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Arginine–pyrimidine conjugates with therapeutic and prophylactic activity in lethal bacterial infections

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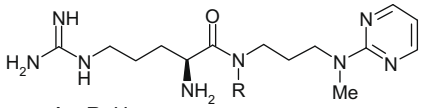
ABSTRACT

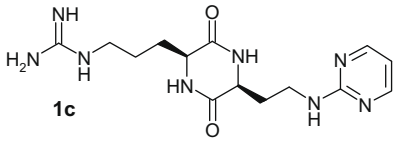
Arginine–pyrimidine conjugates represent a novel class of compounds that exhibits therapeutic and prophylactic activity in lethal infections by Gram-positive and Gram-negative bacteria without showing antibacterial activity in vitro.

The discovery and development of the classical antibacterial agents has been one of medicine's major achievements.[1] But at present, the efficacy of once powerful drugs is continually lessening as more and more antibiotic-resistant bacteria emerge.[2] Innovative non-traditional approaches—alone or in combination with classical antibiotics—could help to complement and secure our future therapeutic options.[3] To better understand the complex interplay of bacterial pathogens and the innate immune defense in infective disease states, it seems wise to also explore host–pathogen interactions[4] and the quorum sensing cross-talk between microbes.[5] Efficient host defense against bacterial pathogens relies on immune recognition and coordination of various signaling pathways. Bacteria respond to adverse environmental conditions by adaptive responses that activate stress-dependent regulatory networks. The biosynthesis of bacterial virulence factors, special proteins that disturb essential processes of immune defense, is triggered by such environmental signals. For example, uropathogenic *Escherichia coli* depends on S fimbrial adhesins to bind to eukaryotic receptors containing sialic acid and to colonize tissues.[6] The associated signal transduction mechanism is a trans-acting process that utilizes a membrane bound signal sensor (transactivator gene product) and a cytoplasmic regulator which after activation binds to regulatory regions of the bacterial DNA. In vitro test systems which use this pathway of bacterial signal transduction to measure adaptive responses have been developed and used to discover novel lead structures.[7]

Here, we report the synthesis and biological profile of novel arginine–pyrimidine conjugates **1** that exhibit therapeutic and prophylactic activity in lethal infections by Gram-positive and Gram-negative bacteria without showing antibacterial activity in vitro (Fig. 1). The conjugates **1** bear some vague structural resemblance to the natural polyamines spermidine and spermine, which due to their polycationic structure interact with negatively charged macromolecules and cellular components interfering with cell proliferation.[8] In addition, polycations such as poly-L-arginine have been used as immunostimulants.[9]



1a: R=H
1b: R=Me



1c

Figure 1. New arginine–pyrimidine conjugates. The initial hit **1a** was discovered in an *E. coli* transactivator assay.

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Initially, conjugate **1a** attracted our attention by reducing the production of intracellular β-galactosidase in an *E. coli* transactivator assay.[6,7] Prototype **1a** showed no antibacterial activity in vitro but exhibited efficacy in vivo in lethal murine sepsis models (infectious dose: LD_{50}–LD_{90}) with Gram-negative and Gram-positive bacteria (Table 1).

Incorporation of an additional *N*-methyl-group provided conjugate **1b** which was more stable in mouse serum and showed a prolonged half-live in vivo.[10] Conjugate **1b** exhibited no antibacterial activity in vitro as well (MIC >250 μg/mL against *E. coli and Staphylococcus aureus*). Intraperitoneal (ip) treatment of lethal infections by *E. coli* in mice with 30 mg/kg 0.5 h and 4 h post infection resulted in 80% survival (Table 1). Notably, the observed efficacy depended on the infective dose (inoculum).

In an *E. coli* sepsis, a 1.5 log reduction (7.8×10^4 to 2.7×10^3 CFU[11]/mL) of bacteria from blood was observed 5 h after ip administration of a single dose of 50 mg/kg of **1b** 0.5 h post infection (pi). Repeated administration at 0.5 h and 4 h pi resulted in a more pronounced reduction of 2.5 log units (7.8×10^4 to 3.7×10^2 CFU/mL). No active metabolites were produced after incubation of **1b** in mouse serum and no antibacterial activity could be detected in the serum of mice which were treated with **1b**. In vivo efficacy in various sepsis models[12] strongly depended on the route of application. While intraperitoneal (ip) and subcutaneous (sc) application of **1b** showed good efficacy, peroral (po) and intravenous (iv) application produced only negligible effects. An active support by the innate immune defense seemed mandatory, since in immunocompromised mice **1b** showed no activity at all.[13] Prophylactic efficacy in a lethal *E. coli* sepsis model after ip treatment with 50 mg/kg of **1b** could be demonstrated up to 3 h before infection (Table 2).

Up to 450 mg/kg (ip) no acute and no subchronic toxic effects were observed in mice over 5 days. Neither an Ames-test nor broad in vitro and in vivo screening of **1b** provided hints for potential pharmacological or toxicological side effects.

An exemplary synthesis of **1b** is shown in Scheme 1. Thus, nucleophilic substitution of 2-chloropyrimidine **3** by *N*,*N*′-dimethylpropane-1,3-diamine (**2**) in dioxane gave diamine **4** that was coupled with tris-benzyloxycarbonyl-protected L-arginine in the presence of EDC and HOBt to afford protected conjugate **5**. Cleavage of the benzyloxycarbonyl groups by HBr in acetic acid and removal of excessive acid by addition of basic ion exchange resin M 600 provided **1b** in three steps and 55% overall yield (Scheme 1).

Structural variations of the guanidine, the pyrimidine and the diamine spacer afforded less active congeners. However, reduction of rotational bonds and integration of conformational constraints in diketopiperazine **1c** were tolerated.[14]

Table 1
Therapeutic efficacy in lethal murine sepsis models after ip treatment with 30 mg/kg 0.5 h and 4 h post infection[a]

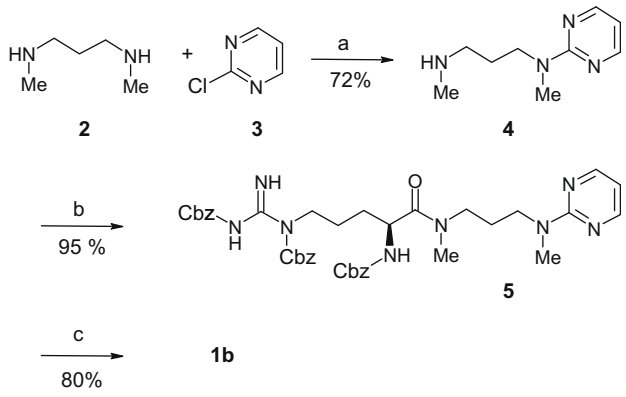
Compound	Survivors (%)	
	S. aureus	*E. coli*
1a	40	40
1b	60	80
Ciprofloxacin	40	60
Control	20	40

[a] Five mice per group.

Table 2
Prophylactic efficacy in a lethal murine *E. coli* sepsis model after ip treatment with 50 mg/kg of **1b** before infection[a]

Time before infection (h)	3	2	1	0.5	Control
Survivors (%)	40	60	80	80	20

[a] Five mice per group.



Scheme 1. Exemplary synthesis of arginine–pyrimidine conjugate **1b**. Reagents and conditions: (a) 1,4-dioxane, 48 h, rt; (b) $(Cbz)_3$Arg-OH, EDC, HOBt, CH_2Cl_2, 16 h, rt; (c) HBr, HOAc, 4 h, rt; basic ion exchange resin M 600. Cbz: benzyloxycarbonyl, EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt: 1-hydroxybenzotriazole.

In summary we have described the synthesis and the preliminary biological profile of novel arginine–pyrimidine conjugates **1** that exhibit therapeutic and prophylactic activity in lethal infections by Gram-positive and Gram-negative bacteria without showing antibacterial activity in vitro. In the presence of conjugates **1** bacteria seemed no longer able to adapt to the host environment and were eradicated by the host immune system. An interference with the bacterial signal transduction pathway could be a possible reason. Additional studies are required to fully understand the role of conjugates **1** in host–pathogen interaction and to elucidate their mode of action.

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10. Conjugate **1b** was completely stable in mouse plasma after 3 h at 37 °C. Pharmacokinetic parameters, mouse: $t_{1/2}$ = 1 h, c_{max} = 56 μg/mL (30 min after ip application of 100 mg/kg **1b**).
11. CFU = colony forming units.
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13. Wound infection by *Pseudomonas aeruginosa* or muscle infection by *Streptococcus pyogenes* in cyclophosphamide-treated (2 × 100 mg/kg ip on day 4 and day 1 before infection) immunocompromised mice.
14. Compared to **1b**, bacteria were more efficiently eliminated from the blood of mice with diketopiperazine **1c**. In preliminary studies a superior efficacy in infection models (*E. coli*, *S. aureus*) was observed.