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Influence of Fluorination at Position 16 of Antibacterial Pristinamycins II#

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Abstract: The influence of a fluorine atom at position C(16) of antibacterial pristinamycins II upon antibacterial, physico-chemical and pharmacokinetics properties is described.

Keywords: Antibacterial · DAST · Fluorination · Pristinamycins II

Introduction

During the last twenty years, resistance among Gram-positive organisms such as *Staphylococcus aureus*, the coagulase-negative staphylococci, *Streptococcus pneumoniae* and the enterococci has slowly evolved into a major concern for public health, especially in hospitals [1]. To face the challenge of multi-resistant Gram-positive bacteria, the streptogramin class of antibiotics constitutes a particularly attractive response. The streptogramins are naturally occurring antibiotics discovered more than forty years ago. They are indeed unique among the antibiotics in that they consist of the association of two structurally unrelated components: the group B components, such as pristinamycin I (PI), members of the macrolide-lincosamides-streptogramin B group (the so-called MLS_B group) are cyclic depsipeptides (Fig. 1) whereas the group A components such as pristinamycin II (PII) are peptidic macrolactones (Fig. 1). The combination of two molecules, one from each group, acts synergistically on the ribosome of bacteria, thereby inhibiting protein synthesis [2]. Streptogramins display *in vitro* bacteriostatic activity against most Gram-positive cocci, certain Gram-negative bacteria (such as *Haemophilus*), certain fastidious bacteria and Gram-positive anaerobes as well as *in vitro* and *in vivo* bactericidal activity against most of these sensitive species. They are also characterized by a long post-antibiotic effect and a very low resistance selection rate (below 1 cell in 10^{10} cells). As a consequence of these unique properties, the prevalence of resistant staphylococci in France is still below 5% [3] though Pyostacine®, a streptogramin made up of a mixture of pristinamycin IA and IIA as major components in a 30/70 ratio by weight, has been extensively used for more than thirty years in this country for respiratory tract infections and for skin or bone infections.

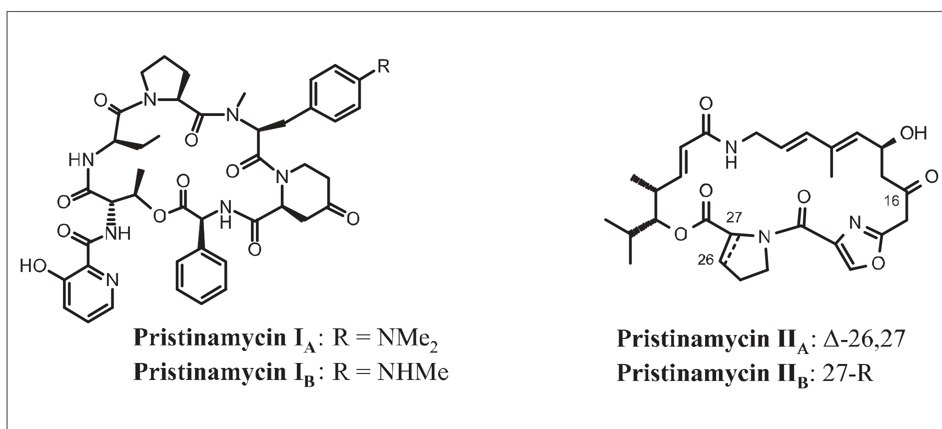

Fig. 1. Structures of representative group A and group B streptogramins

Because of the lack of water solubility of natural pristinamycins that had prevented their use for treating Gram-positive severe infections in hospital, we initiated, in the 80s, a program of semi-synthesis aimed at discovering water-soluble antibacterial pristinamycins. These endeavors culminated with the development of Synercid® (Fig. 2), the first injectable streptogramin that has been approved in the US in 1999 for the treatment of severe Gram-positive infections in hospital [4–6].

After this first success, we continued our efforts to identify the next-generation oral streptogramin. With this objective in mind, we embarked upon a new program of semi-synthesis both in the PI and PII series. Regarding this latter series, we were particularly interested in introducing a fluorine atom at C(16). Based on the well-known bioisosteric relationship between a carbonyl (or a C–OH bond) and a C–F bond, we reasoned that the fluorine atom would retain similar inhibition of the target ribosome compared to natural pristinamycins II while providing an opportunity to overcome the known limitations of the pristinamycins II, namely chemical instability at acidic/basic pHs due mainly to the sensitive β-hydroxy ketone system which is very

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#This publication is dedicated to the memory of my colleague Jean-Claude Barrière.

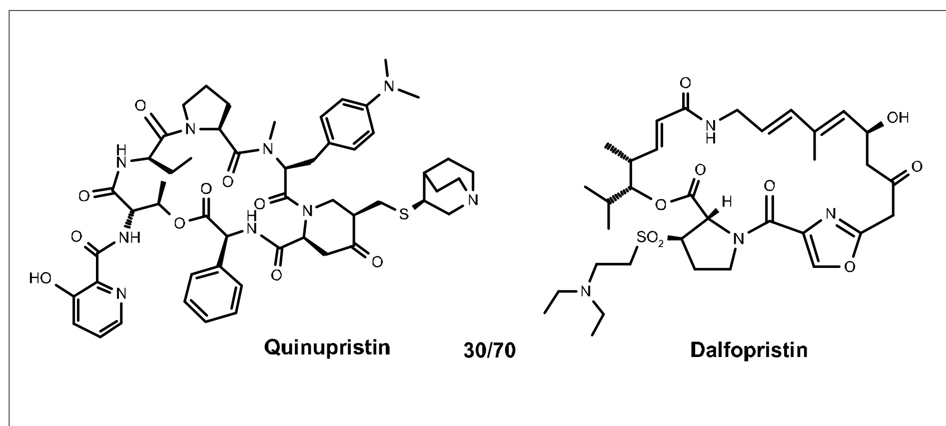
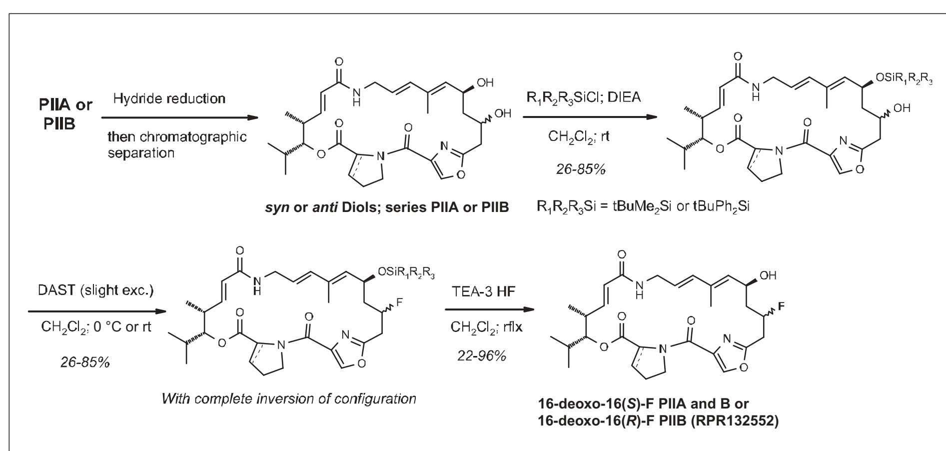


Fig. 2. Structure of the two components of Synercid®



Scheme. Preparation of 16-fluoro pristinamycins II

Table 1. Comparative *in vitro* activities of C(16)-substituted PIIs

16-Substituent (PII series)	Poly(U) (IC_{50}; [µM])	MICs (IP8203) (alone; [µg/ml])	MICs (IP8203) (with PIB; [µg/ml])	Synergy
=*O* (PIIA)	*0.2*	*2*	*0.06*	*Strong*
=*O* (PIIB)	*0.1*	*4*	*0.12*	*Strong*
(*R*)-OH (PIIA)	0.11	1	0.5	Weak
(*S*)-OH (PIIA)	> 10	32	0.5	Strong
(*R*)-OH (PIIB)	0.05	1	0.25	Moderate
(*S*)-OH (PIIB)	6	32	0.5	Strong
(*R*)-F (PIIA)	0.07	0.12	0.03	Moderate
(*R*)-F (PIIB) **(RPR132552)**	0.06	0.25	0.03	Strong
(*S*)-F (PIIB)	0.5	8	0.5	Strong

prone to dehydration to generate inactive trienones, low oral absorption and poor metabolic stability resulting in poor pharmacokinetics (PK) properties. Hereafter, we report the successful preparation of 16-deoxo-16-fluoro pristinamycins IIA and IIB and we discuss the impact of the 16-fluoro substituent upon various properties of the naturals PIIs and of their corresponding diols such as antibacterial activities, aqueous solubility, chemical stability, absorption/disposition/metabolization/excretion (ADME) properties, and pharmacokinetics parameters.

Results and Discussion

The *syn* and *anti* diols PIIs [2][7] (16-deoxo-16(*R*)- and 16(*S*)-hydroxy PIIs, respectively), obtained as mixtures by simple hydride reduction of the corresponding natural PIIs, were obvious precursors of the desired 16-fluoro PIIs, *via* a DAST-mediated substitution of the 16-hydroxy group. Because of the presence of a second hydroxy group at C(14), direct fluorination of the diols only delivered intractable mixtures. This observation prompted us to mask the 14-hydroxy group during the fluorination step by a silyl group that we had previously shown could be introduced efficiently and regioselectively while being cleavable under mild conditions by fluoride ions.

We were delighted to find that DAST-fluorination of 16-deoxo-16-hydroxy-14-silyloxy PIIs proceeded smoothly with complete inversion of configuration at C(16) to afford the corresponding 16-fluoro PIIs and that subsequent desilylation cleanly afforded the expected 16-deoxo-16-fluoro PIIs. In the PIIB series, the overall yields were exceptionally high for pristinamycin II chemistry (above 80%) whereas yields were not as good from 16(*S*)-PIIA diol due to a more pronounced fragility of the 16(*R*)-fluoro derivative under the reaction conditions (Scheme).

All the compounds mentioned above were evaluated for their antibacterial activities: inhibition of the target ribosome as measured by the poly(U) dependent poly(Phe) synthesis test (a cell-free translation assay [8]), *in vitro* activity alone and in association with PIB (minimum inhibitory concentrations: MICs in mg/l [9]), *in vivo* activity, in association with PIB, by the oral (*po*) or subcutaneous (*sc*) routes, in a model of septicemia induced by a representative sensitive *S. aureus* [10]. In terms of inhibition of the ribosome (see column 2 of Table 1), the 16(*R*)-fluoro PIIs (*syn* isomers) displayed IC_{50}s similar to those of the corresponding natural PIIs and to those of the corresponding 16(*R*)-diols. These observations clearly demonstrated that, in the pristinamycin II series, a 16(*R*)-fluoro was an effective bioisosteric replacement of the 16-carbonyl group and of the 16(*R*)-hydroxyl group. The 16(*S*)-fluoro PIIB (*anti* isomer) was less active than the corresponding 16(*R*) epimer and PIIB but much more active than the 16(*S*)-diol PIIB. The same influence of the stereochemistry upon target inhibition was observed in the diol series: whatever the pristinamycin A or B series, the 16(*R*) epimer was much more active than the 16(*S*) epimer.

MICs alone (PII alone; column 3 of Table 1) were roughly correlated to target inhibition: the most potent derivatives on the target also displayed the best MICs alone. However, for a similar level of ribosome inhibition, the MICs could significantly vary. For example, the two 16(*R*)-PII diols were much less active against bacteria than the corresponding 16(*R*)-fluoro PIIs. This was also the case for PIIB compared to

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Table 2. Comparative *in vivo* activities of C(16) substituted PIIs

16-Substituent (PII series)	MIC (IP8203; associated to PIB; [μg/ml])	*In vivo* (septicemia *sc*; *S.a.* IP8203; ED_{50} [μg/kg]; associated to PIB 30/70)	*In vivo* (septicemia *po; S.a.* IP8203; ED_{50} [μg/kg]; associated to PIB 30/70)
=O (PIIA)	*0.06*	*nd*	*130*
=O (PIIB)	*0.12*	*3*	*95*
(*R*)-OH (PIIA)	0.5	8	95
(*S*)-OH (PIIA)	0.5	2.4	100
(*R*)-OH (PIIB)	0.25	4	140
(*S*)-OH (PIIB)	0.5	4.6	95
(*R*)-F (PIIA)	0.03	9.5	110
(*R*)-F *(PIIB)* **(RPR132552)**	0.03	5	40
(*S*)-F (PIIB)	0.5	< 1.5	110

Table 3. Comparative *in vivo* activities and ADME properties of C(16) substituted PIIs

Entry	16-Substituent	*In vivo* (septicemia *po;* IP8203; ED50 [mg/kg]; associated to PIB 30/70)	Metabolic Turnover[a]	Caco-2 % absorbed[b]
1	=*O* (PIIA)	*130*	*48 %*	*4.4*
2	=*O* (PIIB)	*95*	*41 %*	*2.3*
3	(*R*)-OH (PIIA)	95	24 %	0.7
4	(*S*)-OH (PIIA)	100	34 %	0.07
5	(*R*)-OH (PIIB)	140	32 %	0.7
6	(*S*)-OH (PIIB)	95	20 %	nd
7	(*R*)-F (PIIA)	110	66 %	nd
8	(*R*)-F (PIIB) **(RPR132552)**	40	62 %	8
9	(*S*)-F (PIIB)	110	30 %	nd

[a]% of compound metabolized by CD1 mouse hepatic microsomes, 5 μM; incubation time 20 min. [b]Absorption across Caco-2 monolayers apical to basal: % of appearance in the receptor chamber after 180 min; concentration 100 μM. nd: not determined.

the 16(*R*)-fluoro PIIs. These discrepancies probably reflected different capacities of these PIIs to cross the bacterial membranes. For Gram-positive bacteria, increased lipophilicity is expected to favor penetration, which would explain improved MICs for the 16-fluoro derivatives compared to the more hydrophilic diols and natural PIIs (see column 3 of Table 4 for selected logD values at pH 7.4).

Regarding MICs in association with PIB (PIB/PII: 30/70 w/w; see column 4 of Table 1), they did not result from a direct translation of the figures of the MICs alone. The level of synergy (column 5 of Table 1) depended on the nature of the PII component and surprisingly ranged from weak to strong, from a two-fold to a 64-fold factor, whatever the level of inhibition of the ribosome. These observations were difficult to understand on the sole basis of the published hypothesis that postulated that synergy of the streptogramin results from the increased affinity of the group B streptogramin following a conformational change of the ribosome induced by the binding of the group A streptogramin [11]. Our results suggest that complementary explanations are necessary to fully understand the phenomenon of synergy.

In vivo activities, in association with PIB (PIB/PII 30/70, w/w), were again not simply correlated to MICs (in association with PIB 30/70). By the subcutaneous route (column 3 of Table 2), all tested PIIs displayed activities in the same range (1 to 10 mg/kg), whatever the MICs of the combination PIB/PII. In particular, 16(*S*)-diol PIIA and 16(*S*)-fluoro PIIB were the two most potent PIIs though the less active *in vitro* (in combination with PIB). In contrast, the gain of potency observed *in vitro* for the 16(*R*)-fluoro PIIs was totally lost *in vivo sc*. These observations were clearly linked to the metabolic stability of the PII component (see column 4 of Table 3): a good metabolic stability of the PII component was able to compensate for a moderate *in vitro* potency (as for the PII diols or 16(*S*)-fluoro PIIB) and hence afford a better *in vivo sc* activity than for a PII displaying *in vitro* potency associated to metabolic instability (as for 16(*R*)-fluoro PIIB).

By the oral route, in association with PIB (column 4 of Table 3), all compounds, with the notable exception of the 16(*R*)-fluoro PIIB (entry 8), were as active or less active than PIIB.

High *in vitro* potency did not ensure high *in vivo* potency *po* (see in particular the case of PIIA, PIIB and 16(*R*)-fluoro PIIA in entries 1, 2, 7). Understanding these figures required the cross-examination of the ADME properties (column 4 of Table 3: metabolic stability as indicated by mice microsome turnovers and column 5 of Table 3: oral absorption as evaluated by Caco-2 absorption) and of the antibacterial properties of the PIIs. The modest antibacterial diols (entries 3 to 6), though metabolic stable, were poorly permeable and this could explain the modest *in vivo* activities by the oral route. For 16(*R*)-fluoro PIIB (entry 8 of Table 3), a combination of low MICs and high absorption rate resulted in a potent association *in vivo* by the oral route in spite of a high metabolic turnover. These properties were not unexpected based on the physicochemical characteristics of this PII compared to those of the diols and of natural PIIs: improved oral absorption was likely to result from increased lipophilicity associated to a reasonable aqueous solubility (see columns 5–7 of entry 4 in Table 4) while increased metabolization rate could also be a consequence of the higher lipophilicity that favored recognition by cytochrome P450 3A4 which is known to metabolize some PIIs [12]. These ADME properties were confirmed by the pharmacokinetics (PK) properties of 16(*R*)-fluoro PIIB (Table 5). Though still unsatisfactory (see in particular, the high clearance and following oral administration, the low systemic exposure considering the magnitude of the dose), the PK parameters of the fluoro derivative showed a significant improvement compared to PIIB. Absolute bioavailability was close to 2% compared to almost 0% for PIIB. This improvement could explain in part the better *in vivo* activity of 16R-fluoro PIIB compared to PIIB.

Regarding 16(*S*)-fluoro PIIB (entry 9 of Table 3), *in vivo* activity by the oral route was not in line with what we observed by

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Table 4. Comparative physico-chemical properties of C(16) substituted PIIs

Entry	PII	Log D pH 7.4	Chemical stability	Solubility [μg/ml]		
				pH 5	pH 7.4	Water
1	**PIIA**	1.9	Unstable between pH 2–4 and 7–12	50	50	50
2	**PIIB**	1.6	Unstable between pH 2–4 and 7–12	3500	3500	3500
3	**16(*R*) PIIB diol**	1.5	Stable between pH 4 to 13; unstable at pH 1 to 4	> 1000	4500	> 1000
4	**16(*R*)-F-PIIB (RPR132552)**	2.37	Stable between pH 4 and 9; unstable at pH 2 and 12	250	220	980
5	**16(*R*)-F-PIIA**	2.47	Unstable between pH 1–4 and 9–12; slight instability between pH 4 and 7.5	< 10	< 10	< 10

Table 5. Comparative pharmacokinetics properties of **RPR132552** and PIIB

PII	PI Dose [mg/kg]	C_{max} [ng/ml]	t_{max} [h]	F [%]	$AUC_{0-\infty}$ [h.ng/ml]	Terminal $t_{1/2}$ [h]	Cl_T [l/h/kg]	Vdss [l/kg]
PIIB[a]	26; *po*	nd	nd	0	na	na	na	na
16(*R*)-F PIIB[b]	100; *po*	701	0.08	1.8	445	0.6	na	na
16(*R*)-F PIIB	10; *iv*	na	na	na	2408	0.1	4.2	0.7

[a]Data generated following oral administration of PIIB suspended in saline solution containing 0.1% of polysorbate 80. [b]**RPR132552** (Discovery polymorph): iv administration as an aq. soln. containing 30% N-methyl pyrrolidone; oral administration alone by gavage as a suspension in 0.5% methyl cellulose/0.2% Tween 80. na: not applicable; nd: not detected.

the subcutaneous route. We assumed that, though relatively metabolically stable, systemic exposure following oral administration was probably not sufficient to ensure a C_{max} above the modest MIC of this compound (0.5 mg/ml) and hence high *in vivo* activity could not be achieved.

Chemical stability was certainly a complementary factor that contributed to the good *in vivo* activity of 16(*R*)-fluoro PIIB. As shown in Table 4 (entry 4, column 4), this derivative clearly displayed improved chemical stability compared to PIIB (entry 2), between pH 4 and 9. In spite of its good aqueous stability, its moderate metabolization rate and reasonable permeation properties, PIIB was not very potent *in vivo* by the oral route probably as a consequence of its reduced chemical stability under the acidic conditions of the gastro-intestinal tract. For PIIA (entry 1), chemical instability associated to a low aqueous solubility certainly explained its limited oral *in vivo* potency. Table 4 also suggested that chemical instability and low aqueous solubility (results shown for the 16(*R*) PIIB diol only; entry 3) were not operative to understand the moderate oral *in vivo* potencies of PII diols, since for these derivatives stability and solubility were both outstanding compared to natural PIIs.

Tables 3 and 4 also showed that the poor *in vivo* activity by the oral route of 16(*R*)-fluoro PIIA (entry 7 of Table 3 and entry 5 of Table 4) was mainly a consequence of poor aqueous solubility resulting probably in poor oral absorption. The tendency toward a reduced aqueous solubility when going from the PIIB to the PIIA series was also observed for the natural PIIs and for the 16(*R*)-fluoro PIIs. The reduced chemical stability of the 16(*R*)-fluoro PIIA compared to the PIIB analogue could also provide an additional explanation to the reduced oral *in vivo* potency of this derivative. Globally, our results suggested that oral *in vivo* activity of pristinamycins II resulted from a subtle balance between *in vitro* antibacterial activity, ADME/PK properties, chemical stability and aqueous solubility.

Overall, introducing a fluorine atom at C(16) of pristinamycins II had the following consequences: the 16(*R*)-fluoro PIIs were against the target ribosome equipotent to their analogous 16(*R*)-hydroxy or 16-oxo PIIs; more generally, all 16(*R*)-fluoro PIIs displayed improved MICs alone and in combination with PIB against Gram-positive bacteria owing to increased lipophilicity that certainly favored passage through the bacterial membranes; this higher lipophilicity also induced a reduced aqueous solubility and higher metabolization rates but improved oral absorption provided aqueous solubility was not too low. These combined characteristics resulted, in the case of 16(*R*)-fluoro PIIB, in enhanced PK properties and hence, in improved *in vivo* potency by the oral route. Finally, fluorination at C(16) fostered chemical stability by substituting to the very sensitive β-hydroxy ketone system of natural PIIs a more robust β-fluoro alcohol that was only sensitive to strongly acidic conditions due to the allylic nature of the remaining alcohol.

Based on its overall properties, **RPR132552** was selected as the PII component of the new oral streptogramin. In parallel, we selected **RPR202868** as the PI component. The new association **RPR202868/RPR132552** (30/70 w/w; Fig. 3) displayed an *in vitro* spectrum of bacteriostatic activity typical of the streptogramin family, including aerobic Gram-positive cocci (staphylococci including

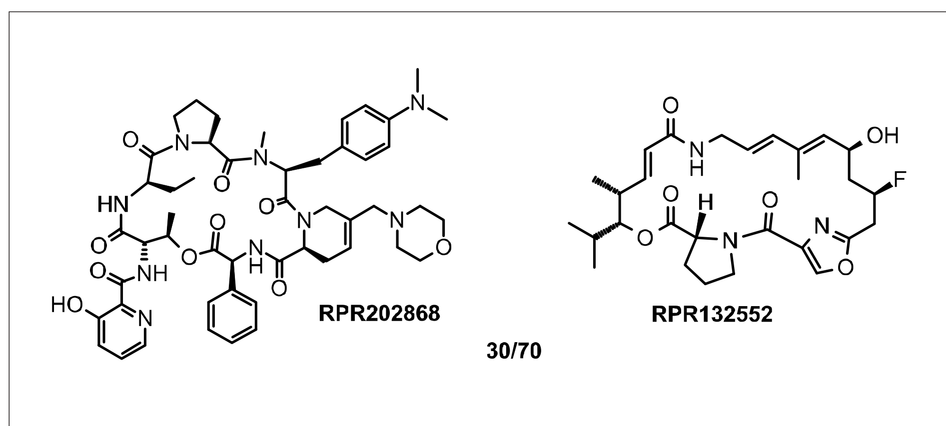

Fig. 3. Structures of both components of the new association **RPR202868/RPR132552** (30/70)

methicillin- MLS_B-resistant strains, streptococci, pneumococci including erythromycin- and/or penicillin-resistant strains, enterococci including vancomycin-resistant strains), certain aerobic Gram-negative bacteria responsible for respiratory tract infections (*Moraxella catarrhalis*, *Neisseria spp*. and *Haemophilus influenzae, Legionella spp*.) and anaerobes.

Its overall *in vitro* activity was generally close or slightly better than that of Synercid®; however, compared to Synercid®, the new association demonstrated enhanced potency against aerobic Gram-negative bacteria responsible for respiratory tract infections and against enterococci, including *E. faecium*. As previously known pristinamycin associations, **RPR202868/RPR132552** (30/70) was rapidly bactericidal against sensitive *S. aureus*, sensitive, erythromycin-resistant *Str. pneumoniae* and sensitive *H influenzae* strains tested. *S. aureus* resistant mutants were selected only at low frequency (10^{-9}–10^{-10}). Moreover, **RPR202868/RPR132552** demonstrated potent *in vivo* activities in all the mouse models of infection studied. This antibacterial profile led to the decision to select **RPR202868/RPR132552** (30/70) for clinical development. Overall, the properties of this new association suggest that this streptogramin might be of interest for the treatment of respiratory tract infections in the community and as an oral relay in hospital to treat severe Gram-positive infections.

Conclusion

We have presented the results of a program aimed at evaluating the impact of fluorination at C(16) of pristinamycins II. The targeted derivatives were prepared by a sequence involving regioselective silylation of the 14-hydroxy group, DAST-mediated substitution of the free 16-hydroxy group and final unmasking of the 14-hydroxy group. 16(*R*) fluorination was shown to lead to substantially improved antibacterial properties, both *in vitro* and *in vivo* by the oral route, as a consequence of retained inhibition of the target ribosome, of higher lipophilicity and of improved chemical stability. Based on these results, we were able to select **RPR202868/RPR132552** (30/70 ratio, w/w) as a new oral streptogramin candidate for clinical development following preliminary favorable antibacterial profiling and toxicological results. The overall properties of this association suggest that this new streptogramin might be of interest for the treatment of respiratory tract infections in the community and as an oral relay in hospital to treat severe Gram-positive infections. Detailed anti-bacterial activities of **RPR202868/RPR132552** will be reported elsewhere.

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[10] Mice 0F1 weighing 20±2 g were used for all experiments (3 to 6 males + 3 to 6 females per group). Experimental systemic infections were established by intraperitoneal injection of bacteria diluted so as to obtain an inoculum between 10 and 100 times the lethal dose. Mice were treated orally or subcutaneously by the new associations PIB/PIIs (30/70 w/w). At 7 days post-infection, the effective dose 50% (ED50) values were calculated as described by G.J. Miraglia, 'Chemotherapy of Infectious Diseases', Ed. H.H. Gadebush, C.R.C. Press, **1976**, 1. The ED50 was defined as the dose that protected 50% of treated mice when all the controls died.

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[12] In-house results for natural PIIs; for RPR132552, this compound was only metabolized by CYP3A4, with an estimated apparent Km of 2.5 μM.