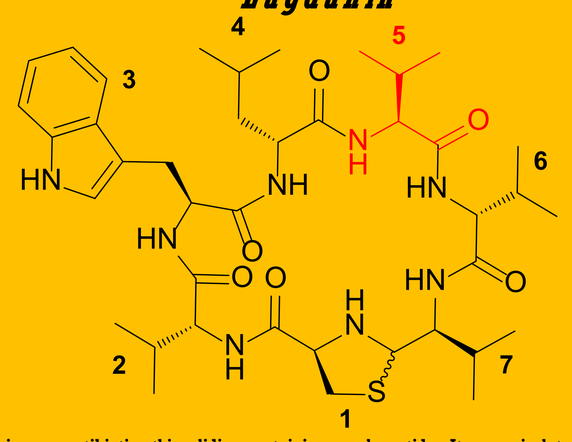
β and γ are more difficult to inhibit with sulfonamides [2]

Dual drug Lugdunin-Acetazolamide



Synthesis of a novel dual drug Lugdunin-Acetazolamide, by replacing L-Val[5] with L-Lys[5] and exploiting the amino function for subsequent coupling.

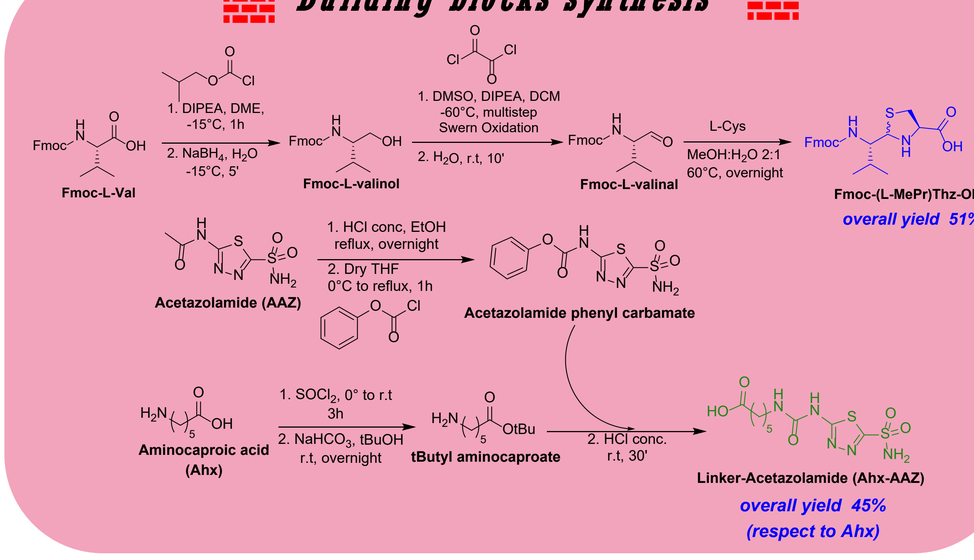
Lugdunin



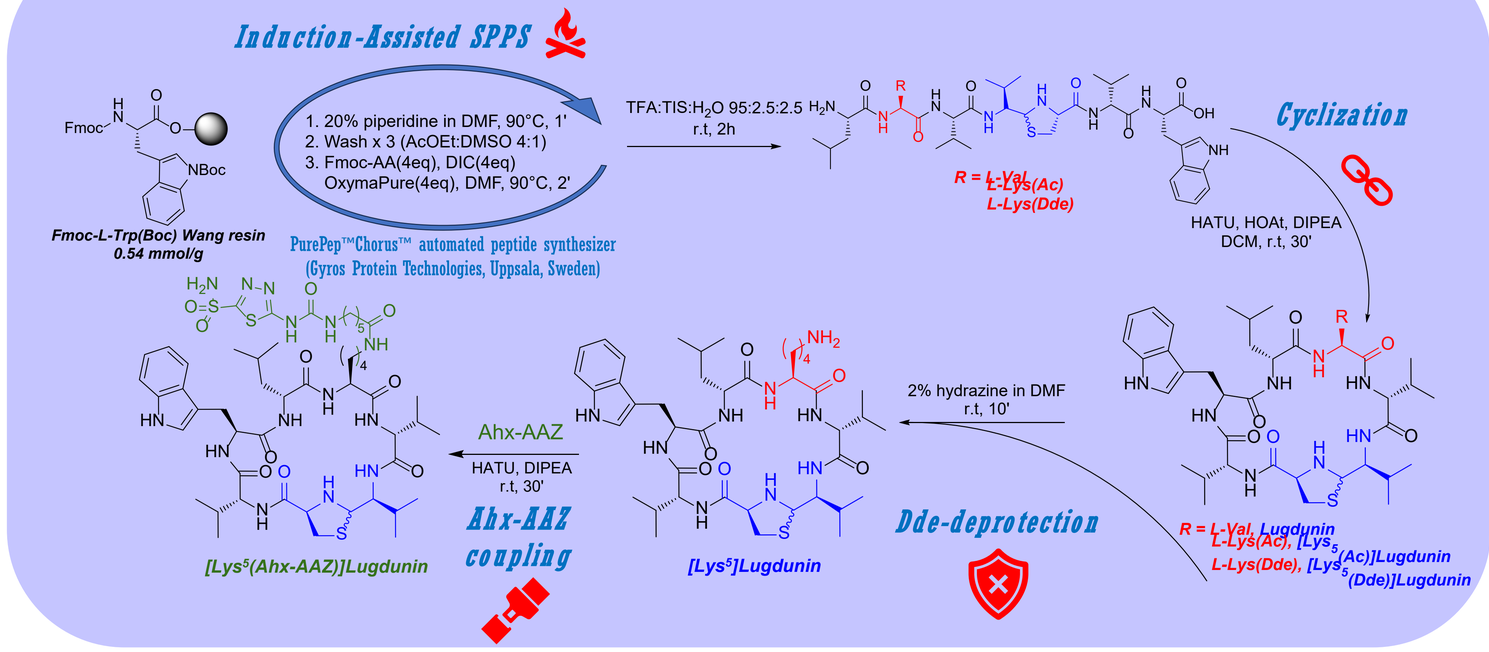
Lugdunin is an antibiotic thiazolidine-containing cyclopeptide. It was isolated in 2016 from *Staphylococcus lugdunensis*. This cyclopeptide displays potent antimicrobial activity against a broad spectrum of Gram-positive bacteria, including challenging-to-treat methicillin-resistant Staphylococcus aureus (MRSA) (MIC = 3.9 μM). In particular, it inhibits target bacteria by dissipating their membrane potential, forming supramolecular peptide channels [3,4].

An Ala-scan performed by Schilling et al. showed that changing L-Val[5] with an L-Ala[5] residue does not influence the antimicrobial activity [5].

Building blocks synthesis



Cyclopeptide synthesis



Pathogenic carbonic anhydrases tested

Carbonic anhydrase	Pathogen	Gram
MtbCA1, CA2, CA3	*Mycobacterium tuberculosis*	+
MscCA	*Mammaliicoccus sciuri*	+
PgiCA	*Porphyromonas gingivalis*	-
EcoCA	*Escherichia coli*	-
HpCA	*Helicobacter pylori*	-
VchCA	*Vibrio cholerae*	-
BpsCA	*Burkholderia pseudomallei*	-
SmuCA	*Streptococcus mutans*	+
NgCA	*Neisseria gonorrhoea*	-
EfCA	*Enterococcus faecalis*	+

Analytical characterization of Lugdunin analogs

Cyclopeptide	HPLC gradient (% B)[a], Rf (min)	HPLC purity (%)	Yield[b] (%)	ESI-MS (m/z) found[c] (calcd)
Lugdunin	30-90, 4.60-4.82	91	60	783.80 (783.45)
[Lys5(Ac)]Lugdunin	30-90, 3.72-3.87	95	45	854.85 (854.49)
[Lys5(Ahx-AAZ)]Lugdunin	30-90, 3.52-3.62	90	20	1132.10 (1132.52)

The cyclopeptides were characterized by RP-HPLC Alliance Chromatography system (Waters, Milford Massachusetts, USA) with a BEH C18 (1.7 μm, 2.1× 50 mm) column at 35°C, 0.6 mL/min, coupled to a single quadrupole ESI-MS Micromass ZQ (Waters, Milford Massachusetts, USA). Eluents: 0.1% (v/v) TFA in H_2O (A) and 0.1% (v/v) TFA in ACN (B), λ 215 nm. Gradient times: [a]5min. [b]Calculated as the ratio of obtained mass to theoretical mass. ESI-MS: detected as [c][M+H]$^+$.

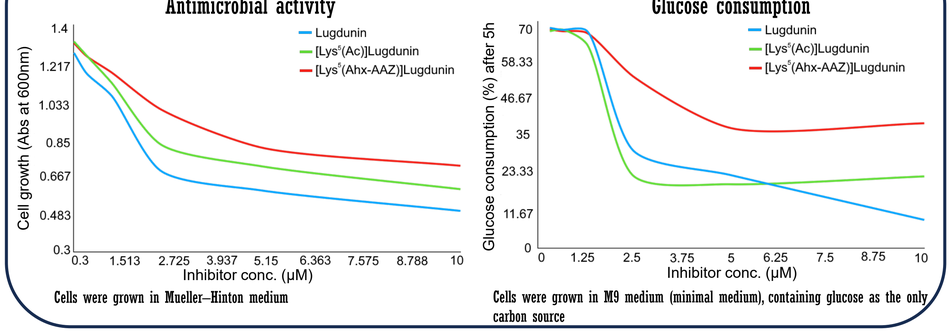
Inhibition by Stopped-Flow technique

Inhibition of different carbonic anhydrases

Inhibitory constant (K_I) expressed in nM concentration, using Acetazolamide (AAZ) as standard drugs. Mean from 3 different assays, by a stopped Flow-Technique (errors were in the range of ± 5-10 % of the reported values).

	α Carbonic anhydrase						β Carbonic anhydrase										γ Carbonic anhydrase				
Inhibitor	**hCA I**	**hCA II**	**HpCAα**	**VchCAα**	**NgCAα**	**EfCAα**	**MtbCA1β**	**MtbCA2β**	**MtbCA3β**	**MscCAβ**	**PgiCAβ**	**EcoCAβ**	**HpCAβ**	**VchCAβ**	**BpsCAβ**	**SmuCAβ**	**PgiCAγ**	**EcoCAγ**	**VchCAγ**	**BpsCAγ**	**EfCAγ**
[Lys5(Ac)]Lugdunin	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000	>100000
[Lys5(Ahx-AAZ)]Lugdunin	332.9	88.9	23.4	19.3	34.9	70.2	373.4	33.6	59.0	184.9	81.4	343.6	70.6	1125	612.4	170.3	478.5	432.5	445.2	288.2	427.1
AAZ	250.0	12.0	21.0	6.8	74.1	56.7	480.0	9.1	104.0	628.0	214.0	227.0	40.0	4512	745.0	344.0	324.0	248.0	473.0	149.0	322.8

In-vitro assays against E.coli



Conclusions

The Lugdunin-acetazolamide conjugate showed promising inhibitory activity in several pathogenic carbonic anhydrases. In particular, compared to acetazolamide, the conjugate shows greater activity in β carbonic anhydrases and comparable activity in α and γ ones.

Regarding the assays against *E.coli*, all three Lugdunin analogs interfered with both bacterial growth and glucose consumption at different concentrations. This demonstrates that Lugdunin-based derivatives are also active against Gram-negative bacteria.

Perspectives

The synthetic Lugdunin analogs will be tested against various pathogenic Gram-positive and Gram-negative bacteria. In addition, the synthesis of Lugdunin analogs with benzensulfonamide replacing sulfonamide will be performed.

In addition, we will explore a triazolyl-based linker between Lugdunin and the inhibitor, formed via Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC).

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