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Short communication

Synthesis of osteotropic hydroxybisphosphonate derivatives of fluoroquinolone antibacterials

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Dedicated to professor Ferenc Fülöp on the occasion of his 60th birthday.

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ABSTRACT

1-Hydroxybisphosphonate derivatives of ciprofloxacin, gatifloxacin and moxifloxacin have been synthesized using Cu(I) catalyzed azide-alkyne 1,3-dipolar cycloaddition reaction. The 1,2,3-triazol linked hydroxybisphosphonate derivative of ciprofloxacin exhibited antibacterial activity comparable to the parent antibiotic and all fluoroquinolone-bisphosphonates displayed osteotropic properties in a bone model.

1. Introduction

Bisphosphonates (BP), structural analogs of pyrophosphate, are strong chelators of metal ions and can adsorb to the hydroxyapatite portion of the bone [1]. Since they can prevent hydroxyapatite dissolution [2] they are used in the medical praxis as anti-osteoporosis drugs [3]. The very high selectivity of bisphosphonates to bone mineral makes possible their use as structural elements of bone targeting medicinal agents [4,5]. Recently we reported a new class of bisphosphonate conjugates [6]: the BP derivatives of fluoroquinolone antibiotics including ciprofloxacin (**1**) (Fig. 1) which retained the *in vitro* antibacterial activity of the ciprofloxacin, albeit at a slightly lower level. **1** remained bound to washed bone powder and in this form, in suspension, completely inhibited the growth of staphylococci. On the influence of weak acid **1** released from bone powder and was able to rebind to it, maintaining the ability to inhibit the growth of staphylococci. Later, in an *in vivo* test, using a rat fracture model of osteomyelitis we have demonstrated the effectiveness of **1** in preventing bacterial burden [7]. In this study, **1** was delivered locally with a synthetic hydroxyapatite (Skelite™) serving as a mineral carrier.

On the basis of our *in vitro* and *in vivo* experiments it can be assumed that the antibacterial activity of such systems is due to adsorption–desorption equilibria of our bisphosphonates between the carrier apatite, the aqueous phase and the bone tissue.

As an extension of these studies, in the present work we report on the synthesis of hydroxybisphosphonate derivatives of fluoroquinolones as well as on the studies of their adsorptive characteristics on nanosized hydroxyapatite.

2. Results and discussion

2.1. Synthesis

1-Hydroxybisphosphonates, having a hydroxy substituent on the carbon connecting the two phosphate moieties, have in general increased adsorption for bone mineral [5,8]. This can be explained

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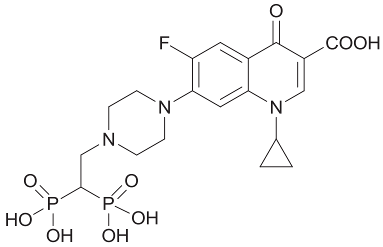

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Fig. 1. Structure of ciprofloxacin bisphosphonate.

by a tridentate binding involving the −OH group [9,10]. As a continuation of the research of bisphosphonate antibiotics, recently we decided to study the influence of the covalently bound hydroxybisphosphonate group on the antibacterial activity and bone targeting capacity of fluoroquinolone antibiotics. For the linking of fluoroquinolones to hydroxybisphosphonate targeting group the copper catalyzed alkyne-azide 1,3-dipolar cycloaddition reaction, the "click" reaction was chosen [11].

The fluoroquinolone antibiotics ciprofloxacin (**2a**), gatifloxacin (**3a**) and moxifloxacin (**4a**) were *N*-propargylated with propargyl bromide in DMF to give compounds **2b**, **3b** and **4b** (Scheme 1). *O*-Silylated 3-azidopropyl-tetraethyl bisphosphonate **5**, synthetic intermediary of alendronate [12] was used as the azido component.

Alkyne derivatives **2b**, **3b** and **4b** were allowed to react with **5** in the presence of a catalytic amount of cuprous iodide to give rise to the bisphosphonate ester derivatives **2c**, **3c** and **4c**. The protective groups were removed by treatment with bromotrimethylsilane obtaining hydroxy-bisphosphonic acids **2d**, **3d** and **4d**.

2.2. Antibacterial activities

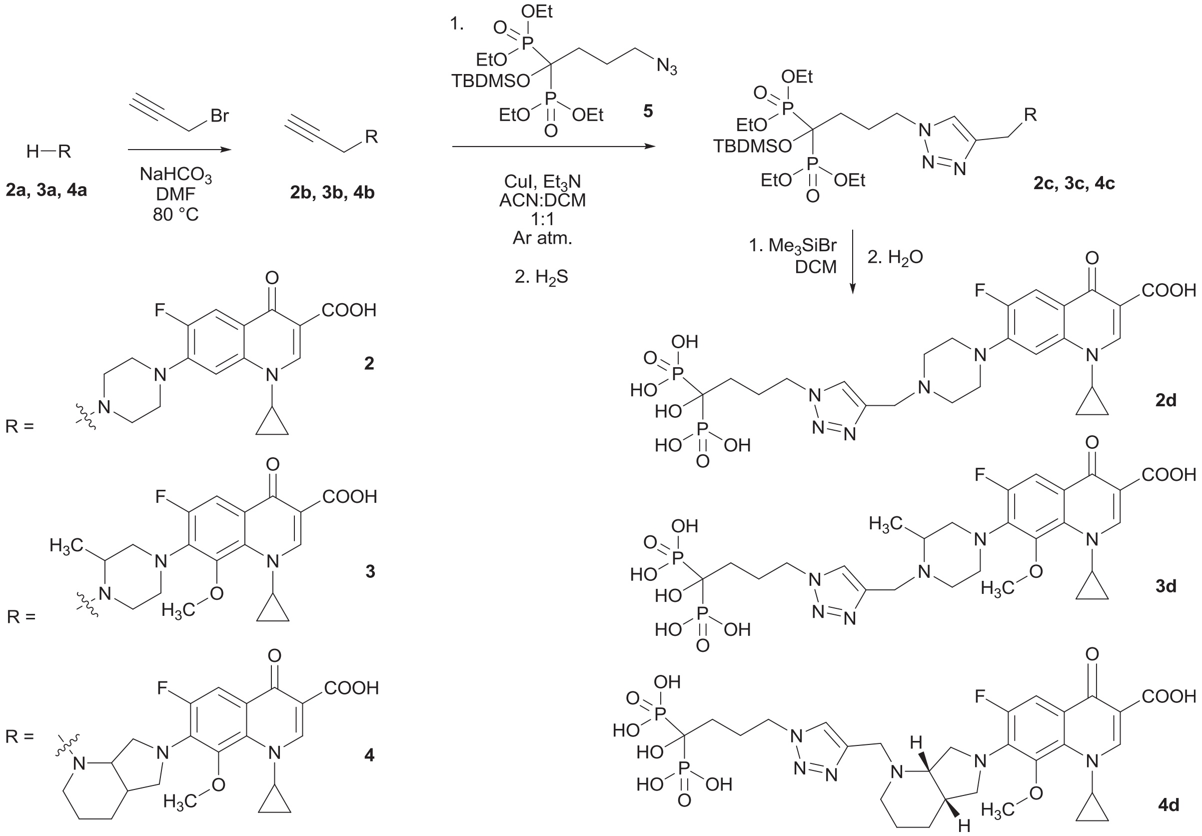
Recently we have demonstrated experimentally that **1** remained bound to washed bone and completely inhibited *Staphylococcus aureus* growth [6]. In this work we wanted to show that hydroxybisphosphonate derivatives of fluoroquinolone antibiotics with stable 1,2,3-triazole linkage could also have high antibacterial activity.

The antibacterial activity of hydroxybisphosphonate derivatives **2d**, **3d** and **4d** has been evaluated and the results are summarized in Table 1. The data demonstrate that OH−BP derivative of ciprofloxacin (**2d**) retained the original bacteriostatic and bactericidal activity of the parent antibiotic **2a** and the activity of **2d** has been improved compared to the simple BP derivative **1**. OH−BP derivatization of gatifloxacin (**3d**) caused a complete loss of activity against Gram negative strains associated with weakened activity against Gram positives, in comparison to that of the parent compound **3a**. Similarly, OH−BP-moxifloxacin (**4d**) was not active against Gram negatives, while activity was maintained against most of the Gram positive test bacteria.

When this work was being performed, two papers of Targanta Therapeutics came to our attention dealing with the synthesis of bisphosphonate prodrugs of fluoroquinolone antibiotics [13,14].

2.3. Adsorption studies

The osteotropic properties of the fluoroquinolone OH−BP-s were determined using NanOss®, a synthetic hydroxyapatite bone model. Interestingly, the fluoroquinolone moiety of the molecules **2d**, **3d** and **4d** strongly influenced their adsorptive properties. While ∼70% of **2d** adsorbed (B_{max}) on NanOss, this value was



Scheme 1. Synthesis of the fluoroquinolone OH−BP-s.

Table 1
Antibacterial activities.

	MIC/MBC (μg/ml)						
	1	**2a**	**2d**	**3a**	**3d**	**4a**	**4d**
Bacillus subtilis ATCC 6633	1/2	2/4	2/4	0.5/0.5	16/32	0.5/2	0.5/4
Staphylococcus aureus MSSA ATCC 29213	4/16	4/4	2/4	0.5/0.5	16/32	0.5/0.5	0.5/0.5
Staphylococcus aureus MRSA ATCC 33591	8/16	2/2	4/4	2/2	16/16	1/1	4/4
Staphylococcus epidermidis ATCC 35984 biofilm+	2/8	1/2	2/8	0.5/0.5	8/16	0.5/1	1/8
Staphylococcus epidermidis mecA+	8/8	2/2	2/2	0.5/0.5	16/16	1/1	4/4
Enterococcus faecalis ATCC 29212	4/256	2/2	2/4	0.5/256	16/128	1/1	16/32
Enterococcus faecalis 15376 vanA+	8/256	4/256	2/128	0.5/256	16/256	2/2	16/128
Enterococcus faecalis ATCC 51299 vanB+	4/256	2/4	4/4	0.5/8	16/128	0.5/1	0.5/64
Escherichia coli ATCC 25922	1/64	0.5/16	2/8	0.5/0.5	256	0.5/2	8/128
Klebsiella pneumoniae ATCC 700603	32/64	8/8	8/64	4/4	256	4/4	256
Proteus vulgaris ATCC 8926	4/4	1/8	8/32	2/8	256	0.5/1	128/256
Pseudomonas aeruginosa ATCC 27853	32/64	4/16	4/4	4/8	256	2/4	256

MIC: Minimum Inhibition Concentration, MBC: Minimum Bactericidal Concentration, ATCC: American Typed Culture Collection, MSSA: Methicillin Sensitive *Staphylococcus aureus*, MRSA: Methicillin Resistant *Staphylococcus aureus*, vanA +: vanA gene positive, vanB +: vanB gene positive.

~90% for **3d** and **4d** adsorbed quantitatively (Fig. 2). The B_{max} value of **2d** was slightly lower than that of **1** indicating that, at least in this case, introduction of OH—BP did not result in a better adsorption.

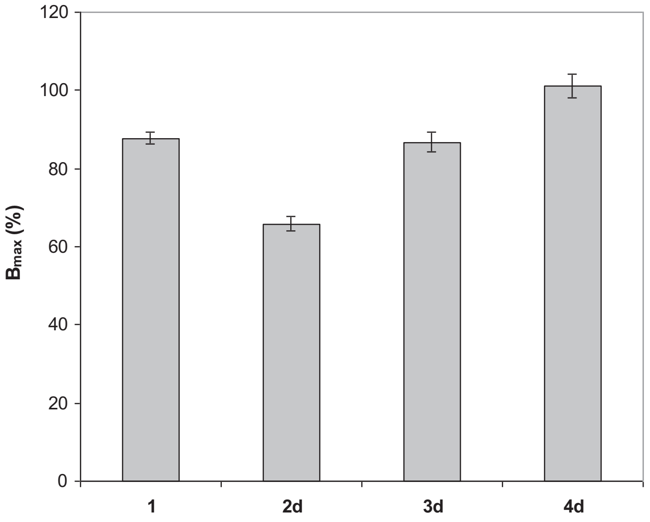


Fig. 2. Maximum adsorption values of the fluoroquinolone BP derivatives on NanOss®.

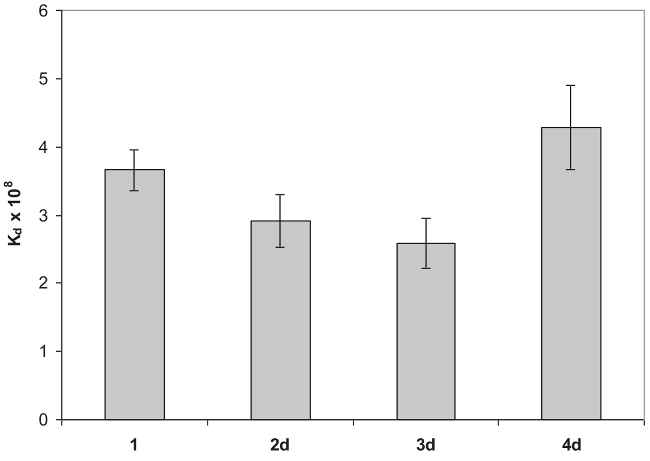


Fig. 3. Dissociation constants of the fluoroquinolone BP derivatives on bone model.

Dissociation constants K_d of the OH—BP-s are very similar to that of the **1** BP derivative (Fig. 3). Since the latter was used formerly for treatment osteomyelitis in an animal model, an analogous application of fluoroquinolone OH—BP-s could be possible. These studies are under investigation.

3. Conclusion

In summary, we have synthesized hydroxybisphosphonate derivatives of three fluoroquinolone antibiotics. Since the stability of the 1,2,3-triazol linker has been proved many times [11], the dissociation of the parent antibiotics **2a**, **3a** and **4a** from **2d**–**4d** can be excluded even in biological milieu. OH—BP derivative of ciprofloxacin (**2d**) possesses improved antibacterial activity compared to that of **1** “normal” bisphosphonate ciprofloxacin conjugate. Since adsorptive capacity of **2d** and on NanOss is satisfactory, **2d** is a candidate for *in vivo* studies.

4. Experimental

4.1. General experimental protocols

All reagents and solvents were of reagent grade or purified according to standard methods before use. Column chromatography was carried using Merck Kieselgel 60 silica (0.063–0.200 mm). NMR spectra were recorded on a Bruker Avance DRX 500 at the given frequency and in the given solvent. Chemical shifts are given in ppm. Mass spectra were obtained on a Bruker Biflex III (MALDI) or Bruker microTOF-Q (ESI QqTOF) spectrometer. Melting points were measured by a Koffler typed hotplate apparatus.

4.2. Synthesis

*4.2.1. Synthesis of **2b**, **3b** or **4b***

To a solution of **2a**, **3a** or **4a** (5 mmol) in abs. dimethylformamide 5 mmol of $NaHCO_3$ and 5 mmol of propargyl bromide were added under vigorous stirring. The mixture was allowed to react at 80 °C overnight. After evaporation the residue was purified by column chromatography using a CH_2Cl_2-—MeOH 98:2 mixture as eluent obtaining **2b**, **4b** or **4c**.

2b: yield 61%; mp. 248–250 °C; MS (MALDI-TOF) *m/z* calcd for $C_{20}H_{20}FN_3O_3Na^+$ $[M + Na]^+$: 392.14, found: 392.2.

3b: yield 68%; mp. 121–123 °C; MS (MALDI-TOF) *m/z* calcd for $C_{22}H_{24}FN_3O_4Na^+$ $[M + Na]^+$: 436.16, found: 436.2.

4b: yield 66%; mp. 180–181 °C; MS (MALDI-TOF) *m/z* calcd for $C_{24}H_{26}FN_3O_4Na^+$ $[M + Na]^+$: 462.18, found: 462.3.

4.2.2. Synthesis of **2c**, **3c** or **4c**

Azido-bisphosphonate compound **5** (100 mg, 0 2 mmol) and propargyl derivative **2b** and **3b** or **4b** (0.2 mmol) was dissolved in a mixture of abs. MeCN (3 ml) and abs. CH_2Cl_2 (3 ml). The solution was purged with argon, then triethylamine (27 μl, 0.2 mmol) and CuI (10 mg) were added and it was allowed to react for 4 h. Hydrogen sulfide was bubbled into the solution for 2 min, the solvent was evaporated and the residue was purified by column chromatography using CH_2Cl_2–MeOH 95:5 mixture as eluent.

2c: yield 24%; MS (MALDI-TOF) *m/z* calcd for $C_{38}H_{61}FN_6O_{10}P_2SiNa^+$ $[M + Na]^+$: 893.36, found: 893.4.

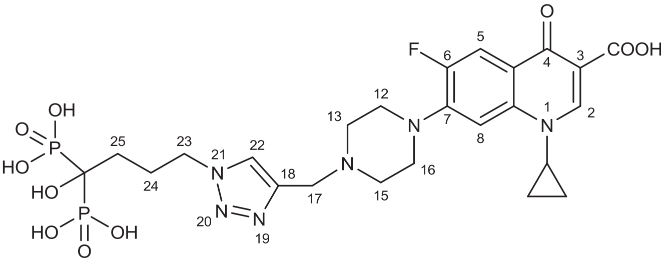
3c: yield 58%; MS (MALDI-TOF) *m/z* calcd for $C_{40}H_{65}FN_6O_{11}P_2SiNa^+$ $[M + Na]^+$: 937.38, found: 937.4.

4c: yield 52%; MS (MALDI-TOF) *m/z* calcd for $C_{42}H_{67}FN_6O_{11}P_2SiNa^+$ $[M + Na]^+$: 963.40, found: 963.4.

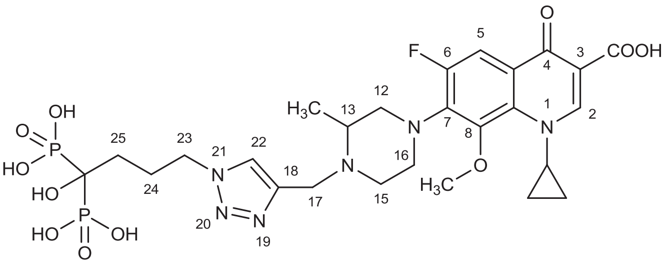
4.2.3. General procedure for the removal of ester protection from the bisphosphonates

1 mmol of **2c**, **3c**, or **4c** was treated with 10 mmol of bromotrimethylsilane for 5 days. After evaporation of the liquid phase 5 ml of water was added and stirred for 1 day. The crystalline product was removed by filtration, washed with water and methanol to give the end-product.

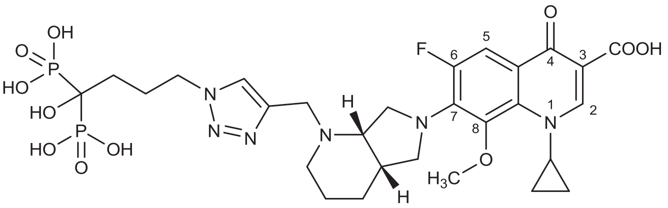
2d: yield 87%; mp. 263–272 °C; δ_H (500 MHz; D_2O; DSS) 1.08 (2H, m, CH_2 cyclopropyl), 1.31 (2H, m, CH_2 cyclopropyl), 1.93 (2H, m, CH_2 chain), 2.22 (2H, m, CH_2 chain), 2.80 (4H, m, $2CH_2$ piperazine), 3.30 (4H, m, $2CH_2$ piperazine), 3.59 (1H, dt, CH cyclopropyl), 3.80 (2H, m, H-17), 4.46 (2H, t, H-23), 7.57 (1H, m, H-22), 7.84 (1H, m, H-8), 8.07 (1H, m, H-5), 8.44 (1H, m, H-2); δ_C (125 MHz; D_2O; DSS) 7.9, 25.7 (t), 31.6, 35.2, 50.0, 51.1, 51.3, 51.8 (t), 74.3 (t), 107.5, 111.8 (d), 117.6, 122.8 (d), 125.9, 139.1, 142.5, 144.4 (d), 147.1, 153.8 (d), 173.3, 175.9; HRMS (ESI-TOF) *m/z* calcd for $C_{24}H_{30}N_6O_{10}FP_2^-$ $[M - H]^-$: 643.1488, found: 643.1498.



3d: yield 86%; mp. 241–251 °C; δ_H (500 MHz; D_2O; DSS) 0.91 (2H, m, CH_2 cyclopropyl), 1.09 (2H, m, CH_2 cyclopropyl), 1.24 (3H, d, CH_3), 1.93 (2H, m, CH_2 chain), 2.22 (2H, m, CH_2 chain), 2.59 (2H, m), 2.89 (1H, d), 3.06 (1H, t), 3.35 (3H, m), 3.69 (3H, s, OMe), 3.92 (2H, m, H-17), 4.03 (1H, m), 4.46 (2H, m, H-23), 7.67 (1H, m, H-22), 8.09 (1H, m, H-5), 8.49 (1H, m, H-2); δ_C (125 MHz; D_2O; DSS) 8.9, 9.0, 15.8, 25.7 (t), 31.6, 40.0, 47.2, 50.5 (d), 51.6, 51.7, 54.8, 57.2, 63.3, 74.2, 107.5 (d), 117.1, 124.4 (d), 126.0 (d), 134.5, 138.5 (d), 141.9, 146.4 (d), 149.5, 156.4 (d), 173.2, 175.7; HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{34}N_6O_{11}FP_2^-$ $[M - H]^-$: 687.1750, found: 687.1809.



4d: yield 97%; mp. 254–260 °C; δ_H (500 MHz; D_2O; DSS) 0.77 (m, 2H, CH_2 cyclopropyl), 1.04 (m, 2H, CH_2 cyclopropyl), 1.22 (t, 1H), 1.35 (m, 1H), 1.49 (m, 2H), 1.63 (m, 1H), 1.87 (m, 2H), 2.19 (m, 3H), 2.45 (m, 1H), 2.65 (m, 1H), 3.12 (m, 1H), 3.21 (m, 1H), 3.31 (m, 2H), 3.41 (s, 3H, OMe), 3.56 (m, 1H), 3.71 (m, 3H), 3.93 (m, 1H), 4.37 (m, 2H), 7.48 (m, 1H, CH triazole), 7.96 (m, 1H, H-5), 8.44 (s, 1H, H-2); δ_C (125 MHz; D_2O; DSS) 8.9, 9.4, 22.5, 23.8, 25.7, 39.9, 47.0, 47.9, 49.5, 49.7, 51.8, 54.9 (d), 55.1, 60.2, 61.5, 74.5 (t), 107.4 (d), 116.1, 124.6, 125.7, 134.8, 136.7 (d), 140.9 (d), 143.0, 149.7, 153.5 (d), 173.1, 175.6; HRMS (ESI-TOF) *m/z* calcd for $C_{28}H_{36}N_6O_{11}FP_2^-$ $[M - H]^-$: 713.1907, found: 713.1938.



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