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(54) Title: BISPHOSPHONATE QUINOLONE CONJUGATES AND USES THEREOF



FIG. 1

(57) Abstract: Described herein are bisphosphonate quinolone compounds, conjugates and pharmaceutical formulations thereof that can include a bisphosphonate (BP) and a quinolone, wherein the BP has an alpha substituent and the alpha substituent is a hydroxy, amino or thiol group and wherein the quinolone is directly or indirectly conjugated to the BP at the germinal carbon alpha substituent (X) of the BP. In one or more embodiments, the BP can be an alpha-OH containing BP and the quinolone can be directly or indirectely reversibly conjugated to the BP at a geminal OH of the BP. Also provided herein are methods of making and methods of using the bisphosphonate quinolone conjugates and pharmaceutical formulations thereof.

[Continued on next page]

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BISPHOSPHONATE QUINOLONE CONJUGATES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to co-pending US Provisional Patent Application No. 62/695,583, filed on July 9, 2018, entitled "BISPHOSPHONATE QUINOLONE CONJUGATES AND USES THEREOF", which is incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under grant number R41DE025789-01 and under grant number R42DE025789-02 awarded by the NIH/NIDCR as well as under grant number R43AR073727 awarded by the NIH/NIAMS. The US government has certain rights in the invention.

BACKGROUND

Bone and joint infections affect millions of adults and children worldwide. The overall incidence in the United States is 3-6 million persons, with specific populations having different risks. For diabetics, the annual incidence of foot ulcers is about 1 in 30, with underlying osteomyelitis in up to two-thirds of the cases. In children, recently reported annual incidence ranges from 1/4000 to 1/15000. However, in the Pediatric Health Information System (PHIS) database of administrative data from U.S. pediatric hospitals, we found 10,245 (0.5%) discharges with a diagnosis of osteomyelitis among 2,247,889 in a 5-year period from 2009-2013, for a rough annual incidence of approximately 1/1100 hospitalizations.

A number of Gram-positive and Gram-negative bacteria, as well as fungi and mycobacteria can cause bone and joint infections. By far the most common organism implicated in bone and joint infections is *Staphylococcus aureus (S.aureus)*, both methicillin-susceptible (MSSA) and methicillin-resistant (MRSA).

The standard of care for bone and joint infections usually requires systemic administration of antibiotics. For acute infections, intravenous antibiotics are generally prescribed for 2 – 6 weeks. Prolonged courses of oral antibiotics may follow for chronic infections, or infections associated with retained implanted hardware. Both for acute and chronic infections, these extended courses of therapy can lead to drug-related adverse events in a significant percentage

of patients – 15% in one estimate for a cohort treated for infections with MSSA. Moreover, it is known that nephrotoxicity with vancomycin, the most common therapy for MRSA infections, occurs in as much as 43% of patients, and increases with duration of therapy.

Persistent bone infections such as jaw osteomyelitis, osteomyelitis at other skeletal sites and osteonecrosis can culminate in significant bone resorption and destruction of bone and hydroxyapatite (HA) mineral. Bone and HA resorption is thought to be induced and mediated not only by bone cells, i.e. osteoclasts, but also microbial biofilm pathogens in combination with host inflammatory responses and osteoclastogenic activity. Biofilms are a complex microbial community composed of one or more bacterial species attached to a substrate and surrounded by a self- produced extracellular matrix. Many different types of microbial infections are known to be caused by organisms growing in a biofilm state. Bacterial biofilms of *Staphylococcus aureus (S. aureus)* are the dominant cause of biofilm- associated infections in health care systems and are associated with serious infections such as osteomyelitis.

Osteomyelitis is associated with significant morbidity and mortality. Surgery and antimicrobial therapy, often intravenous and longer-term antibiotics, are mainstays of osteomyelitis management. Surgery can involve conservative removal of infected bone or more aggressive modalities such as resection. Thus, treatment of infectious bone disease is mainly antimicrobial therapy with or without surgical intervention depending on clinicopathologic factors. Antibiotics, however, have poor bone absorption and pharmacokinetics in vivo. Therefore, any improvement in bone bioavailability of therapeutic antibiotics would be a significant advancement in treating osteomyelitis.

To overcome the many challenges associated with treating bone infections, it has become common practice by clinicians to use local delivery systems for achieving higher therapeutic antibacterial concentrations in bone. For example, polymethylmethacrylate beads represent the majority of non-biodegradable carrier systems used to deliver antibiotics to orthopedic infections, but they require surgical removal upon completion of drug release. They also tend to release antibiotics in an initial burst pattern that quickly depletes the bulk of the drug from the carrier beads, followed by a slow release at lower concentrations that may not be adequate to control infection and may foster development of resistance. These concerns limit the usefulness of this approach in the majority of bone and joint infections.

Dentistry has used local delivery of antimicrobials to treat infected jawbone associated

with conditions like periodontal bone loss, jaw osteomyelitis and osteonecrosis in order to reach high local concentrations of drug, but these modalities are often ineffective without surgical intervention and bone bioavailability of antibiotic is poor. Antibiotic-impregnated cement, used primarily at the time of first debridement of an infected implant to improve control of the infection, is not generally used in the treatment of bone and joint infections of native bone without implanted hardware. Concerns about prolonged sub-therapeutic antibiotic concentrations and selection of resistant organisms also apply to cement.

Localized delivery of antimicrobial agents to bone could be a significant step forward in treating infectious bone disease, but still has penetration limitations and potential eukaryotic cell cytotoxicity; thus, research and the development of more effective and physiologically targeted delivery systems are in high demand. An ideal antibiotic delivery system is one that targets bone tissue without the need for surgical implantation or removal. Such targeting also minimizes systemic doses and exposure of tissues other than bone to antibiotics, therefore reducing the risk of adverse effects or selective pressure facilitating the emergence of resistant organisms. Reduced dosing frequency made possible by achieving prolonged concentrations of the antibiotic at the site of infection is another potential major benefit.

The inadequate efficacy of current antimicrobial treatments for osteomyelitis has been ascribed to the limited access of systemically administered antibiotics to sites where causative bacteria can reside, including as biofilms on bone surfaces, even surfaces within the osteocytic canalicular network, where a class of drugs known as bisphosphonates (BPs) readily gain access.

SUMMARY

Provided herein, in various aspects, are BP quinolone antibiotic compounds, conjugates and formulations, and various methods of use thereof, to address the aforementioned needs.

In any one or more aspects herein, to exploit BP affinity for bone, a “target and release” chemistry approach involving delivery of a quinolone antibiotic to bone or hydroxyapatite (HA) surfaces via BP conjugates is provided, in particular to sites where bone infections have initiated and elevated bone metabolism has taken place. Relatively serum-stable drug- BP linkers can be utilized that metabolize and release the parent quinolone antibiotic most preferentially at the bone surface.

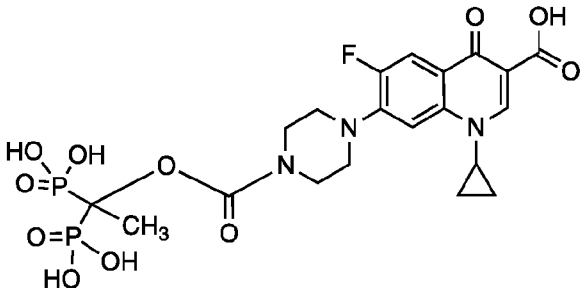
The BP quinolone compounds, conjugates and formulations can contain a bisphosphonate (BP) that can be releasably conjugated to a quinolone compound or analog. In any one or more embodiments herein, the BP has an alpha substituent and the alpha subsitutent is a hydroxy, amino or thiol group. The quinolone can be conjugated directly or indirectly to the BP at the geminal carbon alpha substituent of the BP, a described in any one or more aspects herein. In any one or more embodiments, the quinolone is reversibly coupled or conjugated to a geminal hydroxy, amino or thiol group on the carbon between the two phosphonate groups of the BP. When conjugated in this manner the two phosphonate groups operate to weaken the linkage of the quinolone to the BP, and the BP is activating the linker that reversibly couples or conjugates the quinolone to the BP upon release of the quinolone from the BP. In particular embodiments, the BP can be etidronate, methylene hydroxy bisphosphonate (MHBP) or pamidronate, preferably etidronate or MHBP. In particular embodiments, the BP can be an inactive or a low active BP, as described herein.

In any one or more embodiments, the BP quinolone compound or conjugate can be administered systemically to selectively deliver a quinolone to the skeleton and, in particular, to infected bone sites, or locally when combined with bone grafts or bone graft substitutes (i.e., can target bone, bone infections, or other high bone metabolism sites) in a subject. In any one or more embodiments, the BP quinolone compound or conjugate can release the quinolone, in particular a quinolone compound, substituent or derivative thereof. Also provided herein are methods of synthesizing BP quinolone compounds, conjugates and methods of treating or preventing osteomyelitis or other bone infections with one or more of the BP quinolone compounds, conjugates and/or formulations provided herein.

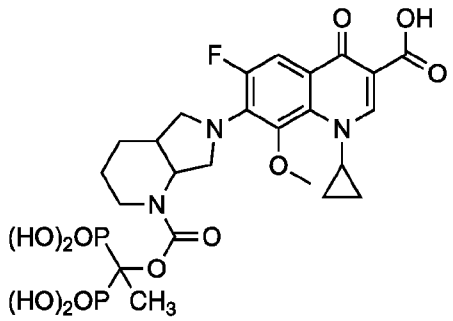
Provided herein, in various aspects, are BP quinolone compounds and conjugates that can contain a bisphosphonate (BP) that can be releasably conjugated to a quinolone. In embodiments, the BP quinolone compound or conjugate can selectively deliver a quinolone to bone, bone grafts, and or bone graft substitutes in particular to sites of higher bone metabolism where bone infections have initiated in a subject. In any one or more embodiments, the BP quinolone compound or conjugate can release the quinolone. Also provided herein are methods of synthesizing BP quinolone compounds and conjugates and methods of treating or preventing osteomyelitis or other bone infections with one or more of the BP quinolone compounds or conjugates provided herein.

In particular embodiments herein, the BP is etidronate conjugated to a fluoroquinolone antibiotic such as ciprofloxacin, moxifloxacin or sitafloxacin. In embodiments, the BP is etidronate

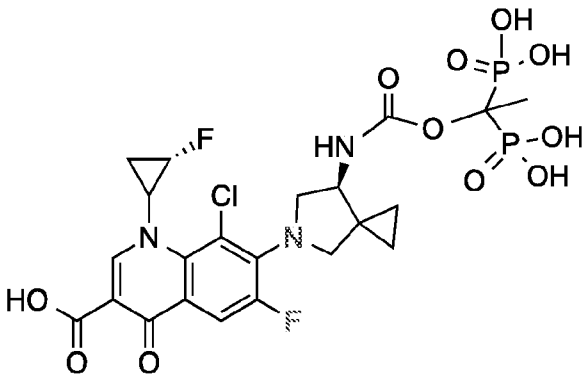
conjugated to a non-fluoroquinolone such as nemonoxacin. For example, the conjugate can be a compound according to Formula **(41)**, Formula **(43)**, Formula (**44**) or Formula (**45**).



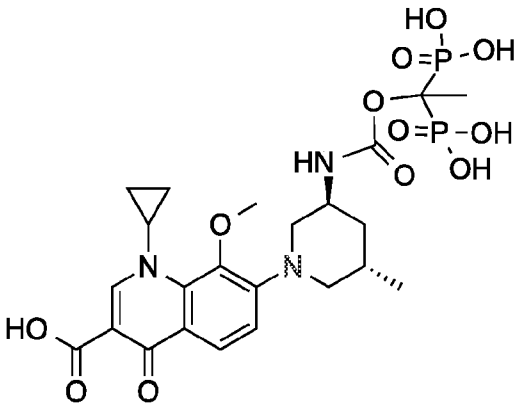
Formula (**41**)



Formula (**43**)



Formula (**44**)



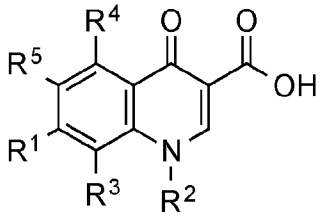
Formula (**45**)

Also provided herein are pharmaceutical compositions or formulations containing a compound according to Formula (**41**), Formula (**43**), Formula (**44**) and/or Formula (**45**), and a pharmaceutically acceptable carrier.

Also provided herein are methods of treating a bone infection in a subject in need thereof that can include the step of administering an amount of the compound according to Formula (**41**), Formula (**43**), Formula (**44**) and/or Formula (**45**), or a pharmaceutical formulation containing a compound according to Formula (**41**), Formula (**43**), Formula (**44**) and/or Formula (**45**), to a subject in need thereof.

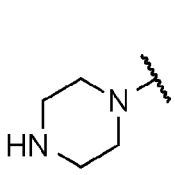
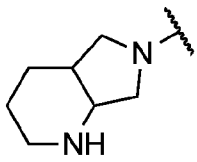
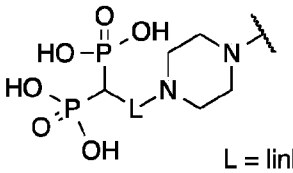
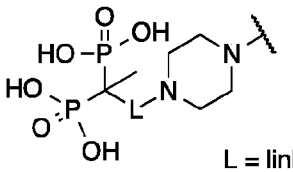
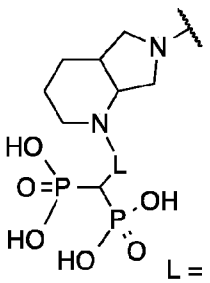
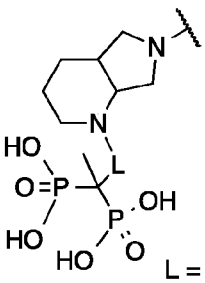
Also provided herein are compounds, conjugates and antimicrobial and antibiotic agents containing a bisphosphonate (BP) and a quinolone compound, wherein the quinolone compound is releasably or reversibly coupled to the bisphosphonate via a linker, as described herein. Preferred releasable linkers, as described herein, are more or less stable in the bloodstream shortly after administration and more or less slowly cleaved in the bone / skeletal compartments of the body to slowly release quinolone antibiotic compounds, substituents or derivatives locally.

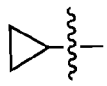
In any one or more aspects, the BP quinolone compound can be comprised of a quinolone antibiotic analog or substituent according to the following structure or Formula (**A**),



Formula (A)

wherein R^1 can be either


or

or

L = linker or

L = linker or

L = linker or

L = linker

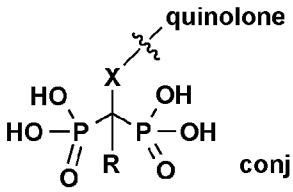
and wherein R^2 can be 

and wherein R^3 can be either H or OCH3,

and where R^4 can be H,

and wherein R^5 can be H or F.

As shown, the quinolone of Formula (**A**) can be linked to a bisphosphonate (BP). In any one or more aspects or embodiments herein, a compound or conjugate comprising a bisphosphonate (BP) and a quinolone compound or analog is provided wherein the BP can have an alpha substituent and the alpha substituent can be a hydroxy, amino or thiol group. The quinolone can be directly or indirectly conjugated to the BP at the germinal carbon alpha substituent (X) of the BP, as illustrated in the formula below.



conjugates between alpha-X containing BP and quinolone

X= O, NH, NR^1, S

R^1 can be alkyl or substituted alkyl, aryl or subsituted aryl groups

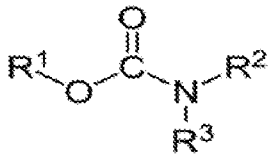
wherein R can be H, substituted and unsubstituted alkyl, alkyl amino, alkyl-aryl, aryl, alkylheteroaryl, or heteroaryl.

Preferred BPs are those that have a geminal hydroxy group on the carbon between the two phosphonate groups. A generic analog of such a BP is illustrated in **Fig. 25**. The BP be an alpha-OH containing BP and wherein the quinolone is directly or indirectly conjugated to the BP at the geminal OH of the BP.

In any one or more aspects, the bisphosphonate can be ethylidenebisphosphonate moiety (etidronate) that can be substituted by hydroxy (an alpha-hydroxy), amino or thiol. In some aspects, the bisphosphonate can include a para-hydroxyphenylethylidene group or derivative thereof. In embodiments, the BP can be a clinically known BP, such as pamidronate, alendronate, risedronate, zoledronate, minodronate, neridronate, and etidronate, which can be unmodified or modified as described herein.

In any one or more preferred embodiments, the BP can be etidronate. Etidronate can be linked to a quinolone to form a quinolone antibiotic etidronate-ciprofloxacin (ECC) conjugate, such as in Formula (**41**) or to form an etidronate moxifloxacin (ECX) conjugate such as in Formula (**43**) herein.

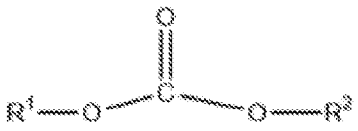
The linker, L, can be a compound that is cleavable, meaning that it reversibly couples the quinolone analog or compound, in particular a quinolone antimicrobial or antibiotic analog or substituent thereof, to the BP. As used herein, the term "cleavable" can mean a group that is chemically or biochemically unstable under physiological conditions. In any one or more aspects, the linker can be a carbamate, having a structure or Formula (**B**) below



Formula (**B**)

for coupling a quinolone, R^2, to a BP, R^1, as described herein, and R^3 can be substituted and unsubstituted alkyl, acetyl, benzoyl or other amides, phenyl and substituted phenyl, preferably H.

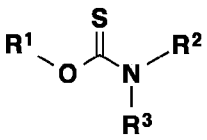
In any one or more aspects, the linker can be a carbonate, having a structure or Formula (**C**) below



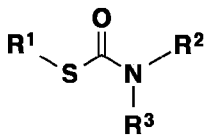
Formula (**C**)

for coupling a quinolone, R^2, to a BP, R^1, as described herein.

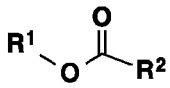
In any one or more aspects of any one or more embodiments herein, the linker can be an alkyl or an aryl carbamate linker. The linker can be an *O*-thioaryl or thioalkyl carbamate linker. The linker can be an *S*-thioaryl or thioalkyl carbamate linker. The linker can be a phenyl carbamate linker. The linker can be a thiocarbamate linker. The linker can be an *O*-thiocarbamate linker. The linker can be an *S*-thiocarbamate linker. The linker can be an ester linker. The linker can be a dithiocarbamate. The linker can be a urea linker. The linker can be part of the R^1 group of Formula **(A)** along with the BP and couple the BP to the quinolone, as described herein. In any one or more aspects, the linker can be exemplified by any one of Formula **(D)** – Formula **(H)** below, wherein: R^2 can be a quinolone or a quinolone substituent or derivative and R^1 can be a BP, both as described herein; and R^3 can be substituted and unsubstituted alkyl, acetyl, benzoyl or other amides, phenyl and substituted phenyl, preferably H.



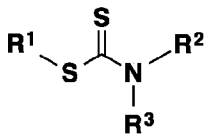
Formula (D)



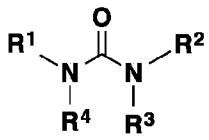
Formula (E)



Formula (F)

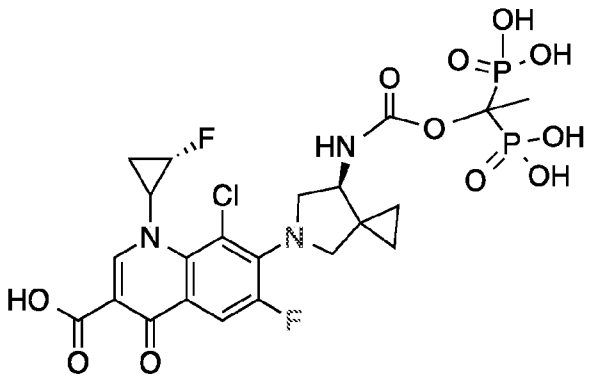


Formula (G)

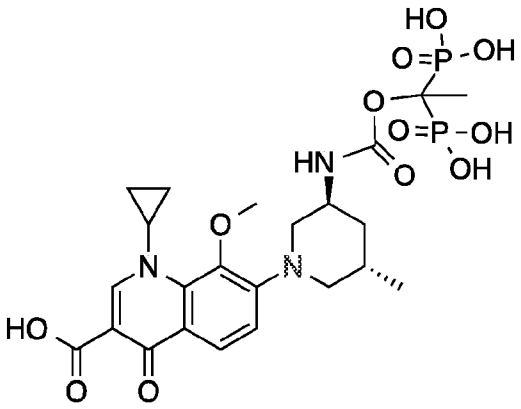


Formula (H)

In some aspects, the BP is etidronate. In some aspects, the quinolone is ciprofloxacin or moxifloxacin. In some aspects, the BP is etidronate, the quinolone is ciprofloxacin and the linker is an alkyl or an aryl carbamate or a linker of Formula (**F**) providing the compound of Formula (**41**). In some aspects, the BP is etidronate, the quinolone is moxifloxacin and the linker is an alkyl or an aryl carbamate or a linker of Formula (**F**) providing the compound of Formula (**43**). In some aspects, the BP is etidronate, the quinolone is sitafloxacin or nemonoxacin and the linker is an alkyl or an aryl carbamate or a linker of Formula (**F**) providing the compound of Formula (**44**) or Formula (**45**) below.



Formula (**44**)



Formula (**45**)

In other aspects, the BP can be another BP described herein, such as pamidronate, neridronate, olpadronate, alendronate, ibandronate, minodronate, risedronate, zoledronate, hydroxymethylenebisphosphonate, and combinations thereof.

Also provided herein are pharmaceutical formulations that can contain a bisphosphonate (BP) and a quinolone compound of Formula (**A**), wherein the quinolone compound is releasably coupled to the bisphosphonate via a linker, L; and a pharmaceutically acceptable carrier. The bisphosphonate (BP) and the linker, L, can be as described herein in any one or more aspects.

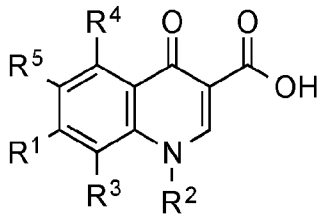
Also provided, in any one or more aspects of any one or more embodiments herein, are compounds and conjugates containing a bisphosphonate (BP) and a quinolone compound, wherein the quinolone compound is releasably coupled to the bisphosphonate via a linker. The BP can be selected from the group of: hydroxyl phenyl alkyl or aryl bisphosphonates, hydroxyl phenyl (or aryl) alkyl hydroxyl bisphosphonates, amino phenyl(or aryl) alkyl bisphosphonates, amino phenyl(or aryl) alkyl hydroxyl bisphosphonates, hydroxyl alkyl bisphosphonates, hydroxyl alkyl hydroxyl bisphosphonates, hydroxyl alkyl phenyl(or aryl) alkyl bisphosphonates, hydroxyl phenyl(or aryl) alkyl hydroxyl bisphosphonates, amino phenyl(or aryl) alkyl bisphosphonates, amino phenyl(or aryl) alkyl hydroxyl bisphosphonates, hydroxyl alkyl bisphosphonates, hydroxyl alkyl hydroxyl bisphosphonates, hydroxypyridyl alkyl bisphosphonates, pyridyl alkyl bisphosphonates, hydroxyl imadazoyl alkyl bisphosphonates, imidazoyl alkyl bisphosphonates, etidronate, pamidronate, neridronate, olpadronate, alendronate, ibandronate, risedronate, zoledronate, minodronate, hydroxymethylenebisphosphonate, and combinations thereof, wherein all the compounds can be optionally further substituted or are unsubsituted.

In any one or more aspects of any one or more embodiments herein, the quinolone compound can be a fluoroquinolone or a non-fluoroquinolone. The quinolone compound can be

selected from the group of: alatrofloxacin, amifloxacin, balofloxacin, besifloxacin, cadazolid, ciprofloxacin, clinafloxacin, danofloxacin, delafloxacin, difloxacin, enoxacin, enrofloxacin, finafloxacin, flerofloxacin, flumequine, gatifloxacin, gemifloxacin, grepafloxacin, ibafloxacin, JNJ-Q2, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, norfloxacin, ofloxacin, orbifloxacin, pazufloxacin, pefloxacin, pradofloxacin, prulifloxacin, rufloxacin, sarafloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin, trvafloxacin, zabofloxacin, nemonoxacin and combinations thereof.

In some aspects, the BP is etidronate. In some aspects, the quinolone is ciprofloxacin or moxifloxacin. In other aspects, the BP can be another BP described herein, such as pamidronate, neridronate, olpadronate, alendronate, ibandronate, minodronate, risedronate, zoledronate, hydroxymethylenebisphosphonate, and combinations thereof.

The quinolone compound can have a structure according to **Formula (A)**,



Formula (A),

where R^1 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups,

where R^2 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted

carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups,

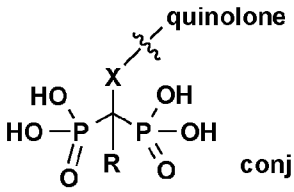
where R^3 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups,

where R^4 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups, and

and wherein R^5 can be H or F.

In any one or more aspects, the linker can be as described in any one or more aspects elsewhere herein. The linker can be attached to the R^1 group of **Formula (A)**.

In any one or more aspects or embodiments herein, the BP can have an alpha substituent and the alpha substituent is a hydroxy, amino or thiol group. The quinolone can be directly or indirectly conjugated to the BP at the germinal carbon alpha substituent (X) of the BP, as illustrated in the formula below.



conjugates between alpha-X containing BP and quinolone

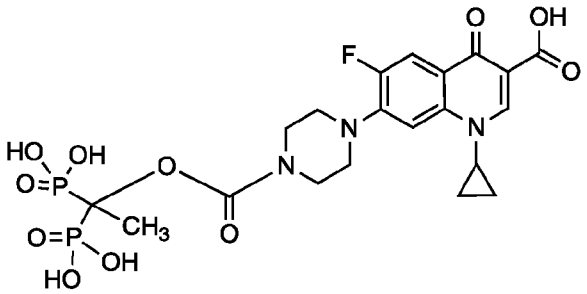
X= O, NH, NR^1, S

R^1 can be alkyl or substituted alkyl, aryl or subsituted aryl groups

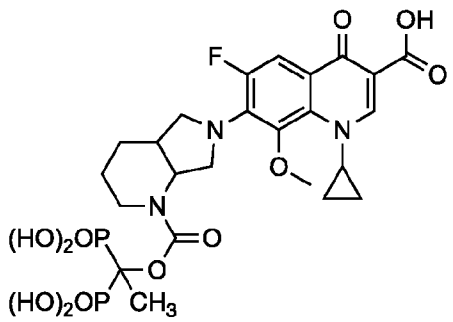
wherein R can be H, substituted and unsubstituted alkyl, alkyl amino, alkyl-aryl, aryl, alkylheteroaryl, or heteroaryl. Preferred BPs are those that have a geminal hydroxy group on the carbon between the two phosphonate groups.

In any one or more aspects or embodiments herein, the bisphosphonate can be an ethylidenebisphosphonate moiety (etidronate) that can be substituted by hydroxy (an alpha-hydroxy), amino or thiol. In some aspects, the bisphosphonate can include a para-hydroxyphenylethylidene group or derivative thereof. In embodiments, the BP can be a clinically known BP, such as pamidronate, alendronate, risedronate, zoledronate, minodronate, neridronate, and etidronate, which can be unmodified or modified as described herein.

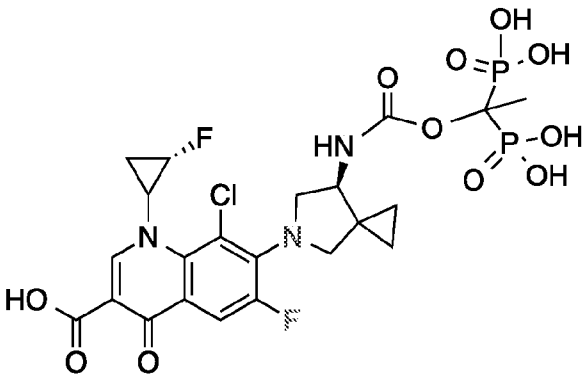
In some aspects, the compound has a formula according to Formula **(41)**, Formula **(43)**, Formula (**44**) or Formula (**45**).



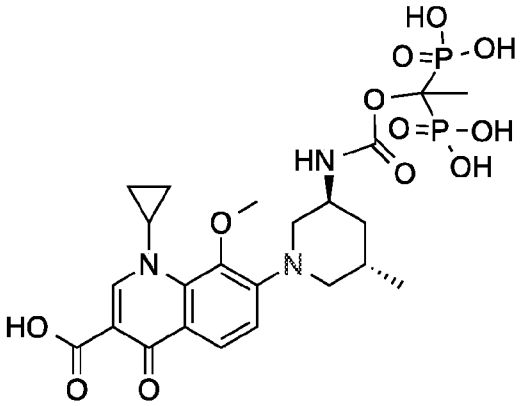
Formula (**41**)



Formula (**43**)



Formula (**44**)



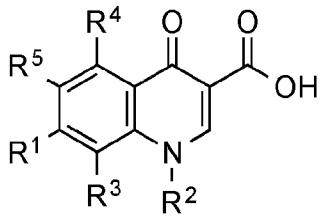
Formula (**45**)

Also provided herein are pharmaceutical formulations that can contain a bisphosphonate and a quinolone compound, wherein the quinolone compound is releasably coupled to the bisphosphonate via a linker; and a pharmaceutically acceptable carrier. The bisphosphonate can be selected from the group of: hydroxyl phenyl alkyl or aryl bisphosphonates, hydroxyl phenyl (or aryl) alkyl hydroxyl bisphosphonates, amino phenyl(or aryl) alkyl bisphosphonates, amino phenyl(or aryl) alkyl hydroxyl bisphosphonates, hydroxyl alkyl bisphosphonates, hydroxyl alkyl hydroxyl bisphosphonates, hydroxyl alkyl phenyl(or aryl) alkyl bisphosphonates, hydroxyl phenyl(or aryl) alkyl hydroxyl bisphosphonates, amino phenyl(or aryl) alkyl bisphosphonates, amino phenyl(or aryl) alkyl hydroxyl bisphosphonates, hydroxyl alkyl bisphosphonates, hydroxyl alkyl hydroxyl bisphosphonates, hydroxypyridyl alkyl bisphosphonates, pyridyl alkyl bisphosphonates, hydroxyl imadazoyl alkyl bisphosphonates, imidazoyl alkyl bisphosphonates,

etidronate, pamidronate, neridronate, olpadronate, alendronate, ibandronate, risedronate, zoledronate, minodronate, hydroxymethylenebisphosphonate, and combinations thereof, wherein all the compounds can be optionally futher substituted or are unsubsituted.

The quinolone compound can be a fluoroquinolone or a non-fluoroquinolone. The quinolone compound can be selected from the group of: alatrofloxacin, amifloxacin, balofloxacin, besifloxacin, cadazolid, ciprofloxacin, clinafloxacin, danofloxacin, delafloxacin, difloxacin, enoxacin, enrofloxacin, finafloxacin, flerofloxacin, flumequine, gatifloxacin, gemifloxacin, grepafloxacin, ibafloxacin, JNJ-Q2, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, norfloxacin, ofloxacin, orbifloxacin, pazufloxacin, pefloxacin, pradofloxacin, prulifloxacin, rufloxacin, sarafloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin, trvafloxacin, zabofloxacin, nemonoxacin and combinations thereof.

The quinolone compound can have a structure according to **Formula (A)**,



Formula (A),

where R^1 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups,

where R^2 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted

carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups,

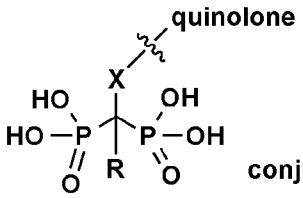
where R^3 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups,

where R^4 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups, and

and wherein R^5 can be H or F.

In any one or more aspects, the linker can be as described in any one or more aspects elsewhere herein. The linker can be attached to the R^1 group of **Formula (A)**.

In any one or more aspects, the BP can have an alpha substituent and the alpha substituent is a hydroxy, amino or thiol group. The quinolone can be directly or indirectly conjugated to the BP at the germinal carbon alpha substituent (X) of the BP, as illustrated in the formula below.



conjugates between alpha-X containing BP and quinolone

X= O, NH, NR^1, S

R^1 can be alkyl or substituted alkyl, aryl or subsituted aryl groups

wherein R can be H, substituted and unsubstituted alkyl, alkyl amino, alkyl-aryl, aryl, alkylheteroaryl, or heteroaryl. Preferred BPs are those that have a geminal hydroxy group on the carbon between the two phosphonate groups.

In any one or more aspects, the bisphosphonate can be an ethylidenebisphosphonate moiety (etidronate) that can be substituted by hydroxy (an alpha-hydroxy), amino or thiol. In some aspects, the bisphosphonate can include a para-hydroxyphenylethylidene group or derivative thereof. In embodiments, the BP can be a clinically known BP, such as pamidronate, alendronate, risedronate, zoledronate, minodronate, neridronate, and etidronate, which can be unmodified or modified as described herein.

In some aspects, the formulation includes a compound that has a formula according to Formula **(41),** Formula **(43)**, Formula (**44**) and/or Formula (**45**) herein.

The amount of the compound or conjugate in the pharmaceutical formulation can be an amount effective to kill or inhibit bacteria. The amount of the compound or conjugate in the pharmaceutical formulation can be an amount effective to treat, inhibit, or prevent a bone disease. The amount of the compound or conjugate in the pharmaceutical formulation can be an amount effective to treat, inhibit, or prevent osteomyelitis, osteonecrosis, peri-implantitis, and/or periodontitis. The amount of the compound or conjugate in the pharmaceutical formulation can be an amount effective for prophylaxis treatment of any of the foregoing.

Further, in one or more embodiments, methods are provided of preparing a bisphosphonate-quinolone compound, conjugate or formulation thereof, comprising linking a bisphosphonate with a quinolone compound or substituent as described in any one or more aspects herein. Methods are also provided for use of any one or more bisphosphonate-quinolone compounds or conjugates in the preparation of a pharmaceutical or medicament for the treatment of any one or more of the diseases mentioned herein.

Also provided herein are methods of treating a bone disease or infection, such as osteomyelitis, in a subject in need thereof that can include the step of administering an amount, in particular an effective amount, of a compound as provided herein or pharmaceutical formulation

thereof to the subject in need thereof. Also provided herein are methods of treating a bone disease, such as a hematogenous or local osteomyelitis including juvenile osteomyelitis and infections related to prosthetic joint replacements or osteonecrosis, in a subject in need thereof that can include the step of administering an amount, in particular an effective amount of a compound as provided herein or pharmaceutical formulation thereof to the subject in need thereof.

Also provided herein are methods of treating peri-implantitis or periodontitis in a subject in need thereof, the method comprising administering an amount of administering an amount, in particular an effective amount, of a compound as provided herein or pharmaceutical formulation thereof to the subject in need thereof.

Also provided herein are methods of treating diabetic foot disease in a subject in need thereof, the method comprising administering an amount, in particular an effective amount, of a compound as provided herein or pharmaceutical formulation thereof to the subject in need thereof. Also provided herein are methods of treating bone infections in diabetic patients including diabetic foot diseases in a subject in need thereof, the method comprising administering an amount, in particular an effective amount, of a compound as provided herein or pharmaceutical formulation thereof to the subject in need thereof. A reduction in related amputations, debridement, of limbs and infected skeletal sites can result from these more powerful localized modes of antibiotic therapies.

Also provided herein are bone graft compositions that can include a bone graft material and a compound as described herein or a pharmaceutical formulation thereof, wherein the compound or pharmaceutical formulation thereof is attached to, integrated with, chemisorbed to, or mixed with the bone graft material. The bone graft material can be autograft bone material, allograft bone material, xenograft bone material, a synthetic bone graft material, or any combination thereof.

Also provided herein are methods that can include the step of implanting the bone graft composition as described herein in a subject in need thereof.

Also provided herein are methods of preventing or prophylaxis treatment of, biofilm infection at an osseous or implant surgical site, or at a surgical site where bone grafting is performed, where the methods can include the step of administering a compound as described herein to a subject in need thereof.

Also provided herein are methods of preventing, or prophylaxis treatment, of biofilm infection at an osseous or implant surgical site, or at a surgical site where bone grafting is

performed, where the method can include the step of implanting a bone graft composition as described herein to a subject in need thereof.

Other compounds, compositions, formulations, methods, features, and advantages of the present disclosure of a fabrication system for nanowire template synthesis, will be or become apparent to one with skill in the art upon examination of the following drawings and detailed description. It is intended that all such additional systems, methods, features, and advantages be included within this description, be within the scope of the present disclosure, and be protected by the accompanying claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Further aspects of the present disclosure will be readily appreciated upon review of the detailed description of its various embodiments, described below, when taken in conjunction with the accompanying drawings.

Fig. 1 shows a scanning electron micrograph (SEM; 100x magnification) of a surgical specimen from a patient with chronic osteomyelitis showing characteristic multi-layered and matrix-enclosed biofilms colonizing bone surfaces internally and externally; inset top right shows high-power view (5000x magnification) of the causative staphylococcal biofilm pathogens. [The sample was processed for SEM, sputter coated with platinum and imaged with an XL 30S SEM (FEG, FEI Co., Hillsboro, OR) operating at 5 kV in the secondary electron mode].

Fig. 2 demonstrates the general BP quinolone conjugate targeting strategy.

Fig. 3 shows an embodiment of a BP-FQ conjugate.

Fig. 4 shows additional BP-Ab conjugate design.

Fig. 5 shows an embodiment of a synthesis scheme for synthesis of BP-Ab conjugates with an *O*-thiocarbamate linker.

Fig. 6 shows an embodiment of a scheme for synthesis of alpha-OH protected BP esters.

Fig. 7 shows an embodiment of a scheme for synthesis of BP 3-linker 3-ciprofloxacin.

Fig. 8 shows a BP-carbamate-moxifloxacin BP conjugate and synthesis scheme.

Fig. 9 shows a BP-carbamate-gatifloxacin BP conjugate and synthesis scheme.

Fig. 10 shows a BP-*p*-Hydroxyphenyl Acetic Acid-ciprofloxacin BP conjugate and synthesis scheme.

Fig. 11 shows a BP-OH-ciprofloxacin BP conjugate and synthesis scheme.

Fig. 12 shows a BP-O-Thiocarbamate-ciprofloxacin BP conjugate and synthesis scheme.

Fig. 13 shows a BP-*S*-Thiocarbamate-ciprofloxacin BP conjugate and synthesis scheme.

Fig. 14 shows a BP-Resorcinol-ciprofloxacin BP conjugate and synthesis scheme.

Fig. 15 shows a BP-Hydroquinone-ciprofloxacin BP conjugate and synthesis scheme.

Fig. 16 shows one embodiment of a genus structure for a genus of BP-Fluoroquinolones.

Fig. 17 shows various BP-fluoroquinolone conjugates.

Fig. 18 shows one embodiment of a genus structure for a genus of a phosphonate containing an aryl group.

Fig. 19 shows various BPs, where X can be F, Cl, Br, or I.

Fig. 20 shows various BP's with terminal primary amines.

Fig. 21 shows various BPs coupled to a linker containing a terminal hydroxyl and amine functional groups where R can be Risedronate, Zoledronate, Minodronate, Pamidronate, or Alendronate.

Fig. 22 shows various BP-pamidronate-ciprofloxacin conjugates.

Fig. 23 shows various BP-Alendronate-ciprofloxacin conjugates.

FIG. 24 depicts examples of pharmacologically inert BPs used in the present conjugation: medium (**A/E**), high (**B/F**), and low (**C/G**) affinity BPs and longer phenylalkyl chain BP (**D/H**).

FIG. 25 depicts examples of pharmacologically low active BPs that can be used in the present conjugation.

FIG. 26 depicts the results of **d**ynamic monitoring of biofilm growth in the presence of different concentrations of conjugates. Culture (-------), *S. aureus* with ECC 2 μg/ml I (-------), ECC 5 μg/ml I (-------), ECC 10 μg/ml (-------), ECX 2 μg/ml I (-------), ECX 5 μg/ml I (-------), ECX 10 μg/ml (-------).

FIG. 27 depicts *S. aureus* MIC_{50} analysis for ECC & ECX. ECC (-------), ECX (-------).

FIG. 28 depicts the results of dynamic monitoring of biofilm growth in the presence of different concentrations of parent antibiotics. Culture (-------), *S. aureus* with Cipro 0.1 μg/ml I (----), Cipro 0.25 μg/ml (-------), Cipro 1 μg/ml (-------), Cipro 2 μg/ml (-------), Moxi 0.05 μg/ml (------), Moxi 0.1 μg/ml (-------), Moxi 0.2 μg/ml (-------), Moxi 0.5 μg/ml (-------).

FIG. 29 depicts *S. aureus* MIC_{50} analysis for Cipro & Moxi. Moxi (-------), Cipro (-------).

FIG. 30 depicts the results of dynamic monitoring of biofilm growth in the presence of Cipro/Moxi+HA(10 μg/ml). Culture (-------), *S. aureus* with Cipro 0.01 μg/ml (-------), Cipro 0.075 μg/ml (-------), Cipro 0.1 μg/ml (-------), Cipro 0.25 μg/ml (-------), Cipro 0.50 μg/ml (-------), Moxi

0.02 μg/ml (-------), Moxi 0.04 μg/ml (-------), Moxi 0.08 μg/ml (-------), Moxi 0.1 μg/ml (-------), Moxi 0.2 μg/ml (-------).

FIG. 31 depicts *S. aureus* MIC_{50} analysis for Cipro/Moxi+HA. Moxi (-------), Cipro (-------).

FIG. 32 depicts the results of dynamic monitoring of biofilm growth in the presence of ECC/ECX+HA(10 μg/ml). Culture (-------), *S. aureus* with ECC 1 μg/ml (-------), ECC 5 μg/ml (----------), ECC 7.5 μg/ml (-------), ECC 10 μg/ml (-------), ECX 1 μg/ml (-------), ECX 2.5 μg/ml (-------), ECX 7.5 μg/ml (-------), ECX 10 μg/ml (-------).

FIG. 33 depicts *S. aureus* MIC_{50} analysis for ECC/ECX+HA. ECX (-------), ECC (-------).

DETAILED DESCRIPTION

Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the

methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided could be different from the actual publication dates that may need to be independently confirmed.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of molecular biology, microbiology, nanotechnology, pharmacology, organic chemistry, biochemistry, botany and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

Definitions

Unless otherwise specified herein, the following definitions are provided.

As used herein, "**about**," "**approximately**," and the like, when used in connection with a numerical variable, generally refers to the value of the variable and to all values of the variable that are within the experimental error (e.g., within the 95% confidence interval for the mean) or within ±10% of the indicated value, whichever is greater.

As used interchangeably herein, "**subject**," "**individual**," or "**patient**," refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. The term "pet" includes a dog, cat, guinea pig, mouse, rat, rabbit, ferret, and the like. The term "farm animal" includes a horse, sheep, goat, chicken, pig, cow, donkey, llama, alpaca, turkey, and the like.

As used herein, "**control**" can refer to an alternative subject or sample used in an experiment for comparison purposes and included to minimize or distinguish the effect of variables other than an independent variable.

As used herein, **"analog" or "analogue,"** such as an analogue of a bisphosphonate described herein, can refer to a structurally close member of the parent molecule or an appended parent molecule such as a bisphosphonate.

As used herein, "**conjugated**" can refer to direct attachment of two or more compounds to one another via one or more covalent or non-covalent bonds. The term "conjugated" as used herein can also refer to indirect attachment of two or more compounds to one another through an intermediate compound, such as a linker.

As used herein, "**pharmaceutical formulation**" refers to the combination of an active agent, compound, or ingredient with a pharmaceutically acceptable carrier or excipient, making the composition suitable for diagnostic, therapeutic, or preventive use in vitro, in vivo, or ex vivo.

As used herein, "**pharmaceutically acceptable carrier or excipient**" refers to a carrier or excipient that is useful in preparing a pharmaceutical formulation that is generally safe, non-toxic, and is neither biologically or otherwise undesirable, and includes a carrier or excipient that is acceptable for veterinary use as well as human pharmaceutical use. A "pharmaceutically acceptable carrier or excipient" as used in the specification and claims includes both one and more than one such carrier or excipient.

As used herein, "**pharmaceutically acceptable salt**" refers to any acid or base addition salt whose counter-ions are non-toxic to the subject to which they are administered in pharmaceutical doses of the salts.

As used herein, "**active agent**" or "**active ingredient**" refers to a component or components of a composition to which the whole or part of the effect of the composition is attributed.

As used herein, "**dose,**" "**unit dose,**" or "**dosage**" refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of a BP conjugate, such as a BP quinolone conjugate, composition or formulation described herein calculated to produce the desired response or responses in association with its administration.

As used herein, "**derivative**" refers to any compound having the same or a similar core structure to the compound but having at least one structural difference, including substituting, deleting, and/or adding one or more atoms or functional groups. The term "derivative" does not mean that the derivative is synthesized from the parent compound either as a starting material or intermediate, although this may be the case. The term "derivative" can include prodrugs, or metabolites of the parent compound. Derivatives include compounds in which free amino groups

in the parent compound have been derivatized to form amine hydrochlorides, p-toluene sulfoamides, benzoxycarboamides, t-butyloxycarboamides, thiourethane-type derivatives, trifluoroacetylamides, chloroacetylamides, or formamides. Derivatives include compounds in which carboxyl groups in the parent compound have been derivatized to form methyl and ethyl esters, or other types of esters, amides, hydroxamic acids, or hydrazides. Derivatives include compounds in which hydroxyl groups in the parent compound have been derivatized to form O-acyl, O-carbamoyl, or O-alkyl derivatives. Derivatives include compounds in which a hydrogen bond donating group in the parent compound is replaced with another hydrogen bond donating group such as OH, NH, or SH. Derivatives include replacing a hydrogen bond acceptor group in the parent compound with another hydrogen bond acceptor group such as esters, ethers, ketones, carbonates, tertiary amines, imine, thiones, sulfones, tertiary amides, and sulfides. "Derivatives" also includes extensions of the replacement of the cyclopentane ring, as an example, with saturated or unsaturated cyclohexane or other more complex, *e.g.,* nitrogen-containing rings, and extensions of these rings with various groups.

As used herein, "**administering**" refers to an administration that is oral, topical, intravenous, subcutaneous, transcutaneous, transdermal, intramuscular, intra-joint, parenteral, intra-arteriole, intradermal, intraventricular, intracranial, intraperitoneal, intralesional, intranasal, rectal, vaginal, by inhalation, or via an implanted reservoir. The term "parenteral" includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, and intracranial injections or infusion techniques.

The term "**substituted**" as used herein, refers to all permissible substituents of the compounds described herein. In the broadest sense, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, but are not limited to, halogens, hydroxyl groups, or any other organic groupings containing any number of carbon atoms, *e.g.* 1-14 carbon atoms, and optionally include one or more heteroatoms such as oxygen, sulfur, or nitrogen grouping in linear, branched, or cyclic structural formats. Representative substituents include alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl,

sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups.

As used herein, **"substituent" or "suitable substituent"** means a chemically and pharmaceutically acceptable group, i.e., a moiety that does not significantly interfere with the preparation of or negate the efficacy of the inventive compounds. Such suitable substituents may be routinely chosen by those skilled in the art. Suitable substituents include but are not limited to the following: a halo, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, C_1-C_6 haloalkoxy, C_2-C_6 alkynyl, C_3-C_8 cycloalkenyl, (C_3-C_8 cycloalkyl)C_1-C_6 alkyl, (C_3-C_8 cycloalkyl)C_2-C_6 alkenyl, (C_3-C_8 cycloalkyl)C_1-C_6 alkoxy, C_3-C_7 heterocycloalkyl, (C_3-C_7 heterocycloalkyl)C_1-C_6 alkyl, (C_3-C_7 heterocycloalkyl) C_2-C_6 alkenyl, (C_3-C_7 heterocycloalkyl)C_1-C_6 alkoxyl, hydroxy, carboxy, oxo, sulfanyl, C_1-C_6 alkylsulfanyl, aryl, heteroaryl, aryloxy, heteroaryloxy, aralkyl, heteroaralkyl, aralkoxy, heteroaralkoxy, nitro, cyano, amino, C_1-C_6 alkylamino, di-(C_1-C_6 alkyl)amino, carbamoyl, (C_1-C_6 alkyl)carbonyl, (C_1-C_6 alkoxy)carbonyl, (C_1-C_6 alkyl)aminocarbonyl, di-(C_1-C_6 alkyl)aminocarbonyl, arylcarbonyl, aryloxycarbonyl, (C_1-C_6 alkyl)sulfonyl, and arylsulfonyl. The groups listed above as suitable substituents are as defined hereinafter except that a suitable substituent may not be further optionally substituted.

The term "**alkyl**" refers to the radical of saturated aliphatic groups (i.e., an alkane with one hydrogen atom removed), including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups.

In some embodiments, a straight chain or branched chain alkyl can have 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chains, and C_3-C_{30} for branched chains). In other embodiments, a straight chain or branched chain alkyl can contain 20 or fewer, 15 or fewer, or 10 or fewer carbon atoms in its backbone. Likewise, in some embodiments cycloalkyls can have 3-10 carbon atoms in their ring structure. In some of these embodiments, the cycloalkyl can have 5, 6, or 7 carbons in the ring structure.

The term "**alkyl**" (or "**lower alkyl**") as used herein is intended to include both "unsubstituted alkyls" and "substituted alkyls," the latter of which refers to alkyl moieties having one or more substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents include, but are not limited to, halogen, hydroxyl, carbonyl (such as a carboxyl, alkoxycarbonyl, formyl, or an acyl), thiocarbonyl (such as a thioester, a thioacetate, or

a thioformate), alkoxyl, phosphoryl, phosphate, phosphonate, phosphinate, amino, amido, amidine, imine, cyano, nitro, azido, sulfhydryl, alkylthio, sulfate, sulfonate, sulfamoyl, sulfonamido, sulfonyl, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths.

It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include halogen, hydroxy, nitro, thiols, amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), $-CF_3$, -CN and the like. Cycloalkyls can be substituted in the same manner.

The term "**heteroalkyl**," as used herein, refers to straight or branched chain, or cyclic carbon-containing radicals, or combinations thereof, containing at least one heteroatom. Suitable heteroatoms include, but are not limited to, O, N, Si, P, Se, B, and S, wherein the phosphorous and sulfur atoms are optionally oxidized, and the nitrogen heteroatom is optionally quaternized. Heteroalkyls can be substituted as defined above for alkyl groups.

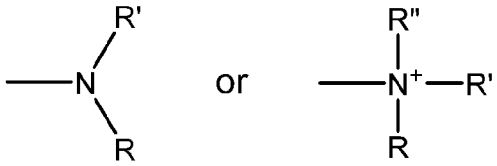
The term "**alkylthio**" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In preferred embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, and -S-alkynyl. Representative alkylthio groups include methylthio, ethylthio, and the like. The term "alkylthio" also encompasses cycloalkyl groups, alkene and cycloalkene groups, and alkyne groups. "Arylthio" refers to aryl or heteroaryl groups. Alkylthio groups can be substituted as defined above for alkyl groups.

The terms "**alkenyl**" and "**alkynyl**", refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The terms "**alkoxyl**" or "**alkoxy**," as used herein, refers to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl is an ether or

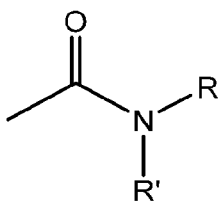
resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, and -O-alkynyl. The terms “aroxy” and “aryloxy”, as used interchangeably herein, can be represented by –O-aryl or O-heteroaryl, wherein aryl and heteroaryl are as defined below. The alkoxy and aroxy groups can be substituted as described above for alkyl.

The terms "**amine**" and "**amino**" (and its protonated form) are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:



wherein R, R’, and R” each independently represent a hydrogen, an alkyl, an alkenyl, -$(CH2)_m$-R_C or R and R’ taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R_C represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or an integer in the range of 1 to 8. In some embodiments, only one of R or R’ can be a carbonyl, e.g., R, R’ and the nitrogen together do not form an imide. In other embodiments, the term “amine” does not encompass amides, e.g., wherein one of R and R’ represents a carbonyl. In further embodiments, R and R’ (and optionally R”) each independently represent a hydrogen, an alkyl or cycloakly, an alkenyl or cycloalkenyl, or alkynyl. Thus, the term "alkylamine" as used herein means an amine group, as defined above, having a substituted (as described above for alkyl) or unsubstituted alkyl attached thereto, i.e., at least one of R and R’ is an alkyl group.

The term "**amido**" is art-recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:



wherein R and R’ are as defined above.

As used herein, "**aryl**" refers to C_5-C_{10}-membered aromatic, heterocyclic, fused aromatic, fused heterocyclic, biaromatic, or bihetereocyclic ring systems. Broadly defined, "aryl", as used herein, includes 5-, 6-, 7-, 8-, 9-, and 10-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine, pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring can be substituted at one or more ring positions with one or more substituents including, but not limited to, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino (or quaternized amino), nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, $-CF_3$, -CN, and combinations thereof. The term "aryl" includes phenyl.

The term "**aryl**" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (i.e., "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic ring or rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocycles. Examples of heterocyclic rings include, but are not limited to, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolinyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolinyl, carbazolyl, 4aH carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, *2H,6H*-1,5,2-dithiazinyl, dihydrofuro[2,3 b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, *1H*-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, 3H-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxindolyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinolizinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl,

thienoimidazolyl, thiophenyl, and xanthenyl. One or more of the rings can be substituted as defined above for "aryl."

The term "**aralkyl**," as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

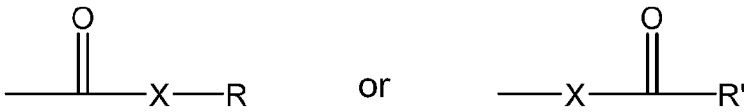
The term "**aralkyloxy**" can be represented by –O-aralkyl, wherein aralkyl is as defined above.

The term "**carbocycle**," as used herein, refers to an aromatic or non-aromatic ring(s) in which each atom of the ring(s) is carbon.

"**Heterocycle**" or "**heterocyclic**," as used herein, refers to a monocyclic or bicyclic structure containing 3-10 ring atoms, and in some embodiments, containing from 5-6 ring atoms, wherein the ring atoms are carbon and one to four heteroatoms each selected from the following group of non-peroxide oxygen, sulfur, and N(Y) wherein Y is absent or is H, O, (C_1-C_{10}) alkyl, phenyl or benzyl, and optionally containing 1-3 double bonds and optionally substituted with one or more substituents. Examples of heterocyclic rings include, but are not limited to, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolinyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolinyl, carbazolyl, 4aH carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2H,6H-1,5,2-dithiazinyl, dihydrofuro[2,3 b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, 1H-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, 3H-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxepanyl, oxetanyl, oxindolyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinolizinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydropyranyl, tetrahydroquinolinyl, tetrazolyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl, and xanthenyl. Heterocyclic groups can optionally be substituted with one or more substituents at one or more positions as defined above for alkyl and

aryl, for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like. The terms "heterocycle" or "heterocyclic" can be used to describe a compound that can include a heterocycle or heterocyclic ring.

The term "**carbonyl**" is art-recognized and includes such moieties as can be represented by the general formula:



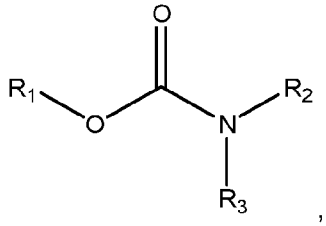
wherein X is a bond or represents an oxygen or a sulfur, and R and R' are as defined above. Where X is an oxygen and R or R' is not hydrogen, the formula represents an "ester". Where X is an oxygen and R is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R is a hydrogen, the formula represents a "carboxylic acid." Where X is an oxygen and R' is hydrogen, the formula represents a "formate." In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiocarbonyl" group. Where X is a sulfur and R or R' is not hydrogen, the formula represents a "thioester." Where X is a sulfur and R is hydrogen, the formula represents a "thiocarboxylic acid." Where X is a sulfur and R' is hydrogen, the formula represents a "thioformate." On the other hand, where X is a bond, and R is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R is hydrogen, the above formula represents an "aldehyde" group.

The term "**heteroatom**" as used herein means an atom of any element other than carbon or hydrogen. Exemplary heteroatoms include, but are not limited to, boron, nitrogen, oxygen, phosphorus, sulfur, silicon, arsenic, and selenium. Heteroatoms, such as nitrogen, can have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. It is understood that "substitution" or "substituted" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, i.e., a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

The term **"hydroxy"** refers to a —OH radical.

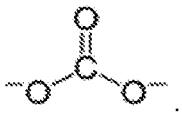
As used herein, the term "**nitro**" refers to -NO_2; the term "halogen" designates -F, -Cl, -Br, or -I; the term "sulfhydryl" refers to -SH; the term "hydroxyl" refers to -OH; and the term "sulfonyl" refers to -SO_2-

As used herein, "**carbamate**" can be used to refer to a compound derived from carbamic acid (NH_2COOH) and can include carbamate esters. "Carbamates" can have the general structure of:



where R_1, R_2, and R_3 can be any permissible substituent.

As used herein, "**carbonate**" can be used to refer to a compound derived from carbonic acid (H_2CO_3) and can include carbonate esters. "Carbonates" can have the general structure of:



As used herein, "**effective amount**" can refer to the amount of a composition described herein or pharmaceutical formulation described herein that will elicit a desired biological or medical response of a tissue, system, animal, plant, protozoan, bacteria, yeast or human that is being sought by the researcher, veterinarian, medical doctor or other clinician. The desired biological response can be modulation of bone formation and/or remodeling, including but not limited to modulation of bone resorption and/or uptake of the BP conjugates, such as the BP quinolone conjugates, described herein. The effective amount will vary depending on the exact chemical structure of the composition or pharmaceutical formulation, the causative agent and/or severity of the infection, disease, disorder, syndrome, or symptom thereof being treated or prevented, the route of administration, the time of administration, the rate of excretion, the drug combination, the judgment of the treating physician, the dosage form, and the age, weight, general health, sex and/or diet of the subject to be treated. "Effective amount" can refer to the

amount of a compositions described herein that is effective to inhibit the growth of or reproduction of a microorganism, including but not limited to a bacterium or population thereof. "Effective amount" can refer to the amount of a compositions described herein that is kill a microorganism, including but not limited to a bacterium or population thereof. "Effective amount" can refer to the amount of a compositions described herein that is effective to treat and/or prevent osteomyelitis in a subject in need thereof.

The terms **"quinolone," "quinolone antimicrobial molecule,"** and **"oxazolidinone antimicrobial agent,"** or "substituents" or "derivatives thereof and related terms, have the same meaning and refer to antimicrobial agents which are part of the well-known class of "quinolones," as described in more detail herein.

As used herein, "**therapeutic**" generally can refer to treating, healing, and/or ameliorating a disease, disorder, condition, or side effect, or to decreasing in the rate of advancement of a disease, disorder, condition, or side effect. The term also includes within its scope enhancing normal physiological function, palliative treatment, and partial remediation of a disease, disorder, condition, side effect, or symptom thereof.

The term **"antibacterial"** includes those compounds that inhibit, halt or reverse growth of bacteria, those compounds that inhibit, halt, or reverse the activity of bacterial enzymes or biochemical pathways, those compounds that kill or injure bacteria, and those compounds that block or slow the development of a bacterial infection.

As used herein, the terms "**treating**" and "**treatment**" can refer generally to obtaining a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of preventing or partially preventing a disease, symptom or condition thereof. And as used herein are intended to mean, at least, the mitigation of a disease condition associated with a bacterial infection in a subject, including mammals, such as a human, that is alleviated by a reduction of growth, replication, and/or propagation of any bacterium such as Gram-positive organisms, and includes curing, healing, inhibiting, relieving from, improving and/or alleviating, in whole or in part, the disease condition.

The term **"prophylaxis"** is intended to mean at least a reduction in the likelihood that a disease condition associated with a bacterial infection will develop in a mammal, preferably a human. The terms **"prevent"** and **"prevention"** are intended to mean blocking or stopping a disease condition associated with a bacterial infection from developing in a mammal, preferably

a human. In particular, the terms are related to the treatment of a mammal to reduce the likelihood **("prophylaxis")** or prevent the occurrence of a bacterial infection, such as bacterial infection that may occur during or following a surgery involving bone reparation or replacement. The terms also include reducing the likelihood **("prophylaxis")** of or preventing a bacterial infection when the mammal is found to be predisposed to having a disease condition but not yet diagnosed as having it. For example, one can reduce the likelihood or prevent a bacterial infection in a mammal by administering a compound of Formula (1) and/or Formula (2), or a pharmaceutically acceptable prodrug, salt, active metabolite, or solvate thereof, before occurrence of such infection.

As used herein, "**synergistic effect**," "**synergism**," or "**synergy**" refers to an effect arising between two or more molecules, compounds, substances, factors, or compositions that is greater than or different from the sum of their individual effects.

As used herein, "**additive effect**" refers to an effect arising between two or more molecules, compounds, substances, factors, or compositions that is equal to or the same as the sum of their individual effects.

The term "**biocompatible**", as used herein, refers to a material that along with any metabolites or degradation products thereof that are generally non-toxic to the recipient and do not cause any significant adverse effects to the recipient. Generally speaking, biocompatible materials are materials which do not elicit a significant inflammatory or immune response when administered to a patient.

As used herein, the term "**osteomyelitis**" can refer to acute or chronic osteomyelitis, and/or diabetic foot osteomyelitis, diabetic chronic osteomyelitis, prosthetic joint infections, periodontitis, peri-implantitis, osteonecrosis, and/or hematogenous osteomyelitis and/or other bone infections.

Discussion

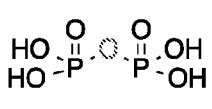
Infectious bone disease, or osteomyelitis, is a major problem worldwide in human and veterinary medicine and can be devastating due to the potential for limb-threatening sequelae and mortality. The treatment approach to osteomyelitis is mainly antimicrobial, and often long-term, with surgical intervention in many cases to control infection. The causative pathogens in most cases of long bone osteomyelitis are infections of *Staphylococcus aureus*, and corresponding biofilms, which are bound to bone in contrast to their planktonic (free-floating) counterparts. Other

bone infections and corresponding biofilms are known to arise from a broad spectrum of both gram positive and gram-negative bacteria.

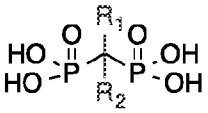
The biofilm-mediated nature of osteomyelitis is important in clinical and experimental settings because many biofilm pathogens are uncultivable and exhibit an altered phenotype with respect to growth rate and antimicrobial resistance (as compared to their planktonic counterparts). The difficulty in eradicating biofilms with conventional antibiotics partly explains why the higher success rates of antimicrobial therapy in general have not yet been realized for orthopedic infections, along with the development of resistant biofilm pathogens, the poor penetration of antimicrobial agents in bone, and adverse events related to systemic toxicity.

To overcome the many challenges associated with osteomyelitis treatment, there is increasing interest in drug delivery approaches using bone-targeting conjugates to achieve higher or more sustained local therapeutic concentrations of antibiotic in bone while minimizing systemic exposure.

Methylenebisphosphonates or substituted methylidine bisphosphonate moieties, commonly referred to as "bisphosphonates" (BPs) are therapeutic agents for the treatment of many bone disorders. The bisphosphonate P-C-P group mimics the P-O-P bond of the naturally-occurring mediator of bone metabolism, inorganic pyrophosphate. The structural relationship of pyrophosphate and methylene bisphosphonates in acid form is shown below. Individual BPs can be defined by the covalently attached substituents R_1, and R_2.



Pyrophosphate



Bisphosphonate

The bridging carbon of the bisphosphonate can be substituted with modifying groups (R_1, R_2) to confer specific biological properties on the derivative. BPs exhibit strong binding affinity to HA, the major inorganic material found in bone, particularly at sites of high bone turnover, and they are exceptionally stable to both chemical and biological degradation. It is often underappreciated that BPs also traverse through soft and hard tissues of the body (e.g. endothelium, periosteum, HA) to target bone and the canalicular network and vascular canals within bones. These highly specific bone-targeting properties of BPs make them ideal carriers to deliver drugs or macromolecules to bone surfaces.

Quinolone, and in particular fluoroquinolone, antibiotics conjugated to bisphosphonates (BPs), for example osteoadsorptive BPs, represents a promising approach because of the long clinical track-record of safety of each constituent, and their advantageous biochemical properties. In early investigations of the fluoroquinolone family in this context, ciprofloxacin demonstrated the best binding and microbiological properties when bound to a BP. Ciprofloxacin has several advantages for repurposing in this context: it can be administered orally or intravenously with relative bioequivalence, it has broad spectrum antimicrobial activity that includes the most commonly encountered osteomyelitis pathogens, it demonstrates bactericidal activity in clinically achievable doses, and it is the least expensive drug in the fluoroquinolone family.

The specific bone-targeting properties of the BP family makes them ideal carriers for introducing antibiotics to bone in osteomyelitis pharmacotherapy. BPs form strong bidentate and tridentate bonds with calcium and as a result concentrate in hydroxyapatite (HA), particularly at sites of active metabolism or infection and inflammation. BPs also exhibit exceptional stability against both chemical and biological degradation. The concept of targeting ciprofloxacin to bone via conjugation with a BP has been discussed in a number of reports over the years.

Despite these positive attributes of BPs and quinolones, such as ciprofloxacin, current attempts at generating prodrugs containing BPs and quinolones have been unsuccessful. Most attempts resulted in either systemically unstable prodrugs or non-cleavable conjugates that were found to mostly inactivate either component of the conjugate by interfering with the pharmacophoric requirements. For example, Delorme et al. (WO 2007/138381) describe use of acyloxy chemical neighbors to activate an alkyl carbamate linker for cleavage of an oxazolidinone from a bisphosphonate, but this appears to be too actively cleaved in the bloodstream. Similar findings have been described by Houghton et al 2008 J. Medicinal Chemistry, 51:6955-6969. And it has been shown by Morioka et. al ("Design, synthesis, and biological evaluation of novel estradiol–bisphosphonate conjugates as bone-specific estrogens," Bioorganic & Medicinal Chemistry 18 (2010) 1143–1148) and by Arns et. al ("Design and synthesis of novel bone-targeting dual-action pro-drugs for the treatment and reversal of osteoporosis," Bioorganic & Medicinal Chemistry 20 (2012) 2131–2140) that alkyl carbamates are too stable to be useful as a "target and release" linkage for bone targeted, active conjugates.

With the deficiencies of current BP quinolone conjugates in mind, described herein are BP quinolone conjugates that can contain a BP that can be releasably conjugated to a quinolone, such as ciprofloxacin, moxifloxacin, sitafloxacin or nemonoxacin. In embodiments, the BP quinolone conjugate can selectively deliver a quinolone to bone, bone grafts, and or bone graft

substitutes (i.e. can target bone, bone grafts, or bone graft substitutes) in a subject. In some embodiments, the BP quinolone conjugate can release the quinolone. Also provided herein are methods of synthesizing BP quinolone conjugates and methods of treating or preventing osteomyelitis or other bone infections with one or more of the BP quinolone conjugates provided herein.

The compositions provided herein can employ a "target and release linker" strategy, where a releasable and bone-specific targeting bisphosphonate-antibiotic (BP-Ab) conjugate can be made by attaching the antibiotic or antimicrobial agent, e.g., a quinolone, to a BP. In any one or more aspects, the BP can be a pharmacologically inactive BP or pharmacologically low active BP using a cleavable or reversible linker, such as carbamate, thiocarbamate, hydrazone, ~~hydrazone, et al.,~~ or carbonate, so that the antimicrobial agent can be released upon binding to bone surfaces by the decreased pH and/or enzymatic environment which is typically found at active sites of bone resorption or infection.

In any one or more aspects, the quinolone can be attached to a hydroxy BP, directly or indirectly, to the geminal hydroxyl group on the carbon between the two phosphonate groups of the BP. This is in contrast to use of an aryl carbamate to otherwise attach or link a quinolone to the BP at a site other than an alpha hydroxyl, alpha thiol, or alpha amino site. To activate the attachment or linker enough for cleavage (the "target and release" concept) the present disclosure utilizes carbamates (and relatives) that are uniquely activated by the alpha carbon or substituent of bisphosphonates for adequate release. In any one or more aspects herein, all analogs of known clinically used BPs are preferred. Etidronate and MHDP or methylene hydroxy BP may be most preferred.

The present chemistry and conjugate design also allows the cleavage to release two known (clinically used) drugs, the quinolone and the BP (in particular clinically used BPs). Carbon dioxide is the only other released component from the linker. Thus, no new safety questions exist for the released components. Previously unknown was whether a linkage to the geminal hydroxy group of a bisphosphonate would cleave appropriately for this use to accomplish this ("target and release" based efficacy) goal in vivo. Previously, unknown BPs, that have not been used clinically, were used to create aryl carbamate linkages at sites other than the alpha carbon of the BP. These BPs, that had not previously been studied in a human subject, allowed creation of an aryl carbamate-based conjugate to allow a release rate/cleavage rate that was useful for bioactivity. Here it has been found in vitro that release still occurs at a useful rate because a carbamate, via linkage to the geminal hydroxy group is sufficiently activated by the adjacent phosphonate groups to cleave adequately. Since this has now been found to occur, the present compounds,

conjugates and formulations offer the opportunity to use many clinically known bisphosphonates within the conjugate because most have a geminal hydroxy group.

An exemplary BP-quinolone release mechanism is depicted in **FIG. 2** using ciprofloxacin as an exemplary quinolone. However, a non-fluoroquinolone can also be conjugated to the BP as described herein. This BP-Ab conjugate can have the ability to deliver and release the antimicrobial agent specifically to the infectious osteolytic site where higher bone metabolism is occurring. Use of an inactive or low active BP can also offer a unique treatment option by providing a higher concentration of antimicrobial agent at the disease site and relatively lower systemic levels than with higher active BPs. Other BP-quinolone compounds and conjugates, as described herein, can have the same or similar activity.

Also provided herein are formulations that can include an amount of a compound, conjugate or composition described herein and an additional compound (such as, but not limited to, a carrier, diluent, or other active agent or ingredient). The formulation can be a pharmaceutical formulation that can contain a pharmaceutically acceptable carrier. The compositions and/or formulations can be administered to a subject. The subject can have a bone infection. The compositions and formulations provided herein can be used to treat and/or prevent bone infection. The compositions and formulations provide herein can provide, in some embodiments, bone specific delivery of an antimicrobial agent.

The general concept of targeting active drug species to the bone compartment with BP has been discussed in a number of reports. However, no drugs have yet been developed, as early attempts led to either systemically unstable prodrugs or non-cleavable conjugates that were found mostly to inactivate either component of the conjugate by interfering with the pharmacophoric requirements. This suggests that target and release strategies are likely chemical class-dependent (taking into consideration compatibilities of the functional groups of each component) as well as biochemical target-dependent, and the design for any particular chemical class must be customized for its use. Therefore, provided herein are embodiments of a novel approach to develop a bone targeted antibiotic or antimicrobial agent with a linkage that can be metabolically stable in the bloodstream and metabolically labile on bone to facilitate appropriate release.

In particular, in any one or more aspects herein, the linkages utilized herein are designed to allow maximum local antibacterial efficacy at the site of an infection where higher bone turnover exists, while also limiting exposure of lower turnover skeletal sites, non-skeletal sites, and distant compartments throughout the body from any adverse effect due to antibiotic or bisphosphonate

components or conjugate. Thus, for example, the alpha hydoxy carbamate linkers and other related alpha carbon oriented linkers herein are specifically selected to have maximum stability in the bloodstream, while still having sensitivity to chemically cleave and release quinolone antibiotics at skeletal sites of bacterial infection, due to their sensitivity to the enzymatic processes and pH characteristics of that environment. Furthermore, select, but not all, embodiments in this disclosure, include the use of bisphosphonates that do not have significant pharmacological activity for the targeting component of these drug conjugates. These "nonantiresorptive" or weak antiresorptive bisphosphonates have the characteristics of only targeting the antibiotic to the bone compartments described and do not have properties to otherwise affect bone metabolism directly. Examples include aryl carbamates and aryl thiocarbamates derived from substituted and unsubstituted 2-[4-aminophenyl]ethane 1,1 bisphosphonate and 2-[4-hydroxyphenyl]ethane 1,1 bisphosphonate. Also, included are carbamates derived from substituted and unsubstituted 2-[3-aminophenyl]ethane 1,1 bisphosphonate and 2-[3-hydroxyphenyl]ethane 1,1 bisphosphonate substituted and unsubstituted 2-[2-aminophenyl]ethane 1,1 bisphosphonate and 2-[2-hydroxyphenyl]ethane 1,1 bisphosphonate. Furthermore, aryl dithiocarbamates, derived from substituted and unsubstituted 2-[4-thiophenyl]ethane 1,1 bisphosphonate, 2-[3-thiophenyl]ethane 1,1 bisphosphonate, and 2-[2-thiophenyl]ethane 1,1 bisphosphonate.

In any one or more aspects, the BP of the conjugate can be a pharmacologically inert or inactive BP. Examples of pharmacologically inert or inactive BPs that can be conjugated with a quinolone as described herein are shown in **FIG. 24**.

As an example, the inert or inactive BP series for the conjugation can be 4-hydroxyphenylethylidene BP (**FIG. 24A**) or 4-aminophenylethylidene BP (**FIG. 24E**) having a medium mineral affinity. Further analogs such as hydroxy BP (**FIGS. 24B and 24F**) (higher mineral affinity) and methyl BP (**FIGS. 24C and 24G**) (lower mineral affinity) can be used to adjust the concentration of the BP-Ab conjugates at bone. Phenyl alkyl BPs with different chain lengths such as in **FIGS. 24D and 24H** (propyl or butyl vs. ethyl phenyl) can also be utilized to optimize the conjugation chemistry yields and conjugate stability.

In any one or more aspects, the BP of the conjugate can be a pharmacologically low active BP. Examples of pharmacologically low active BPs that can be conjugated with a quinolone as described herein are shown in **FIG. 25**. By "low active BP" we mean either the bisphosphonate or the dosage level of the bisphosphonate is not so high as to effect bone metabolism. However, in some aspects, a higher active BP may be desired where it is desired to both effect bone

metabolism and to deliver a quinolone antibiotic to inhibit and/or kill/effect bacteria on bone.

Other compositions, compounds, methods, features, and advantages of the present disclosure will be or become apparent to one having ordinary skill in the art upon examination of the following drawings, detailed description, and examples. It is intended that all such additional compositions, compounds, methods, features, and advantages be included within this description, and be within the scope of the present disclosure.

Bisphosphonate (BP) Quinolone Conjugates and Formulations Thereof

BP Quinolone Conjugates

Provided herein are BP quinolone compounds, conjugates and formulations thereof. A BP can be conjugated to a quinolone via a linker. In embodiments, the linker is a releasable linker. The quinolone can be releasably attached via a linker to the BP. Thus, in some embodiments, the BP quinolone conjugate can selectively deliver and release the quinolone at or near bone, bone grafts, or bone graft substitutes **(Fig. 2)**. In other words, for example, a BP fluoroquinolone conjugate can provide targeted delivery of a fluoroquinolone to bone and/or the areas proximate to bone.

The BP of the BP quinolone conjugates provided herein can be conjugated to any BP including but not limited to, hydroxyl phenyl alkyl or aryl bisphosphonates, hydroxyl phenyl (or aryl) alkyl hydroxyl bisphosphonates, amino phenyl(or aryl) alkyl bisphosphonates, amino phenyl(or aryl) alkyl hydroxyl bisphosphonates, hydroxyl alkyl bisphosphonates, hydroxyl alkyl hydroxyl bisphosphonates hydroxyl alkyl phenyl(or aryl) alkyl bisphosphonates, hydroxyl phenyl(or aryl) alkyl hydroxyl bisphosphonates, amino phenyl(or aryl) alkyl bisphosphonates, amino phenyl(or aryl) alkyl hydroxyl bisphosphonates, hydroxyl alkyl bisphosphonates, hydroxyl alkyl hydroxyl bisphosphonates, all of the former being further unsubstituted or substituted. In particular, the BP can be etidronate, pamidronate, neridronate, olpadronate, alendronate, ibandronate, minodronate, risedronate, zoledronate, hydroxymethylenebisphosphonate, and combinations thereof. The bisphosphonate may also be substituted for phosphono phosphinic acid or phosphono carboxylic acid. In embodiments, the BP can be pamidronate, alendronate, risedronate, zoledronate, minodronate, neridronate, etidronate, which can be unmodified or modified as described herein. In preferred embodiments, the BP is etidronate, MHBP, or

pamidronate, unmodified or modified.

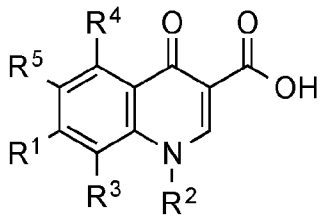
The BP can contain, or be modified to contain, linkages to an alpha subsitutent and the alpha subsitutent can be a hydroxy, amino or thiol group. A antibiotic quinolone compound or analog can be conjugated directly or indirectly to the BP at a geminal carbon substituent of the BP. The quinolone and/or a linker can also be coupled to a BP having an anti-resorptive effect that is significantly reduced or eliminated.

In BPs containing an aryl or phenyl, the aryl or phenyl can be substituted with a suitable substitutent at any position on the ring. In some embodiments, the aryl or phenyl ring of the BP is substituted with one or more electron donating species (e.g. F, N, and Cl).

Non-pharmacologically active BP variants may also be used for the purpose of quinolone delivery absent BP action.

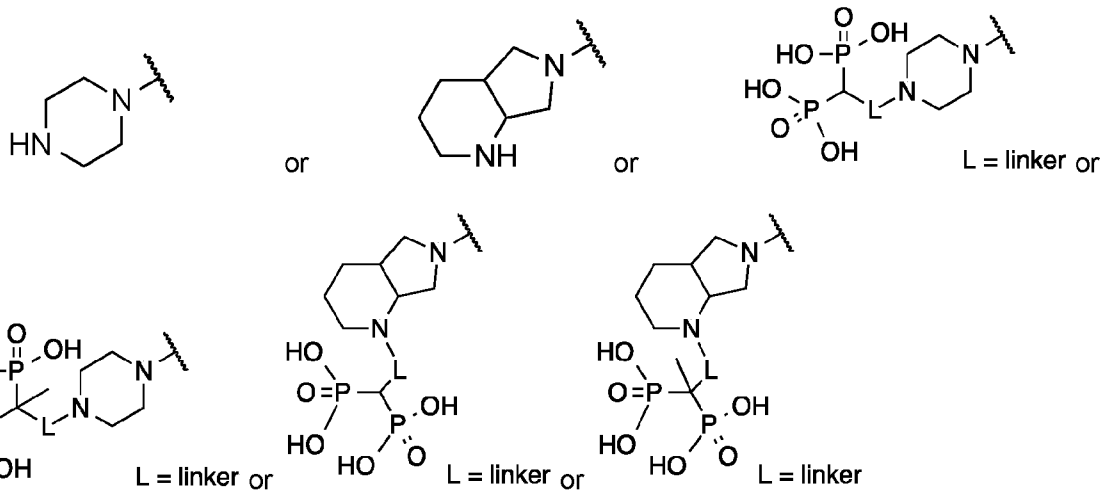
The quinolone can be any quinolone, a fluoroquinolone or a non-fluoroquinolone including but not limited to alatrofloxacin, amifloxacin, balofloxacin, besifloxacin, cadazolid, ciprofloxacin, clinafloxacin, danofloxacin, delafloxacin, difloxacin, enoxacin, enrofloxacin, finafloxacin, flerofloxacin, flumequine, gatifloxacin, gemifloxacin, grepafloxacin, ibafloxacin, JNJ-Q2, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, norfloxacin, ofloxacin, orbifloxacin, pazufloxacin, pefloxacin, pradofloxacin, prulifloxacin, rufloxacin, sarafloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin, trvafloxacin, zabofloxacin, nemonoxacin and any combination thereof. In particular, the quinolone can be a fluoroquinolone, preferably ciprofloxacin or moxifloxacin.

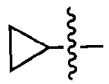
In any one or more aspects or embodiments herein, the BP quinolone compound can be comprised of a quinolone analog or substituent according to the following structure or Formula (**A**),



Formula (**A**)

wherein R^1 can be either



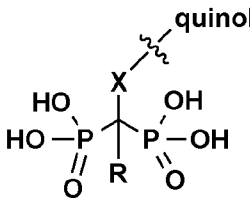
and wherein R^2 can be 

and wherein R^3 can be either H or OCH3,

and where R^4 can be H,

and wherein R^5 can be H or F.

As shown, the quinolone of Formula (**A**) can be linked to a bisphosphonate (BP). In any one or more aspects or embodiments herein, the BP can have an alpha substituent and the alpha substituent is a hydroxy, amino or thiol group. The quinolone can be directly or indirectly conjugated to the BP at the germinal carbon alpha substituent (X) of the BP, as illustrated in the formula below.



conjugates between alpha-X containing BP and quinolone

X= O, NH, NR^1, S

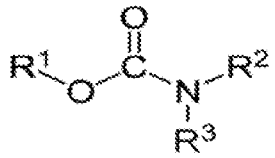
R^1 can be alkyl or substituted alkyl, aryl or subsituted aryl groups

wherein R can be H, substituted and unsubstituted alkyl, alkyl amino, alkyl-aryl, aryl, alkylheteroaryl, or heteroaryl.

Preferred BPs are those that have a geminal hydroxy group on the carbon between the two phosphonate groups. A generic analog of such a BP is illustrated in **Fig. 25**._In any one or more aspects, the bisphosphonate can be ethylidenebisphosphonate moiety (etidronate) that can be substituted by hydroxy (an alpha-hydroxy), amino or thiol. In some aspects, the bisphosphonate can include a para-hydroxyphenylethylidene group or derivative thereof. In embodiments, the BP can be a clinically known BP, such as pamidronate, alendronate, risedronate, zoledronate, minodronate, neridronate, and etidronate, which can be unmodified or modified as described herein.

In any one or more embodiments, the BP can be etidronate. Etidronate can be linked to a quinolone to form a quinolone antibiotic etidronate-ciprofloxacin (ECC) conjugate, such as in Formula (**41**) or to form an etidronate moxifloxacin (ECX) conjugate such as in Formula (**43**) herein.

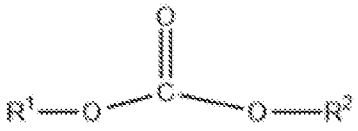
The linker, L, can be a compound that is cleavable, meaning that it reversibly couples the quinolone analog or compound, in particular a quinolone antimicrobial or antibiotic analog or substituent thereof, to the BP. As used herein, the term "cleavable" can mean a group that is chemically or biochemically unstable under physiological conditions. In any one or more aspects, the linker can be a carbamate, having a structure or Formula (**B**) below



Formula (**B**)

for coupling a quinolone, R^2, to a BP, R^1, as described herein, and R^3 can be substituted and unsubstituted alkyl, acetyl, benzoyl or other amides, phenyl and substituted phenyl, preferably H.

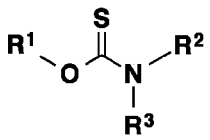
In any one or more aspects, the linker can be a carbonate, having a structure or Formula (**C**) below



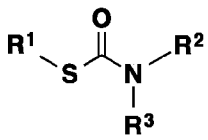
Formula (C)

for coupling a quinolone, R^2, to a BP, R^1, as described herein.

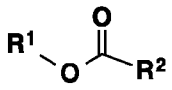
In any one or more aspects, the linker can be an alkyl or an aryl carbamate linker. The linker can be an *O*-thioaryl or thioalklyl carbamate linker. The linker can be an *S*-thioaryl or thioalkyl carbamate linker. The linker can be a phenyl carbamate linker. The linker can be a thiocarbamate linker. The linker can be an *O*-thiocarbamate linker. The linker can be an *S*-thiocarbamate linker. The linker can be an ester linker. The linker can be a dithiocarbamate. The linker can be a urea linker. The linker can be part of the R^1 group of Formula **(A)** along with the BP and couple the BP to the quinolone, as described herein. In any one or more aspects, the linker can be exemplified by any one of Formula **(D)** – Formula **(H)** below, wherein: R^2 can be a quinolone or a quinolone substituent or derivative and R^1 can be a BP, both as described herein; and R^3 can be substituted and unsubstituted alkyl, acetyl, benzoyl or other amides, phenyl and substituted phenyl, preferably H.



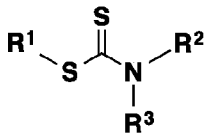
Formula (D)



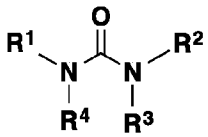
Formula (E)



Formula (F)



Formula (G)



Formula (H)

In some aspects, the BP is etidronate. In some aspects, the quinolone is ciprofloxacin or moxifloxacin. In some aspects, the BP is etidronate, the quinolone is ciprofloxacin and the linker is an aryl or alkyl carbamate or a linker of Formula (**F**) providing the compound of Formula (**41**). In some aspects, the BP is etidronate, the quinolone is moxifloxacin and the linker is an aryl or alkyl carbamate or a linker of Formula (**F**) providing the compound of Formula (**43**). In some

aspects, the BP is etidronate, the quinolone is sitafloxacin or nemonoxacin and the linker is an alkyl or aryl carbamate or a linker of Formula (**F**) providing the compound of Formula (**44**) or Formula (**45**) herein.

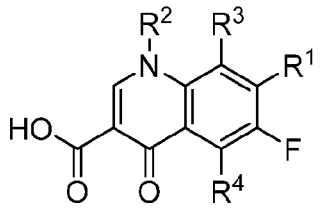
In other aspects, the BP can be another BP described herein, such as pamidronate, neridronate, olpadronate, alendronate, ibandronate, minodronate, risedronate, zoledronate, hydroxymethylenebisphosphonate (HMBP), and combinations thereof.

In any one or more aspects, the bisphosphonate can have an alpha substituent that is substituted by hydroxy (an alpha-hydroxy), amino, or thiol. In any one or more aspects, the bisphosphonate can be an ethylidenebisphosphonate moiety (etidronate) that can be substituted by hydroxy (an alpha-hydroxy), amino or thiol. In some aspects, the bisphosphonate can include a para-hydroxyphenylethylidene group or derivative thereof. In embodiments, the BP can be a clinically known BP, such as pamidronate, alendronate, risedronate, zoledronate, minodronate, neridronate, and etidronate, which can be unmodified or modified as described herein.

Also provided herein are pharmaceutical formulations that can contain a bisphosphonate (BP) and a quinolone compound of Formula (**A**), wherein the quinolone compound is releasably coupled to the bisphosphonate via a linker, L; and a pharmaceutically acceptable carrier. The bisphosphonate (BP) and the linker, L, can be as described herein in any one or more aspects.

In any one or more embodiments and aspects herein, the quinolone can have a generic structure according to Formula (**A**), where R^1 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups, where R^2 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted

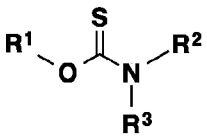
amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups, where R^3 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups, and where R4 can be substituents including alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C_3-C_{20} cyclic, substituted C_3-C_{20} cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups, and wherein R^5 can be H or F.



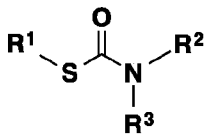
Formula (A)

The BP can be conjugated to the quinolone, either a fluoroquinolone or a non-fluoroquinolone, preferably a fluoroquinolone, via a releasable linker. In any one or more aspects, the linker can be an alkyl or an aryl carbamate linker. The linker can be an *O*-thioaryl or thioalkyl

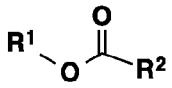
carbamate linker. The linker can be an *S*-thioaryl or thio alkyl carbamate linker. The linker can be a phenyl carbamate linker. The linker can be a thiocarbamate linker. The linker can be an *O*-thiocarbamate linker. The linker can be an *S*-thiocarbamate linker. The linker can be an ester linker. The linker can be a dithiocarbamate. The linker can be a urea linker. The linker can be part of the R^1 group of Formula **(A)** along with the BP and couple the BP to the quinolone, as described herein. In any one or more aspects, the linker can be exemplified by any one of Formula **(D)** – Formula **(H)** below, wherein: R^2 can be a quinolone or a quinolone substituent or derivative and R^1 can be a BP, both as described herein; and R^3 can be substituted and unsubstituted alkyl, acetyl, benzoyl or other amides, phenyl and substituted phenyl, preferably H.



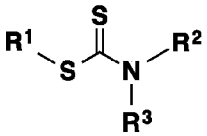
Formula (D)



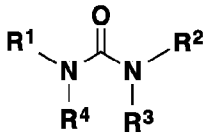
Formula (E)



Formula (F)



Formula (G)

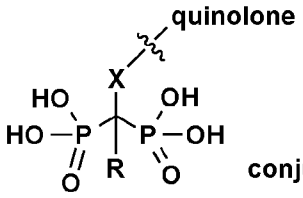


Formula (H)

In some aspects, the BP is etidronate. In some aspects, the quinolone is ciprofloxacin or moxifloxacin. In some aspects, the BP is etidronate, the quinolone is ciprofloxacin and the linker is an alkyl or an aryl carbamate or a linker of Formula (**F**) providing the compound of Formula (**41**) herein. In some aspects, the BP is etidronate, the quinolone is moxifloxacin and the linker is an alkyl or an aryl carbamate or a linker of Formula (**F**) providing the compound of Formula (**43**) herein. In some aspects, the BP is etidronate, the quinolone is sitafloxacin or nemonoxacin and the linker is an alkyl or an aryl carbamate or a linker of Formula (**F**) providing the compound of Formula (**44**) or Formula (**45**) herein.

In any one or more aspects or embodiments herein, the BP has an alpha substituent and the alpha substituent is a hydroxy, amino or thiol group and the quinolone is directly or indirectly

conjugated to the BP at the germinal carbon alpha substituent (X) of the BP, as illustrated in the formula below.



conjugates between alpha-X containing BP and quinolone

X= O, NH, NR^1, S

R^1 can be alkyl or substituted alkyl, aryl or subsituted aryl groups

wherein R can be H, substituted and unsubstituted alkyl, alkyl amino, alkyl-aryl, aryl, alkylheteroaryl, or heteroaryl.

In any one or more aspects, the BP is an alpha-OH containing BP that can be conjugated to the quinolone, such as a fluoroquinolone, at a geminal OH group on the BP as shown below. In various aspects the quinolone, such as a fluoroquinolone, can be directly conjugated (e.g., without use of a linker other than C=O) to a geminal OH group of the BP. In various aspects, the quinolone can be indirectly conjugated via a linker at the geminal OH group of the BP.

In some aspects, the compound can have a formula according to Formula **(41)**, Formula **(43)**, Formula (**44**) or Formula (**45**) herein.

BP Quinolone Conjugate Pharmaceutical Formulations

Also described herein are formulations, including pharmaceutical formulations, which can contain an amount of a BP quinolone compound or conjugate described elsewhere herein in any one or more aspects or embodiments. The amount can be an effective amount. The amount can be effective to inhibit the growth and/or reproduction of a bacterium. The amount can be effective to kill a bacterium. Formulations, including pharmaceutical formulations can be formulated for delivery via a variety of routes and can contain a pharmaceutically acceptable carrier. Techniques and formulations generally can be found in *Remmington's Pharmaceutical Sciences*, Meade Publishing Co., Easton, Pa. (20th Ed., 2000), the entire disclosure of which is herein incorporated by reference. For systemic administration, an injection is useful, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the therapeutic compositions of the invention can be formulated in liquid solutions, for example in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the BP quinolone conjugates and/or components thereof can be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. Formulations, including pharmaceutical

formulations, of the BP quinolone conjugates can be characterized as being at least sterile and pyrogen-free. These formulations include formulations for human and veterinary use.

Suitable pharmaceutically acceptable carriers include, but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxyl methylcellulose, and polyvinyl pyrrolidone, which do not deleteriously react with the BP quinolone conjugate.

The pharmaceutical formulations can be sterilized, and if desired, mixed with auxiliary agents, such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances, and the like which do not deleteriously react with the BP quinolone conjugate.

Another formulation includes the addition of the BP quinolone conjugates to bone graft material or bone void fillers for the prevention or treatment of osteomyelitis, peri-implantitis or peri-prosthetic infections, and for socket preservation after dental extractions.

A pharmaceutical formulation can be formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Formulations, including pharmaceutical formulations, suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers can include physiological saline, bacteriostatic water, Cremophor EM™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). Injectable pharmaceutical formulations can be sterile and can be fluid to the extent that easy syringability exists. Injectable pharmaceutical formulations can be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and

fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid polyetheylene glycol, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In some embodiments, it can be useful to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition.

Sterile injectable solutions can be prepared by incorporating any of the BP quinolone conjugates described herein in an amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions can be prepared by incorporating the BP quinolone conjugate into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders for the preparation of sterile injectable solutions, examples of useful preparation methods are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated can be used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fluidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the BP quinolone conjugates can be formulated into ointments, salves, gels, or creams as generally known in the art. In some embodiments, the BP quinolone conjugates can be applied via transdermal delivery systems, which can slowly release the BP quinolone conjugates for percutaneous absorption. Permeation enhancers can be used to facilitate transdermal penetration of the active factors in the conditioned media. Transdermal patches are described in for example, U.S. Pat. No. 5,407,713; U.S. Pat. No. 5,352,456; U.S. Pat. No. 5,332,213; U.S. Pat. No. 5,336,168; U.S. Pat. No. 5,290,561; U.S. Pat. No. 5,254,346; U.S. Pat. No. 5,164,189; U.S. Pat. No. 5,163,899; U.S. Pat. No. 5,088,977; U.S. Pat. No. 5,087,240; U.S. Pat. No. 5,008,110; and U.S. Pat. No. 4,921,475.

For oral administration, a formulation as described herein can be presented as capsules, tablets, powders, granules, or as a suspension or solution. The formulation can contain

conventional additives, such as lactose, mannitol, cornstarch or potato starch, binders, crystalline cellulose, cellulose derivatives, acacia, cornstarch, gelatins, disintegrators, potato starch, sodium carboxymethylcellulose, dibasic calcium phosphate, anhydrous or sodium starch glycolate, lubricants, and/or or magnesium stearate.

For parenteral administration (i.e., administration through a route other than the alimentary canal), the formulations described herein can be combined with a sterile aqueous solution that is isotonic with the blood of the subject. Such a formulation can be prepared by dissolving the active ingredient (e.g. the BP quinolone conjugate) in water containing physiologically-compatible substances, such as sodium chloride, glycine and the like, and having a buffered pH compatible with physiological conditions, so as to produce an aqueous solution, then rendering the solution sterile. The formulation can be presented in unit or multi-dose containers, such as sealed ampoules or vials. The formulation can be delivered by injection, infusion, or other means known in the art.

For transdermal administration, the formulations described herein can be combined with skin penetration enhancers, such as propylene glycol, polyethylene glycol, isopropanol, ethanol, oleic acid, N-methylpyrrolidone and the like, which increase the permeability of the skin to the nucleic acid vectors of the invention and permit the nucleic acid vectors to penetrate through the skin and into the bloodstream. The formulations and/or compositions described herein can be further combined with a polymeric substance, such as ethylcellulose, hydroxypropyl cellulose, ethylene/vinyl acetate, polyvinyl pyrrolidone, and the like, to provide the composition in gel form, which can be dissolved in a solvent, such as methylene chloride, evaporated to the desired viscosity and then applied to backing material to provide a patch.

For inclusion in bone graft substitutes or bone void fillers to prevent local post-operative infection or graft failure after surgery, and to provide sustained local release of antibiotic at the graft site, the formulations described herein can be combined with any xenograft (bovine), autograft (self) or allograft (cadaver) material or synthetic bone substitute. For example, a powder formulation can be premixed by the treating surgeon or clinician bedside/chairside with any existing bone graft substitute on the market or with an autologous graft. This formulation can be further combined with any previously described formulation, and can be combined with products containing hydroxyapatites, tricalcium phosphates, collagen, aliphatic polyesters (poly(lactic) acids (PLA), poly(glycolic)acids (PGA), and polycaprolactone (PCL), polyhydroxybutyrate (PHB), methacrylates, polymethylmethacrylates, resins, monomers, polymers, cancellous bone allografts, human fibrin, platelet rich plasma, platelet rich fibrin, plaster of Paris, apatite, synthetic hydroxyapaptite, coralline hydroxyapatite, wollastonite (calcium silicate), calcium sulfate,

bioactive glasses, ceramics, titanium, devitalized bone matrix, non-collagenous proteins, collagen, and autolyzed antigen extracted allogenic bone. In this embodiment the bone graft material combined with BP quinolone conjugate can be in the formulation of a paste, powder, putty, gel, hydrogel, matrix, granules, particles, freeze-dried powder, freeze-dried bone, demineralized freeze-dried bone, fresh or fresh-frozen bone, corticocancellous mix, pellets, strips, plugs, membranes, lyophilized powder reconstituted to form wet paste, spherules, sponges, blocks, morsels, sticks, wedges, cements, or amorphous particles; many of these may also be in injectable formulations or as a combination of two or more aforementioned formulations (e.g. injectable paste with sponge).

In another embodiment, the BP-quinolone conjugate can be combined with factor-based bone grafts containing natural or recombinant growth factors, such as transforming growth factor-beta (TGF-beta), platelet-derived growth factor (PDGF), fibroblast growth factors (FGF), and/or bone morphogenic protein (BMP). In another embodiment, the BP quinolone conjugate can be combined with cell-based bone grafts used in regenerative medicine and dentistry including embryonic stem cells and/or adults stem cells, tissue-specific stem cells, hematopoietic stem cells, epidermal stem cells, epithelial stem cells, gingival stem cells, periodontal ligament stem cells, adipose stem cells, bone marrow stem cells, and blood stem cells. Therefore, a bone graft with the property of osteoconduction, osteoinduction, osteopromotion, osteogenesis, or any combination thereof can be combined with the BP quinolone conjugate for clinical or therapeutic use.

Dosage forms

The BP quinolone compounds, conjugates and formulations thereof described herein in any one or more aspects or embodiments can be provided in unit dose form such as a tablet, capsule, single-dose injection or infusion vial, or as a predetermined dose for mixing with bone graft material as in formulations described above. Where appropriate, the dosage forms described herein can be microencapsulated. The dosage form can also be prepared to prolong or sustain the release of any ingredient. In some embodiments, the complexed active agent can be the ingredient whose release is delayed. In other embodiments, the release of an auxiliary ingredient is delayed. Suitable methods for delaying the release of an ingredient include, but are not limited to, coating or embedding the ingredients in material in polymers, wax, gels, and the like. Delayed release dosage formulations can be prepared as described in standard references such as "Pharmaceutical dosage form tablets," eds. Liberman et. al. (New York, Marcel Dekker, Inc., 1989), "Remington – The science and practice of pharmacy", 20th ed., Lippincott Williams & Wilkins, Baltimore, MD, 2000, and "Pharmaceutical dosage forms and drug delivery systems", 6th

Edition, Ansel et al., (Media, PA: Williams and Wilkins, 1995). These references provide information on excipients, materials, equipment, and processes for preparing tablets and capsules and delayed release dosage forms of tablets and pellets, capsules, and granules. The delayed release can be anywhere from about an hour to about 3 months or more.

Coatings may be formed with a different ratio of water soluble polymer, water insoluble polymers, and/or pH dependent polymers, with or without water insoluble/water soluble non polymeric excipient, to produce the desired release profile. The coatings can be either performed on the dosage form (matrix or simple) which includes, but is not limited to, tablets (compressed with or without coated beads), capsules (with or without coated beads), beads, particle compositions, "ingredient as is" formulated as, but not limited to, suspension form or as a sprinkle dosage form.

Examples of suitable coating materials include, but are not limited to, cellulose polymers such as cellulose acetate phthalate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, and hydroxypropyl methylcellulose acetate succinate; polyvinyl acetate phthalate, acrylic acid polymers and copolymers, and methacrylic resins that are commercially available under the trade name EUDRAGIT® (Roth Pharma, Westerstadt, Germany), zein, shellac, and polysaccharides.

Effective Amounts

The formulations can contain an effective amount of a BP quinolone compound or conjugate (effective for inhibiting and/or killing a bacterium) described herein in any one or more aspects or embodiments. In some embodiments, the effective amount ranges from about 0.001 pg to about 1,000 g or more of the BP quinolone conjugate described herein. In some embodiments, the effective amount of the BP quinolone conjugate described herein can range from about 0.001 mg/kg body weight to about 1,000 mg/kg body weight. In yet other embodiments, the effective amount of the BP quinolone conjugate can range from about 1% w/w to about 99% or more w/w, w/v, or v/v of the total formulation. In some embodiments, the effective amount of the BP quinolone conjugate is effective at killing a bacterium that is the causative agent of osteomyelitis and all its subtypes (e.g. diabetic foot osteomyelitis), jaw osteonecrosis, and periodontitis including, but not limited to any strain or species of Staphylococcus, Pseudomonas, Aggregatibacter, Actinomyces, Streptococcus, Haemophilus, Salmonella, Serratia, Enterobacter, Fusobacterium, Bacteroides, Porphyromonas, Prevotella, Veillonella, Campylobacter, Peptostreptococcus, Eikenella, Treponema, Dialister, Micromonas, Yersinia, Tannerella, and Escherichia.

Methods of Using the BP Quinolone Conjugates

An amount, including an effective amount, of the BP quinolone compounds, conjugates and formulations thereof described herein in any one or more aspects or embodiments can be administered to a subject in need thereof. In some embodiments the subject in need thereof can have a bone infection, disease, disorder, or a symptom thereof. In some embodiments, the subject in need thereof can be suspected of having or is otherwise predisposed to having a bone infection, disease, disorder, or a symptom thereof. In some embodiments, the subject in need thereof may be at risk for developing an osteomyelitis, osteonecrosis, peri-prosthetic infection, and/or peri-implantitis. In embodiments, the disease or disorder can be osteomyelitis and all its subtypes, osteonecrosis, peri-implantitis or periodontitis. In some embodiments the subject in need thereof has a bone that is infected with a microorganism, such as a bacteria. In some embodiments, the bacteria can be any strain or species of *Staphylococcus*, *Pseudomonas, Aggregatibacter, Actinomyces, Streptococcus, Haemophilus, Salmonella, Serratia, Enterobacter, Fusobacterium, Bacteroides, Porphyromonas, Prevotella, Veillonella, Campylobacter, Peptostreptococcus, Eikenella, Treponema, Dialister, Micromonas, Yersinia, Tannerella, and Escherichia*. In some embodiments, the bacteria can form biofilms. In some embodiments, osteomyelitis can be treated in a subject in need thereof by administering an amount, such as an effective amount, of BP quinolone conjugate or formulation thereof described herein to the subject in need thereof. In some embodimnets, the compositions and compounds provided herein can be used in osteonecrosis treatment and/or prevention, distraction osteogenesis, cleft repair, repair of critical supra-alveolar defects, jawbone reconstruction, and any other reconstructions or repair of a bone and/or joint.

Administration of the BP quinolone compounds or conjugates is not restricted to a single route, but can encompass administration by multiple routes. For instance, exemplary administrations by multiple routes include, among others, a combination of intradermal and intramuscular administration, or intradermal and subcutaneous administration. Multiple administrations can be sequential or concurrent. Other modes of application by multiple routes will be apparent to the skilled artisan.

The pharmaceutical formulations can be administered to a subject by any suitable method that allows the agent to exert its effect on the subject in vivo. For example, the formulations and other compositions described herein can be administered to the subject by known procedures including, but not limited to, by oral administration, sublingual or buccal administration, parenteral administration, transdermal administration, via inhalation, via nasal delivery, vaginally, rectally,

and intramuscularly. The formulations or other compositions described herein can be administered parenterally, by epifascial, intracapsular, intracutaneous, subcutaneous, intradermal, intrathecal, intramuscular, intraperitoneal, intrasternal, intravascular, intravenous, parenchymatous, and/or sublingual delivery. Delivery can be by injection, infusion, catheter delivery, or some other means, such as by tablet or spray. Delivery can also be by a carrier such as hydroxyapatite or bone in the case of anti-infective bone graft material at a surgical site. Delivery can be via attachment or other association with a bone graft material.

EXAMPLES

Now having described the embodiments of the present disclosure, in general, the following Examples describe some additional embodiments of the present disclosure. While embodiments of the present disclosure are described in connection with the following examples and the corresponding text and figures, there is no intent to limit embodiments of the present disclosure to this description. On the contrary, the intent is to cover all alternatives, modifications, and equivalents included within the spirit and scope of embodiments of the present disclosure.

Introduction

Infectious bone disease, or osteomyelitis, is a major problem worldwide in human and veterinary medicine and can be devastating due to the potential for limb-threatening sequelae and mortality (Lew, et al., Osteomyelitis. Lancet 2004;364:369-79; Desrochers, et al, Limb amputation and prosthesis. Vet Clin North Am Food Anim Pract 2014;30:143-55; Stoodley, et al., Orthopaedic biofilm infections. Curr Orthop Pract 2011;22:558-63; Huang, et al., Chronic osteomyelitis increases long-term mortality risk in the elderly: a nationwide population-based cohort study. BMC Geriatr 2016;16:72). The treatment approach to osteomyelitis is mainly antimicrobial, and often long-term, with surgical intervention in many cases to control infection. The causative pathogens in most cases of long bone osteomyelitis are biofilms of *Staphylococcus aureus*; by definition these microbes are bound to bone **(Fig. 1)** in contrast to their planktonic (free-floating) counterparts (Wolcott, et al., Biofilms and chronic infections. J Am Med Assoc 2008;299:2682–2684).

The biofilm-mediated nature of osteomyelitis is important in clinical and experimental settings because many biofilm pathogens are uncultivable and exhibit an altered phenotype with respect to growth rate and antimicrobial resistance (as compared to their planktonic counterparts) (Junka, et al., Microbial biofilms are able to destroy hydroxyapatite in the absence of host

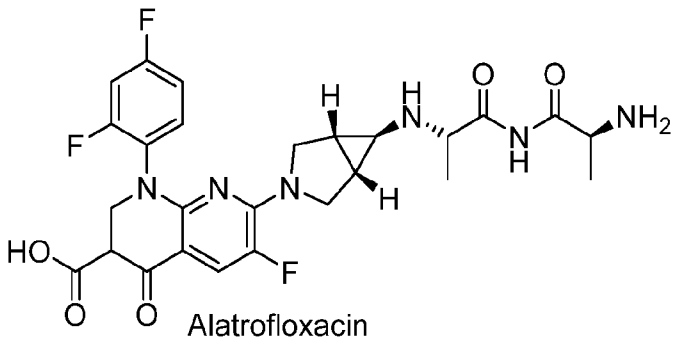
immunity in vitro. J Oral Maxillofac Surg 2015;73:451-64; Herczegh, et al., Osteoadsorptive bisphosphonate derivatives of fluoroquinolone antibacterials. J Med Chem 2002; 45:2338-41). The difficulty in eradicating biofilms with conventional antibiotics partly explains why the higher success rates of antimicrobial therapy in general have not yet been realized for orthopedic infections, along with the development of resistant biofilm pathogens, the poor penetration of antimicrobial agents in bone, and adverse events related to systemic toxicity (Buxton, et al., Bisphosphonate-ciprofloxacin bound to Skelite is a prototype for enhancing experimental local antibiotic delivery to injured bone. Br J Surg 2004;91:1192-6).

To overcome the many challenges associated with osteomyelitis treatment, there is increasing interest in drug delivery approaches using bone-targeting conjugates to achieve higher or more sustained local therapeutic concentrations of antibiotic in bone while minimizing systemic exposure (Panagopoulos, et al., Local Antibiotic Delivery Systems in Diabetic Foot Osteomyelitis: Time for One Step Beyond? Int J Low Extrem Wounds 2015;14:87-91; Puga, et al., Hot melt poly-epsilon-caprolactone/poloxamine implantable matrices for sustained delivery of ciprofloxacin. Acta biomaterialia 2012;8:1507-18). Fluoroquinolone antibiotics conjugated to osteoadsorptive bisphosphonates (BPs) represents a promising approach because of the long clinical track-record of safety of each constituent, and their advantageous biochemical properties (Buxton, et al., Bisphosphonate-ciprofloxacin bound to Skelite is a prototype for enhancing experimental local antibiotic delivery to injured bone. Br J Surg 2004;91:1192-6). In early investigations of the fluoroquinolone family in this context, ciprofloxacin demonstrated the best binding and microbiological properties when bound to BP (Herczegh, et al., Osteoadsorptive bisphosphonate derivatives of fluoroquinolone antibacterials. J Med Chem 2002;45:2338-41). Ciprofloxacin has several advantages for repurposing in this context: it can be administered orally or intravenously with relative bioequivalence, it has broad spectrum antimicrobial activity that includes the most commonly encountered osteomyelitis pathogens, it demonstrates bactericidal activity in clinically achievable doses, and it is the least expensive drug in the fluoroquinolone family (Houghton, et al., Linking bisphosphonates to the free amino groups in fluoroquinolones: preparation of osteotropic prodrugs for the prevention of osteomyelitis. J Med Chem 2008;51:6955-69).

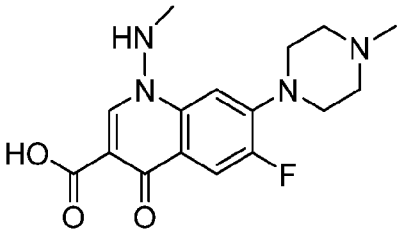
Example 1:

Non-limiting examples of quinolones that can be included in the BP conjugates herein include the following quinolones.

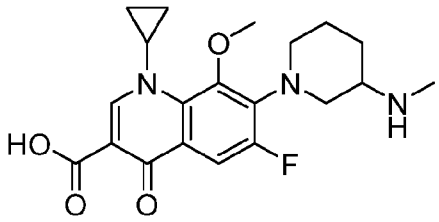
Fluorinated Quinolones



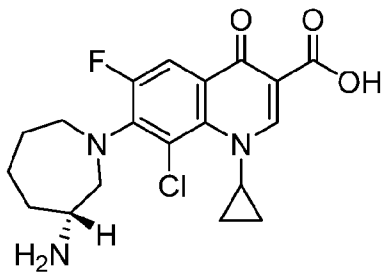
Alatrofloxacin



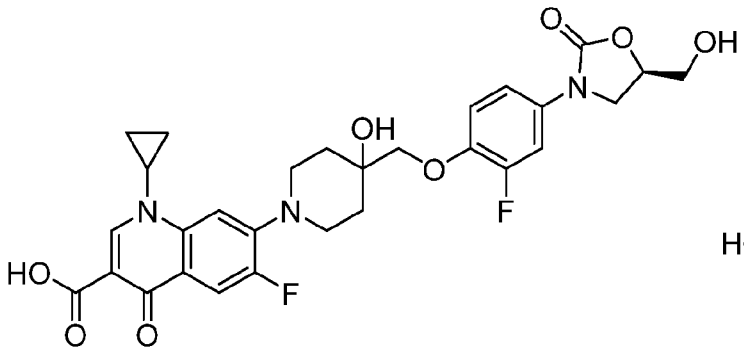
Amifloxacin



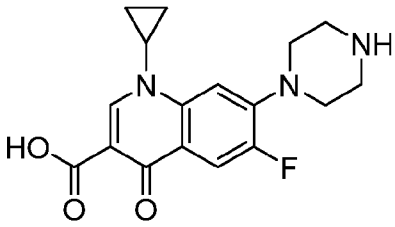
Balofloxacin



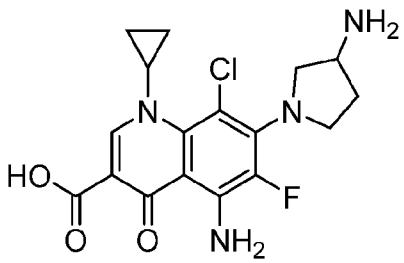
Besifloxacin



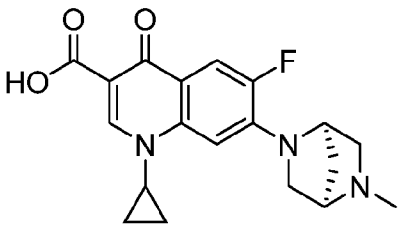
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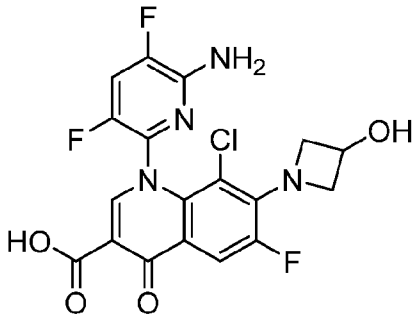
Ciprofloxacin



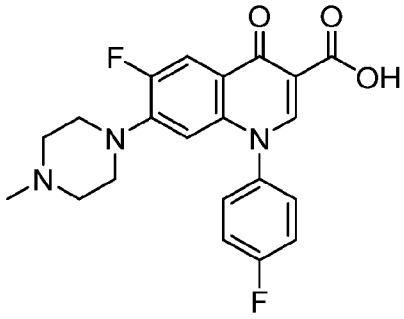
Clinafloxacin



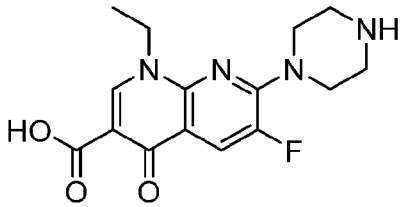
Danofloxacin



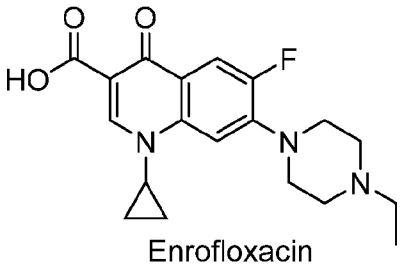
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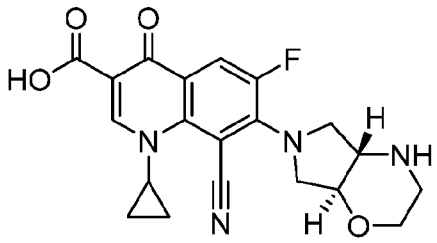
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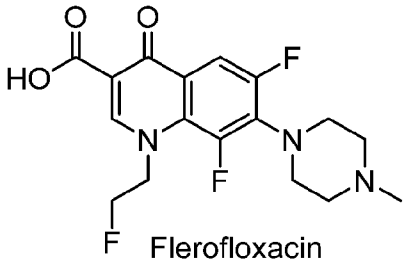
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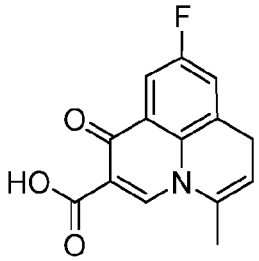
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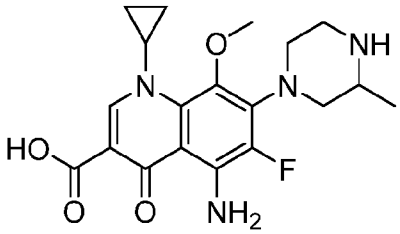
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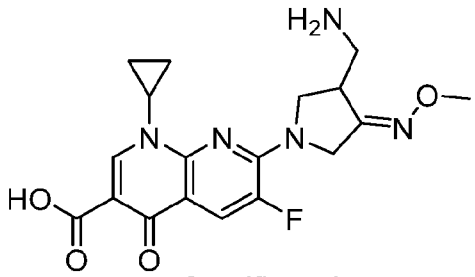
Flerofloxacin



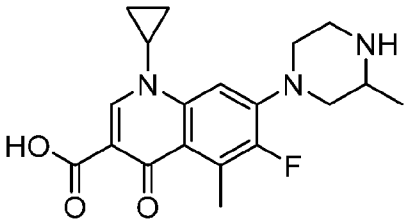
Flumequine



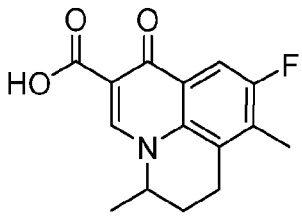
Gatifloxacin



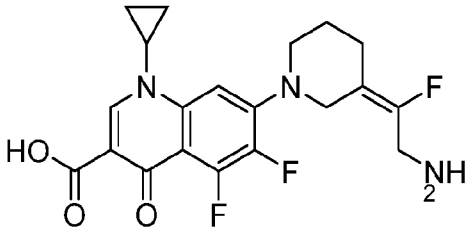
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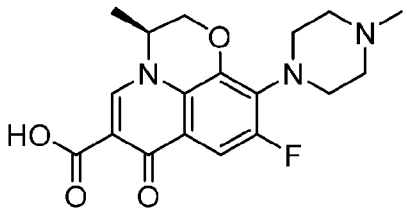
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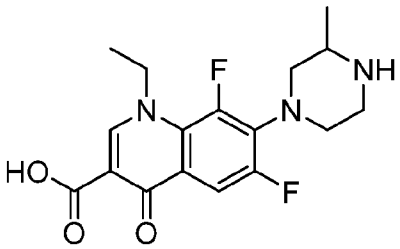
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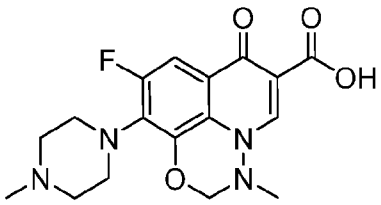
JNJ-Q2



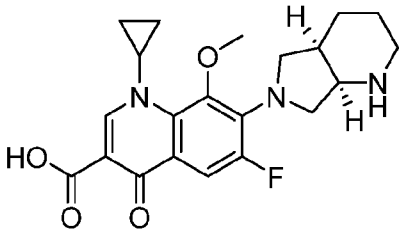
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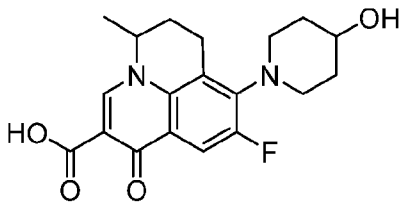
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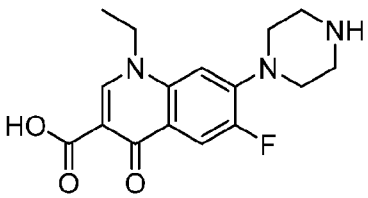
Marbofloxacin



Moxifloxacin

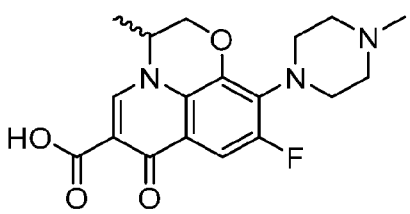


Nadifloxacin

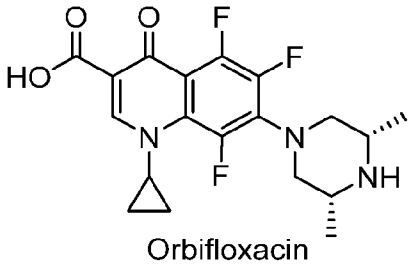


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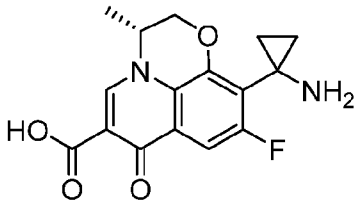
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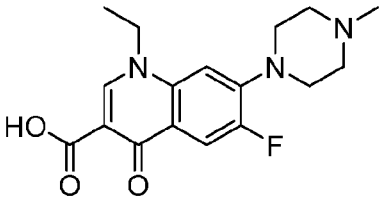
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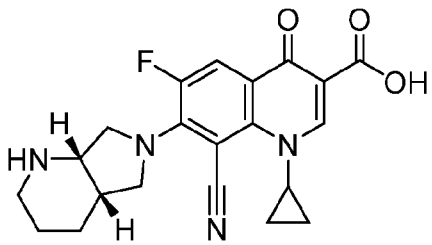
Orbifloxacin



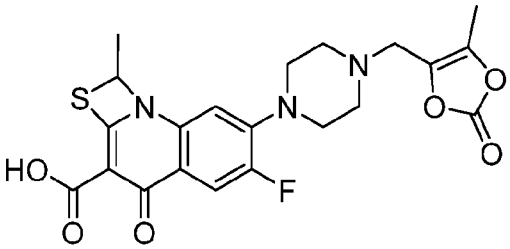
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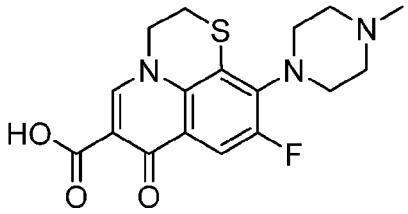
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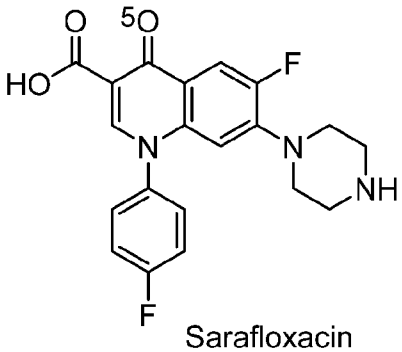
Pradofloxacin



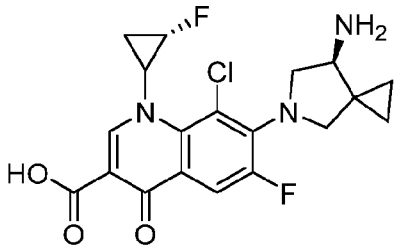
Prulifloxacin



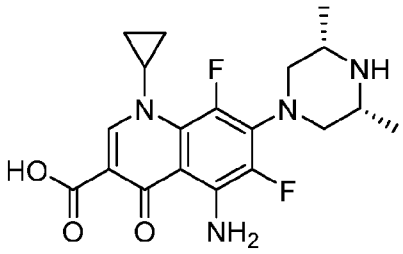
Rufloxacin



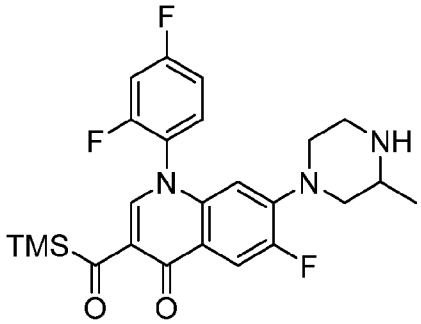
Sarafloxacin



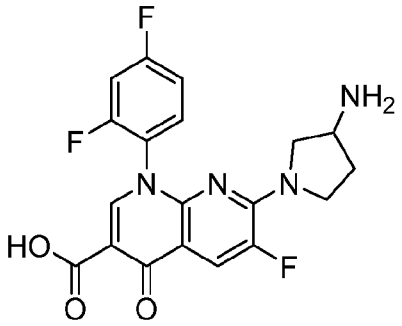
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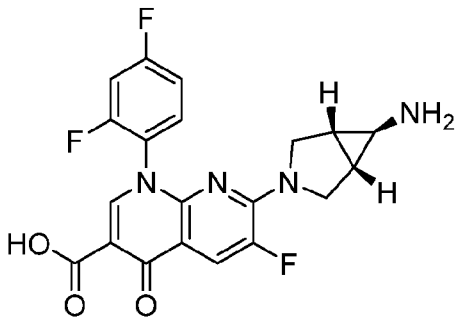
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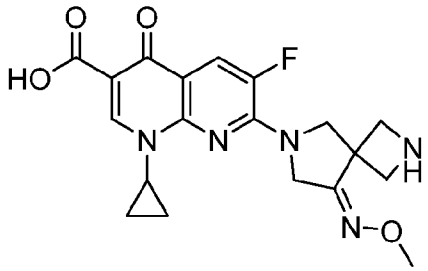
Temafloxacin



Tosufloxacin

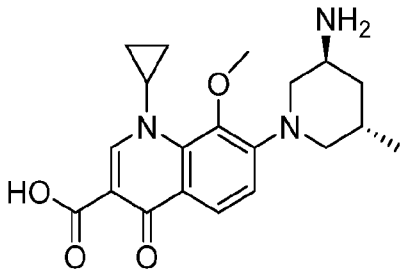


Trovafloxacin



Zabofloxacin

The following is an example of a non-flouronated quinolone.



Nemonoxacin

Example 2:

Dental implants are a critical part of modern dental practice and it is estimated that up to 35 million Americans are missing all of their teeth in one or both jaws. The overall market for these implants to replace and reconstruct teeth is expected to reach $4.2 billion by 2022. While the majority of implants are successful, some of these prosthetics fail due to *peri-implantitis*, leading to supporting bone destruction. Peri-implantitis has a bimodal incidence, incluiding early stage (<12 months) and late stage (>5 years) failures; both of these critical failure points are largely the result of bacterial biofilm infections on and around the implant. Peri-implantitis is a common reason for implant failure. Dental implants failures are generally caused by biomechanical or biological/microbiological reasons. The prevalence of peri-implantitis, the most severe form of microbiological-related implant disease leading to the destruction of supporting bone is difficult to ascertain from the current literature. However, recent studies indicate that peri-implantites is a growing problem with increasing prevalence[4]. A recent study of 150 patients followed 5 to 10 years showed a rate of peri-implantitis of approximately 17% and 30% respectively, indicating that it is a significant issue[5]. Early implant failure or lack of osseointegration is a separate problem and occurs in roughly 9% of implanted jaws[6]. This is more prevalent in the maxilla[6] and is associated with bacterial infection during surgery or from a nearby site (e.g. periodontitis) as well as other well-recognized and modifiable risk factors such as smoking, diabetes, excess cement, and poor oral hygiene[2].

Biofilm infection can be involved in the etiophathogeneiss of peri-implantitis. Biofilm infections represent a unique problem for treatment and are often difficult to diagnose, resistant to standard antibiotic therapy, resistant to host immune responses, and lead to persistent intractable infections[7].The biofilm hypothesis of infection has been steadily expanded since the early elucidation that bacteria live in matrix supported communities[8,9]. It is now established that over 65% of chronic infections are caused by bacteria living in biofilms[7]. This implies that approximately 12 million people in the US are affected by, and almost half a million people die in the US each year, from these infections. Peri-implantitis and periodontitis are among the most common biofilm infections encountered. Peri-implantitis has been found to be a comparatively simpler infection with less diverse communities (and keystone pathogens) than periodontitis infections[10]. Typically, gram negative species predominate[11]. Other orthopedic or osseous infections including those of the jaw, are also caused by bacterial biofilm communities[12] making the technology developed here amenable for use in these diseases as well.

Currently treatment approaches to peri-implantits have their limitations. While peri-implantitis has several causes, the predominant etiology is bacterial biofilm. There are no universally accepted guidelines or protocols for peri-implantitis therapy, many of the clinical regimens for bacterial peri-implantitis treatment comprise local and systemic antibiotic delivery[13] and surgical debridement of the lesion, including restorative grafting with bone graft substitutes[14,15]. Clinical experience has shown, however, that it is difficult to advance even a local antibiotic delivery device to the bottom of a deep peri-implant pocket and to infected jawbone, or to get systemic antibiotics to penetrate adequately into infected jawbone to kill biofilm pathogens[16], which is largely due to the intrinsic *poor bone (and peri-implant) biodistribution or pharmacokinetics of the antibiotics*[17]. In previous long-term studies, even when infected implants were cleaned locally with an antiseptic agent and systemic antibiotics were administered, there was additional loss of supporting bone in more than 40% of the advanced peri-implantitis lesions[15].

In addition, longer-term systemic antibiotic therapy could result in systemic toxicity or adverse effects, and also resistance. Therefore, it has become common practice by clinicians to use local delivery systems for achieving higher therapeutic antibacterial concentrations in bone. For example, dentists use chairside mixing of minocycline or doxycycline powder (e.g. Arestin®), or chlorhexidine solution (e.g. PerioChip®), with bone graft material for local delivery[18]. Such approaches are merely a slurry and do not represent a strong binding between the antibiotic and the bone substitute as in the BioVinc approach, and thus suffer from comparatively earlier washout and less efficient pharmacokinetics as previously discussed. In addition, investigators have also used several biodegradable and non-biodegradable local antibiotic delivery systems[19]. However, these approaches have several limitations, e.g., non-biodegradable approaches (e.g. polymethylmethacrylate cements) require a second surgery to remove the antibiotic loaded device, are incompatible with certain antibiotics, and suffer from inefficient release kinetics; in some cases, <10% of the total delivered antibiotic is released[17]. Biodegradable materials including fibers, gels, and beads are receiving increasing interest, however, their clinical efficacy for the treatment of peri-implantitis is not well-documented[3]. Even when effective antimicrobials/antiseptics are used to treat peri-implantitis in the jaw, such as local chlorhexidine delivery, there is minor influence on treatment outcomes as demonstrated in prospective animal and human studies[15,17]. These data taken together further support the poor pharmacokinetics of antibiotics in bone as previously mentioned, and highlight the need for bone-binding/bone-targeted and sustained antibiotic release strategies.

BP-Conjugates

Considering the limitations of current treatment approaches, it is a significant advance in the field to develop a bone/biofilm-targeting antimicrobial agent. The BP-antibiotic (BP-Ab) conjugates provided herein can overcome many challenges associated with poor antibiotic pharmacokinetics or bioavailability in bone and within bone-bound biofilms. These componds can reduce infection via a "targeting and release approach," which can reduce concern with systemic toxicity and/or drug exposure in other (e.g. non-infected) tissues. The BP-Ab conjugates can be integrated into a bone graft substitute. The BP-Ab can be a BP-fluoroquinolone conjugate. In some instances, the BP-Ab can be a bisphosphonate-carbamate-ciprofloxacin (BCC, compound **6**), as shown in **Fig. 3**. The exemplary structure of **Fig. 3** is also referred to herein as BCC (compound **6)**. When integrated into a bone graft the BP-Ab bone graft material can also be referred to as a BP-Ab-bone graft. For example, when the antiboiotic is a fluoroquinolone, it can be referred to as a BP-FQ-bone graft. These compound(s) can effectively adsorb to hydroxyapatite (HA)/bone, and can achieve a sustained release and antimicrobial efficacy against biofilm pathogens over time. The compounds and graft material integrating the compound(s) provided herein can be used as an anti-infective bone graft substitute for adjunct treatment or prevention of peri-implantitis. The conjugate will be released locally from the graft material with sustained release kinetics and cleaved in the presence of bacterial or osteoclastic activity as we have previously demonstrated, in vitro and in vivo, in other results provided elsewhere herein. In this way the grafts can provide greater local concentrations of the FQ, such as ciprofloxacin, as compared to current delivery routes. In sum the compounds and bone-graft materials provided herein can contain an antibiotic that is conjugated to a safe or pharmacologically inactive (non-antiresorptive) BP moiety bound to calcium/HA in the graft material via strong polydentate electrostatic interactions, and the antibiotic releases over time; it does not simply represent a topical antibiotic that is merely mixed in as a slurry with existing bone graft material as some current clinical approaches in this context. This chemisorbed drug attached to calcium phosphate mineral (HA) is therefore a major advance in the field and overcomes many of the limitations in antibiotic delivery to peri-implant bone for effective bactericidal activity against biofilm pathogens.

The general concept of targeting bone by linking active drug molecules to BPs has been discussed in a review[30]. However, as of this time no FDA approved drugs have been developed, as early attempts led to either systemically unstable prodrugs or non-cleavable conjugates that were found mostly to inactivate either component of the conjugate by interfering with the pharmacophoric requirements. In the quinolone field a prominent example was described by

Herczegh where antibacterial properties of the fluoroquinolone were diminished upon conjugation with a stable BP-linked congener[31-32]. Therefore, a *target and release linker strategy* is needed.

Recently, medicinal chemistry strategies exploiting less stable linking techniques start to emerge. Others have linked fluoroquinolones via the carboxylic acid group to several different BP moieties. They found that glycolamide ester prodrugs of the antibiotics moxifloxacin and gatifloxacin reduced infection when used prophylactically in a rat osteomyelitis model[33]. This same group has used acyloxycarbamate and phenylpropanone based linkers to tether the same antibiotics via amine functionality to simple BP systems[34]. They show using the same prophylactic rat model that these conjugates are also better than the parent antibiotic at inhibiting the establishment of infection. The Targanta team[33] has carried several of these prodrug strategies on into use with the glycopeptide antibiotic oritavancin[35]. This dual function drug seems to be somewhat effective in preventing infection. However, to date they have not published studies showing that they can treat an established infection and they also have not published pharmacokinetics of the prodrug. It is believed that these analogs are too labile in the bloodstream to fully realize success with this therapeutic approach as their drug candidate selection was based in part on plasma instability. Thus, it is believed that these compounds developed by these groups fail to achieve effective local concentrations of the antibiotic.

The BCC compound(s) **(Fig. 3)** can incorporate the phenyl moity of the phenyl carbamate linker directly into the BP portion of the molecule. Release kinetics can be modified or tuned via modification of the phenyl ring with electron withdrawing or donating groups, which can alter the liability of the linker. Additionally, the BP core lacks effectiveness as an antiresporptive agent, and thus, does not carry the risk of medication-related osteonecrosis of the jaw like the more potent nitrogen-containing BP drugs (e.g., zoledronate[39,40]. It is demonstrate herein and in other Examples herein that this target and release strategy using the phenyl carbamate linker very likely releases the active drug directly into the bacterial biofilm in the bone milieu. The bone targeting is so effective that it works better than ciprofloxacin against biofilms grown on HA bone matrix surrogate than on planktonic cultures grown in plastic vessels. An analog conjugate made with a non-cleavable amide linkage (bisphosphonate-amide-ciprofloxacin, leaving out the phenolic oxygen of the carbamate, was found to have very little effect on bacterial growth under any circumstances, demonstrating that active cleavage of the conjugate is required for antimicrobial activity.

Example 3

Design and synthesis of additional BP-Ab conjugates (**Fig. 4**). Additional BP-Ab conjugates can be designed using, for example, ciprofloxacin and moxifloxacin conjugated to BPs (e.g. 4-hydroxyphenylethylidene BP (BP 1, **Fig. 4**), its hydroxy-containing analog (BP 2, **Fig. 4**, with higher bone affinity) and pamidronate (BP 3, **Fig. 4**), via carbamate based linkers (e.g. carbamate, *S*-thiocarbamate, and *O*-thiocarbamate). **Fig. 5** shows an exemplary synthesis scheme for synthesis of BP-Ab conjugates with an *O*-thiocarbamate linker. Conjugates with S-thiocarbamate linkage (slightly more labile) can be obtained by isomerization of conjugates with O-thiocarbamate linkage via the Newman-Kwart rearrangement (ref. 47, 48). Preliminary chemistry has already been conducted to demonstrate the feasibility of the quick synthesis of these targets. Adding bone affinity is therefore well demonstrated using the α-OH containing BPs (49). Added bone affinity will enhance concentrations of the conjugate at the bone surface and facilitate higher local concentrations of drug short term and long term. For the synthesis of conjugates with α-OH containing BPs (BP 2 and pamidronate, **Fig. 4**), since the α-OH bisphosphonate ester is prone to rearrangement to a phosphonophosphate, the α-OH can be protected with the tert-butyldimethylsilyl (TBS) group (Scheme 2, **Fig. 6**) (50). Then the α-O-TBS BP 2 ester are activated by 4-nitrophenyl chloroformate and reacted with ciprofloxacin or moxifloxacin similarly as in **Fig. 5**. For α-O-TBS BP 3 ester, a linker with phenol group (e.g., linker 1 (resorcinol), linker 2 (hydroquinone), linker 3 (4-hydroxyphenylacetic acid), **Figure 20**) are used to tether BP and antimicrobial agents, and the synthesis route using linker 3 is illustrated as an example here (**Scheme 3, Fig. 7**). All BP-Ab conjugates are characterized by 1H, 31P, 13C NMR, MS, HPLC, and elemental analysis to assure identity.

The mineral binding affinity of the BP-Ab conjugates can be determined. Briefly, Anorganic bovine bone large particle size (uniformly 1-2mm) can be accurately weighed (1.4-1.6 mg) and suspended in a 4 mL clear vial containing the appropriate volume of assay buffer [0.05% (wt/vol) Tween20, 10μM EDTA and 100mM HEPES pH=7.4] for 3hr. This bone material can then be incubated with increasing amounts of BP-Ab (0, 25, 50, 100, 200 and 300μM). Samples can be gently shaken for 3h at 37°C in the assay buffer. Subsequent to the equilibrium period, the vials can be centrifuged at 10,000 rpm for 5 min to separate solids and supernatant. The supernatant (0.3 mL) can be collected and the concentration of the equilibrium solution are measured using a Shimadzu UV-VIS spectrometer (275nm wavelength). Fluorescent emission can also be used to calculate binding parameters. Nonspecific binding can be measured with a similar procedure in the absence of HA as control. The amount of parent drug/BP-Ab conjugates bound to HA is deduced from the difference between the input amount and the amount recovered in the supernatants after binding. Binding parameters (K_d and Bmax represent the equilibrium

dissociation constant and maximum number of binding sites, respectively) can be calculated using the PRISM program (Graphpad, USA) and measured in 5 independent experiments. Compounds with an equilibrium dissociation constant (K_d) lower than 20 μM (~ 2x K_d of parent BPs) can be preferred. A two-sample t-test can be used to evaluate the binding parameters of the BP-Abs. The sample size (n=5) in each group can be used to detect the effect size 1.72 for this hypothesis at a power of 80% and a one-side Type I error of 0.05.

The linkage-stability of the BP-Ab conjugates can be determined. Briefly, the linker stability of each BP-Ab conjugate can be tested in PBS buffers with different pH (pH = 1, 4, 7.4, 10) and human or canine serum. BP-Ab can be suspended in 400 μL of above-mentioned PBS or in 400 μL of 50% (v/v in PBS) human or canine serum. The suspension/solution can be incubated for 24 h at 37 °C and centrifuged at 13000 rpm for 2 min, and the supernatant can be recovered. Methanol (5X volume relative to supernatant) can be added to each supernatant, and the mixture can be vortexed for 15 min to extract released fluoroquinolone. The mixture can be then centrifuged at 10000 rpm for 15 min to pellet the insoluble material. The supernatant containing the extracted fluoroquinolone can be recovered and evaporated to dryness. The dried pellets can be resuspended in PBS, and the amount of released fluoroquinolone can be determined by UV-VIS measurements as described previously. The percentage of fluoroquinolone drug released can then be calculated based on the input amount and the measured amount of released drug. The identity of released drug can be confirmed by LC-MS analysis and/or NMR if the concentrations are sufficient.

The in vitro inhibition of biofilm growth on HA discs can be determined. Briefly, for custom disc manufacturing, commercially available HA powder can be used. Powder pellets of 9.6mm in diameter can be pressed without a binder. Sintering can be performed at 900 °C. The tablets can be compressed using the Universal Testing System for static tensile, compression, and bending tests (Instron model 3384; Instron, Norwood, MA). The quality of the manufactured HA discs can be checked by means of confocal microscopy and microcomputed tomography (micro-CT) using an LEXT OLS4000 microscope (Olympus, Center Valley, PA) and Metrotom 1500 microtomograph (Carl Zeiss, Oberkochen, Germany), respectively. HA discs can then be introduced to the following concentrations [mg/mL] of each BP-Ab conjugate and ciprofloxacin/moxifloxacin: 800, 400, 200, 100, 50, 25, 10, 5, 1 and left for 24h/37°C. After incubation, HA discs can be removed and introduced to 1 mL of PBS and left for 5 min in gentle rocker shaker; 3 subsequent rinsings are performed this way. After rinsing, 1mL of *Aa* suspension can be introduced to discs and left for 24h/37°C. Discs can then be rinsed to remove non-bound

bacteria and subjected to vortex shaking. The serial dilutions of suspension obtained can then be culture plated on modified TSB agar plates and colony growth is counted after 24h.

The oseeointegration effect of the BP-FQ-bone grafts on critical size can be evaluated in supra-alveolar peri-implant defect model for bone grafting. Briefly, in this split mouth design, mandibular PM2-PM4 are bilaterally extracted in 6 beagle dogs (3 males, 3 females) and are allowed to heal for 12 weeks. Crestal incision are made followed by mucoperiosteal flap reflection. Ostectomy are performed to create a 6mm supra-alveolar defect. Implant site osteotomy preparations are made in each of the premolar regions by sequential cutting with internally irrigated drills in graduated diameters under copious irrigation. Implants (Astra Tech Osseospeed Tx® 3 x 11 mm) are placed in the position of PM2-PM4 on each side in such manner that the implants are positioned 4mm supracrestally in relation to the created defect and at the same distance from the buccal cortical bone plate. Dogs are divided randomly into 3 different groups (2 dogs per group):

1. Anorganic bovine bone (1g large particle size 1-2mm) chemisorbed with BP-fluoroquinolone are used on the right side and collagen plugs (negative control) are used on the left side.

2. Anorganic bovine bone (1g large particle size 1-2mm, positive control) are used on the right side and collagen plugs (negative control) are used on the left side.

3. Bio-Oss® (1g large particle size 1-2mm) chemisorbed with BP-fluoroquinolone are used on the right side and Bio-Oss® (1g large particle size 1-2mm, positive control) are used on the left side.

Chemistry and antimicrobial assay results from experiments described above can inform calculations of the ideal standardized quantity of the conjugate for adsorption to graft material for use in all in vivo experiments described here. Early calculations predicated based on the preliminary results indicate that 5mg or less of conjugate adsorbed to 1g of graft material will provide 2-3 orders of magnitude bactericidal activity above the MIC of tested pathogens. Our BP-fluoroquinolone conjugate can be applied in a range of bone graft materials including commercially available ones, e.g., Bio-Oss®; thus we choose house-made anorganic bovine bone and BioOss as a positive control in the study for a demonstration of wide applications of the conjugate. All defects are filled (depending on the groups above) with a standardized amount of biomaterial up to the platform of each implant on both sides, and Bio-Gide® membranes are used to cover the graft and the implants for improved stability. The flaps are closed in a tension free manner with the use of periosteal releasing incisions, internal mattress and finally marginal single interrupted sutures (PTFE 4,0, Cytoplast, USA). MicroCT are acquired at this point and animals

are monitored clinically for inflammation and adverse events. Additionally, as described in the experiments to follow, these animals undergo PK studies to assess for any systemic exposure to the components within the graft material (e.g. intact conjugate, BP, antibiotic, or linker). Animals are sacrificed after 12 weeks and the mandibles are resected and examined by micro-CT followed by histologic preparation. Baseline micro-CT scans of the jaws are taken for comparison to post-experimental scans. Quantitative 3D volumetric micro-CT and histomorphometric analyses are performed to examine the volume of new bone present in peri-implant sites, as well as first bone-to-implant contact, total defect area, regenerated area, regenerated area within total defect area, regenerated bone, residual bone substitute material, percentage of mineralized tissue, soft tissue, and void. Finally, necropsy are performed for post-mortem evaluation of organs and systems for gross and microscopic signs of tolerability issues from local oral therapy.

Antimicrobial efficacy of the BP-FQ-bone grafts can be evaluated in a canine peri-implantitis model. Briefly, in this split mouth design, mandibular PM2-PM4 are extracted bilaterally in 8 beagle dogs (4 males, 4 females; 48 teeth total) using minimally traumatic technique. After 3 months of healing mucoperiosteal flaps are elevated on both sides of the jaw and osteotomy preparations are made in each of the premolar regions by sequential cutting with internally irrigated drills in graduated diameters under copious external irrigation. Using a non-submerged technique, implants (Astra Tech Osseospeed Tx® 3 x 11 mm) are installed at each site. The sequence of implant placement are identical in both sides but randomized with a computer generated randomization scheme between dogs. Healing abutments are connected to the implants and flaps approximated with resorbable sutures. A plaque control regimen comprising brushing with dentifrice is then initiated four times a week. Twelve weeks after implant placement just prior to initiation of experimental peri-implantitis, microbiological samples are obtained from all peri-implant sites with sterile paper points (Dentsply, Maillefer, size 35, Ballaigues, Switzerland) and placed immediately in Eppendorf tubes (Starlab, Ahrensburg, Germany) for microbiological analysis. Microbiologic analysis are performed as we have previously detailed via DNA extraction and 16S rRNA PCR amplification.(55) PCR amplicons are sequenced using the Roche 454 GS FLX platform and data analyzed with the Quantitative Insights into Microbial Ecology (QIIME) software package (56). Colony forming unit counts (CFU/mL) are determined from samples as in our Phase I study as described earlier. At this point experimental peri-implantitis are initiated as follows. Aggregatibacter actinomycetemcomitans (Aa) biofilm, a keystone periodontal pathogen, which is not endogenous to canine flora, are initiated on the healing abutments in vitro as performed in our previous experiment in a rat animal model and also in our previous animal peri-implantitis study. The biofilm inoculated healing abutments are placed

on the implants and cotton ligatures are placed in a submarginal position around the neck of implants. After 10 weeks of bacterial infection, microbial sampling and analysis are done again as before and micro-CT scans are taken as the baseline for the peri-implantitis defect. Treatment of this experimental peri-implantitis model are initiated by surgical debridement of all implant sites by raising full-thickness buccolingual flaps, removing any existing calculus from implant surfaces using an air-powder abrasion device, and wiping of the implant surfaces with gauze soaked in chlorhexidine gluconate 0.12%. The animals are divided into 4 groups as follows (2 dogs per group):

1. Anorganic bovine bone (1g large particle size 1-2mm) with chemisorbed BP-fluoroquinolone are used on the right side and collagen plugs (negative control) are used on the left side.

2. Anorganic bovine bone (1g large particle size 1-2mm, positive control) are used on the right side and collagen plugs (negative control) are used on the left side.

3. Anorganic bovine bone (1g large particle size 1-2mm) with chemisorbed BP-fluoroquinolone are used on the right side and an antimicrobial releasing device (100 mg topical minocycline, positive control) are used on the left side.

4. Bio-Oss® (1g large particle size 1-2 mm) with chemisorbed BP-fluoroquinolone (positive control) are used on the right side and an antimicrobial releasing device (100 mg topical minocycline, positive control) are used on the left side.

Treatment group assignments are blinded to future investigators for data analysis. Standardized and comparable amounts of antimicrobials are used in treatment groups. After treatment, flaps are repositioned and sutured (PTFE 4,0, Cytoplast, USA) and oral hygiene measures reinstituted after 1 week following suture removal. Clinical and micro-CT scan examinations are performed again at 3 months after surgery and also microbiological samples are acquired at this time point for analysis as described above. Six months after peri-implantitis surgery animals are euthanized and micro-CT scans are performed, and the jaws are resected for assessment of histopathologic parameters as detailed in the section "critical size supra-alveolar peri-implant defect model." An inflammatory score are determined from histologic sections as previously detailed (ref. 57) for correlation with clinical and radiologic findings.

Statistical analysis: Statistical calculations are performed with SPSS 22.0 (IBM, Armonk, NY) and Excel 2016 (Microsoft Corporation, Redmond, WA). Power analyses were performed to determine sample size estimations for all animal studies using G Power 3 software[58]. Following data collection from these animal studies, quantitative outcomes are analyzed first with descriptive statistics to understand the distribution of the data (parametric or non-parametric) and to generate

the mean, standard error, standard deviation, kurtosis and skewness, and 95% confidence levels. The data are analyzed using the Kruskall-Wallis test, ANOVA, or mixed linear models as applicable and statistical significance are carried out at α=0.05 level when comparing groups. Post-hoc testing using unpaired t-tests and Dunnett's test for multiple comparisons are also performed to further validate findings. All animal experiments are described using the ARRIVE guidelines for reporting on animal research to ensure the quality, reliability, validity, and reproducibility of results[59].

The drug compound and component stability and in vitro ADME of BCC (**6**) can be evaluated. This data can help establish if there is likely to be any large differences in human metabolism vs. experimental animals. Incubation of **6** with human, rat, and dog liver microsomes and hepatocytes followed by LC/MS analysis of the metabolite mixture are performed. The metabolic profile of ciprofloxacin is known[62,63], and so our focus are on any metabolites of the BP portion of the molecule and of the parent (e.g. piperazine ring cleavage as is known for ciprofloxacin). Once metabolites have been determined in vitro, plasma samples from other in vivo experiments described abvoe are used to determine these compounds at steady state in vivo.

The toxicology of the BCC (**6**) can be evaluated in rat and dog to determine NOAEL. In order to determine the NOAEL and maximum tolerated dosage (MTD) in rat and dog we first carry out dose ranging studies. Groups of 6 rats (3 males, 3 females), are given a single intravenous dose of 10 mg/kg for 6, or based on our best assessment at the time. The dose are escalated by doubling until acute toxicity is noted (MTD) then this dose are reduced by 20% sequentially until no effects are seen, this will be the NOAEL for the compound. Toxicity are assessed as mild, moderate or substantial, and moderate toxicity in ≥2 or substantial toxicity in ≥1 animal define the MTD[64]. Animals are followed for body weight and clinical observations for 5 days. After 5 days, animals are euthanized and necropsy performed to assess for organ weight and histology (15 sections to include liver and kidney based on clinical BP toxicology). A similar dose range study are carried out in dogs (1/sex, starting at the equivalent dose as determined from allometric scaling 4 mg/kg assuming 250 g rats and 10 kg dogs) and include hematology and clinical chemistry in addition to identical terminal studies as in rat. This can use a total of 4-6 cohorts.

An expanded acute toxicity testing in groups of animals including toxicokinetics and recovery testing at the NOAEL and the MTD can be performed. Gropus of 48 rats including 10/sex can be used for each dose for assessment of toxicity and 9/sex for toxicokinetics and 5/sex for recovery. Toxicokinetics are determined at 6 time points (3 rats/time point chosen randomly from male or female) following administration of each dose. Time points are 5, 30, 60, 120 mins, 12

hrs, and 24 hrs post dosing. Recovery animals are observed for 14 days followed by assessment of organ weight and histology as in the above study. From the toxicokinetic study, PK parameters are determined by non-compartmental analysis (NCA) including Cmax, AUC and half-life. An identical experiment was carried out in canines but including 10 total animals (3/sex for dosing and 2/sex for recovery) with multiple blood draws from each animal at the same time points as for the rats. The AUC at the NOAEL for canines are used to calculate the maximum allowable exposure from the bone graft/BP-fluoroquinolone conjugate as described in aim 2 and PK experiments in canines are used to determine if there is systemic exposure above 1/100 of this level.

For population modeling, a unique 3-compartment (blood/urine/bone) mathematical model of BP pharmacokinetics which has been validated clinically and are applied to the current project[65]. From the canine study, in each animal at the time of euthanasia, we sample bone (jaw and femur), tendon (gastrocnemius) for determination of BP and fluoroquinolone concentrations. We combine these data and our model to describe the time course in dogs. From this model we can simulate the expected exposure of bone and cartilage to both BP and fluoroquinolone with alternative dosing or repeated dosing. This can inform subsequent human dosing. The nonparametric adaptive grid (NPAG) algorithm with adaptive gamma implemented within the Pmetrics package for R (Laboratory of Applied Pharmacokinetics and Bioinformatics, Los Angeles, CA) are used for all PK model-fitting procedures as previously described[66-68]. Assay error (SD) is accounted for using an error polynomial as a function of the measured concentration, and comparative performance evaluation are completed using Akaike's information criterion, a regression of observed versus predicted concentrations, visual plots of PK parameter-covariate regressions, and the rule of parsimony.

Example 4

The BP-Ab conjugates can be integrated into grafts and grafting devices. In embodiments, one or more of the BP-Ab conjugates can be integrated into an already approved bone graft product, such as the bovine bone materials from BioOss® (Geistlich Pharma AG, Switzerland) or MinerOss® (BioHorizons, Birmingham, AL) to name a few. The BP-Ab conjugate(s) can be admixed with a support material for use as a dental bone graft substitute. The product will comprise the conjugate adsorbed to anorganic bovine bone material. This material will allow the local delivery of antibiotic to the region of bone graft implantation to reduce bacterial infection rates and associated dental pathology such as peri-implantitis and other infections. The dental applications for our product could include not only peri-implantitis treatment, but also socket

preservation after tooth extraction, ridge or sinus augmentation, periodontitis prevention or treatment, osteomyelitis or osteonecrosis treatment or prevention, or other oral and periodontal surgery applications where such a bone graft could be beneficial. The BP-fluoroquinolone conjugate material will be intimately adsorbed on the bone graft substitute and our preliminary data show sustained release into the area of bone destruction in the case of infections, which allows our product to more effectively deliver antibiotic to the site of infection with negligible to no systemic exposure to either component of the conjugate compound.

The grafting material can also be beneficial for non-dental grafting procedures, such as sinus grafting procedures.

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Example 5:

This Example demonstrates various BP conjugate compounds and synthesis schemes. BP-carbamate-moxifloxacin BP conjugate and synthesis scheme is demonstrated in **Fig. 8. Fig. 9** shows a BP-carbamate-gatifloxacin BP conjugate and synthesis scheme. **Fig. 10** shows a BP-*p*-Hydroxyphenyl Acetic Acid-ciprofloxacin BP conjugate and synthesis scheme. **Fig. 11** shows a BP-OH-ciprofloxacin BP conjugate and synthesis scheme. **Fig. 12** shows a BP-O-Thiocarbamate-ciprofloxacin BP conjugate and synthesis scheme. **Fig. 13** shows a BP-*S*-Thiocarbamate-ciprofloxacin BP conjugate and synthesis scheme. **Fig. 14** shows a BP-Resorcinol-ciprofloxacin BP conjugate and synthesis scheme. **Fig. 15** shows a BP-Hydroquinone-ciprofloxacin BP conjugate and synthesis scheme.

Fig. 16 shows one embodiment of a genus structure for a BP-fluoroquinolone conjugate, where W can be O or S or N, X can be O, S, N, CH_2O, CH_2N, or CH_2S, Y can be H, CH_3, NO_2, F,

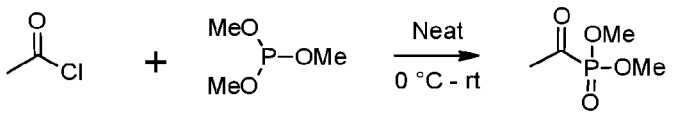
Cl, Br, I, or CO_2H, Z can be H, CH_3, OH, NH_2, SH, F, Cl, Br, or I, and n can be 1-5. **Fig. 17** shows various BP-fluoroquinolone conjugates.

Fig. 18 shows one embodiment of a genus structure for a genus of a phosphonate containing an aryl group, where X can be H, CH_3, OH, NH_2, SH, F, Cl, Br, or I, Y can be PO_3H_2, or CO_2H. Z can be OH, NH_2, SH, or N_3, and n can be 1 or 2. **Fig. 19** shows various BPs, where X can be F, Cl, Br, or I and n can be 1 or 2.

Fig. 20 shows various BP's with terminal primary amines. **Fig. 21** shows various BPs coupled to a linker containing a terminal hydroxyl and amine functional groups where R can be Risedronate, Zoledronate, Minodronate, Pamidronate, or Alendronate. **Fig. 22** shows various BP-pamidronate-ciprofloxacin conjuagtes. **Fig. 23** shows various BP-Alendronate-ciprofloxacin conjuagtes.

Example 6.

1. Dimethyl acetylphosphonate **(37)**

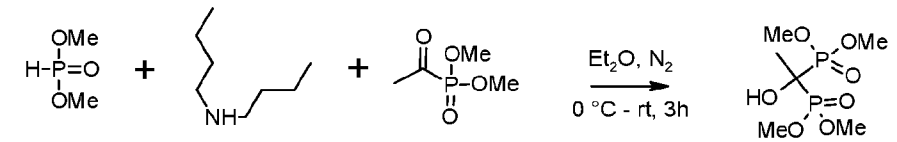


C_2H_3ClO 78.49 g/mol | $C_3H_9O_3P$ 124.07 g/mol | $C_4H_9O_4P$ 152.08 g/mol

(37)

Trimethyl phosphite (2.36 mL, 20 mmol) was added to ice-cold acetyl chloride (1.44 mL, 20.2 mmol) under N_2 over a period of 20 mins. The colorless solution was warmed to room temperature, stirred for 30 mins, and concentrated under vacuum to afford 2.89 g (94%) product as colorless oil which was used in next reaction as is. ^{1}HNMR (300 MHz, CDCl3): δ 3.84 (d, J = 12 Hz, 6H), 2.46 (d, J = 5.4 Hz, 3H). ^{31}PNMR (121 MHz, $CDCl_3$): δ -1.10.

2. Tetramethyl(1-hydroxyethylidene)-bisphosphonate **(38)**

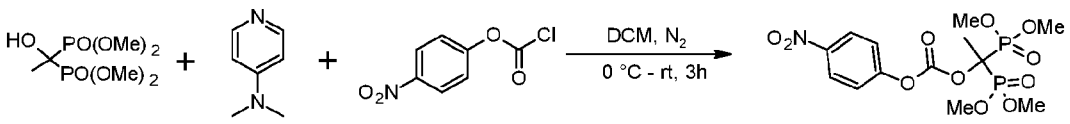


$C_2H_7O_3P$ 110.04 g/mol | $C_8H_{19}N$ 129.24 g/mol | $C_4H_9O_4P$ 152.08 g/mol | $C_6H_{16}O_7P_2$ 262.13 g/mol

(37) **(38)**

Dimethyl acetylphosphate **(37)** (2.2 g, 14. 44 mmol) was added dropwisely to an ice-cold solution of dimethyl phosphite (1.63 mL, 15.91 mmol) and dibutylamine (0.767 mL, 1.44 mmol) in dry ether (30 mL) under N_2. The ice bath was removed, and the mixture was stirred at room temperature for 3h. The resulting precipitate was filtered, washed with ether, and dried under vacuum overnight to afford 3.24 g (85%) of product as white solid. ^{1}HNMR (300 MHz, $CDCl_3$): δ 3.94 – 3.82 (m, 12H), 3.44 (t, J = 8.4 Hz, 1H), 1.68 (t, J = 16.2 Hz, 3H). ^{31}PNMR (121 MHz, $CDCl_3$): δ 22.21. MS-ESI: 263.1 [M+H]+.

3. Tetramethyl (1-{[(4-nitrophenoxy)carbonyl]oxy}ethane-1,1-diyl)bis(phosphonate) **(39)**

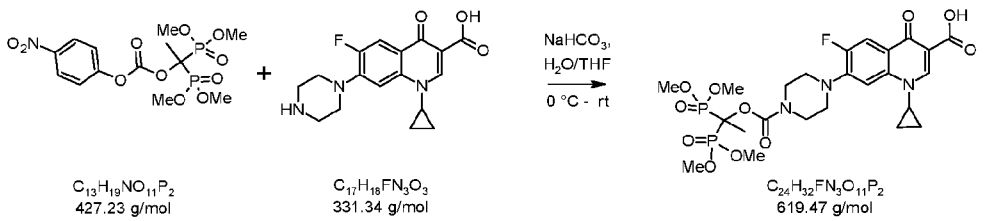


$C_6H_{16}O_7P_2$ 262.13 g/mol | $C_7H_{10}N_2$ 122.16 g/mol | $C_7H_4ClNO_4$ 201.56 g/mol | $C_{13}H_{19}NO_{11}P_2$ 427.23 g/mol

(39)

p-nitrophenyl chloroformate (768 mg, 3.81 mmol) was added to an ice-cold solution of DMAP (466 mg, 3.81 mmol) in DCM (20 mL) under N_2. After stirring for 10 mins, the tetramethyl(1-hydroxyethylidene)-bisphosphonate (1 g, 3.81 mmol) was added in one portion. The ice-bath was removed, and the mixture was stirred at room temperature for 3h. Next, the reaction mixture was extracted with 20 mL each of cold aqueous 0.1 N HCl (2x), water, brine, dried over $MgSO_4$, and concentrated. The crude mixture was separated by column chromatography using EtOAc/MeOH (1-3%) to afford 1.16g (71%) light-yellow oil. ^{1}HNMR (300 MHz, CDCl3): δ 8.27 (d, J = 9 Hz, 2H), 7.40 (d, J = 9 Hz, 2H), 3.97 – 3.87 (m, 12H), 2.02 (t, J = 15.6 Hz, 3H). ^{31}PNMR (121 MHz, $CDCl_3$): δ 17.98. MS-ESI: 445.3 [M+NH4]+.

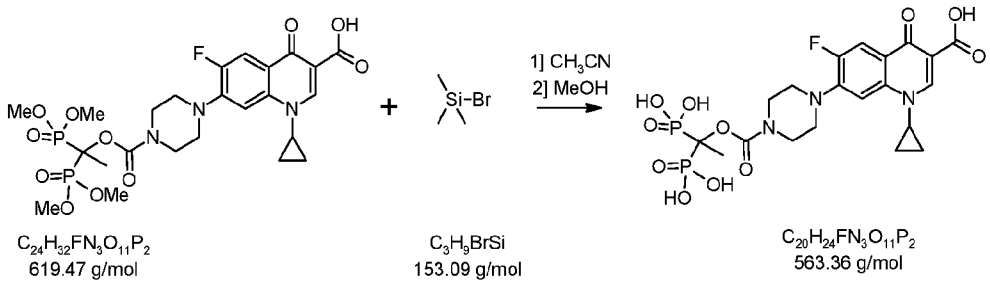
4: Ciprofloxacin carbamoyl etidronate tetramethyl ester **(40)**



(40)

To a solution of $NaHCO_3$ (239.5 mg, 2.85 mmol) in H_2O (20 mL) was added ciprofloxacin (899.6 mg, 2.71 mmol) and the suspension was cooled in ice-bath. Next, the tetramethyl (1-{[(4-nitrophenoxy)carbonyl]oxy}ethane-1,1-diyl)bis(phosphonate) dissolved in THF (20 mL) was added dropwisely over a period of 20 mins. The yellow suspension was stirred overnight (14 h) at room temperature. The reaction mixture was concentrated, and the crude mixture was separated by column chromatography using DCM/MeOH (1-5%) to provide 832 mg (49%) of light-yellow solid. ^{1}HNMR (300 MHz, $CDCl_3$): δ 8.77 (s, 1H), 8.03 (d, J = 12.6 Hz, 1H), 7.36 (d, J = 6.9 Hz, 1H) 3.98 – 3.80 (m, 12H), 3.79 – 3.68 (br s, 4H), 3.58 – 3.50 (m, 1H), 3.30 (t, J = 9.6 Hz, 4H), 1.95 (t, J = 15.6 Hz, 3H), 1.40 (q, J = 6.8 Hz, 2H), 1.23 – 1.16 (m, 2H). ^{31}PNMR (121 MHz, CDCl3): δ 20.19. MS-ESI: 620.3 [M+H]+.

5. Etidronate-carbamate-Ciprofloxacin **(41)**

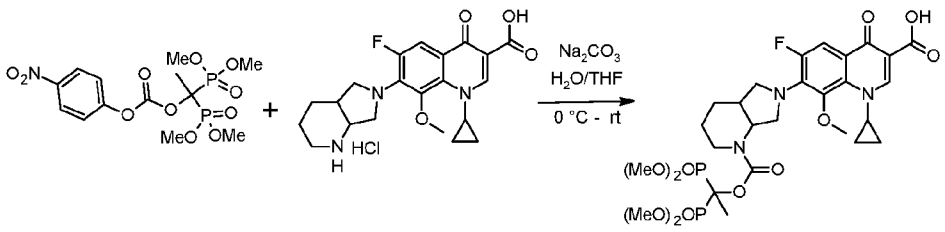


(41)

A mixture of tetramethyl etidronate-carbamate-ciprofloxacin (775 mg, 1.25 mmol) and bromotrimethylsilane (1.53 g, 10 mmol) in ACN (28 mL) was stirred for 2 h. The volatiles were evaporated under vacuum and MeOH (28 mL) was added to the residue. After stirring for 30mins the resulting suspension was filtered, washed with MeOH (10 mL x 2), and dried under vacuum overnight to afford 662 mg (93%) off-white solid. ^{1}H NMR (300 MHz, 20% CD_3CN in DMSO-d6) δ 8.66 (s, 1H), 7.92 (d, J = 13.2 Hz, 1H), 7.57 (d, J = 7.4 Hz, 1H), 3.78 (p, J = 3.1 Hz, 1H), 3.64 (br

d, J = 32.1 Hz, 4H), 3.32 (br s, 4H), 1.82 (t, J = 15.1 Hz, 3H), 1.32 (d, J = 6.5 Hz, 2H), 1.16 (s, 2H). ^{31}PNMR (121 MHz, 20% CD3CN in DMSO-d6): δ 15.57. MS-ESI: 564.2 [M+H]+.

6. Moxifloxacin carbamoyl etidronate tetramethyl ester **(42)**



$C_{13}H_{19}NO_{11}P_2$ 427.23 g/mol

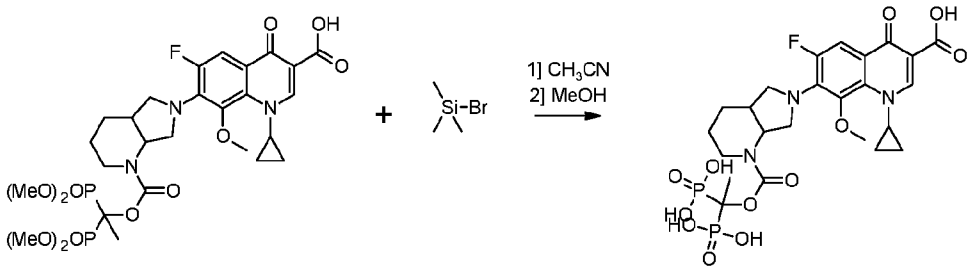
$C_{21}H_{25}ClFN_3O_4$ 437.89 g/mol

$C_{28}H_{38}FN_3O_{12}P_2$ 689.56 g/mol

(42)

Moxifloxacin HCl was added to a solution of Na2CO3 in H2O (20 mL) and the solution was cooled in ice bath. Next, the tetramethyl (1-{[(4-nitrophenoxy)carbonyl]oxy}ethane-1,1-diyl)bis(phosphonate) dissolved in THF (20 mL) was added dropwisely over 30 min. The ice bath was removed, the flask was covered with aluminum foil, and the reaction was stirred for 20 h at room temperature. Next, the reaction mixture was concentrated, and the crude purified by column chromatography using DCM/MeOH (1-5%) to afford 624 mg (29%) of product as off-white foam. ^{1}H NMR (300 MHz, Chloroform-d) δ 8.78 (s, 1H), 7.81 (d, J = 13.8 Hz, 1H), 4.82 (br s, 1H), 4.16 - 4.04 (m, 2H), 4.02 - 3.92 (m, 2H), 3.92 - 3.80 (m, 12H), 3.56 (s, 3H), 3.48 (t, J = 10.5 Hz, 1H), 3.24 (d, J = 10.5 Hz, 1H), 3.00 (br s, 1H), 2.40 - 2.24 (m, 1H), 1.94 (t, J = 15.9 Hz, 3H), 1.87 - 1.74 (m, 2H), 1.60 - 1.44 (m, 2H), 1.35 - 1.21 (m, 1H), 1.17 – 1.01 (m, 2H), 0.88 - 0.75 (m, 1H). ^{31}PNMR (121 MHz, CDCl3): δ 20.36. MS-ESI: 690.4 [M+H]+

7. Etidronate-carbamate-Moxifloxacin **(43)**



$C_{28}H_{38}FN_3O_{12}P_2$ 689.56 g/mol

C_3H_9BrSi 153.09 g/mol

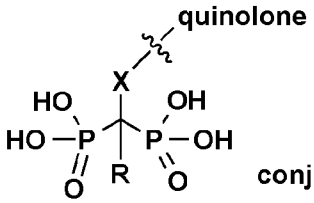
$C_{24}H_{30}FN_3O_{12}P_2$ 633.45

(43)

A mixture of tetramethyl etidronate-carbamate-moxifloxacin tetramethyl ester (764 mg, 1.10 mmol) and bromotrimethylsilane (1.35 g, 8.86 mmol) in ACN (25 mL) was stirred for 2h. The volatiles were evaporated under vacuum and MeOH (25 mL) was added to the residue. After stirring for 30 mins, the solvent was evaporated, and the residue was triturated with minimum volume of DCM for 30mins. The solid was filtered, and dried under high vacuum to afford 757 mg of product (quantitative yield). ^{1}H NMR (300 MHz, Methanol-d4) δ 8.98 (s, 1H), 7.79 (d, J = 14.5 Hz, 1H), 4.39 - 4.25 (m, 1H), 4.24 - 4.07 (m, 2H), 4.01 (t, J = 10.3 Hz, 1H), 3.65 (s, 3H), 3.61 - 3.51 (m, 2H), 3.41 (d, J = 10.7 Hz, 1H), 3.04 (br s, 1H), 2.43 – 2.27 (m, 1H), 1.90 (t, J = 15.2 Hz, 3H), 1.83 - 1.71 (m, 2H), 1.55 (q, J = 10.8 Hz, 2H), 1.43 - 1.32 (m, 1H), 1.30 - 1.18 (m, 1H), 1.17 - 1.03 (m, 1H), 1.01 - 0.83 (m, 1H). ^{31}PNMR (121 MHz, Methanol-d4): δ 16.60. MS-ESI: 634.2 [M+H]+.

Example 7.

The following is a general structure of BP-quinolone as can be described in one or more aspects herein.



conjugates between alpha-X containing BP and quinolone

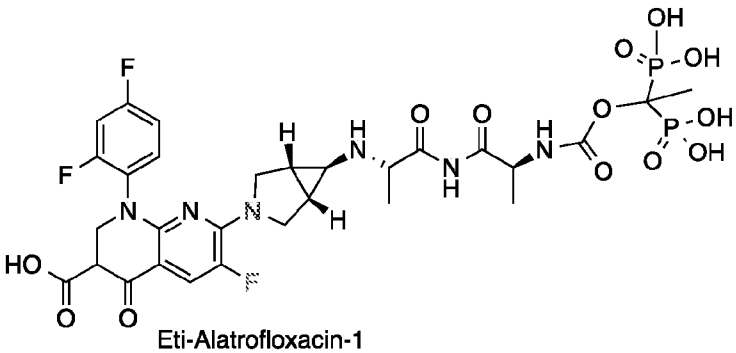
X= O, NH, NR^{1}, S

R^{1} can be alkyl or substituted alkyl, aryl or subsituted aryl groups

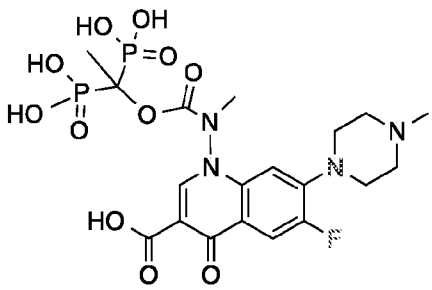
wherein R can be H, substituted and unsubstituted alkyl, alkyl amino, alkyl-aryl, aryl, alkylheteroaryl, or heteroaryl.

Example 8.

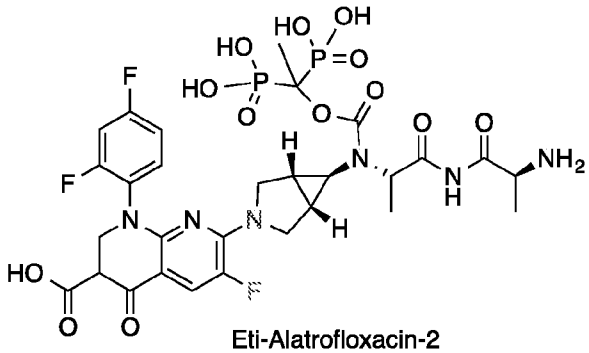
The following are non-limiting examples of BP-quinolone conjugates as described in one or more aspects herein.



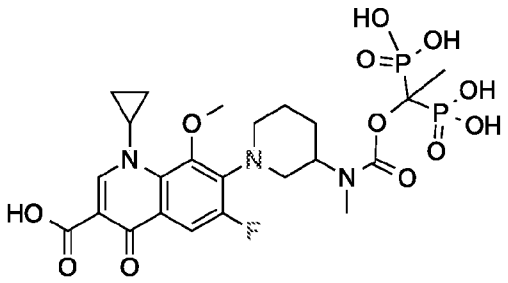
Eti-Alatrofloxacin-1



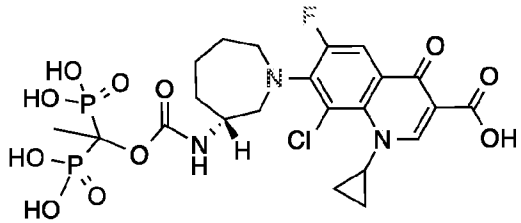
Eti-Amifloxacin



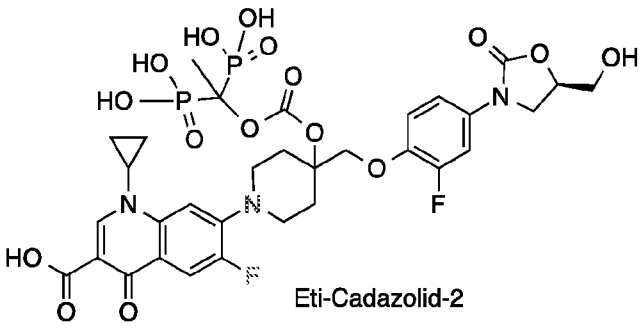
Eti-Alatrofloxacin-2



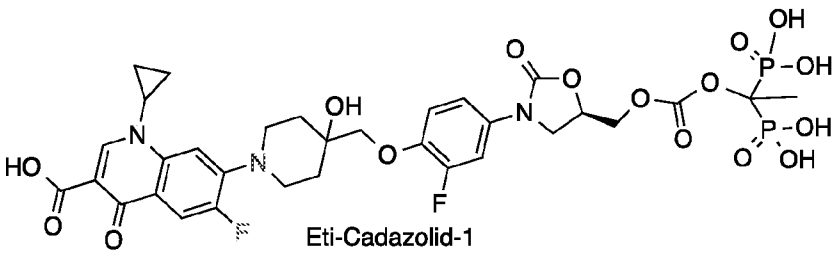
Eti-Balofloxacin



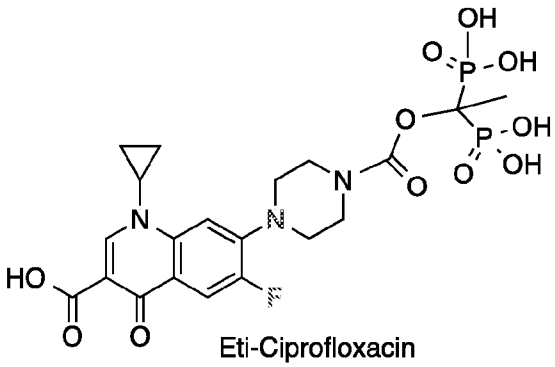
Eti-Besifloxacin



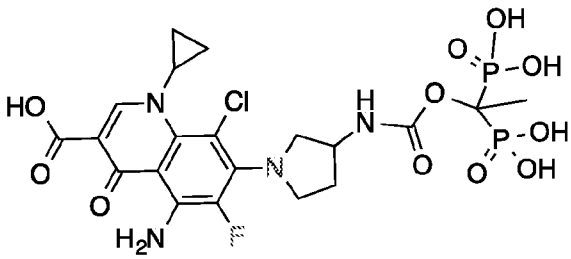
Eti-Cadazolid-2



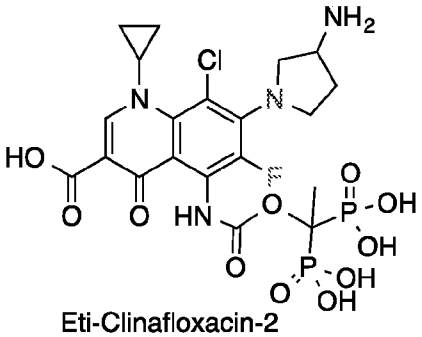
Eti-Cadazolid-1



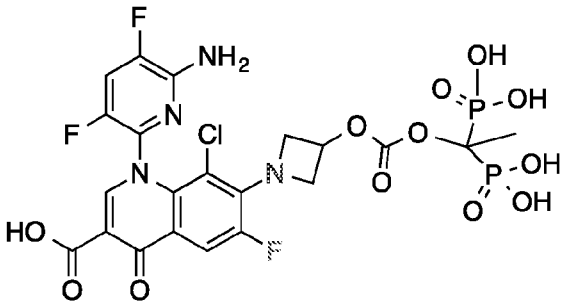
Eti-Ciprofloxacin



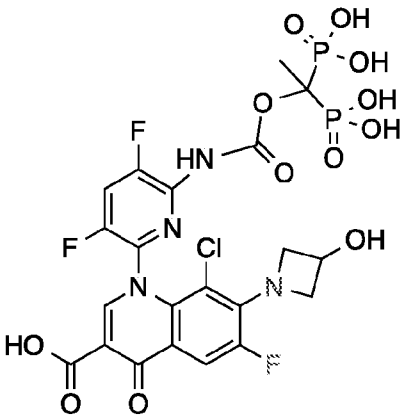
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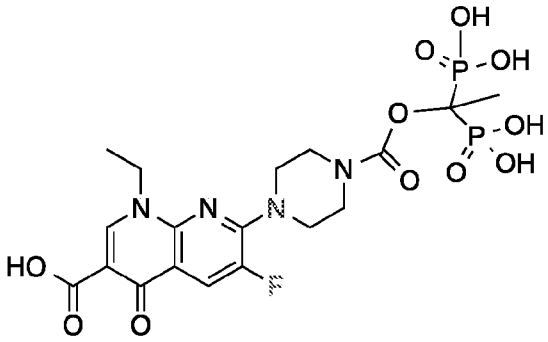
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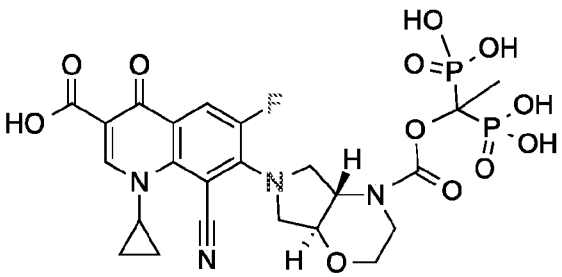
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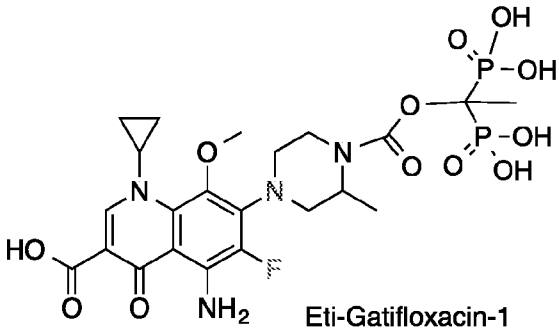
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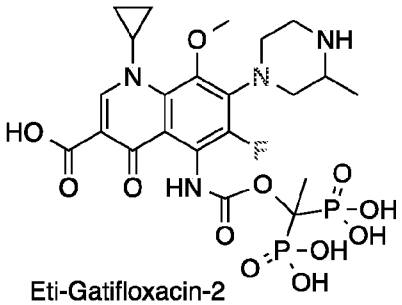
Eti-Enoxacin



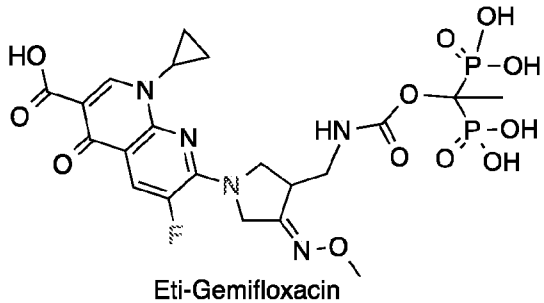
Eti-Finafloxacin



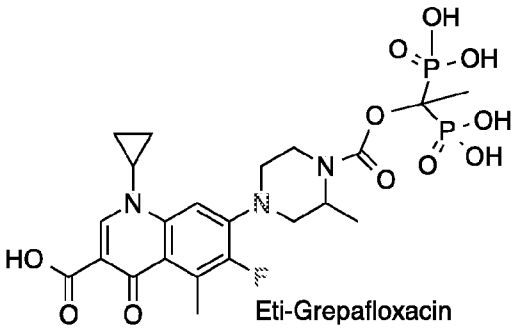
Eti-Gatifloxacin-1



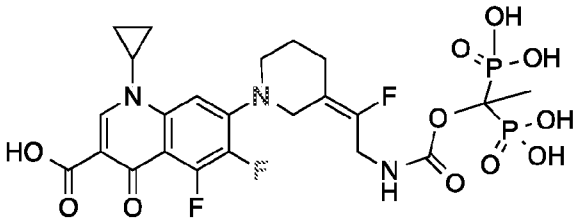
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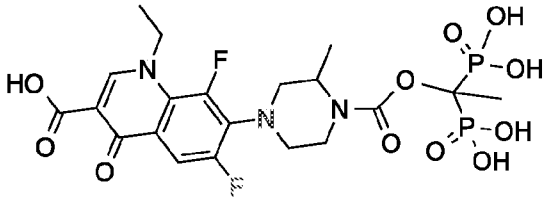
Eti-Gemifloxacin



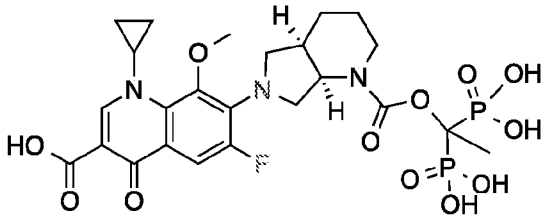
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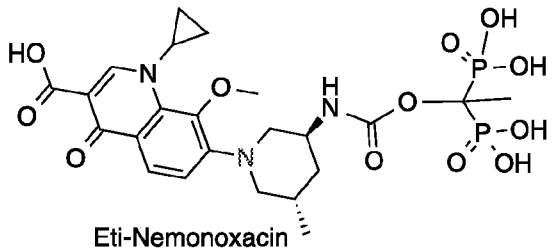
Eti-JNJ-Q2



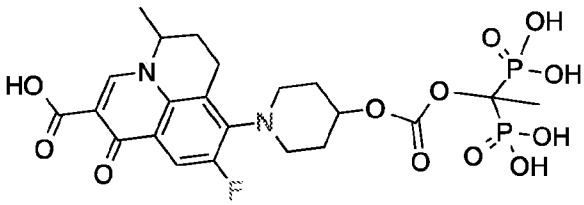
Eti-Lomefloxacin



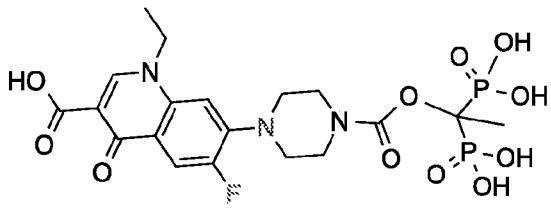
Eti-Moxifloxacin



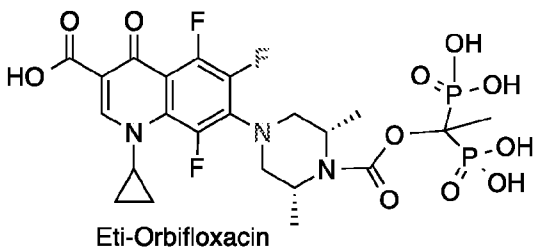
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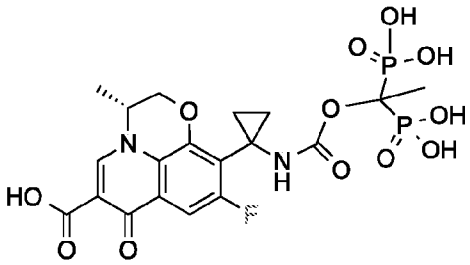
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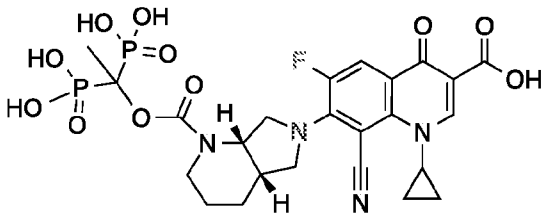
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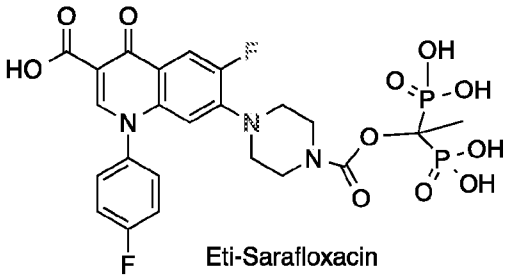
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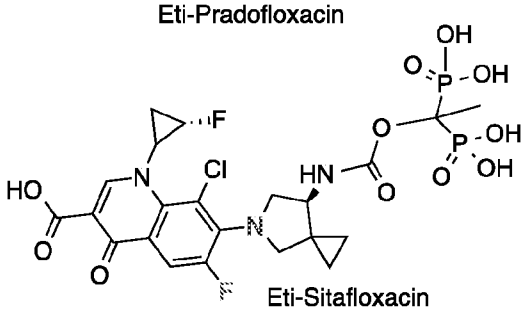
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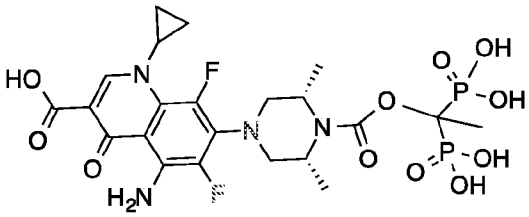
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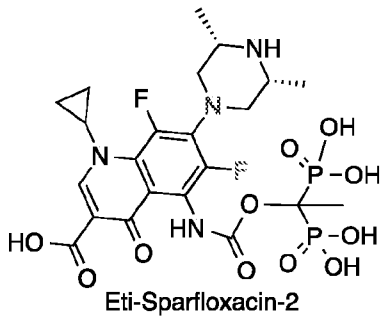
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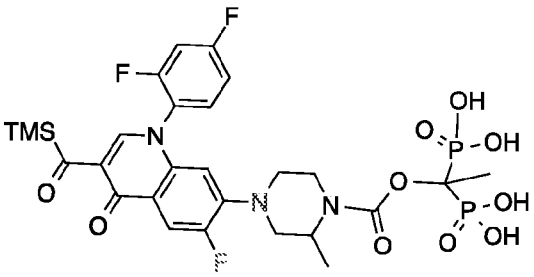
Eti-Sitafloxacin



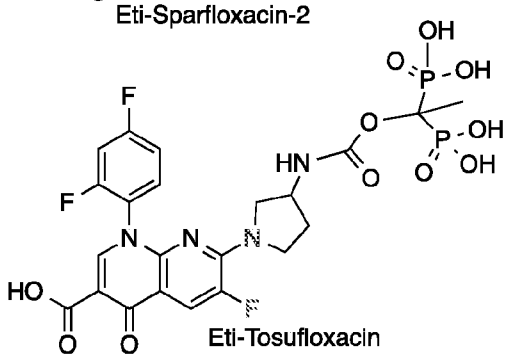
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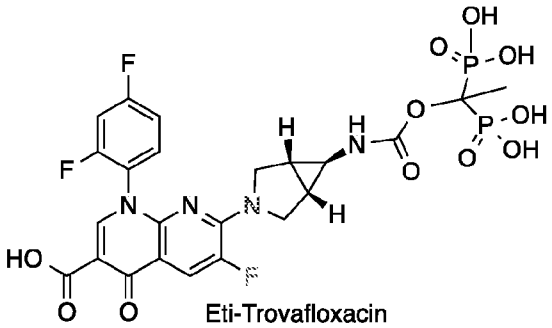
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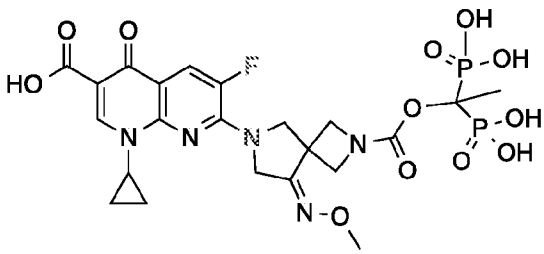
Eti-Temafloxacin



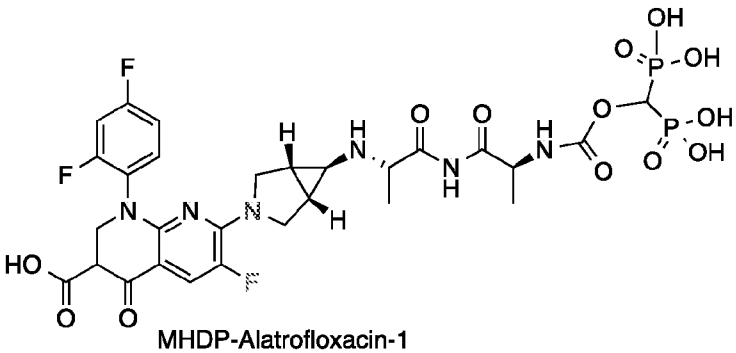
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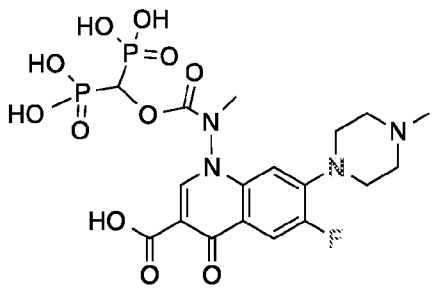
Eti-Trovafloxacin



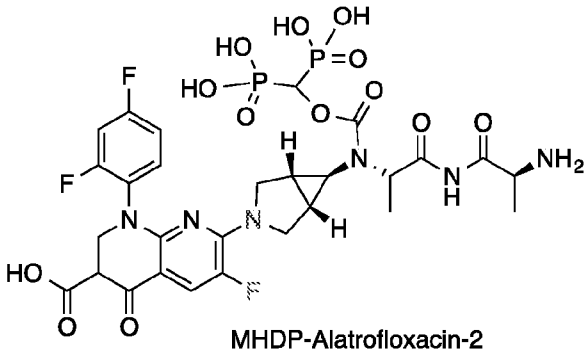
Eti-Zabofloxacin



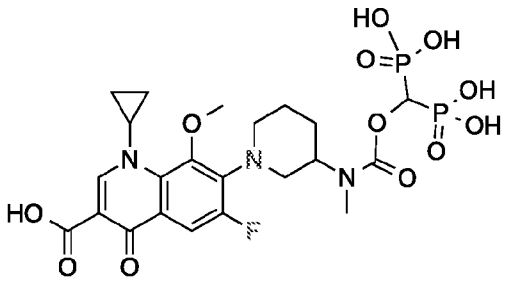
MHDP-Alatrofloxacin-1



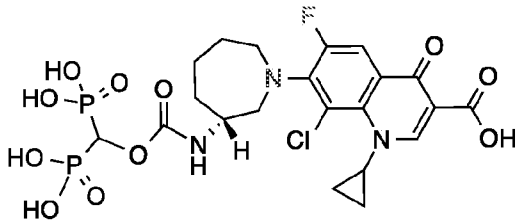
MHDP-Amifloxacin



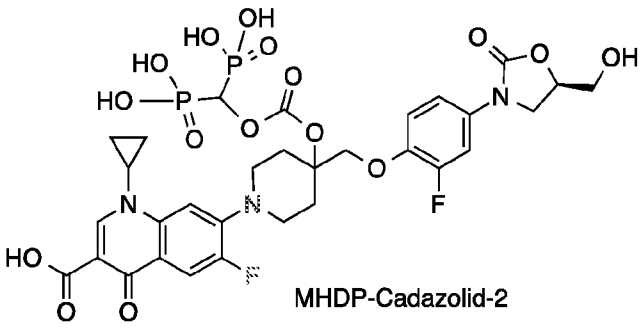
MHDP-Alatrofloxacin-2



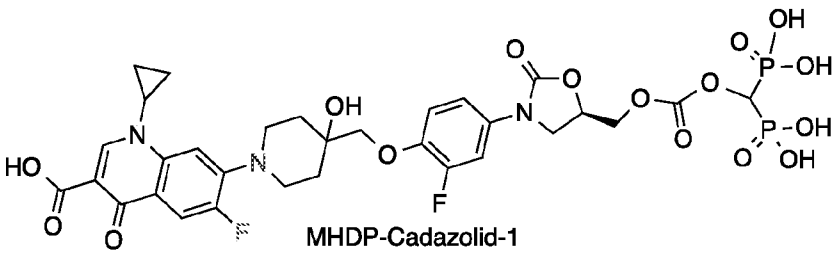
MHDP-Balofloxacin



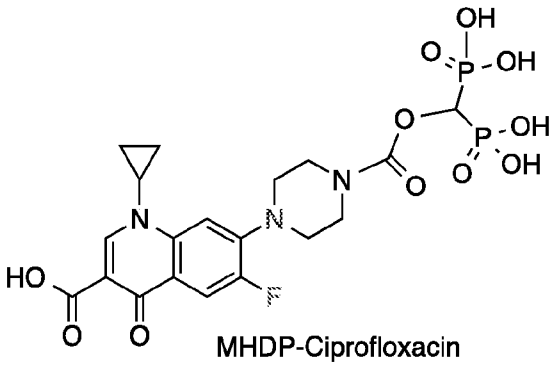
MHDP-Besifloxacin



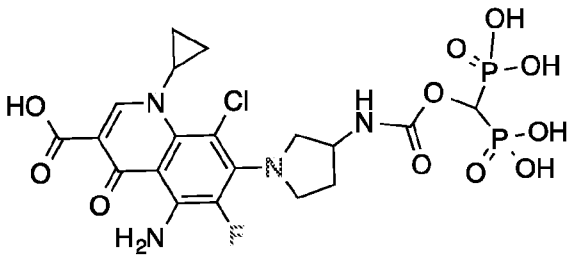
MHDP-Cadazolid-2



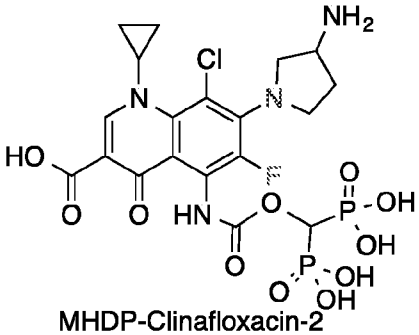
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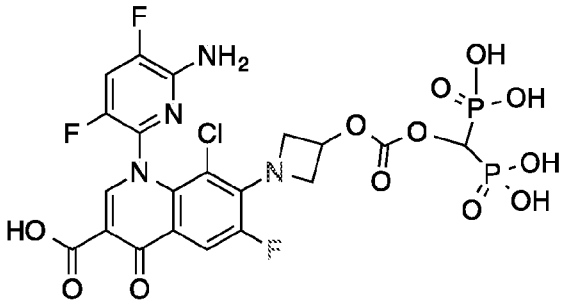
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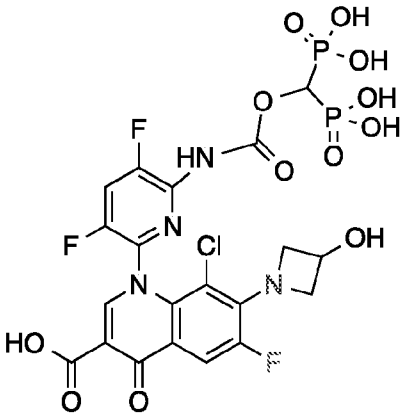
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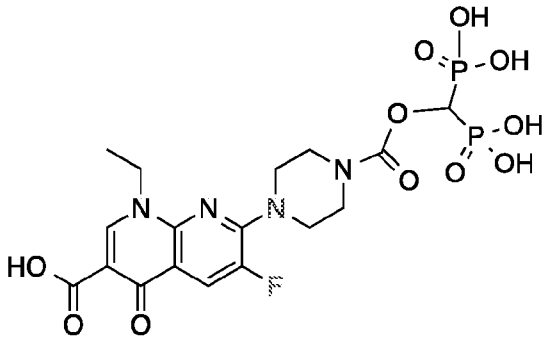
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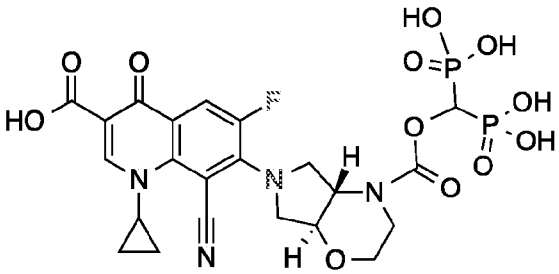
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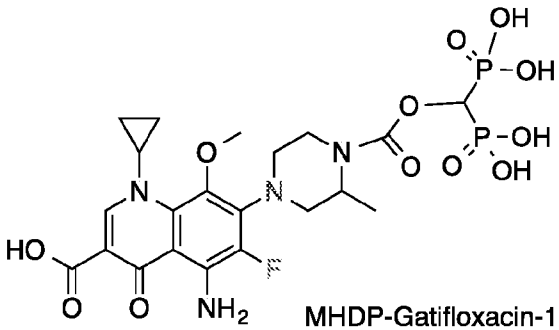
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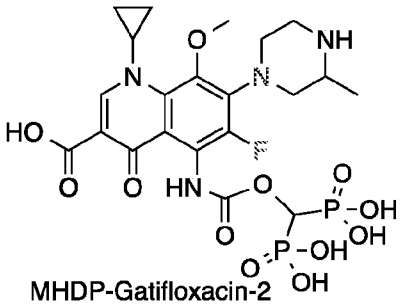
MHDP-Enoxacin



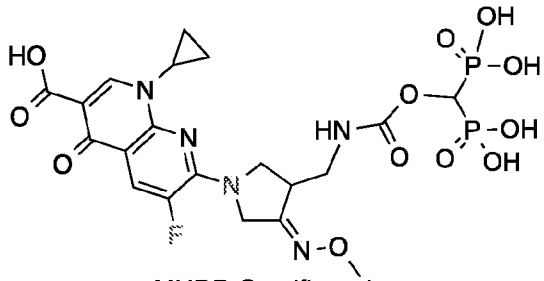
MHDP-Finafloxacin



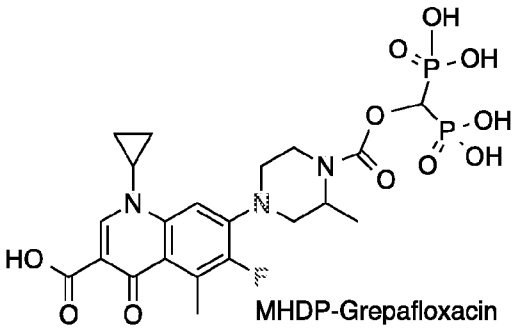
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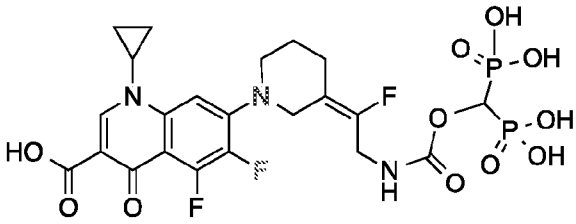
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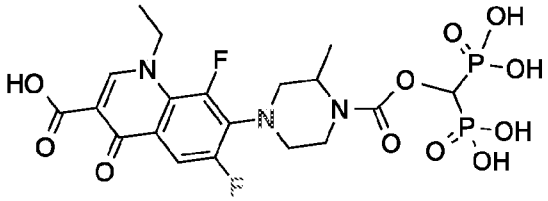
MHDP-Gemifloxacin



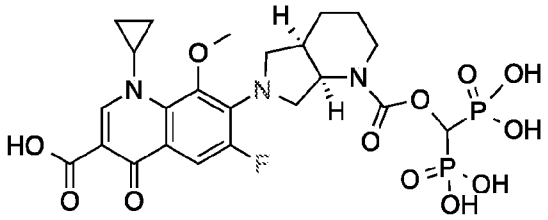
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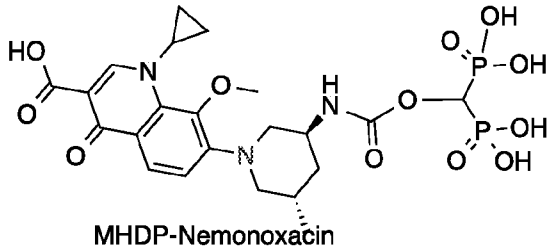
MHDP-JNJ-Q2



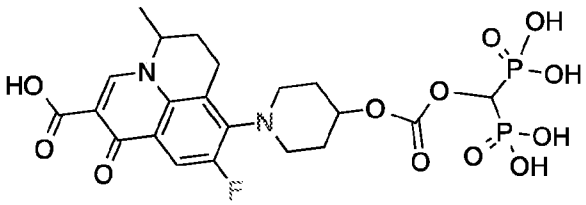
MHDP-Lomefloxacin



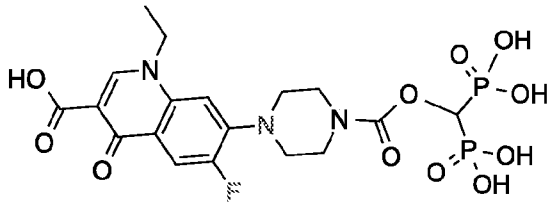
MHDP-Moxifloxacin



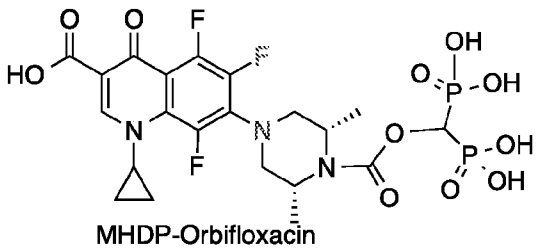
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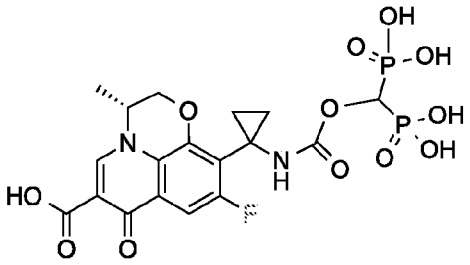
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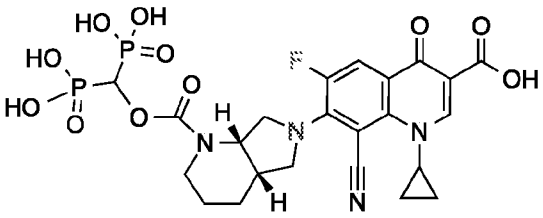
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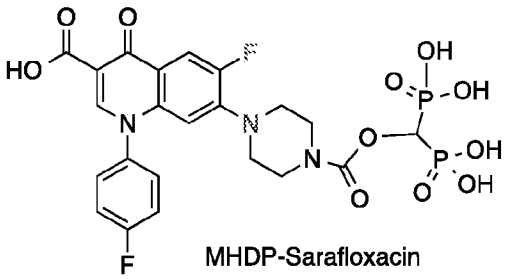
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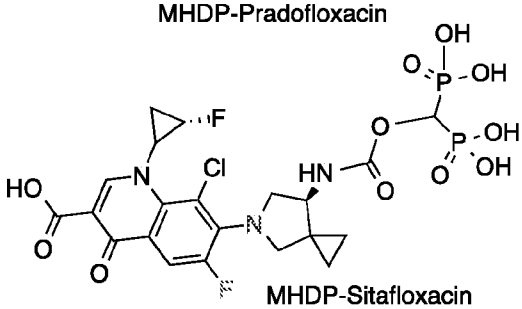
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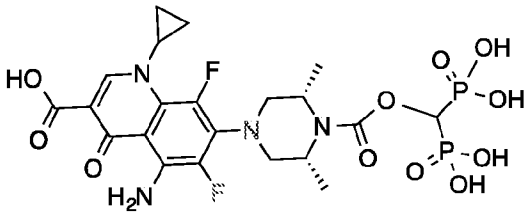
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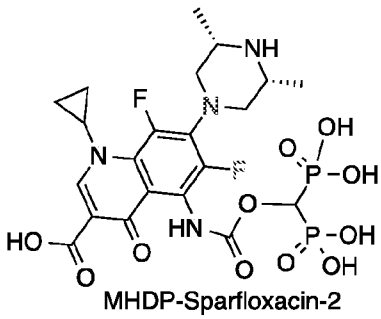
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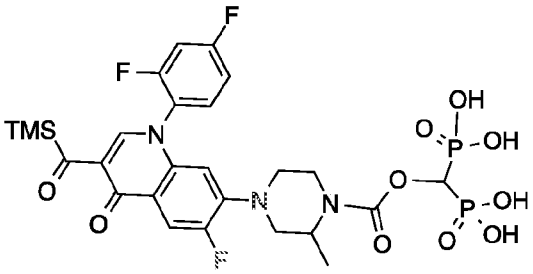
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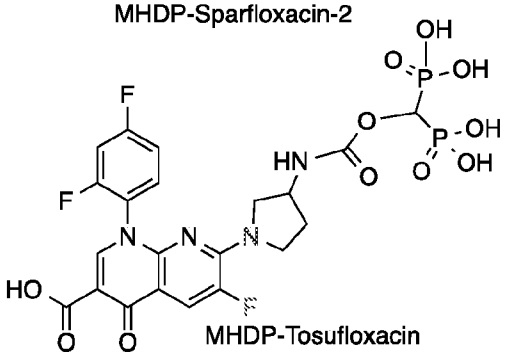
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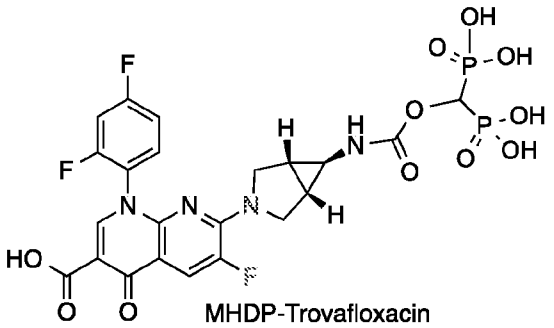
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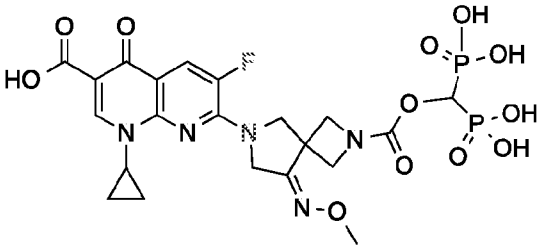
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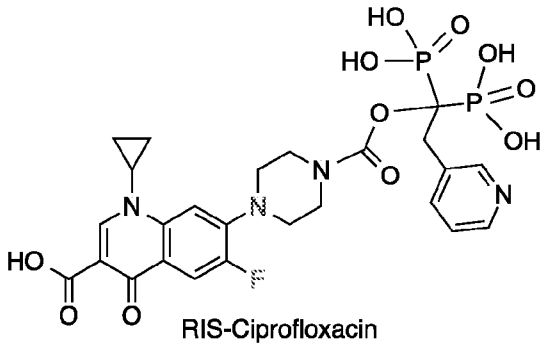
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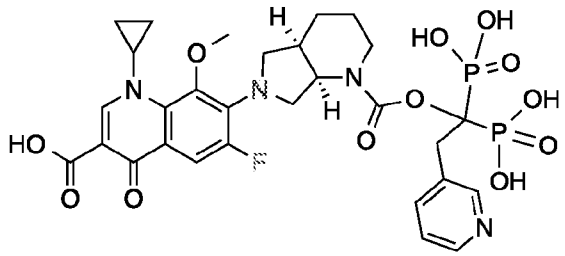
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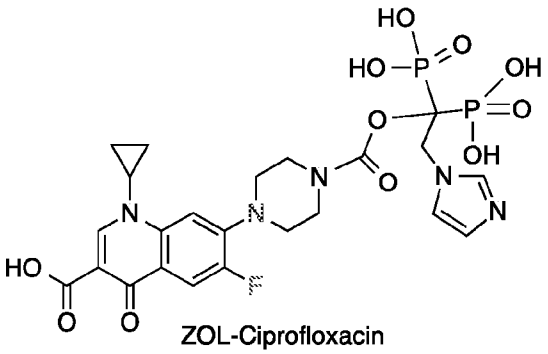
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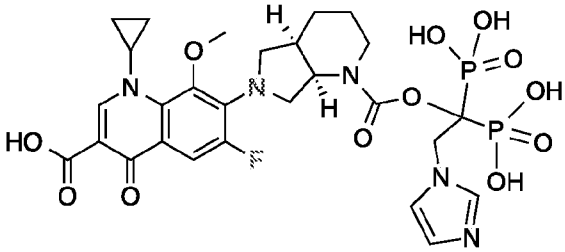
RIS-Ciprofloxacin



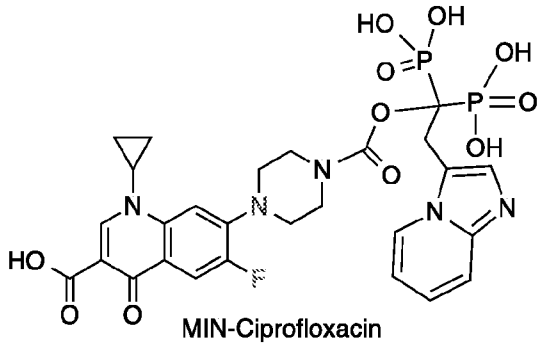
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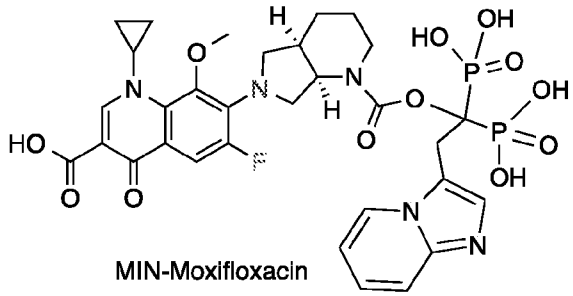
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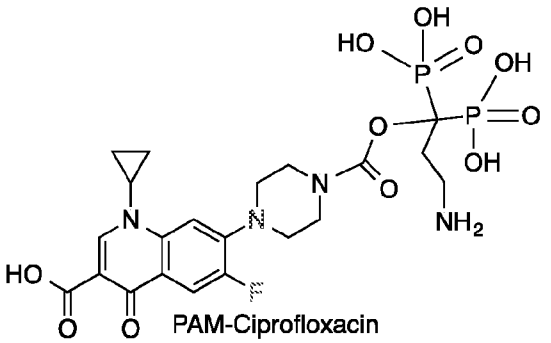
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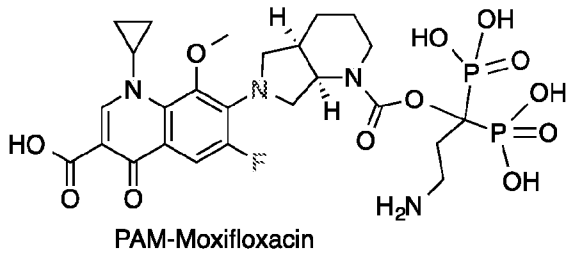
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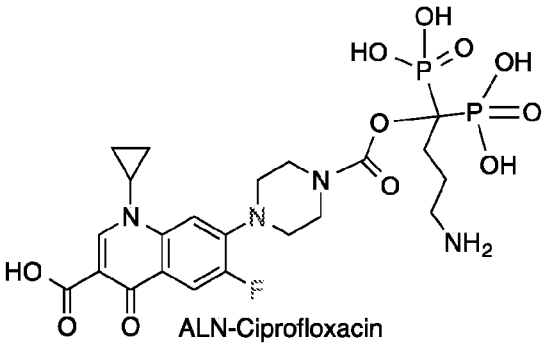
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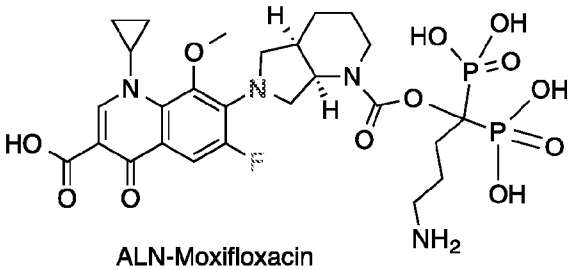
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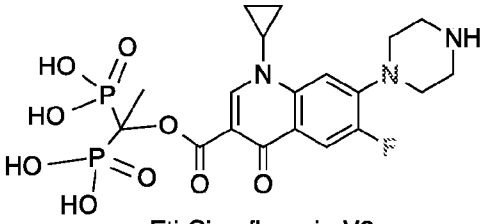
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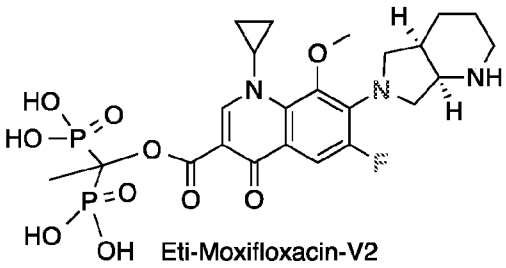
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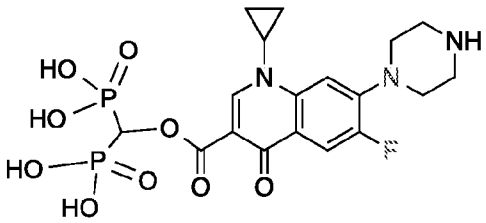
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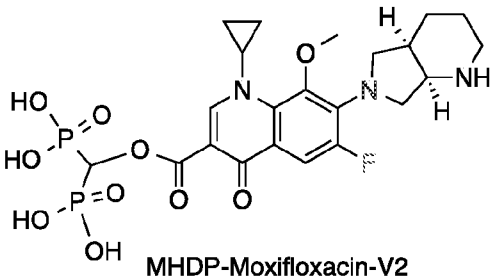
Eti-Ciprofloxacin-V2



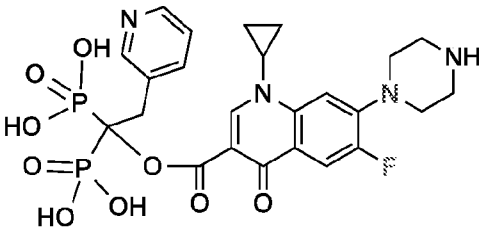
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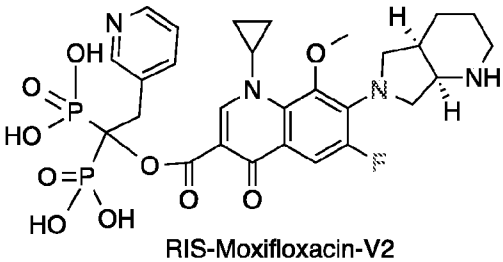
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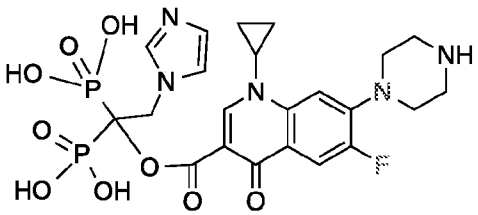
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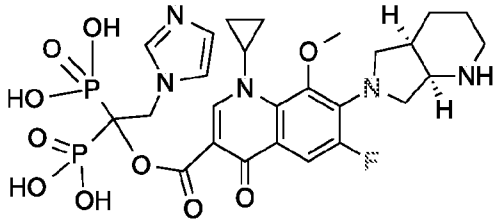
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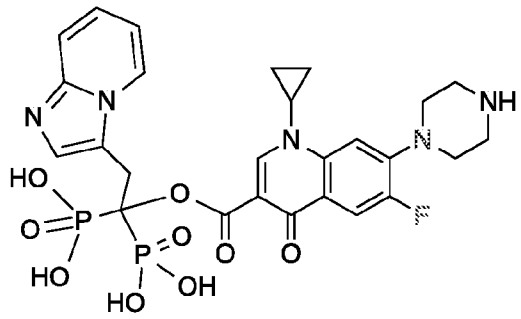
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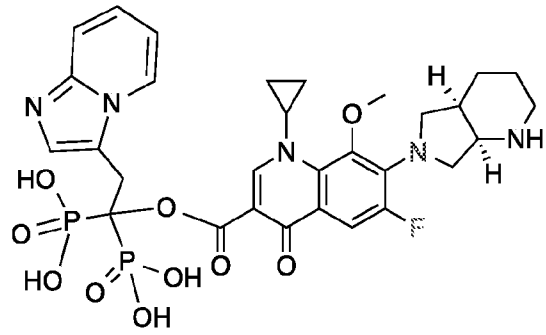
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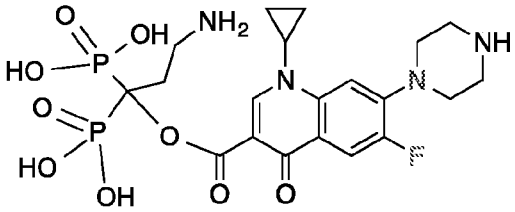
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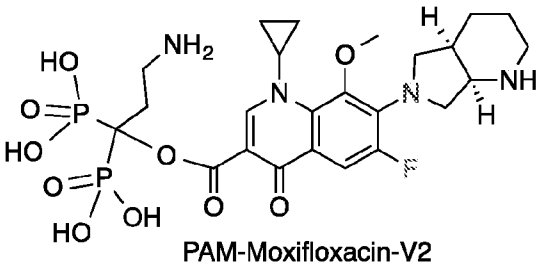
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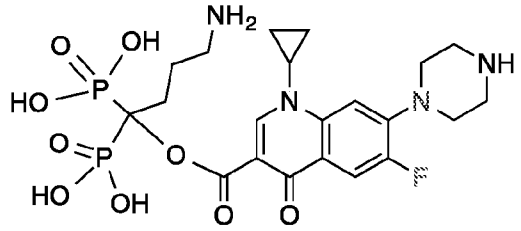
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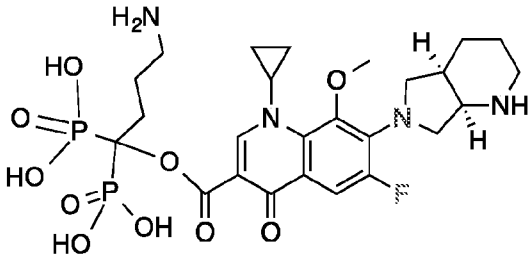
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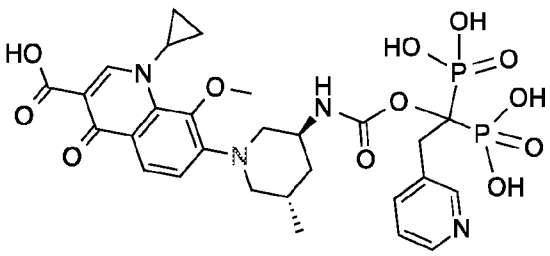
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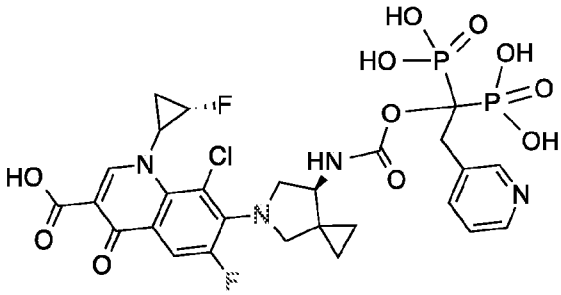
ALN-Ciprofloxacin-V2



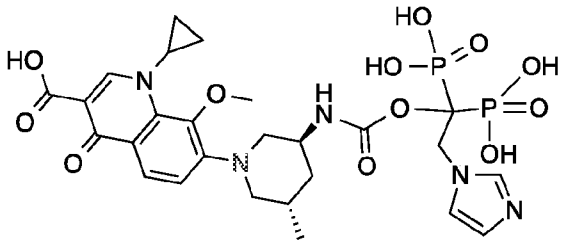
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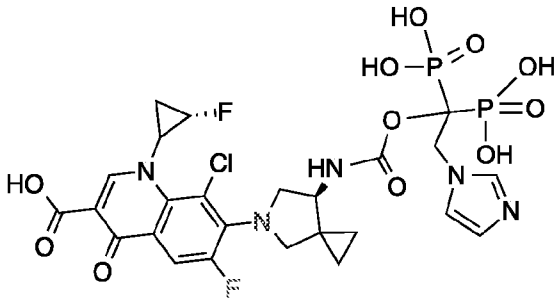
RIS-Nemonoxacin



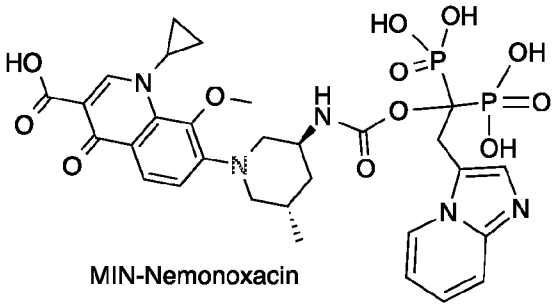
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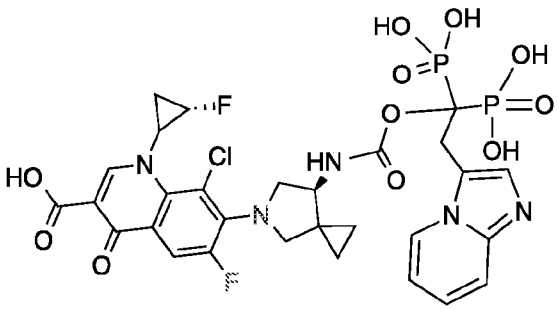
ZOL-Nemonoxacin



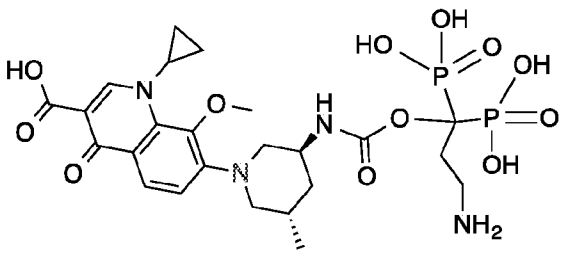
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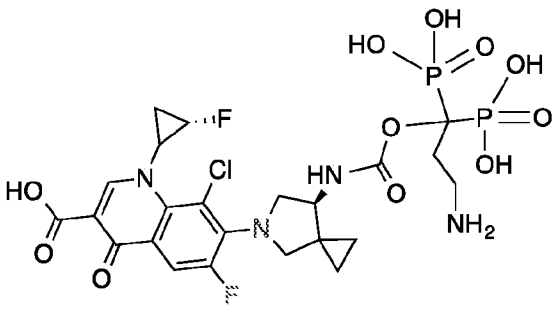
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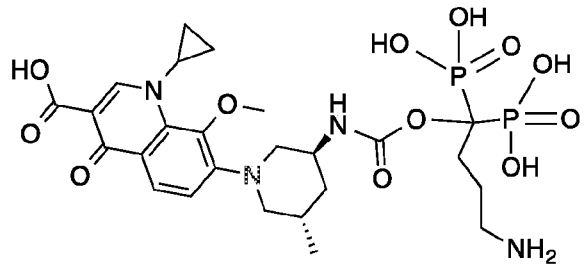
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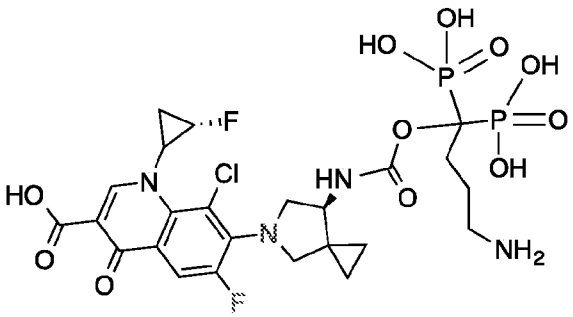
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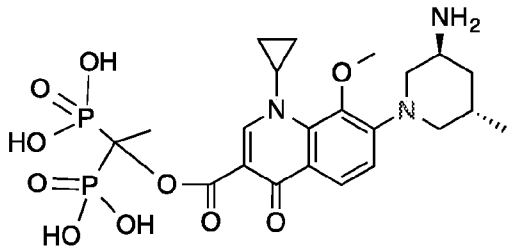
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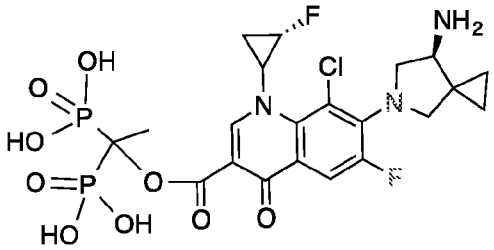
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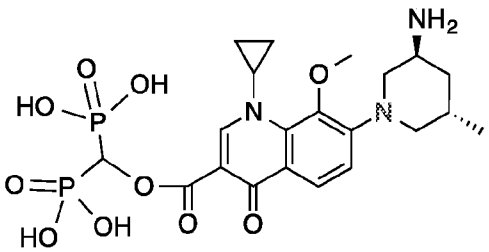
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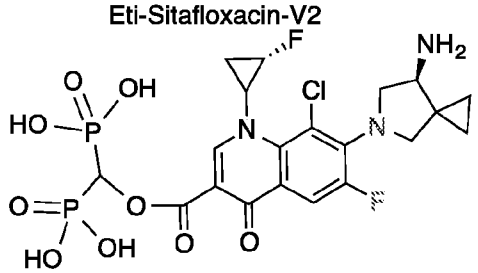
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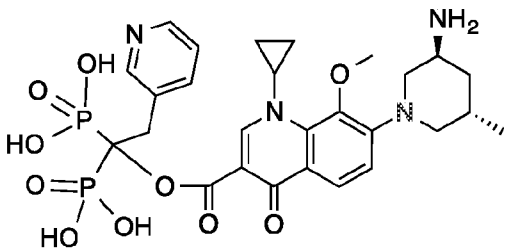
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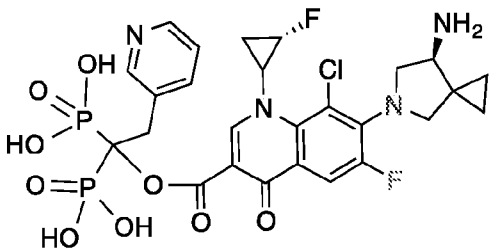
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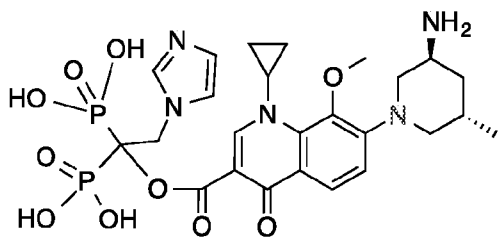
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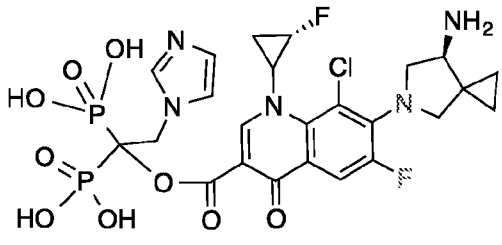
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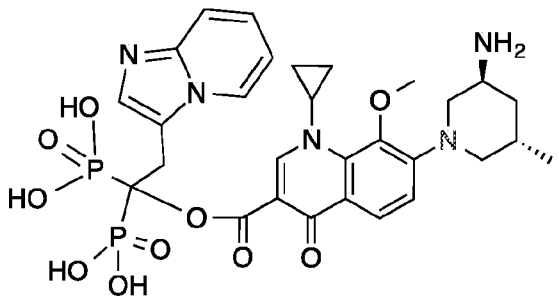
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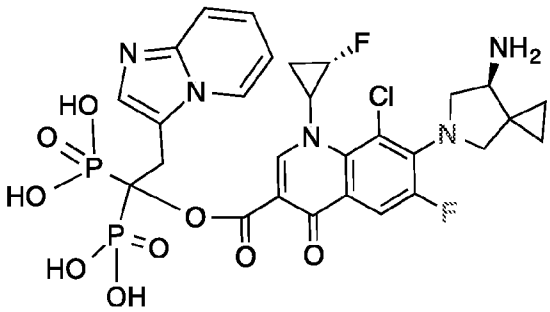
ZOL-Nemonoxacin-V2



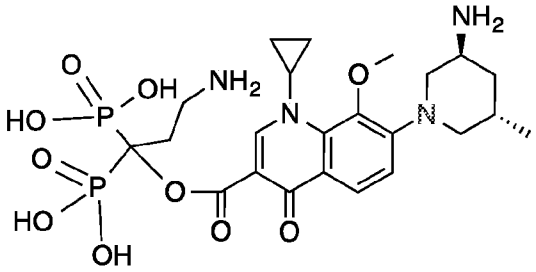
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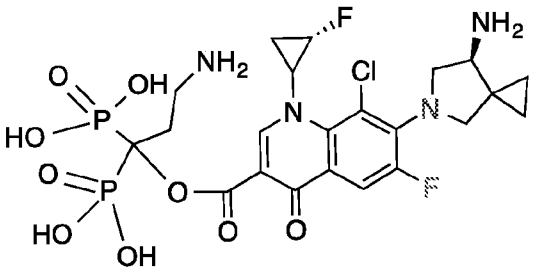
MIN-Nemonoxacin-V2



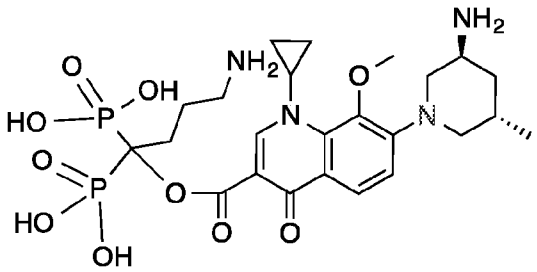
MIN-Sitafloxacin-V2



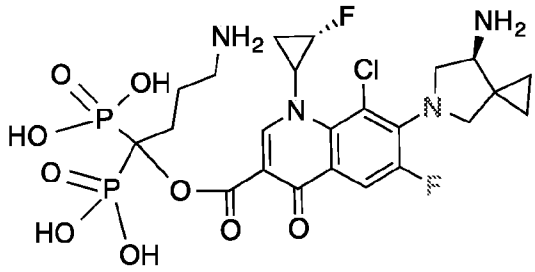
PAM-Nemonoxacin-V2



PAM-Sitafloxacin-V2



ALN-Nemonoxacin-V2



ALN-Sitafloxacin-V2

Example 9:

To exploit BP affinity for bone, a "target and release" chemistry approach was investigated involving delivery of antibiotics to bone or hydroxyapatite (HA) via BP conjugates. Serum-stable drug-BP linkers were utilized that metabolize and release the parent antibiotic at the bone surface. Designed, synthesized, and tested were novel quinolone antibiotic etidronate-ciprofloxacin (ECC) conjugate, BV81022, and etidronate-moxifloxacin (ECX) conjugate, BV81051, for activity against *S. aureus* biofilms which are causative in the majority of osteomyelitis cases.

Problems for public health like osteomyelitis caused by bacterial biofilms have emphasized the lack of information about biofilm development. Several methods are available to study biofilms in vitro or ex vivo, including quantification of sessile bacteria after detachment from the surface by scraping, vortexing or sonication, and observations by microscopy techniques to

monitor biofilm progression. However, these applications are limited by high labor intensity, intrusive sampling and/or long time lags from sampling to obtaining a result.[1] Hence, improved biofilm-monitoring assays are essential for biofilm behavior research and biomedical applications. Therefore, sensitive, accurate, reproducible and fast methods are desirable for real-time monitoring of biofilms. In this regard, promising advances in impedance technology based on the ability of cells to impede an electrical current when adhered to MTP with gold electrodes have been developed in recent years.

In the present study, electrode impedance measurements were applied to test the therapeutic efficacy and effect on biofilm formation and growth of our novel ECC and ECX conjugates in comparison to ciprofloxacin and moxifloxacin against *S. aureus*. Real-time measurements are able to detect whether these biofilms are unaffected, inhibited, or induced during antimicrobial therapy. [2]

Microbiolgy: For experimental purposes, a robust biofilm forming and well-studied *S. aureus* strain ATCC 6538 was used. The following parent antibiotics were tested: ciprofloxacin (C), moxifloxacin (X); the following experimental conjugates were tested: etidronate-ciprofloxacin (ECC) and etidronate-moxifloxacin (ECX). Real-time biofilm assays were performed with an xCELLigence RTCA SP instrument according to the manufacturer's instructions.[4] For monitoring biofilm formation and RTCA sensitivity assays, 80 µl of TSBYE was added to each well of non-reusable 16X microtiter E-plates (ACEA Biosciences) for the impedance background measurement using the standard protocol provided by the software. 1 µl of bacteria suspension in a total of 120 µl of TSBYE was then added to the 16 E-plate wells. Each sample was run in duplicate. E-plates were positioned in the xCELLigence Real-Time Cell Analyzer MP, incubated at 37°C and monitored on the RTCA system at 15-min time intervals for 24 h. Cell-sensor impedance was expressed as a unit called cell index (CI) according to the manufacturer's instructions. The CI at each time point is defined as (ZnZb)/15, where Zn is the cell-electrode impedance of the well when it contains cells and Zb is the background impedance with growth media alone. Standard deviations of duplicates or triplicates of wells were analyzed with the RTCA Software

Affinity of antibiotics to HA. 1 µg/mL of each compound was added to a solution containing 10 µg/mL of HA powder and incubated for 4h/37°C under magnetic stirring. Next, HA powder was allowed to sediment for 1h/4°C. After this time, the content of antibiotic in the supernatant was assessed using HPLC (Shimadzu Prominence). To evaluate quantity of conjugates bound to HA,

we used methodology described in more detail in our previous work.[3] Affinity of compounds to HA powder was estimated as follows: 100% - peak area of tested compound detected / peak area of control sample *100%.

Infection preventative experiment. 80-µl of TSBYE was added to each well to measure background impedance. 1 µl of bacteria suspension in a total of 120 µl of TSBYE containing a variety of concentrations toward antibiotics was then added to the 16 E-plate wells. Two replicates of each antibiotic concentration and negative controls without antibiotic were also included. Cells were monitored for 24 h and analysis was done to calculate MIC_{50} values.

Infection preventative experiment with HA. Different concentrations of each compound were added to a solution containing 10 µg/mL of HA powder and incubated for 2h/37°C under magnetic stirring. Then 80-µl of TSBYE was added to each well to measure background impedance. 1 µl of bacteria suspension in a total of 120 µl of TSBYE containing a gradient of concentrations toward antibiotics plus HA was then added to the 16 E-plate wells. Two replicates of each antibiotic concentration and negative controls without antibiotic were also included. Cells were monitored for 24 hrs and analysis was performed to calculate MIC_{50} values.

Results: HPLC results evaluating the binding affinity of tested compounds to HA indicate that the conjugates had high binding affinity and retention to HA in comparison to the unconjugated antibiotics. Electrode impedance measurements were applied to test the therapeutic efficacy and effect on biofilm formation and growth of the novel ECC and ECX conjugates in comparison to ciprofloxacin and moxifloxacin against S. aureus. Real-time measurements were able to detect whether these biofilms are unaffected, inhibited, or induced during antimicrobial therapy (see, **Figs. 26, 28, 30, 32**).

In the infection preventative experiment using real time monitoring MIC_{50} has been calculated for each conjugate and parent antibiotic as shown in **FIGs. 27 and 29**. The *S. aureus* MIC_{50} for ciprofloxacin, moxifloxacin, ECC, and ECX are: 0.09, 0.11, 4.88, and 5.10 µg/ml respectively.

In the infection preventative experiment in the presence of HA using real time monitoring MIC_{50} has been calculated for each conjugate and parent antibiotic as shown in **FIGs 31 and 33.** The *S. aureus* MIC_{50} for ciprofloxacin, moxifloxacin, ECC, and ECX are: 0.24, 0.09, 9.60, and 28 µg/ml respectively.

Conclusion: Thus, by using impedance measurements in microtiter plates with gold electrodes the antibiotic effect on S. aureus bacterial biofilm growth were assessed in real time. Real-time biofilm analysis allowed detection of decreases or increases in microbial mass over time during antimicrobial therapy, which can be used to evaluate antibiotic susceptibility and efficacy in biofilm-mediated infections clinically. The novel etidronate-fluoroquinolone conjugates designed and tested in this study (ECC, ECX) retained the bone binding properties of the parent BP drug, and also the antimicrobial activity of the parent antibiotic in the presence or absence of HA albeit at lower levels due to the nature of the chemical modification and possible partial cleavage at the tested conditions. This class of conjugates using BP drugs as biochemical vectors for the delivery of antibiotic agents to bone (where osteomyelitis biofilm pathogens reside) represents an advantageous approach to the treatment of osteomyelitis by providing improved bone pharmacokinetics while minimizing systemic exposure (toxicity) of these drugs.

References for Example 9:

1) Coenye T, Nelis H.J. In vitro and in vivo model systems to study microbial biofilm formation. *J Microbiol Methods 2010; 83*, 89-105.

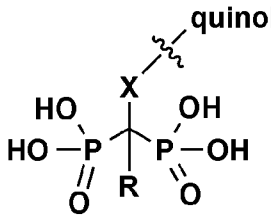
2) Saginur R, Stdenis M, Ferris W, Aaron, S.D, Chan F, Lee C, Ramotar K. Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. *Antimicrob Agents Chemother 2006;* 50, 55-61.

3) Sedghizadeh PP and Ebetino FH *et al*. Design, synthesis, and antimicrobial evaluation of a novel bone-targeting bisphosphonate-ciprofloxacin conjugate for the treatment of osteomyelitis biofilms. *J Med Chem* 2017; 60, 2326-43.

4) Atienza J.M, Zhu J, Wang X, Xu X, Abassi Y. Dynamic monitoring of cell adhesion and spreading on microelectronic sensor arrays. *J Biomol Screen* 2005; 10, 795–805.

We claim:

1. A compound comprising a bisphosphonate (BP) and a quinolone, wherein the BP has an alpha substituent and the alpha substituent is a hydroxy, amino or thiol group and wherein the quinolone is directly or indirectly conjugated to the BP at the germinal carbon alpha substituent (X) of the BP of the following formula



conjugates between alpha-X containing BP and quinolone

X= O, NH, NR^1, S

R^1 can be alkyl or substituted alkyl, aryl or subsituted aryl groups

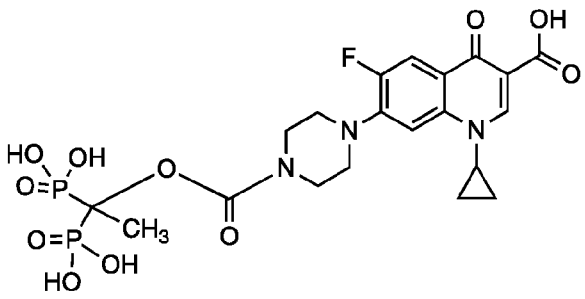
wherein R can be H, substituted and unsubstituted alkyl, alkyl amino, alkyl-aryl, aryl, alkylheteroaryl, or heteroaryl.

2. The compound of claim 1, wherein the BP is an alpha-OH containing BP and wherein the quinolone is directly or indirectly conjugated to the BP at the geminal OH of the BP.

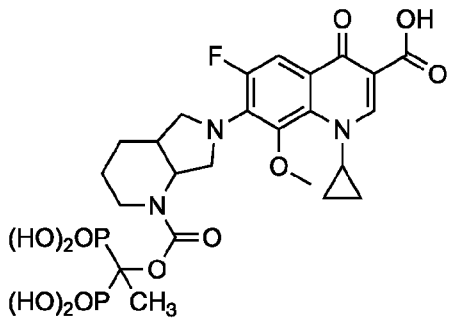
3. The compound of claim 1 or 2, wherein the quinolone is a fluoroquinolone.

4. The compound of any one of claim 1 or 2, wherein the quinolone is selected from the group consisting of alatrofloxacin, amifloxacin, balofloxacin, besifloxacin, cadazolid, ciprofloxacin, clinafloxacin, danofloxacin, delafloxacin, difloxacin, enoxacin, enrofloxacin, finafloxacin, flerofloxacin, flumequine, gatifloxacin, gemifloxacin, grepafloxacin, ibafloxacin, JNJ-Q2, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, norfloxacin, ofloxacin, orbifloxacin, pazufloxacin, pefloxacin, pradofloxacin, prulifloxacin, rufloxacin, sarafloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin, trvafloxacin, zabofloxacin, nemonoxacin and combinations thereof.

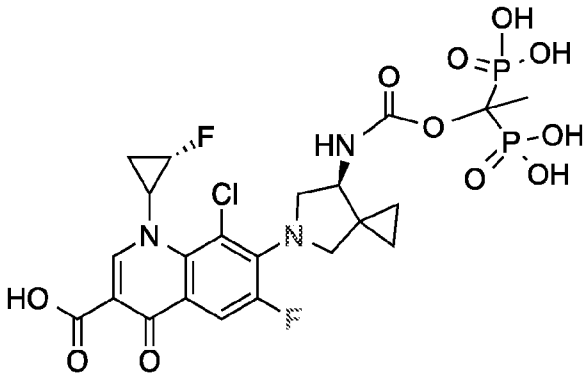
5. The compound of claim 1 or 2, according to Formula (**41**), Formula (**43**), Formula (**44**) or Formula (**45**)



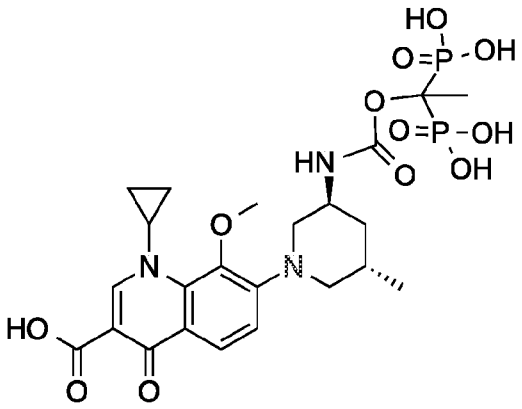
Formula (**41**),



Formula (**43**),

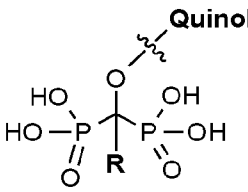


Formula (**44**), or



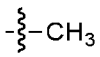
Formula (**45**).

6. The compound of claim 1, according to the following formula



conjugates between alpha-OH containing BP and quinolone

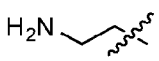
R =



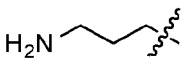
Etidronate



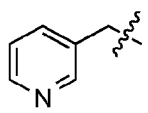
methylene hydroxyl bisphosphonic acid (MHDP)



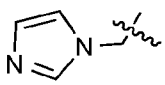
Pamidronate



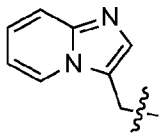
Alendronate



Risedronate

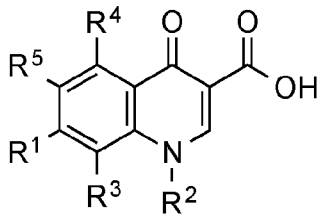


Zoledronate



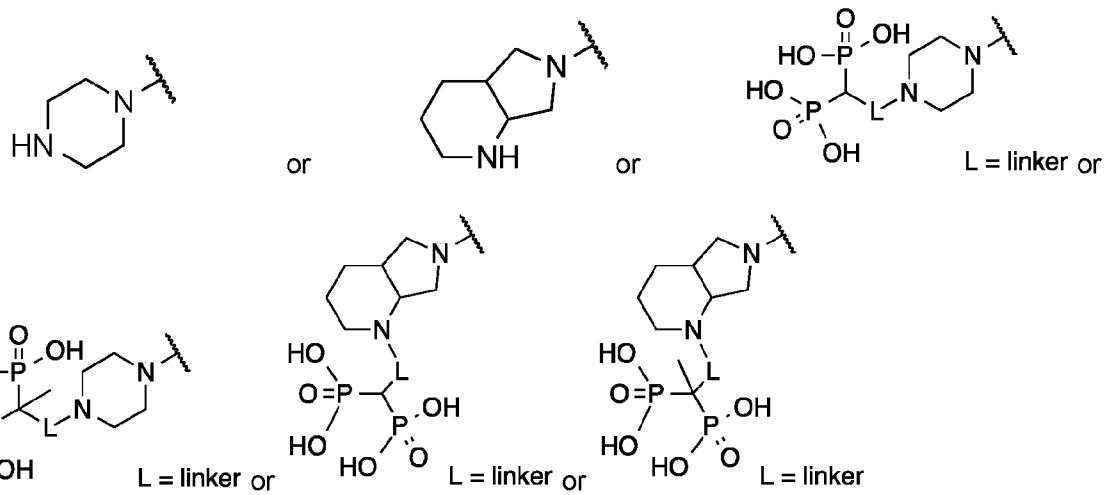
Minodronate

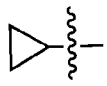
7. The compound of claim 1, wherein the quinolone or the BP quinolone compound is comprised of a quinolone antibiotic analog or substituent according to Formula (**A**),



Formula (**A**)

wherein R^1 can be either



and wherein R^2 can be 

and wherein R^3 can be either H or OCH3,

and where R^4 can be H,

and wherein R^5 can be H or F.

8. The compound of claim 1, wherein the bisphosphonate is selected from the group consisting of: etidronate, methylenehydroxybisphosphonate (MHBP), risedronate, zoledronate,

minodronate, neridronate, pamidronate, alendronate, modified or unmodified, and combinations thereof.

9. The compound of claim 7 or claim 8, wherein the quinolone compound is a fluoroquinolone.

10. The compound of claim 7 or claim 8, wherein the quinolone compound is selected from the group consisting of: alatrofloxacin, amifloxacin, balofloxacin, besifloxacin, cadazolid, ciprofloxacin, clinafloxacin, danofloxacin, delafloxacin, difloxacin, enoxacin, enrofloxacin, finafloxacin, flerofloxacin, flumequine, gatifloxacin, gemifloxacin, grepafloxacin, ibafloxacin, JNJ-Q2, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, norfloxacin, ofloxacin, orbifloxacin, pazufloxacin, pefloxacin, pradofloxacin, prulifloxacin, rufloxacin, sarafloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin, trvafloxacin, zabofloxacin, nemonoxacin and combinations thereof.

11. The compound of claim 7 or claim 8, wherein the quinolone compound is ciprofloxacin, moxifloxacin, sitafloxacin or nemonoxacin.

12. The compound of any one of claims 7-11, wherein the linker is a carbamate, a thiocarbamate, a hydrazine, or a carbonate or ester, or a urea.

13. The compound of any one of claims 7-11, wherein the linker is a carbamate linker or ester linker.

14. The compound of any one of claims 7-11, wherein the linker is an alkyl or an aryl carbamate linker.

15. The compound of any one of claims 7-11, wherein the linker is an *O*-thioaryl or thioalkyl carbamate linker.

16. The compound of any one of claims 7-11, wherein the linker is an *S*-thioaryl or thioalkyl carbamate linker.

17. The compound of any one of claims 7-11, wherein the linker is a phenyl carbamate linker.

18. The compound of any one of claims 7-11, wherein the linker is a thiocarbamate linker.

19. The compound of any one of claims 7-11, wherein the linker is an *O*-thiocarbamate linker.

20. The compound of any one of claims 7-11, wherein the linker is an *S*-thiocarbamate linker.

21. A pharmaceutical formulation comprising:
an amount of a compound as set forth in any one of claims 1-20; and
a pharmaceutically acceptable carrier.

22. The pharmaceutical formulation of claim 21, wherein the amount of the compound is an amount effective to kill or inhibit bacteria.

23. The pharmaceutical formulation of claim 21, wherein the amount of the compound is an amount effective to treat or prevent bone diseases with abnormal bone resorption, osteoporosis, bone infections, osteomyelitis, osteonecrosis, peri-implantitis, and periodontitis.

25. A method of treating a bone infection in a subject in need thereof, the method comprising:
administering an amount of the compound of any one of claims 1-20 or a pharmaceutical formulation thereof to the subject in need thereof.

26. A method of treating osteomyelitis in a subject in need thereof, the method comprising:
administering an amount of a compound as in any one of claims 1-20 or a pharmaceutical formulation thereof to the subject in need thereof.

26. A method of treating peri-implantitis or periodontitis in a subject in need thereof, the method comprising administering an amount of a compound as in any one of claims 1-20 or a pharmaceutical formulation thereof to the subject in need thereof.

27. A method of treating diabetic foot in a subject in need thereof, the method comprising administering an amount of a compound as in any one of claims 1-20 or a pharmaceutical formulation thereof to the subject in need thereof.

28. A method comprising:

administering an amount of a compound as in any one of claims 1-20 or a pharmaceutical formulation thereof to a subject.

29. A bone graft composition comprising:

a bone graft material and a compound as in any one of claims 1-20 or a pharmaceutical formulation thereof, wherein the compound or pharmaceutical formulation is attached to, integrated with, chemisorbed to, or mixed with the bone graft material.

30. The bone graft composition of claim 29, wherein the bone graft material is autograft bone material, allograft bone material, xenograft bone material, a synthetic bone graft material, or any combination thereof.

31. A method comprising:

implanting the bone graft composition of any one of claims 29-30 into a subject in need thereof.

32. A method of preventing biofilm infection at an osseous or implant surgical site, or at a surgical site where bone grafting is performed, where the method comprises:

administering a compound as in any one of claims 1-20 or a pharmaceutical formulation thereof to a subject in need thereof.

33. A method of preventing biofilm infection at an osseous or implant surgical site, or at a surgical site where bone grafting is performed, where the method comprises:

implanting a bone graft composition as in any one of claims 29-30 to a subject in need thereof.

34. Use of the composition of any one of claims 1-20 for the manufacture of a pharmaceutical formulation for the treatment a bone infection in a subject in need thereof.

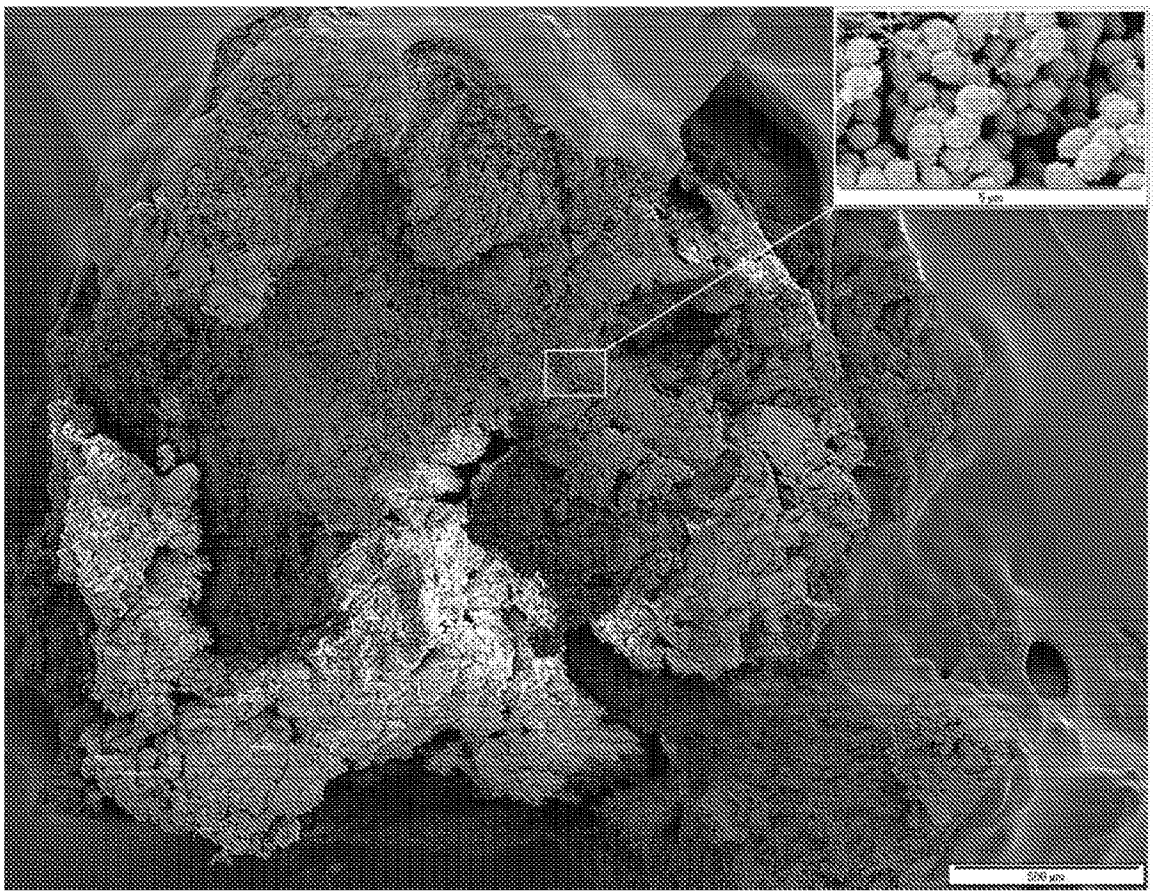


FIG. 1

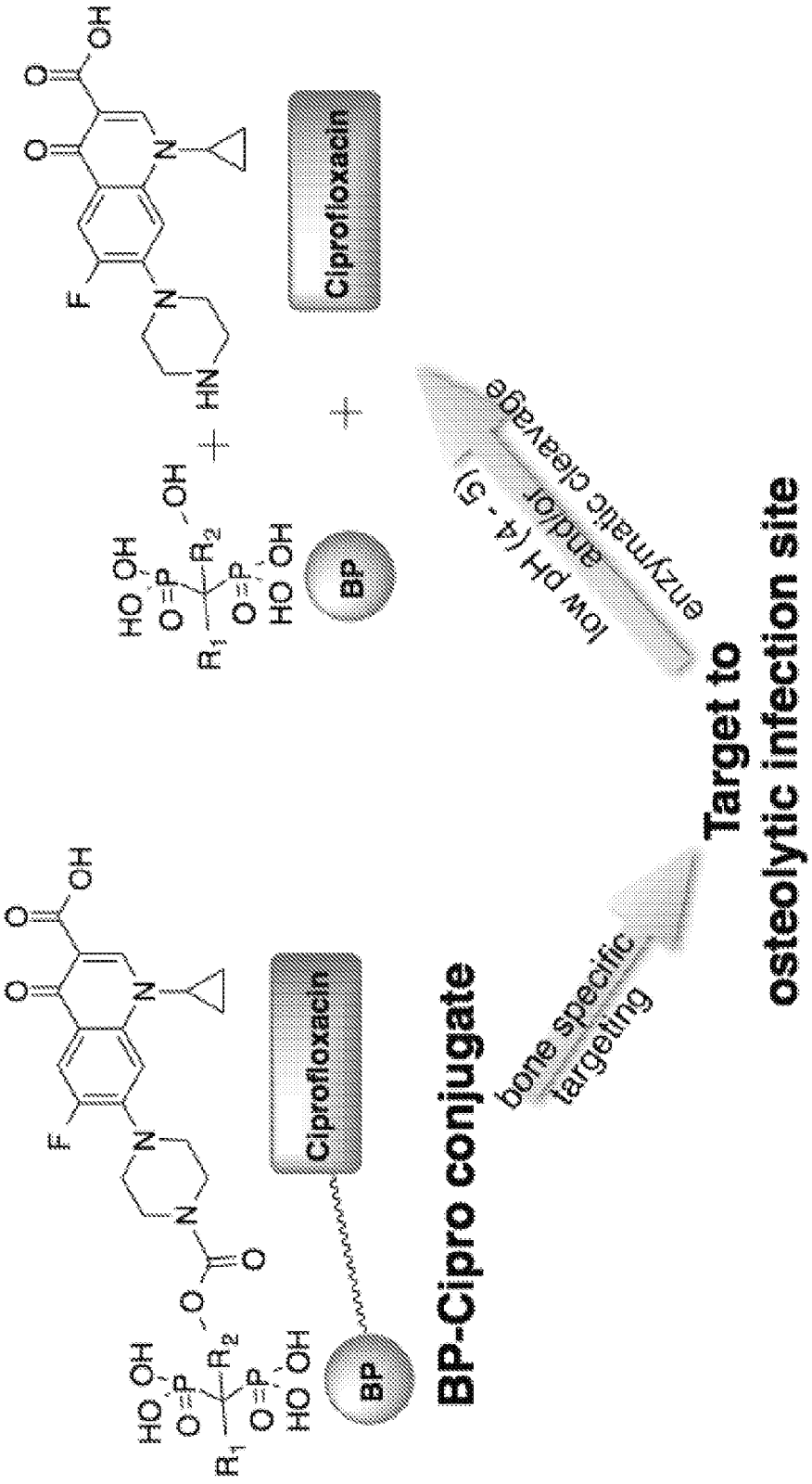

Ciprofloxacin
BP
low pH (4 - 5)
and/or
enzymatic cleavage
Target to
osteolytic infection site
BP-Cipro conjugate
bone specific
targeting
Ciprofloxacin
BP

FIG. 2

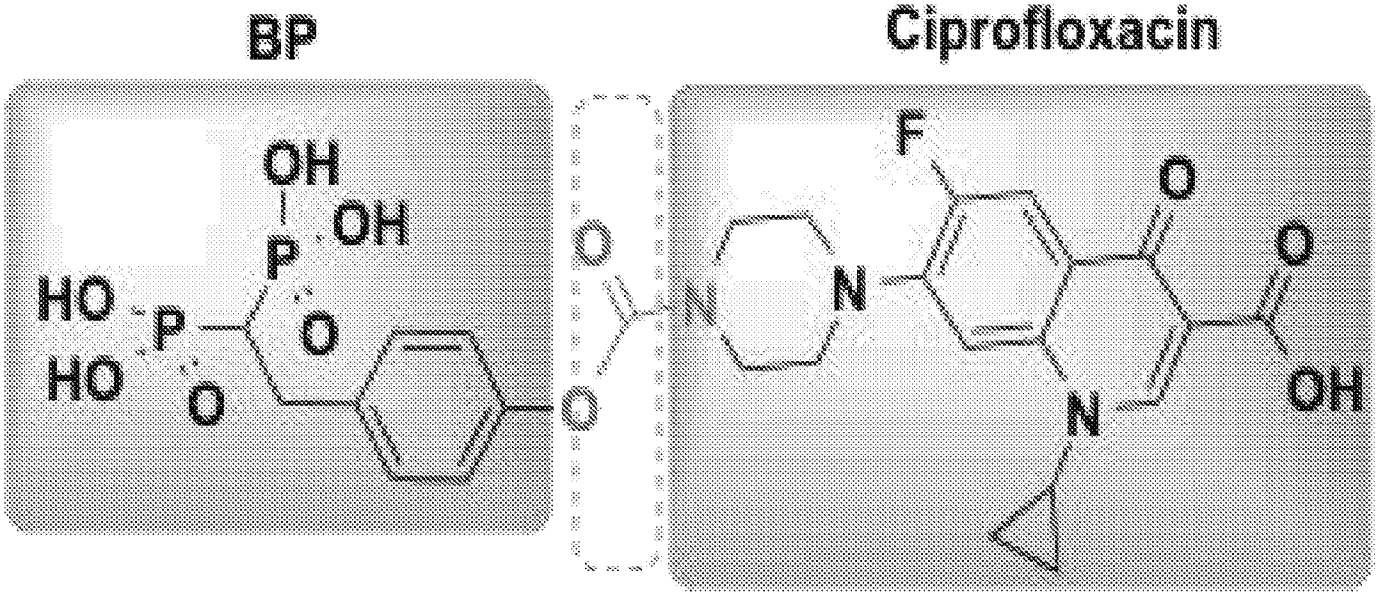


FIG. 3

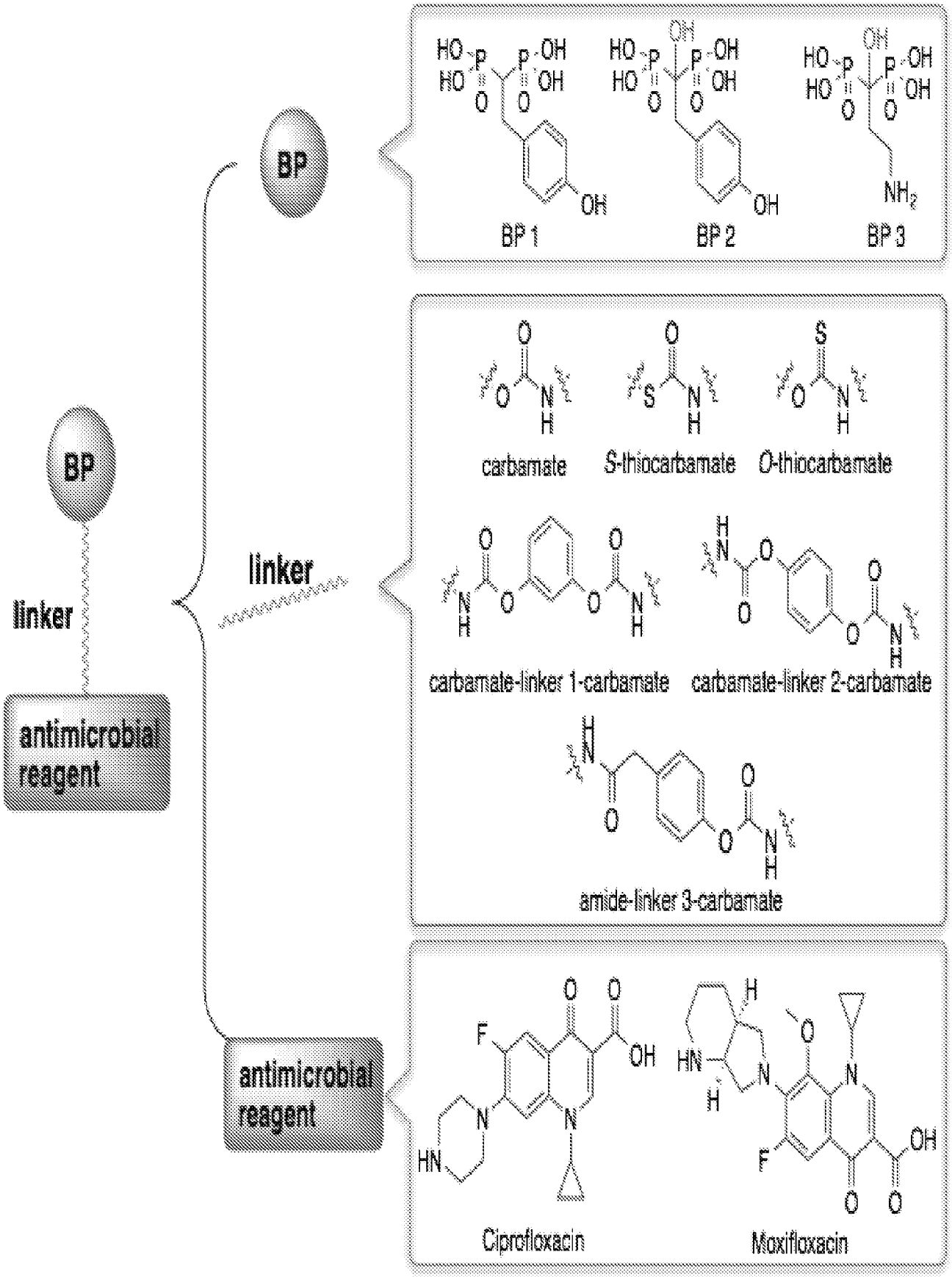

BP
linker
antimicrobial reagent
BP
linker
antimicrobial reagent
BP 1
BP 2
BP 3
carbamate
S-thiocarbamate
O-thiocarbamate
carbamate-linker 1-carbamate
carbamate-linker 2-carbamate
amide-linker 3-carbamate
Ciprofloxacin
Moxifloxacin

FIG. 4

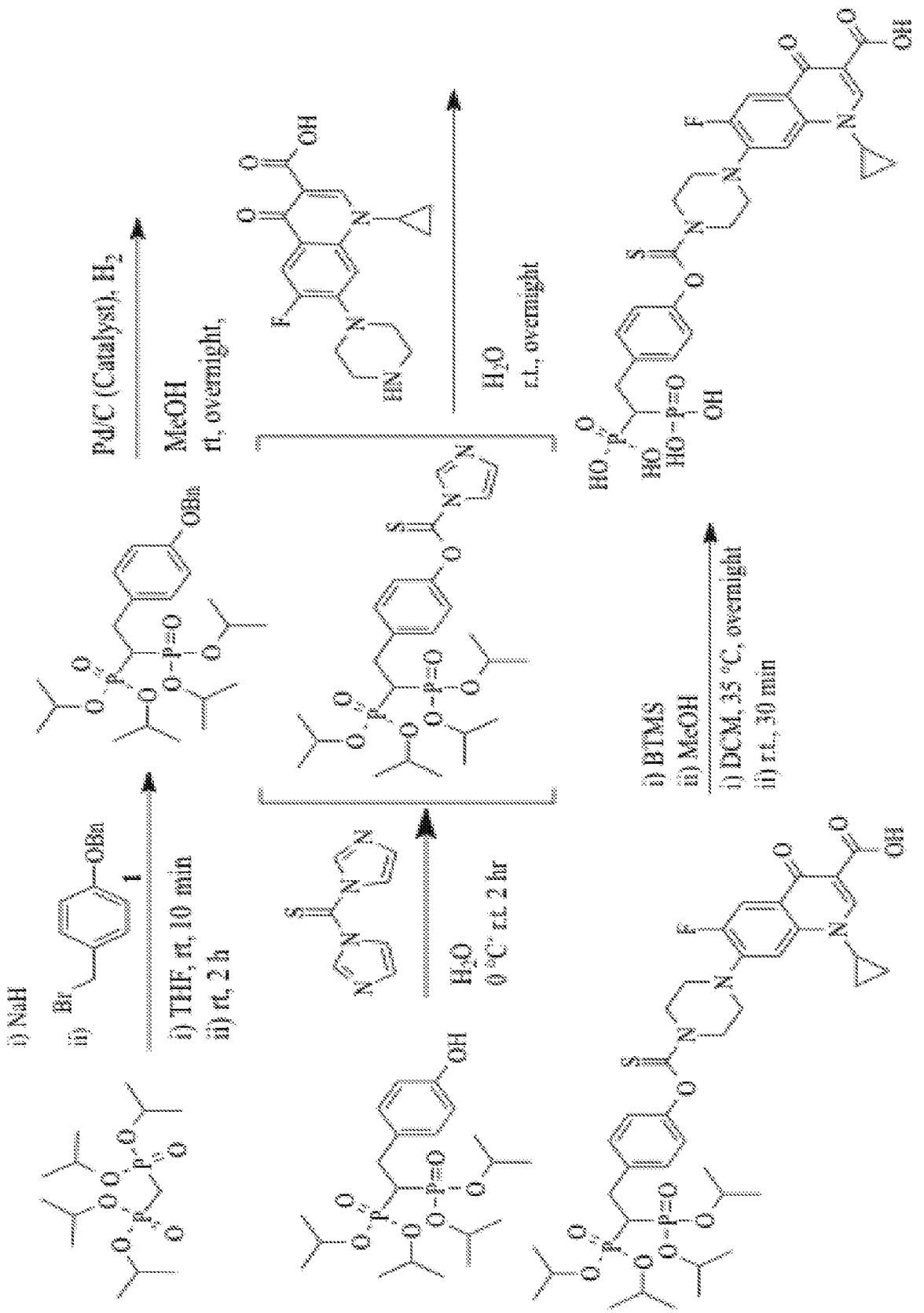

i) NaH
ii)
Br
OBn
1
i) THF, rt, 10 min
ii) rt, 2 h
Pd/C (Catalyst), H2
MeOH
rt, overnight,
H2O
0 °C r.t. 2 hr
H2O
r.t., overnight
i) BTMS
ii) MeOH
i) DCM, 35 °C, overnight
ii) r.t., 30 min
HN
F
OH
HO-P=O

FIG. 5

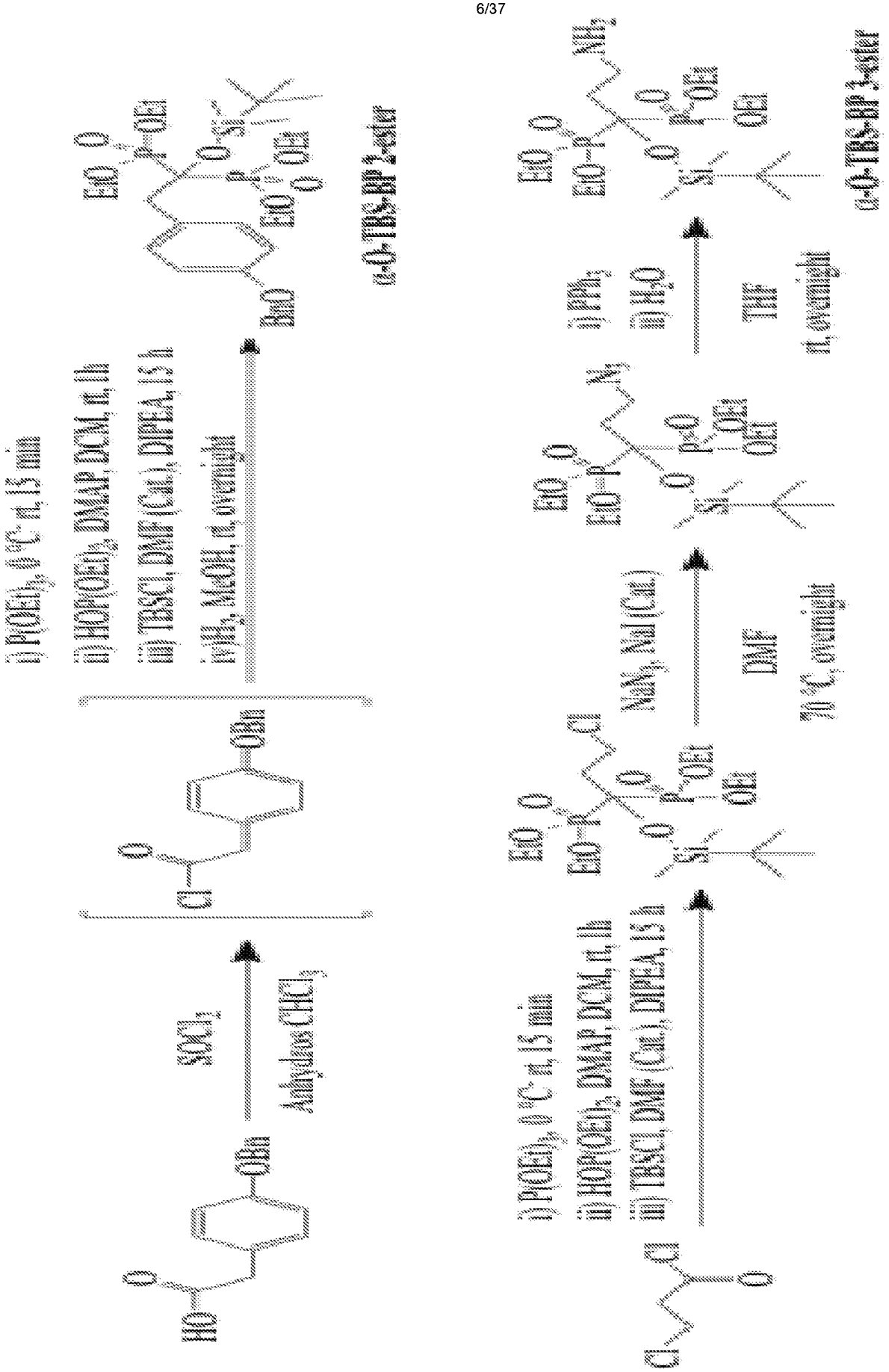


FIG. 6

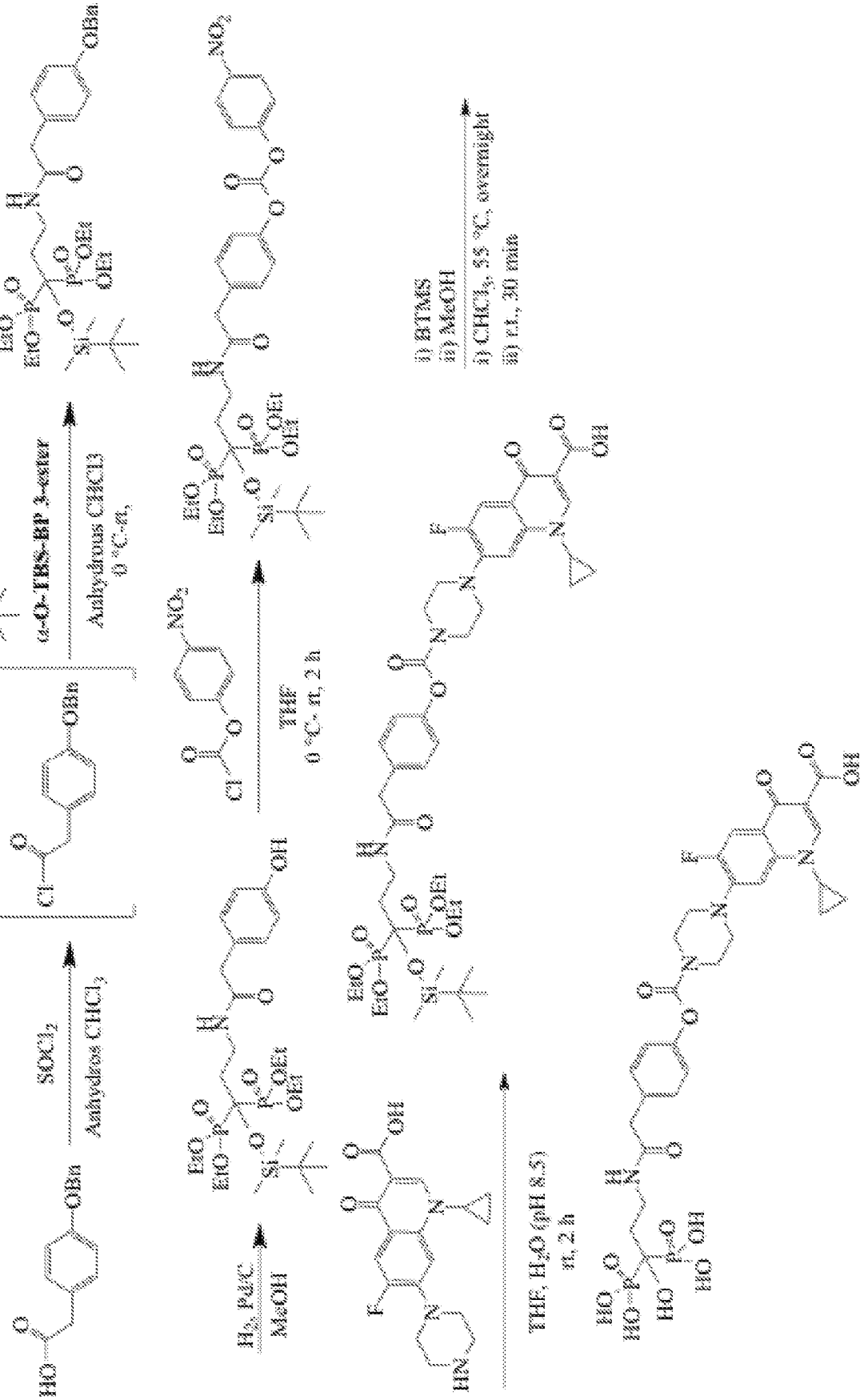


FIG. 7

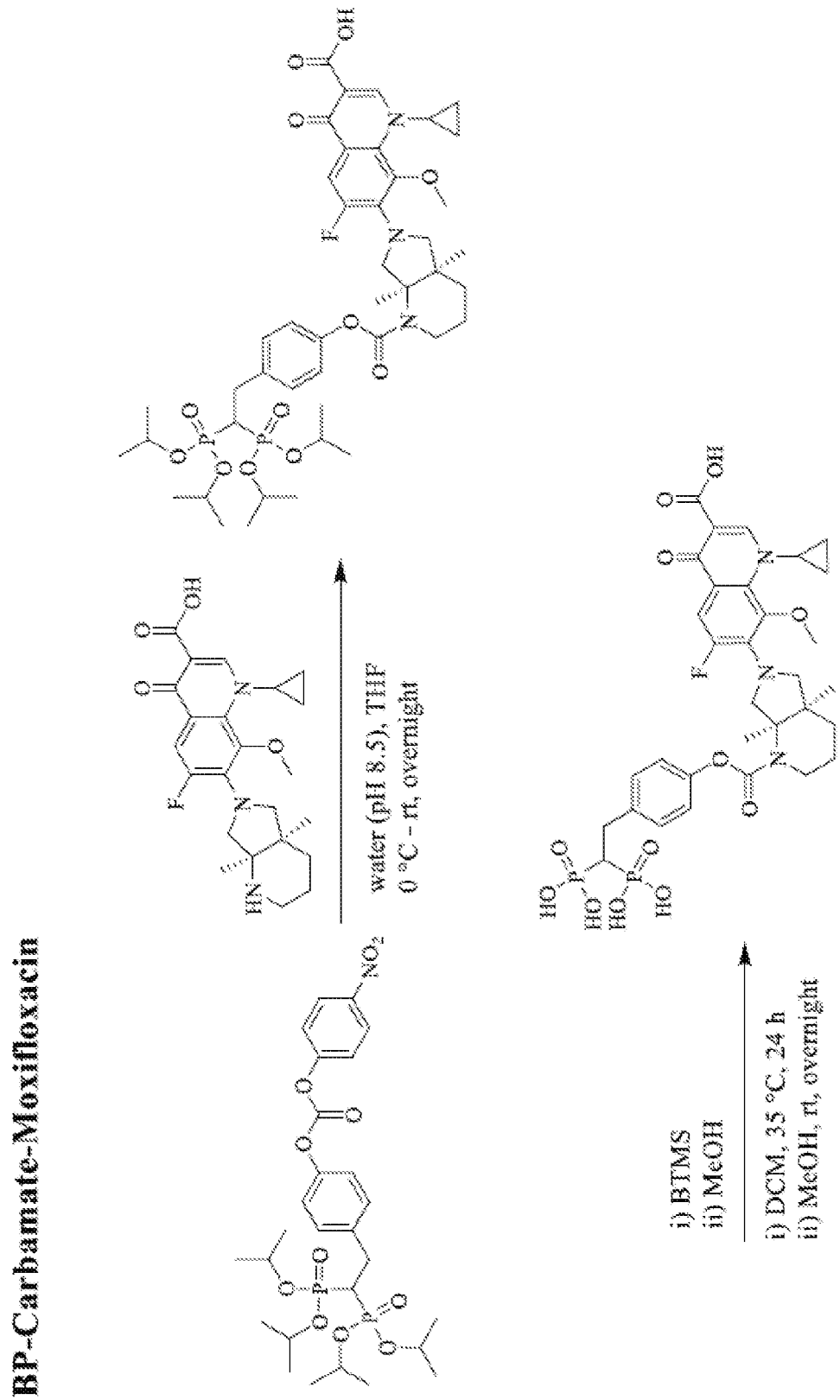

BP-Carbamate-Moxifloxacin
water (pH 8.5), THF
0 °C – rt, overnight
i) BTMS
ii) MeOH
i) DCM, 35 °C, 24 h
ii) MeOH, rt, overnight

FIG. 8

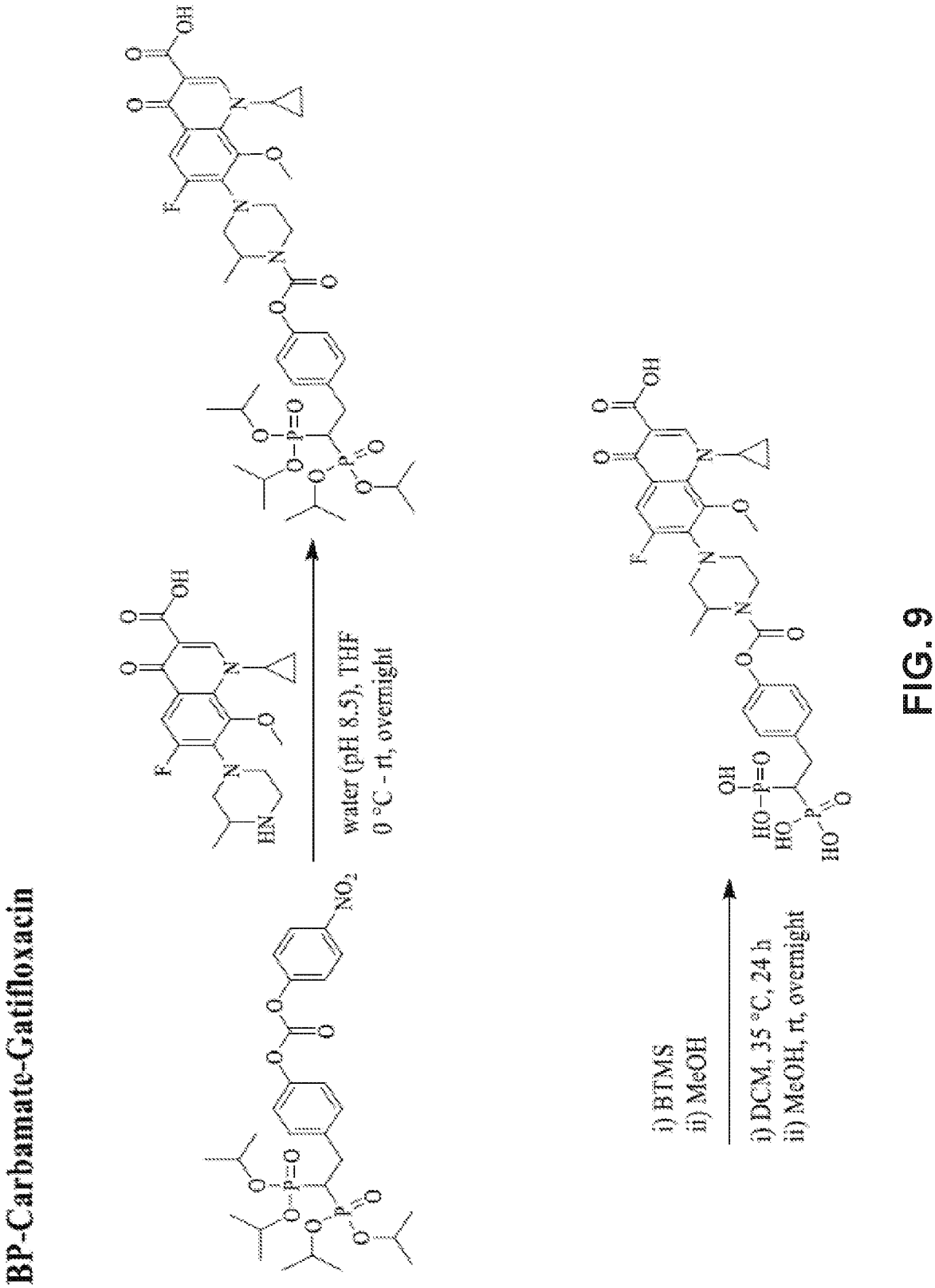

BP-Carbamate-Gatifloxacin
water (pH 8.5), THF
0 °C - rt, overnight
i) BTMS
ii) MeOH
i) DCM, 35 °C, 24 h
ii) MeOH, rt, overnight

FIG. 9

BP-p-Hydroxyphenyl Acetic Acid-Cipro

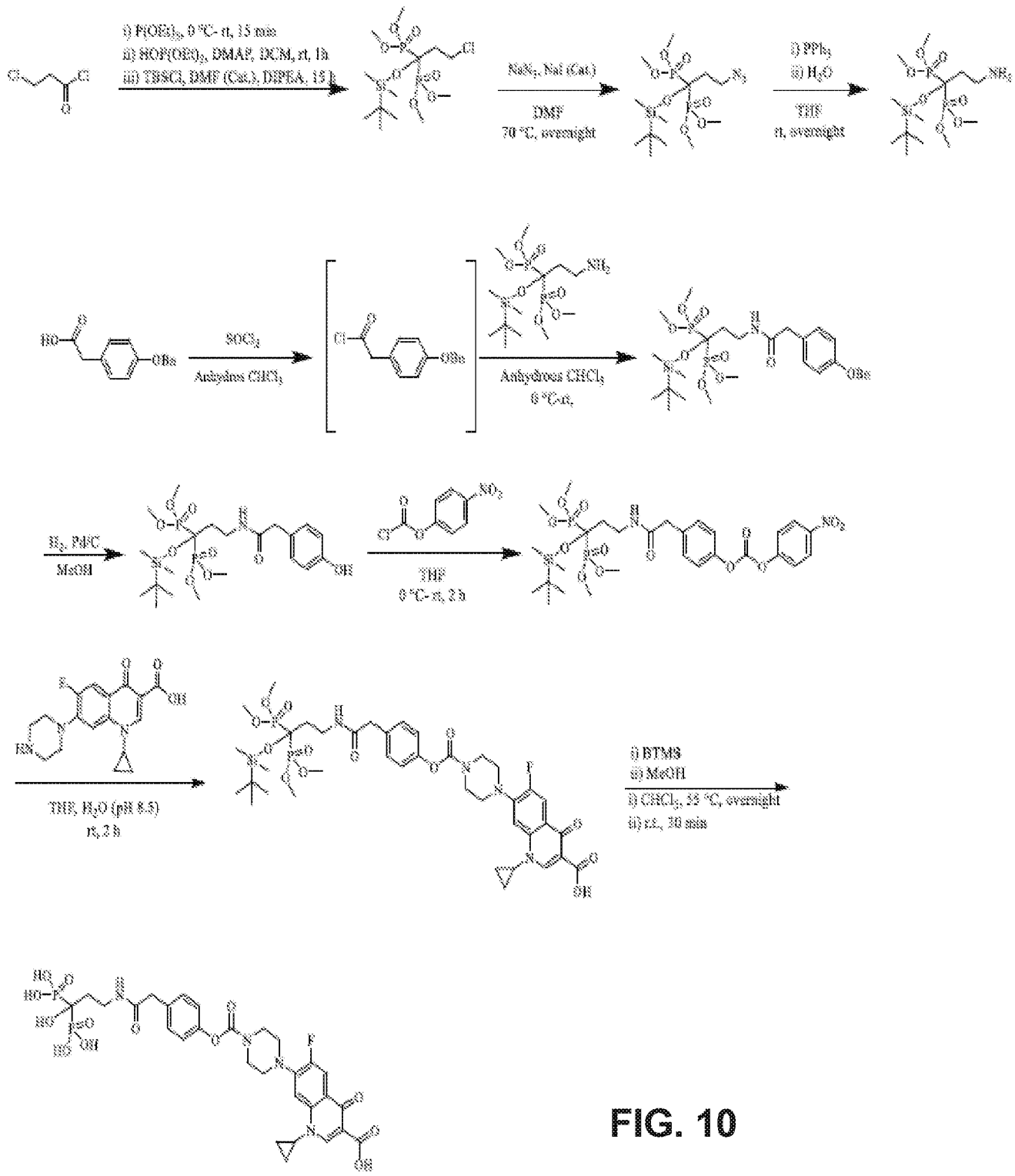


FIG. 10

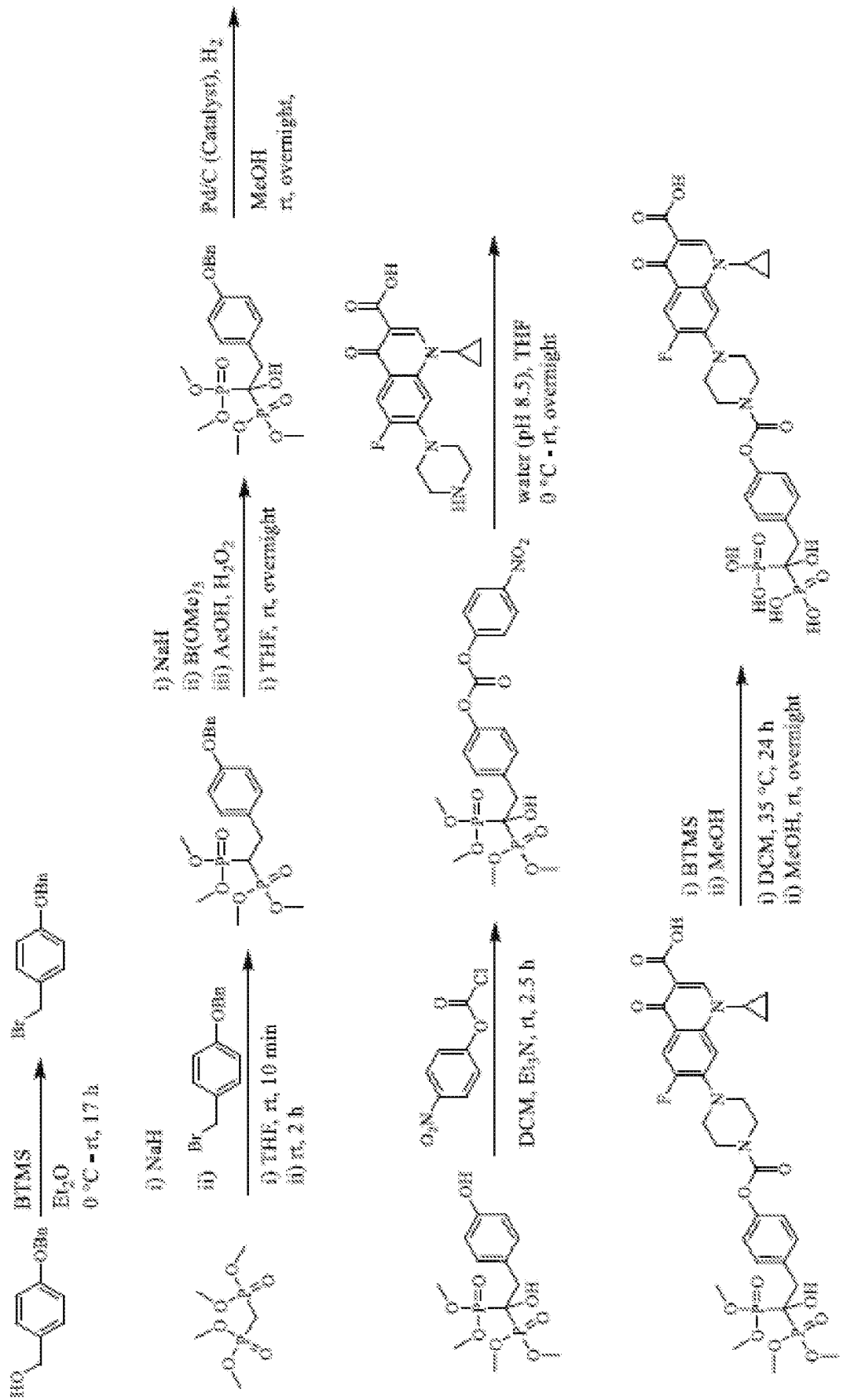


FIG. 11

BP-*O*-Thiocarbamate-Cipro

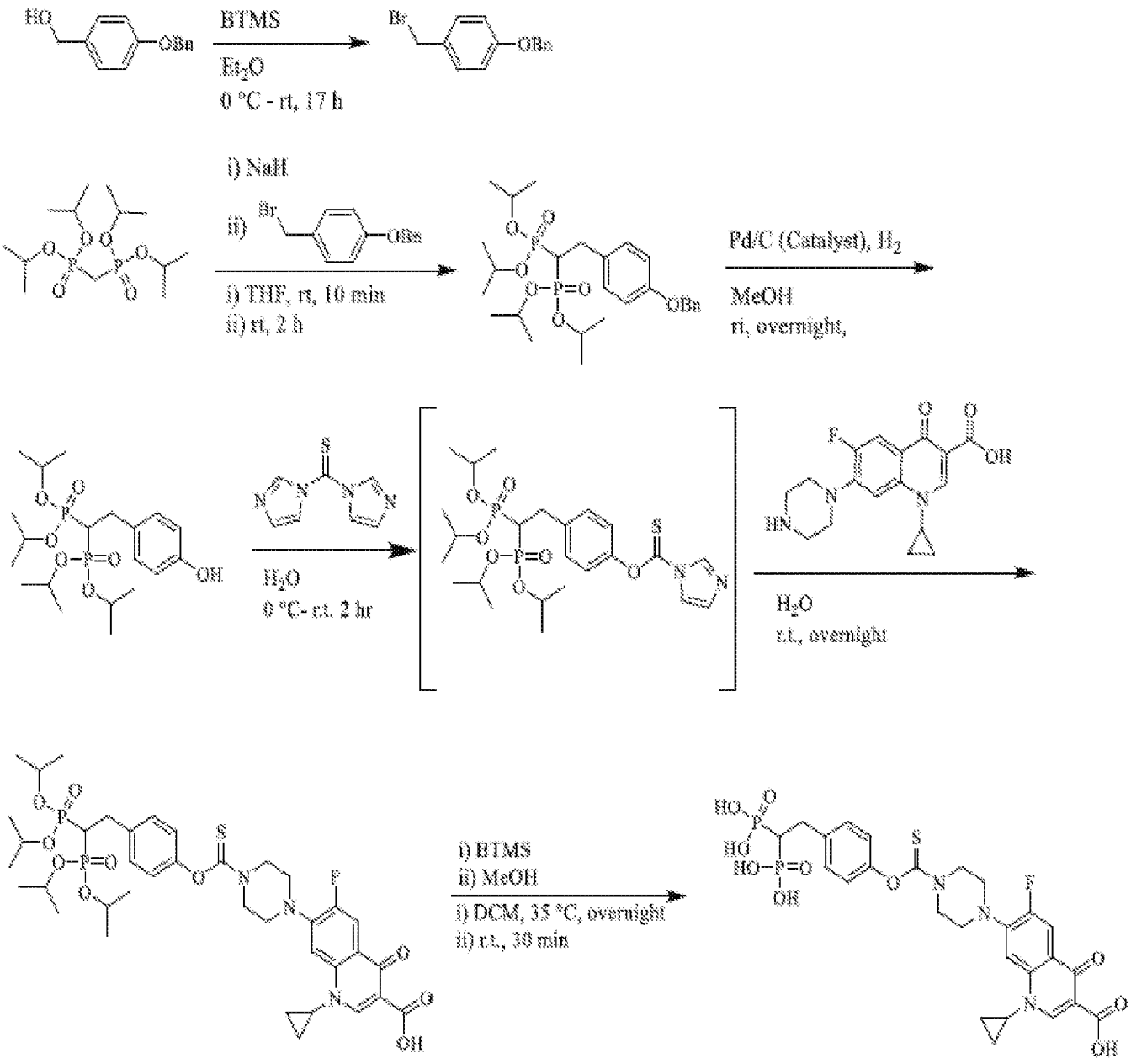


FIG. 12

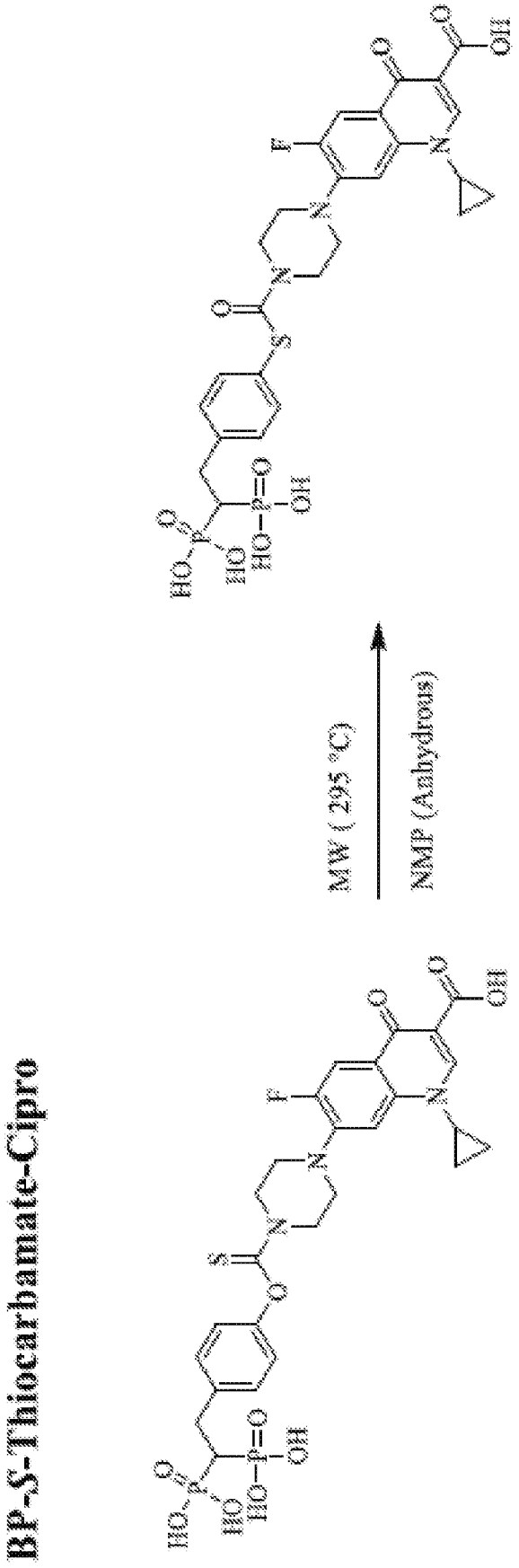


FIG. 13

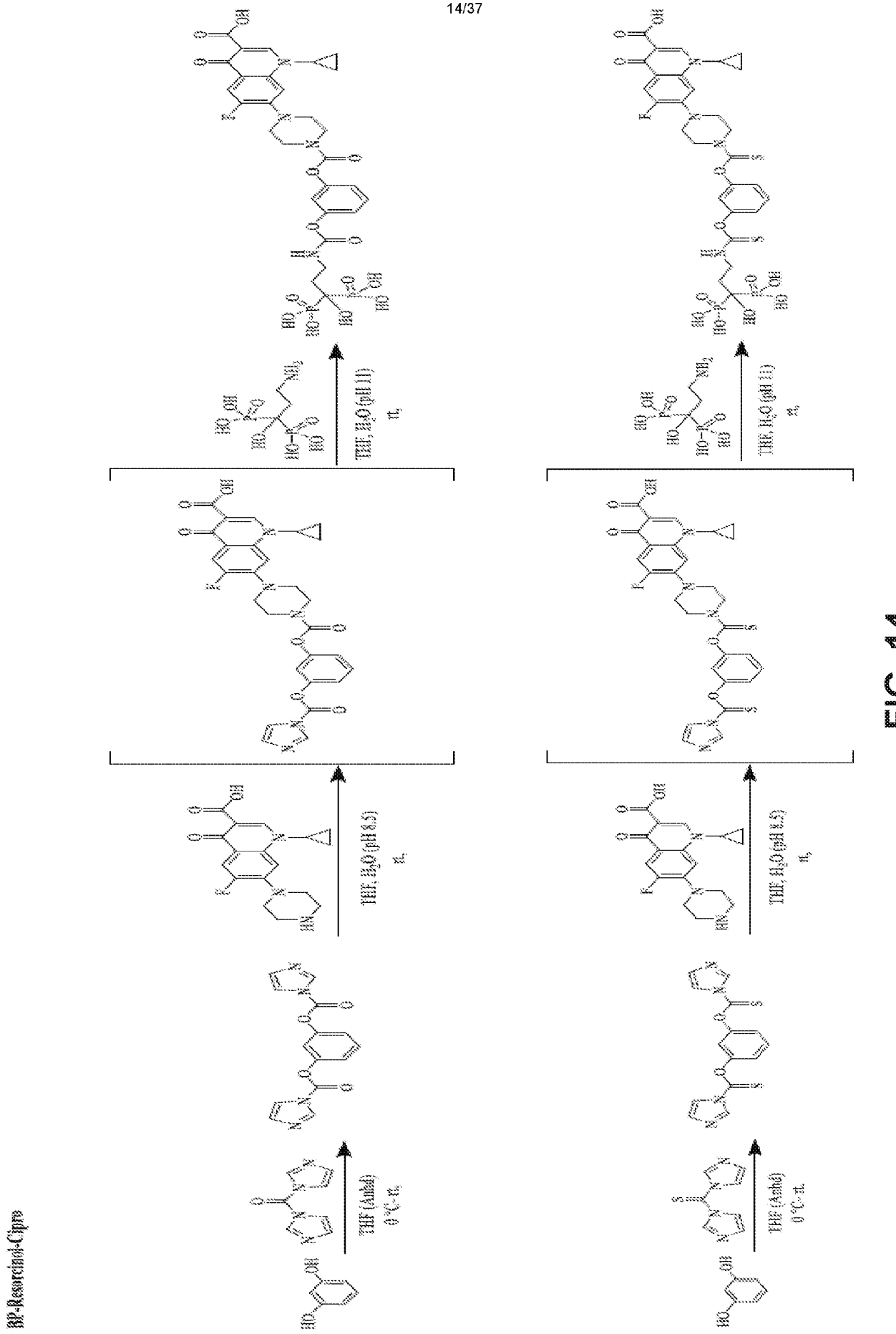

BP-Rosuvastatin-Cipro
THF (Anhd)
0 °C, rt,
THF, H2O (pH 8.5)
rt,
THF, H2O (pH 11)
rt,

FIG. 14

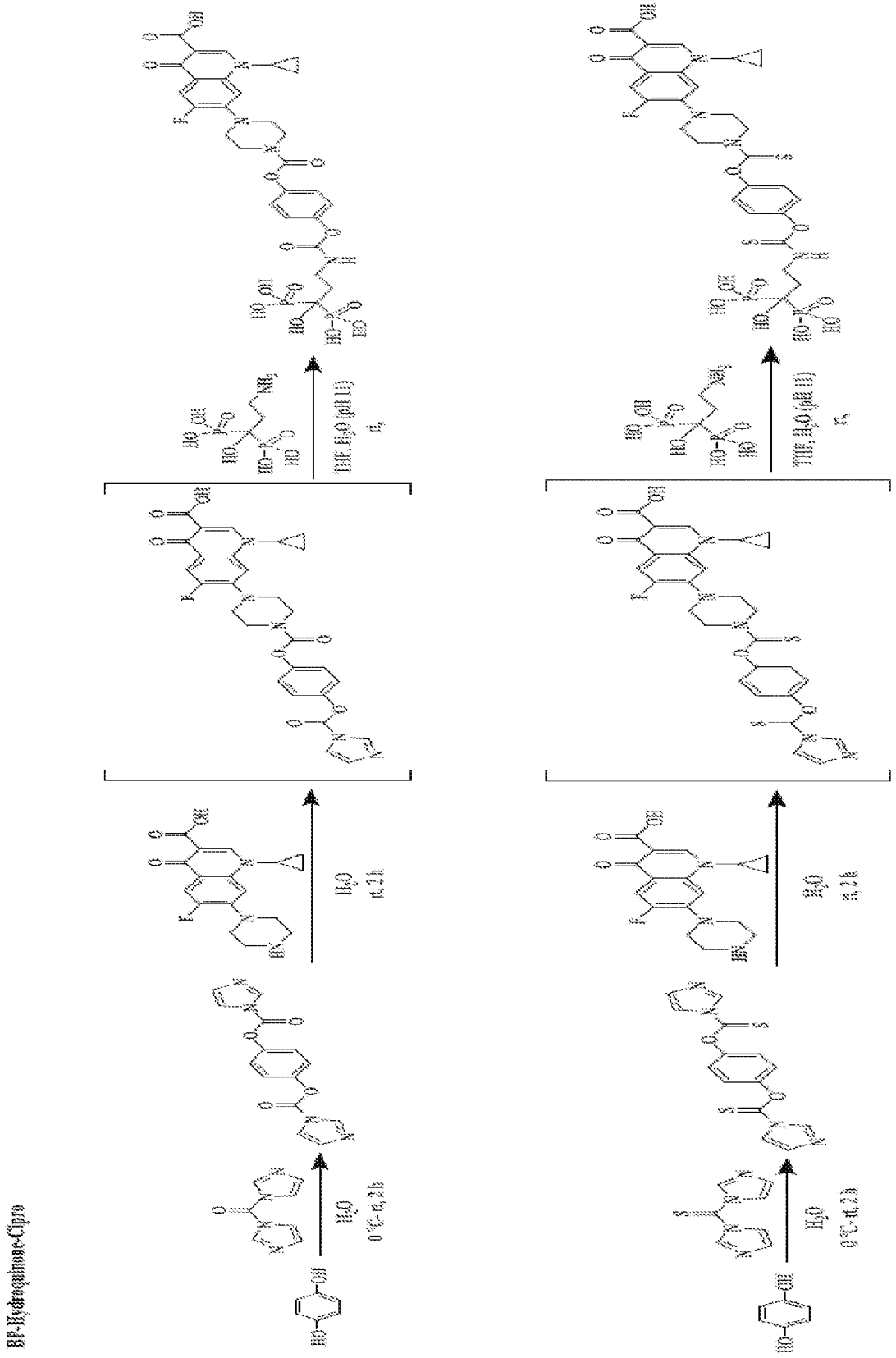


FIG. 15

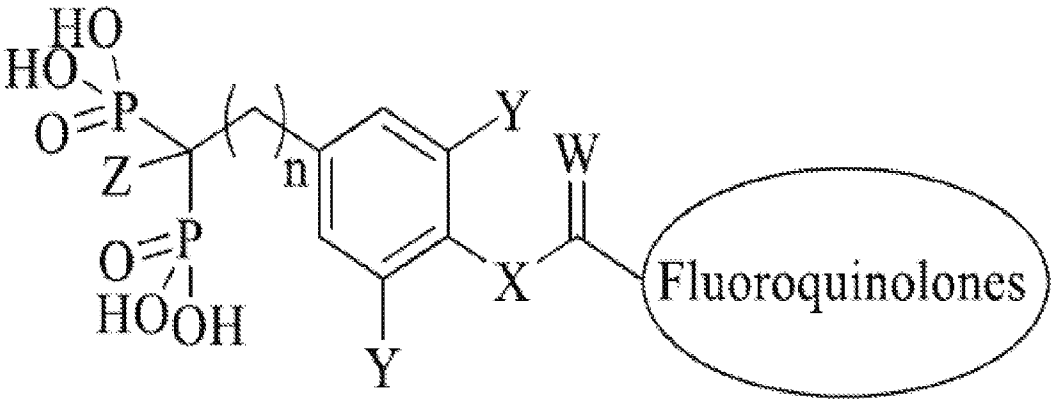

HO
HO
O=P
Z
O=P
HO OH
n
Y
W
X
Y
Fluoroquinolones

FIG. 16

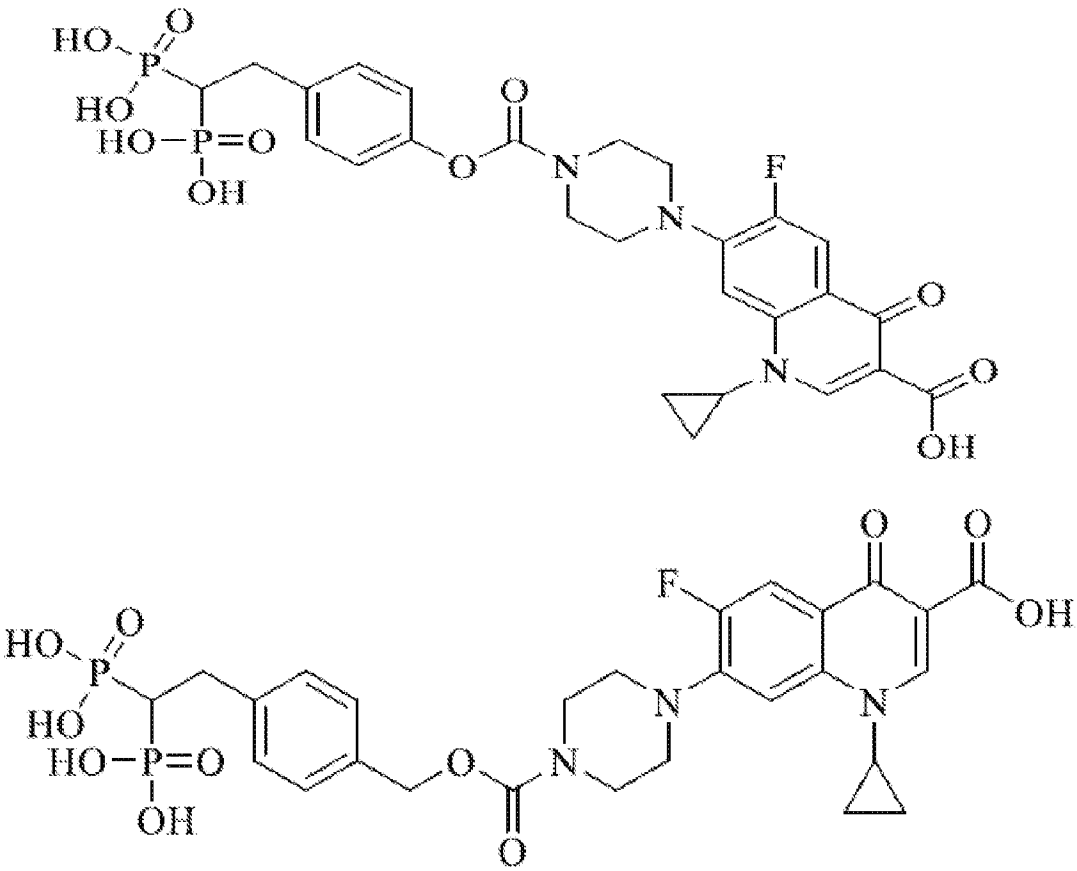

HO
HO
HO—P=O
OH
O
N
N
F
O
N
O
OH
HO
HO
HO—P=O
OH
O
O
F
N
N
O
N
O
O
OH

FIG. 17

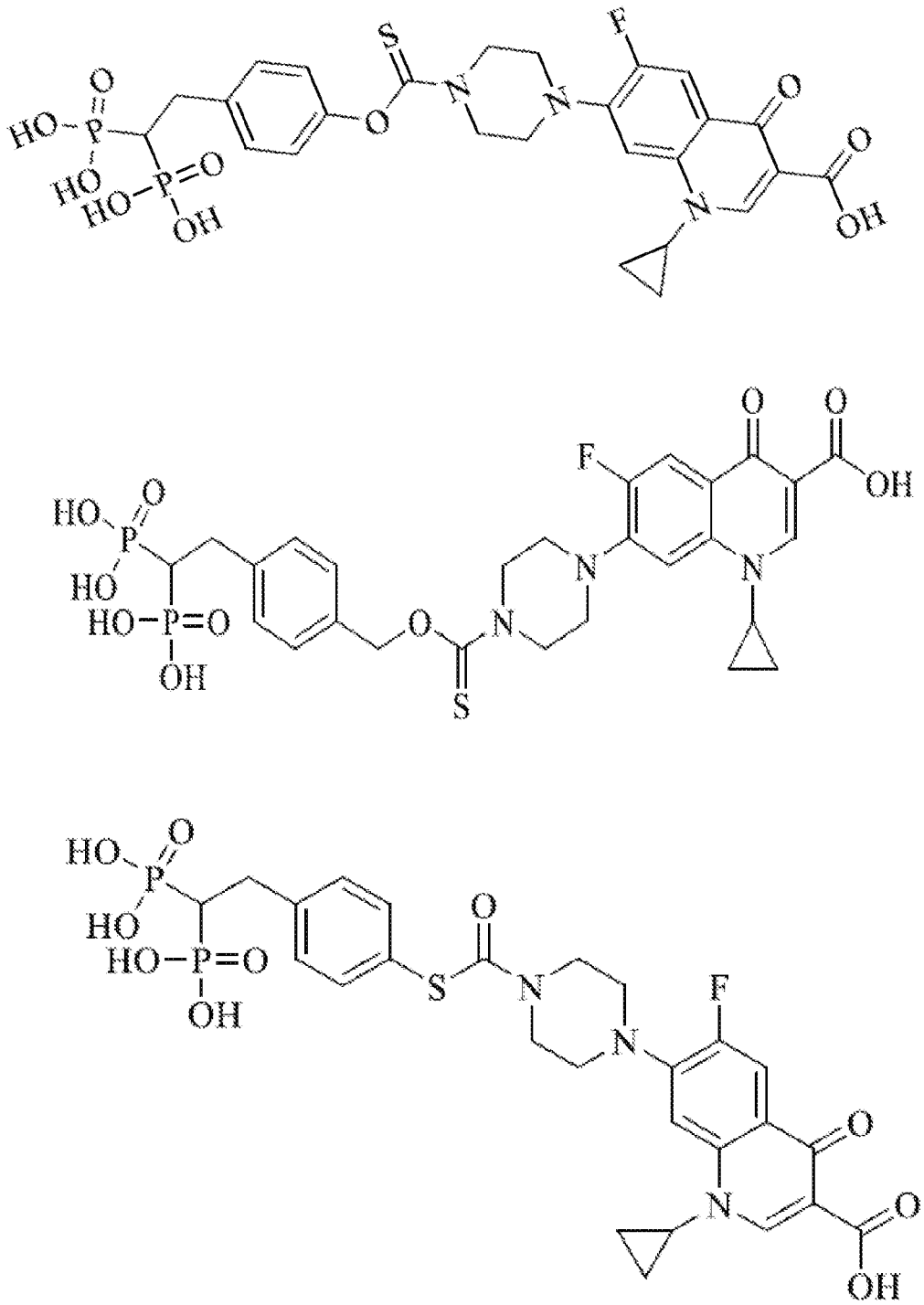


FIG. 17 ctd.

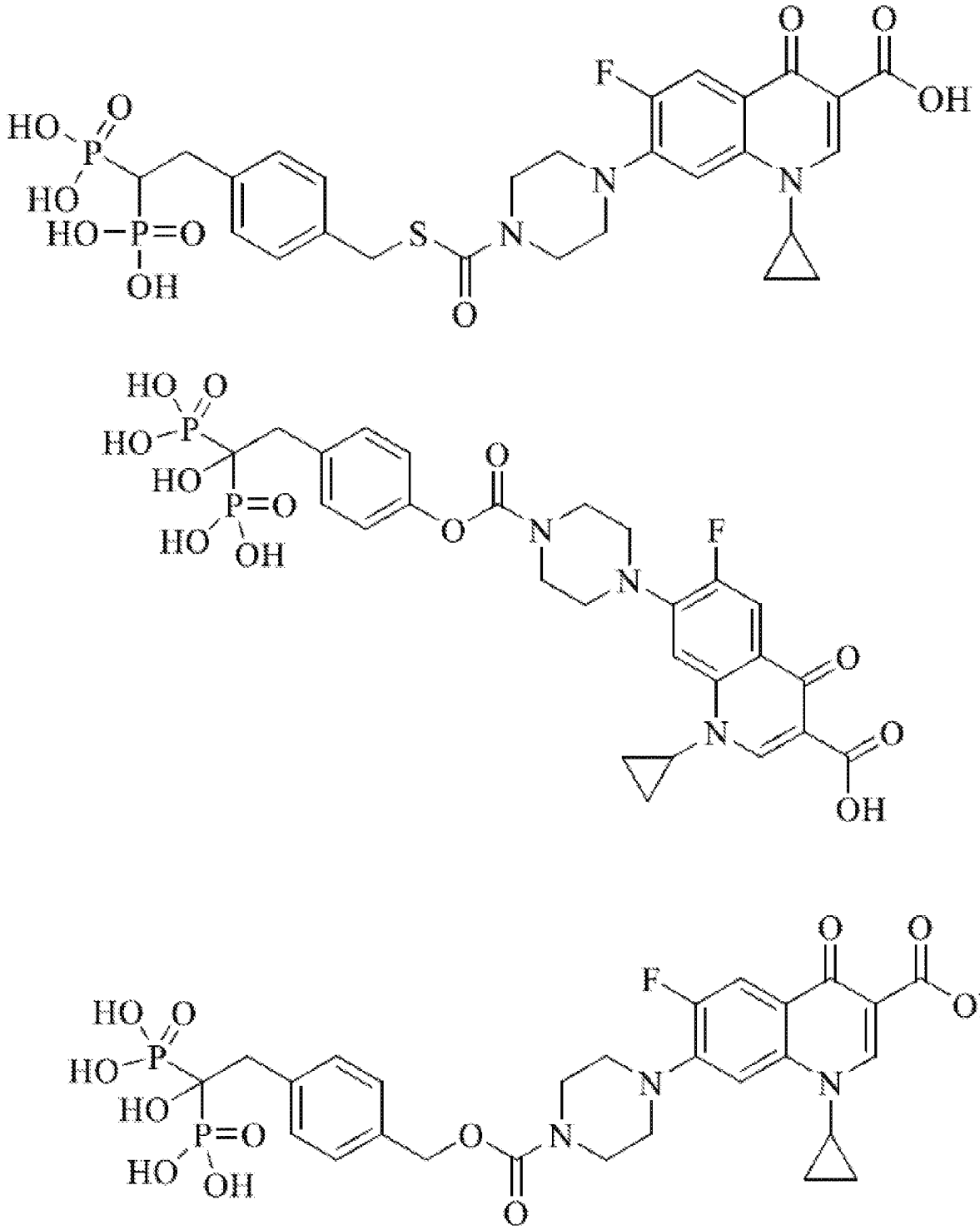


FIG. 17 ctd.

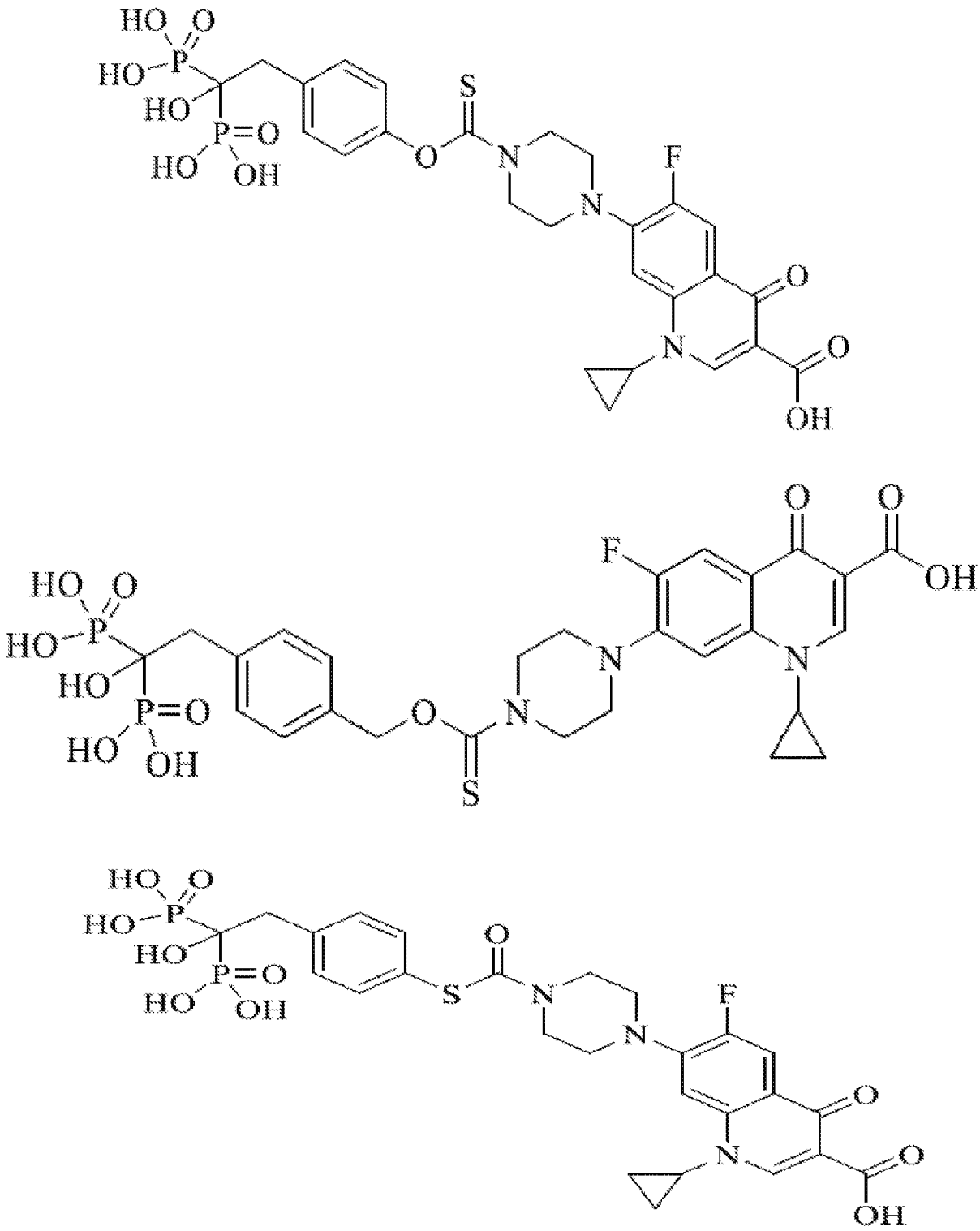


FIG. 17 ctd.

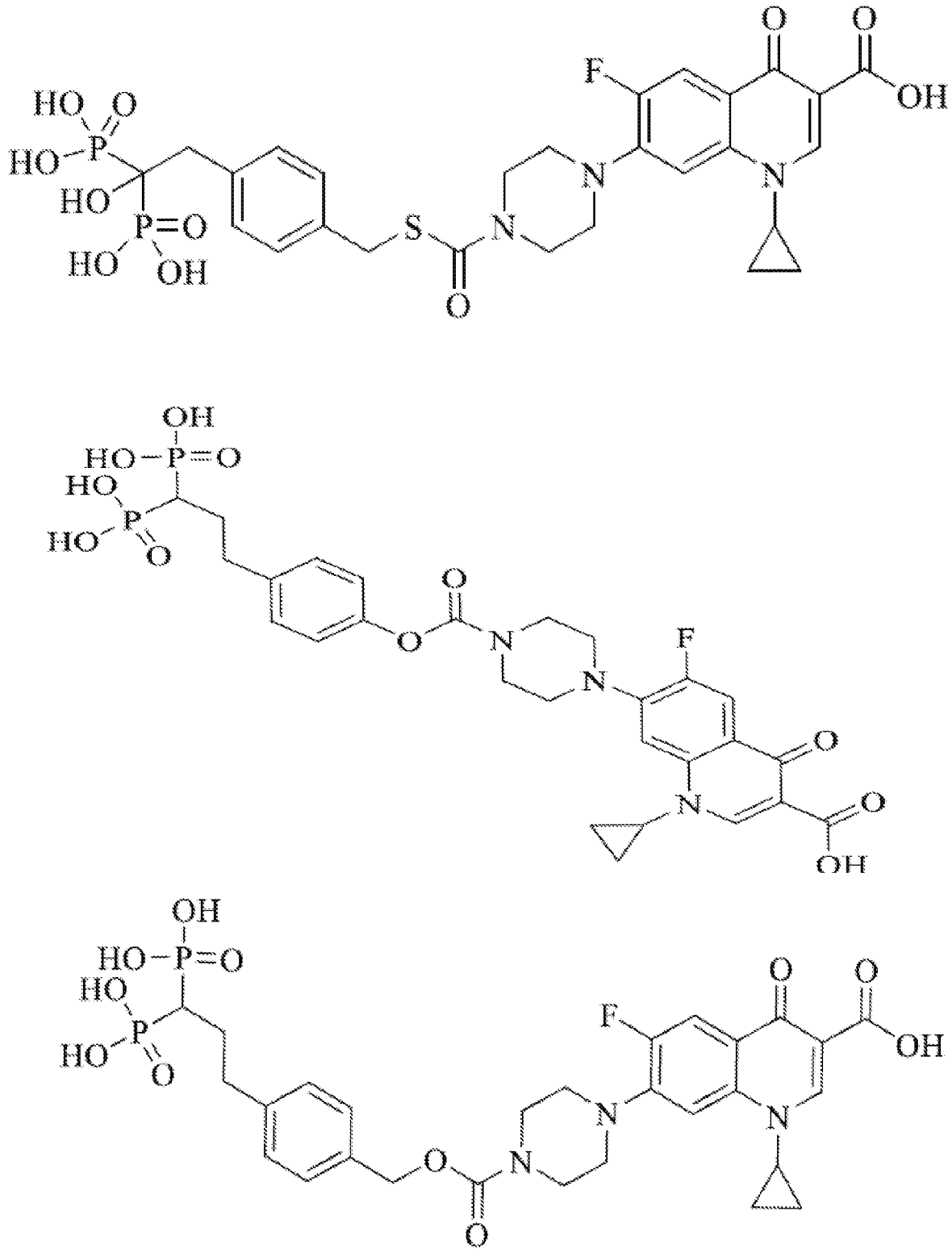
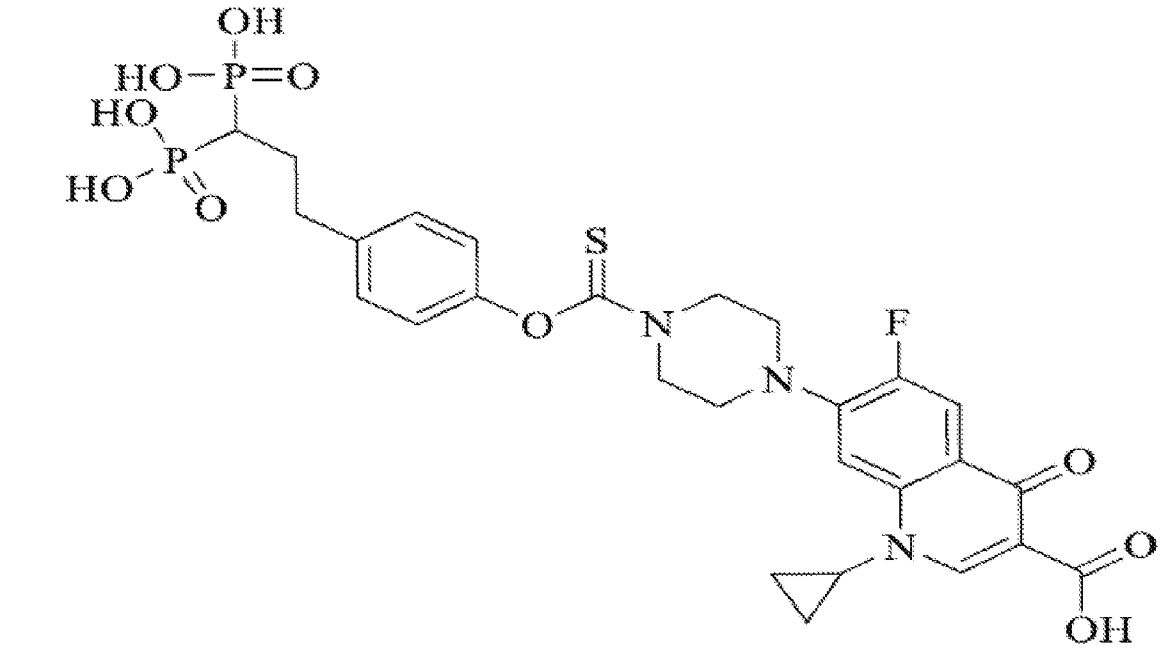


FIG. 17 ctd.



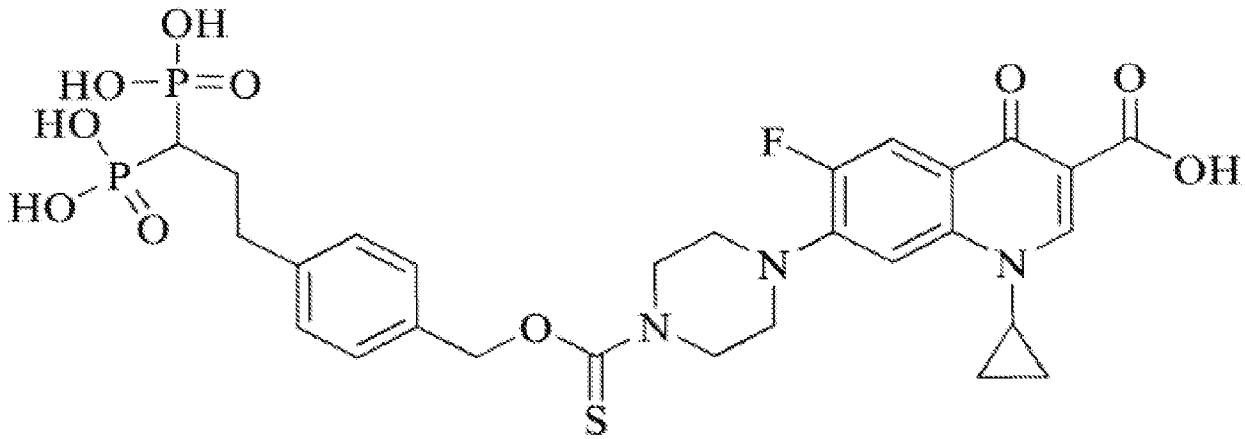
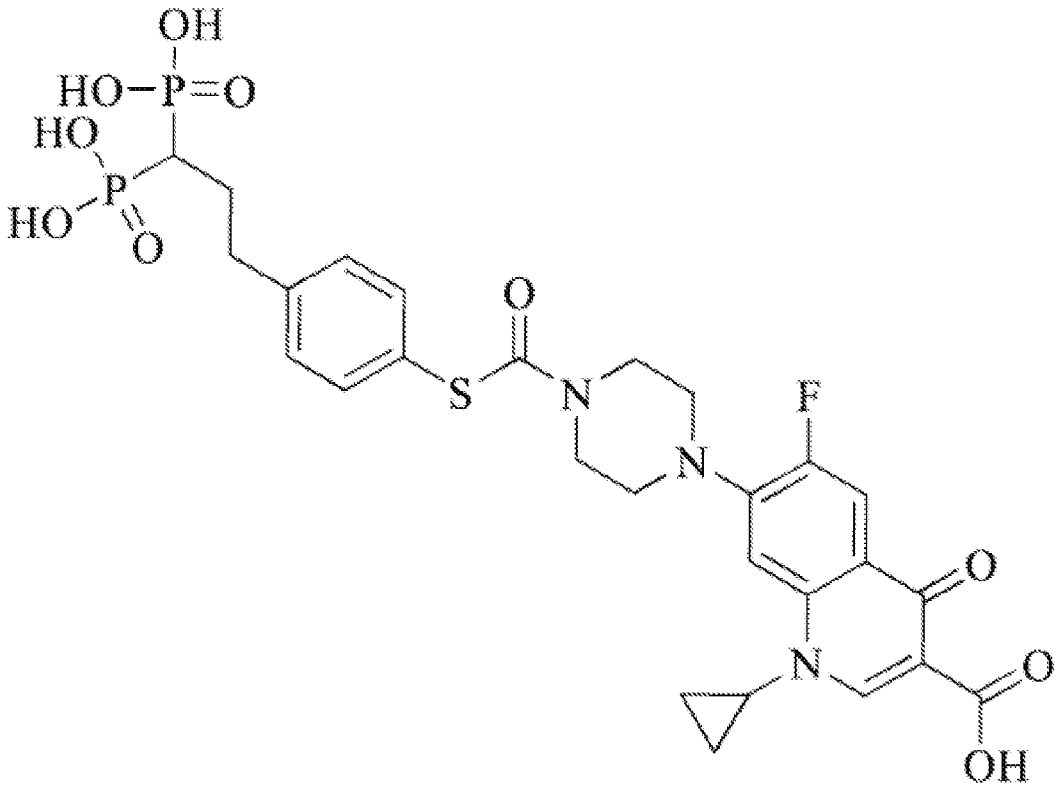


FIG. 17 ctd.



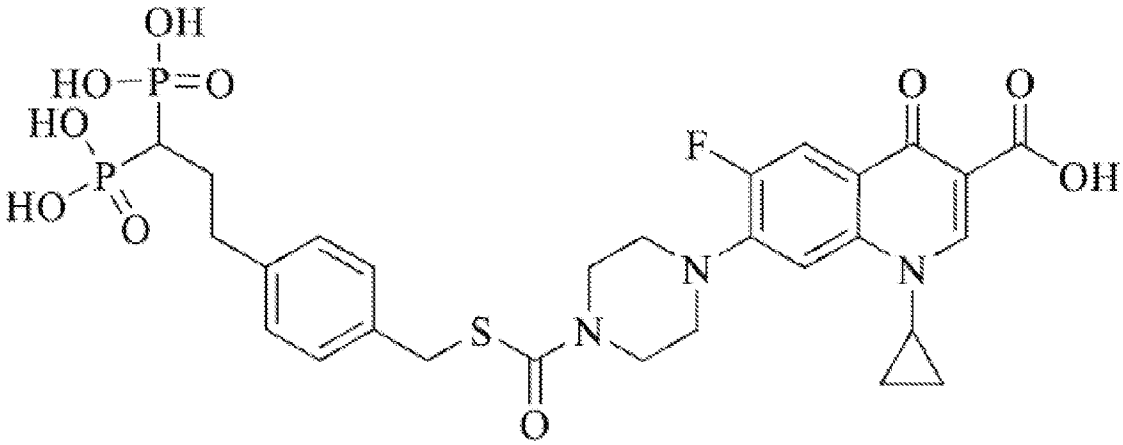


FIG. 17 ctd.

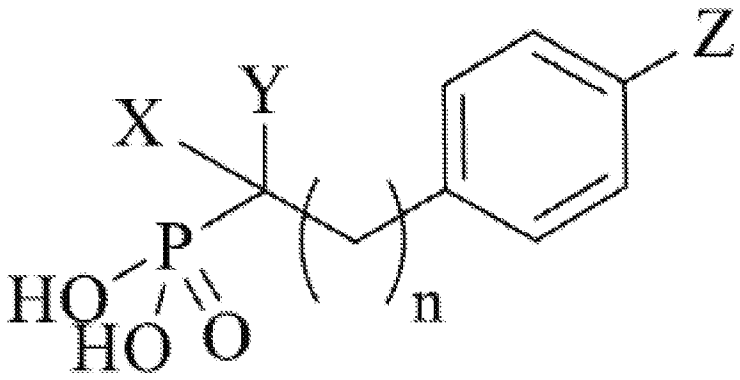

Z
Y
X
P
HO
HO
O
n

FIG. 18

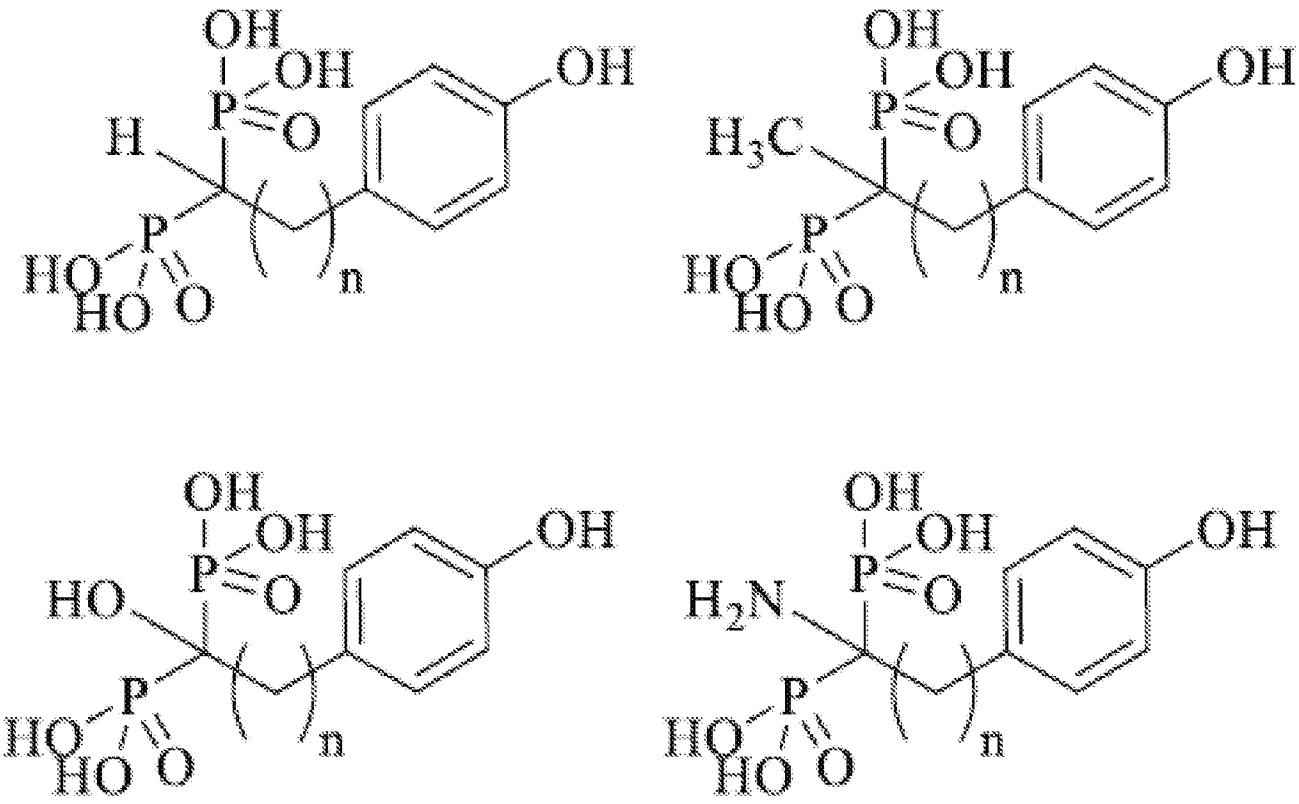

OH
OH
OH
P
O
H
P
HO
HO
O
n
OH
OH
OH
P
O
H3C
P
HO
HO
O
n
OH
OH
OH
P
O
HO
P
HO
HO
O
n
OH
OH
OH
P
O
H2N
P
HO
HO
O
n

FIG. 19

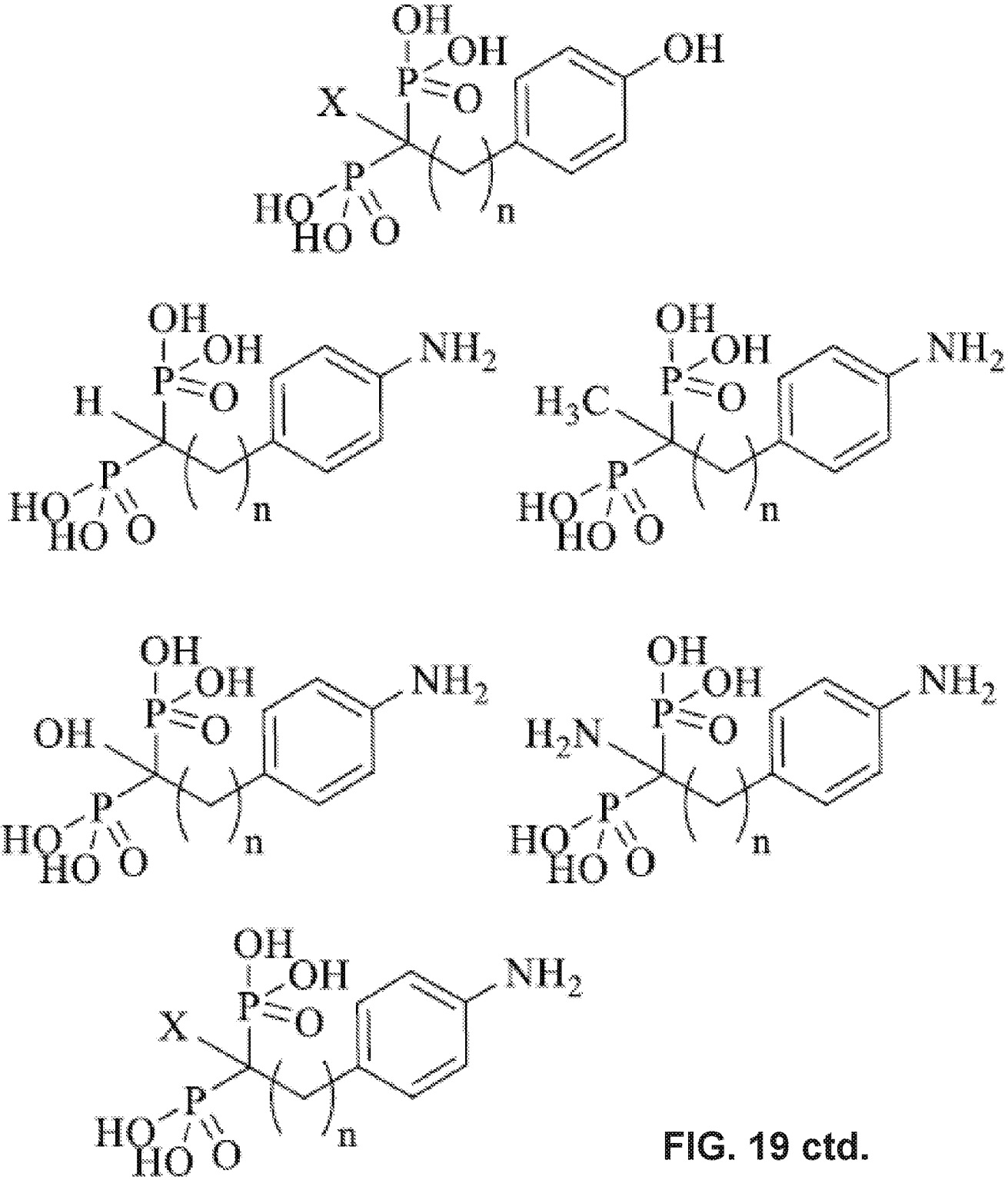


FIG. 19 ctd.

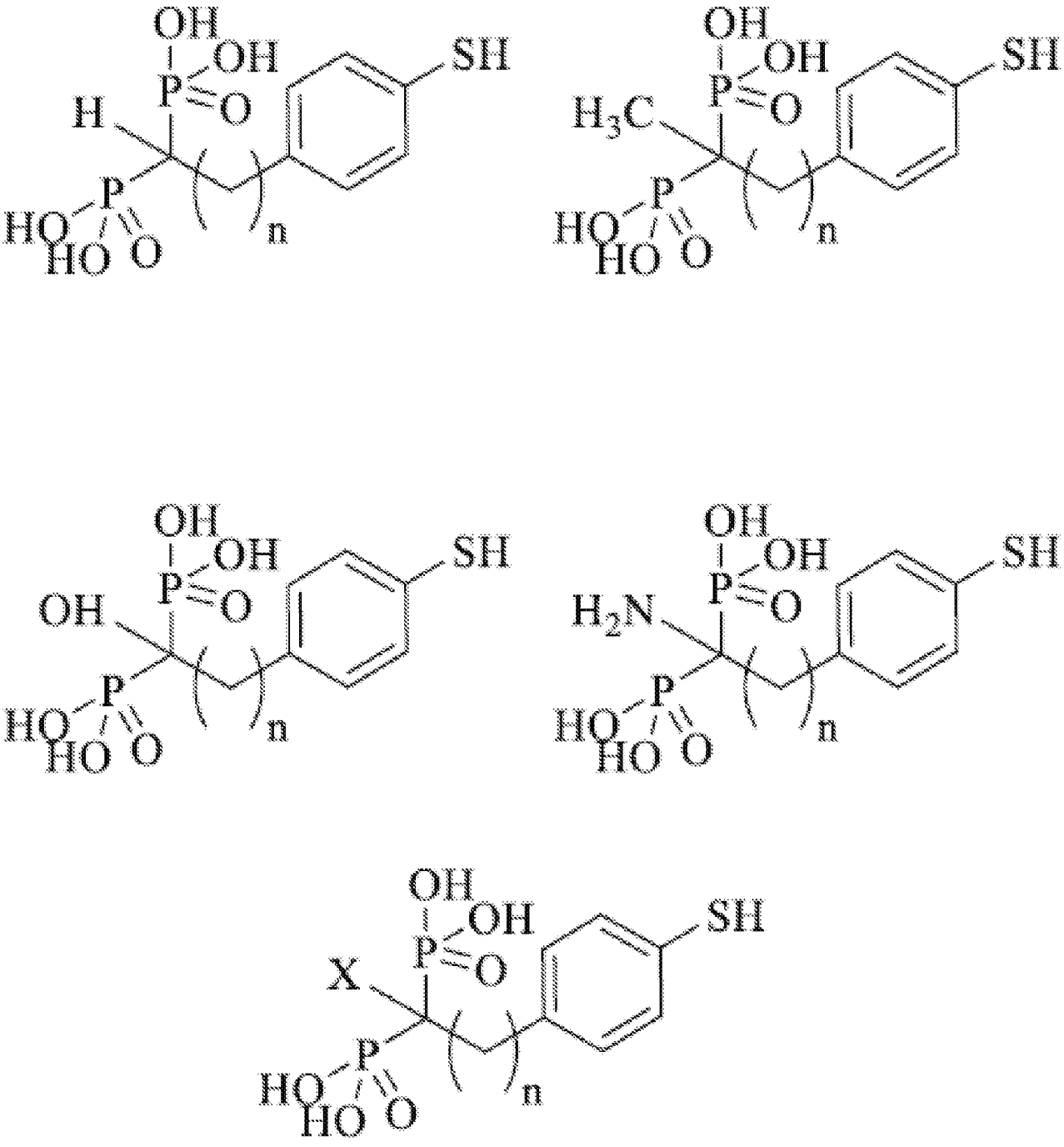

OH
OH
P
O
SH
H
HO
HO
O
n
OH
OH
P
O
SH
H3C
HO
HO
O
n
OH
OH
P
O
SH
OH
HO
HO
O
n
OH
OH
P
O
SH
H2N
HO
HO
O
n
OH
OH
P
O
SH
X
HO
HO
O
n

FIG. 19 ctd.

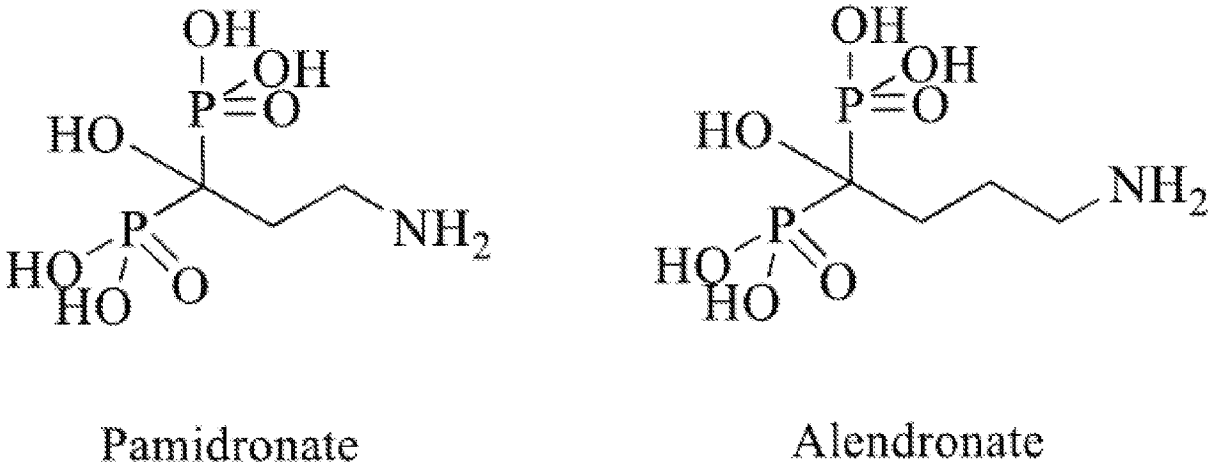

Pamidronate
Alendronate

FIG. 20

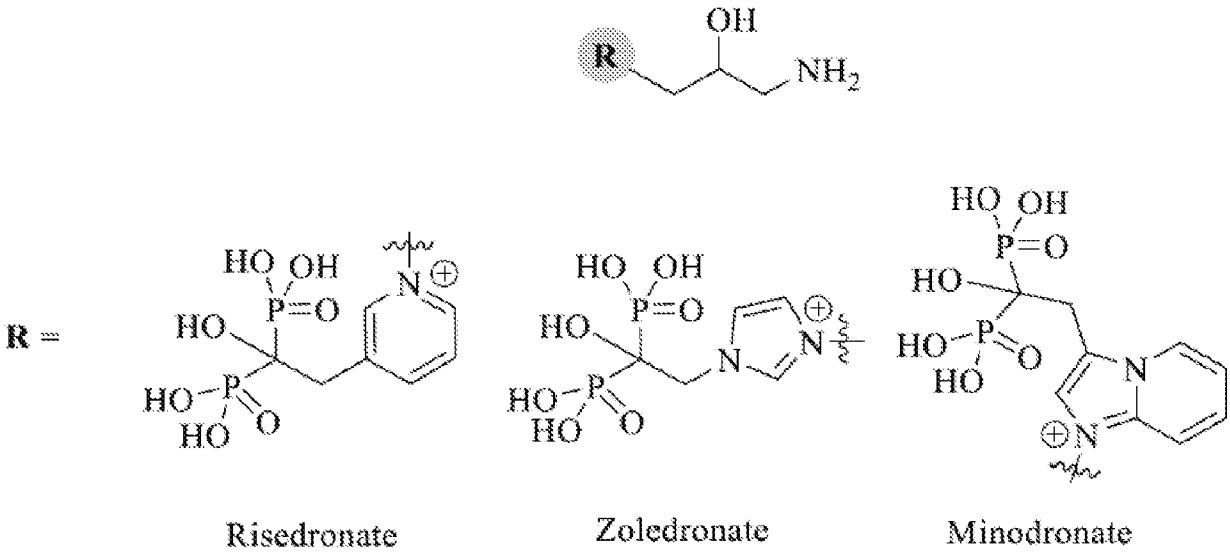
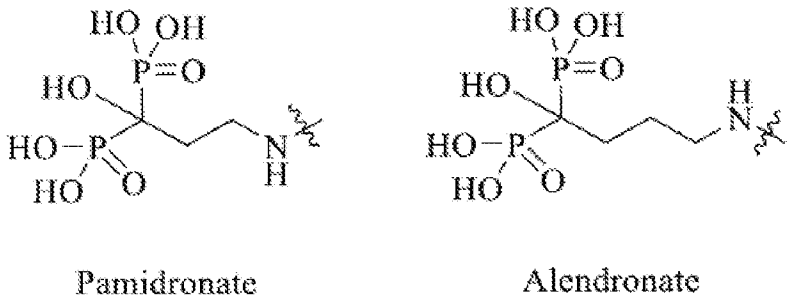

R =
Risedronate
Zoledronate
Minodronate

FIG. 21


Pamidronate
Alendronate

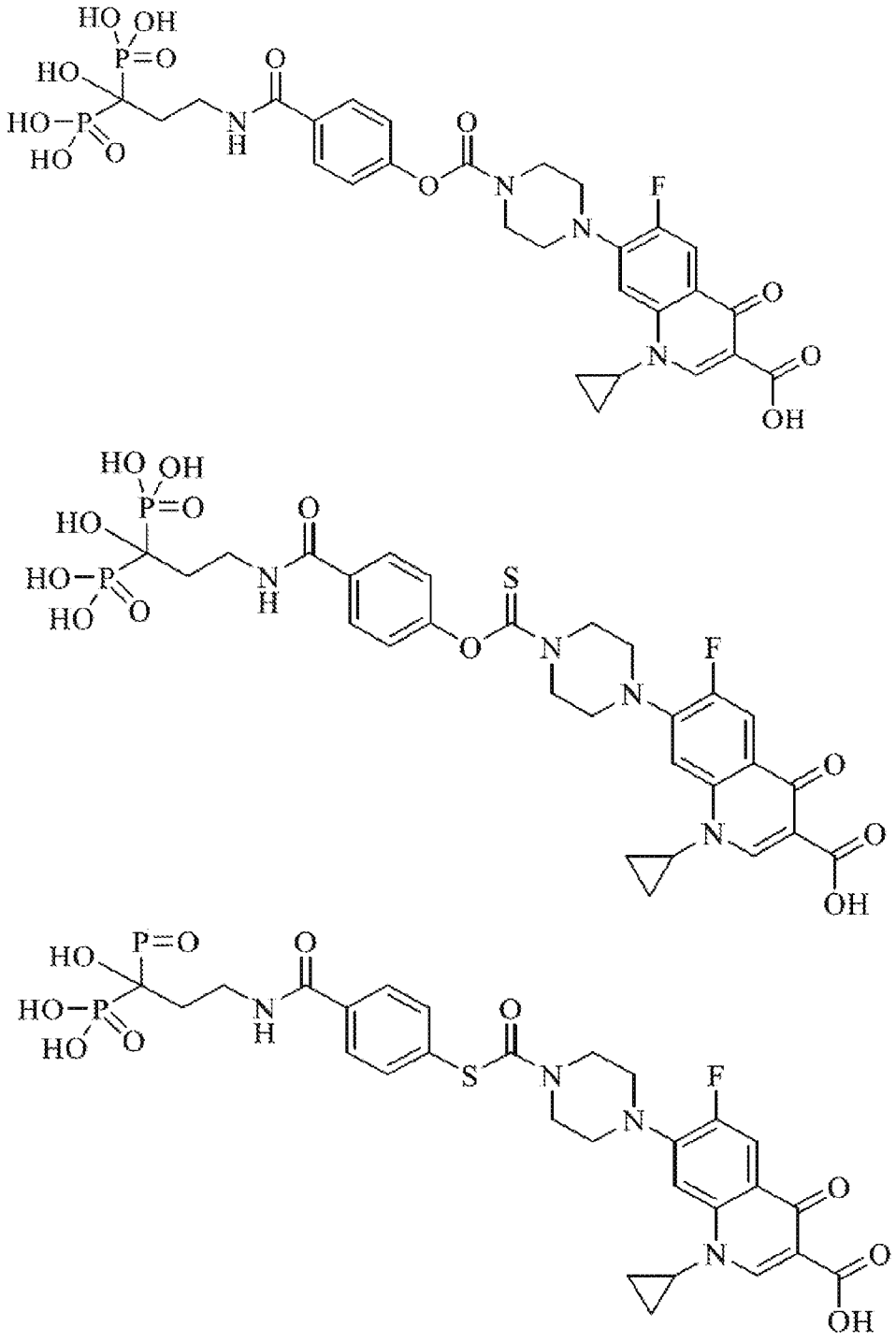


FIG. 22

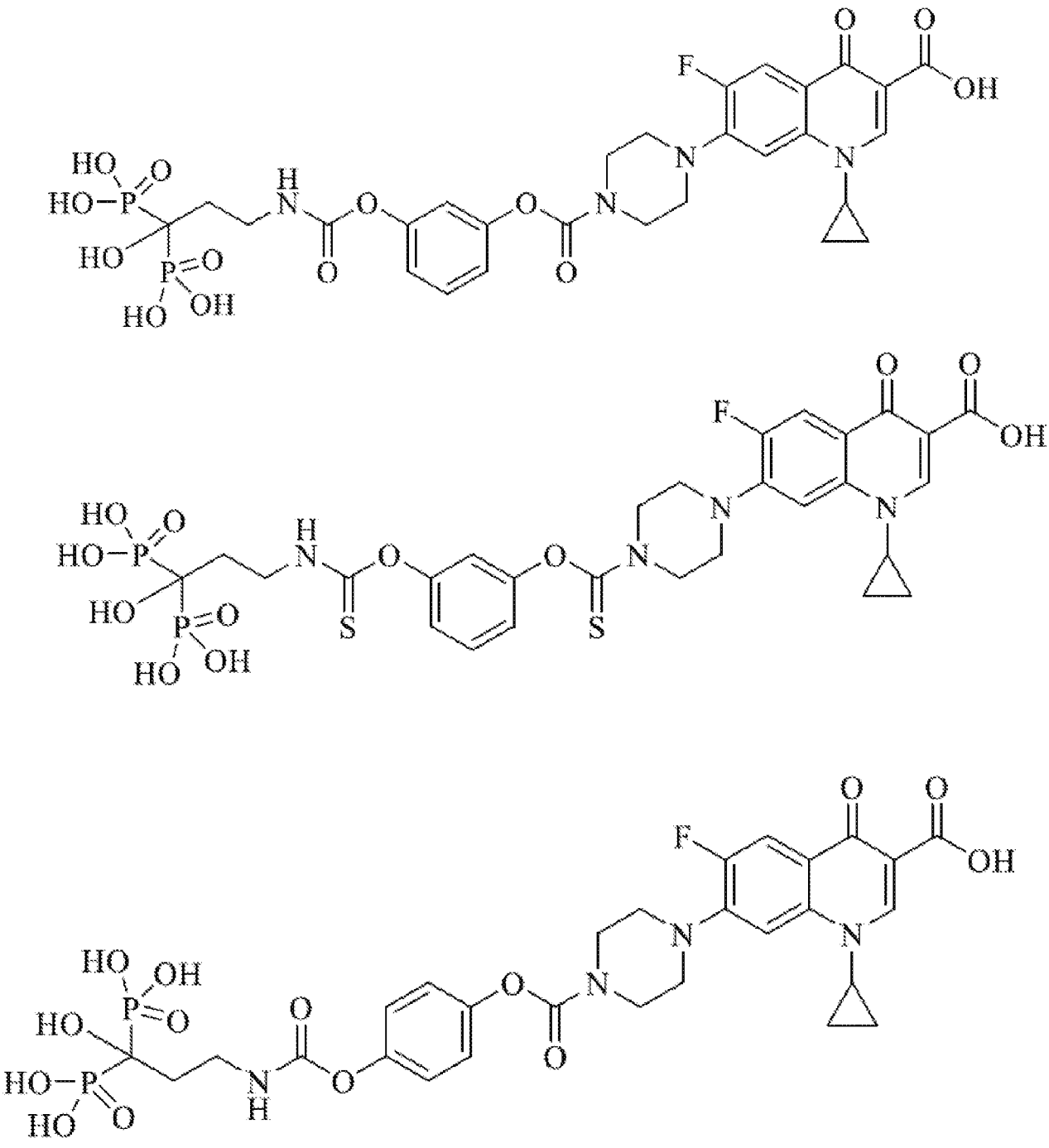


FIG. 22 ctd.

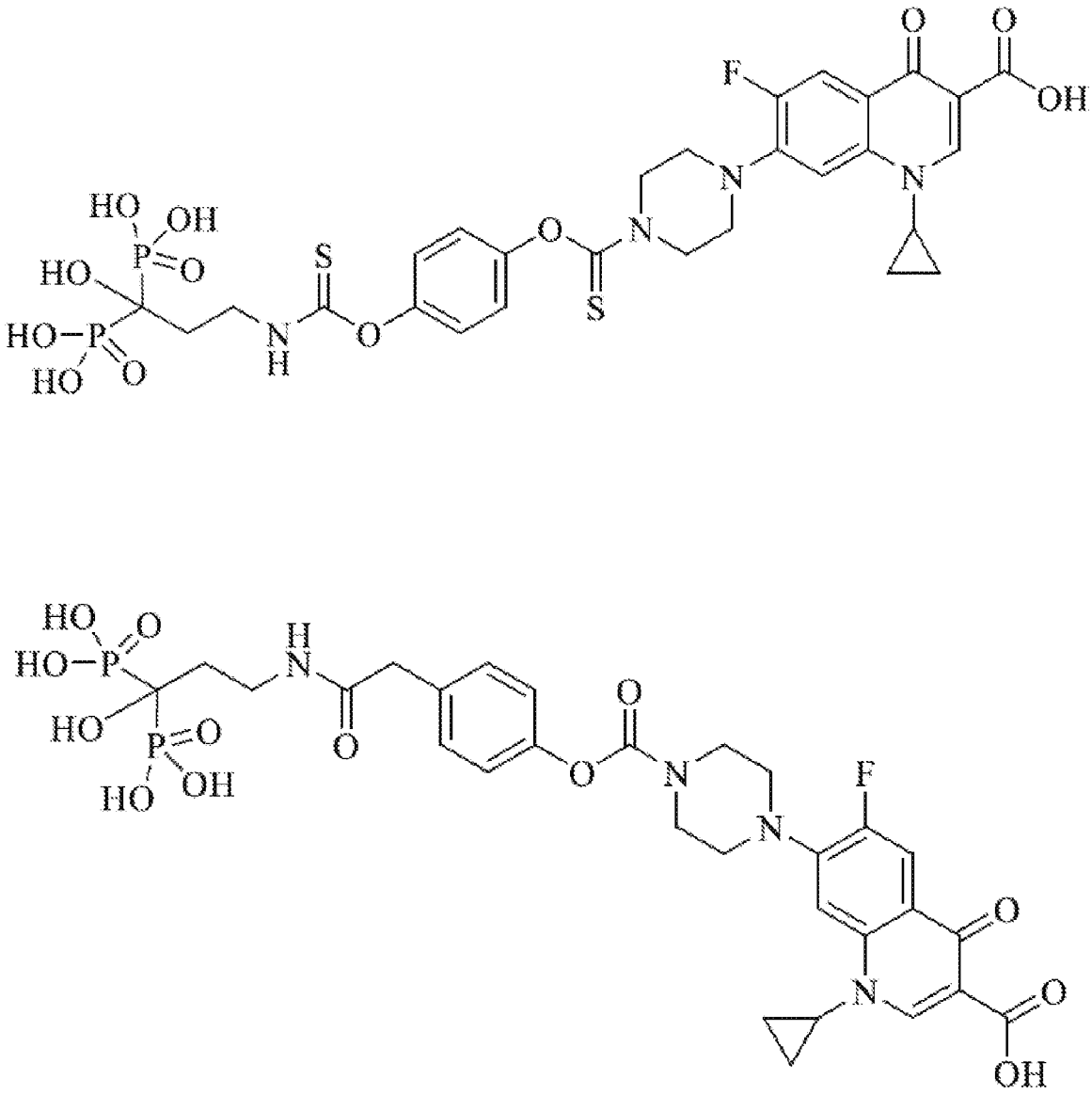


FIG. 22 ctd.

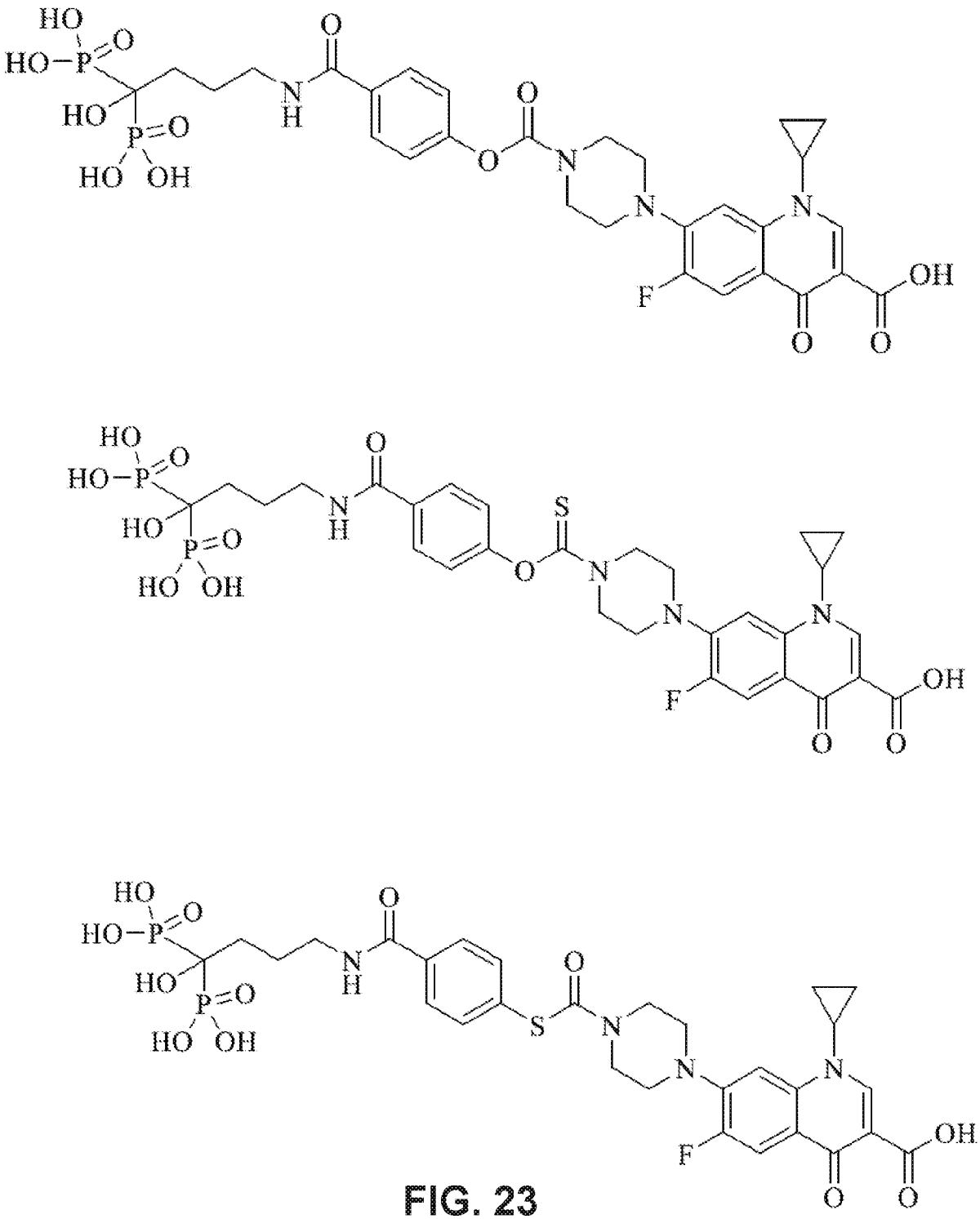


FIG. 23

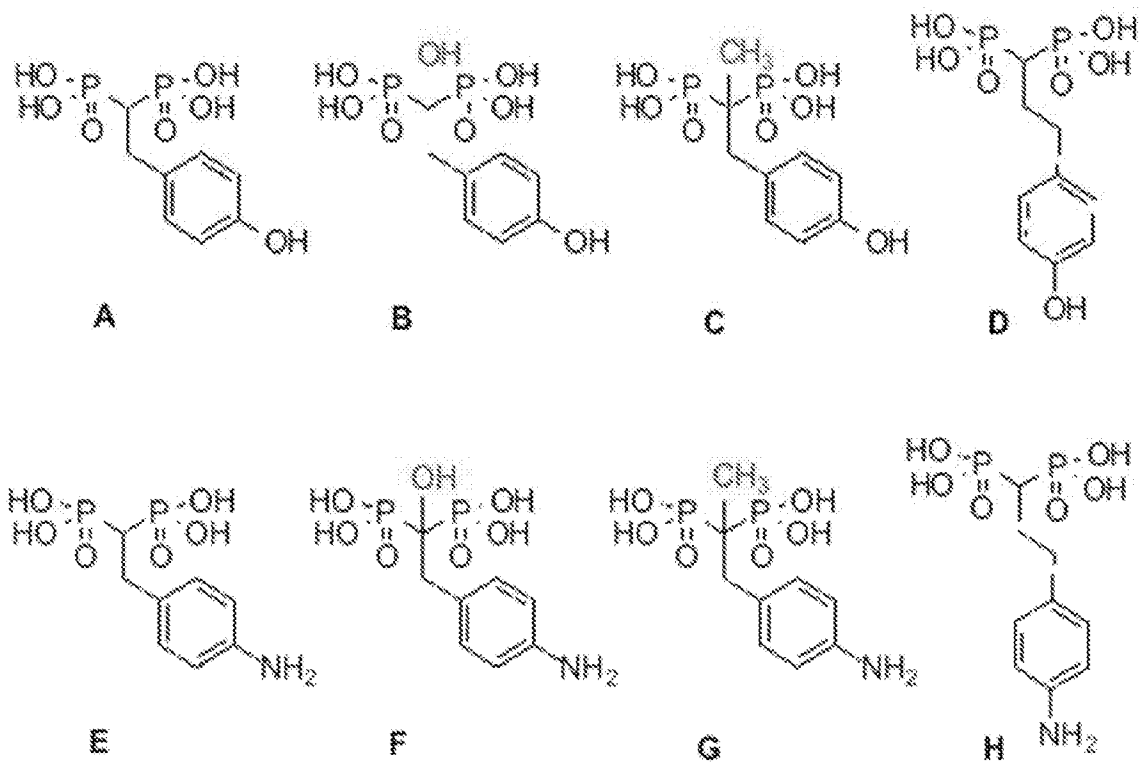


FIG. 24

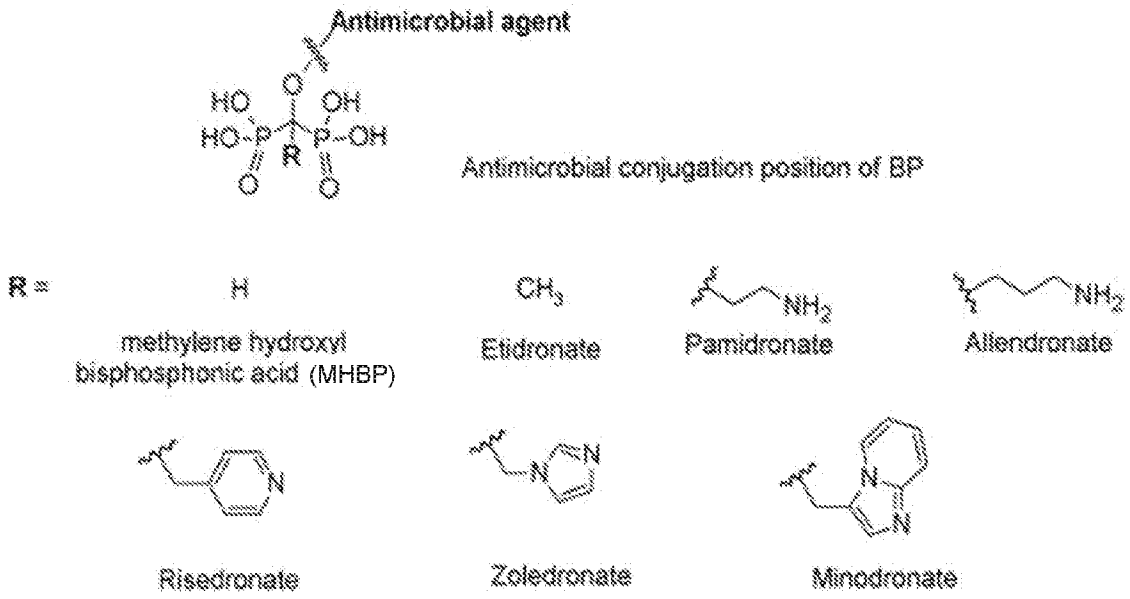

Antimicrobial agent
HO
OH
HO-P
P-OH
R
O
O
Antimicrobial conjugation position of BP
R =
H
methylene hydroxyl bisphosphonic acid (MHBP)
CH3
Etidronate
NH2
Pamidronate
NH2
Alendronate
N
Risedronate
N
N
Zoledronate
N
N
Minodronate

FIG. 25

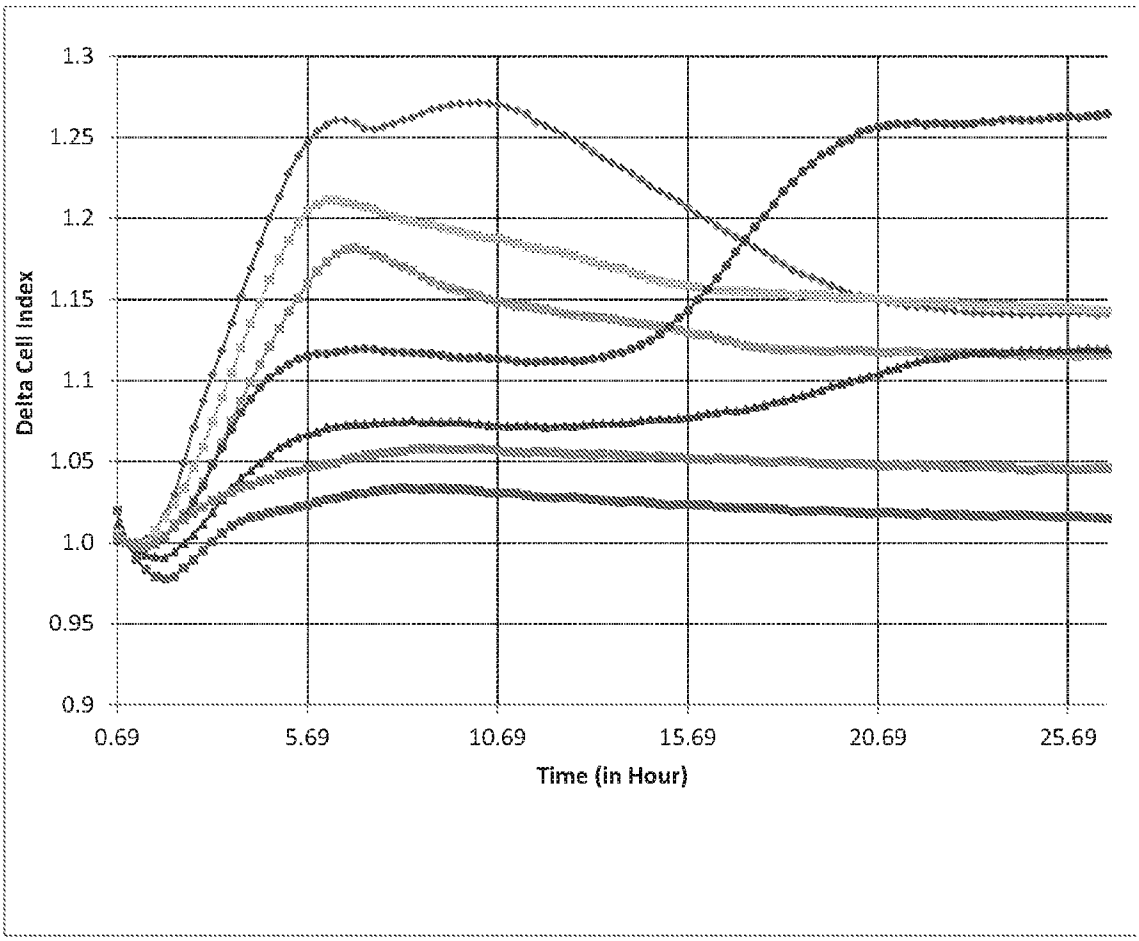

1.3
1.25
1.2
1.15
1.1
1.05
1.0
0.95
0.9
Delta Cell Index
0.69
5.69
10.69
15.69
20.69
25.69
Time (in Hour)

FIG. 26

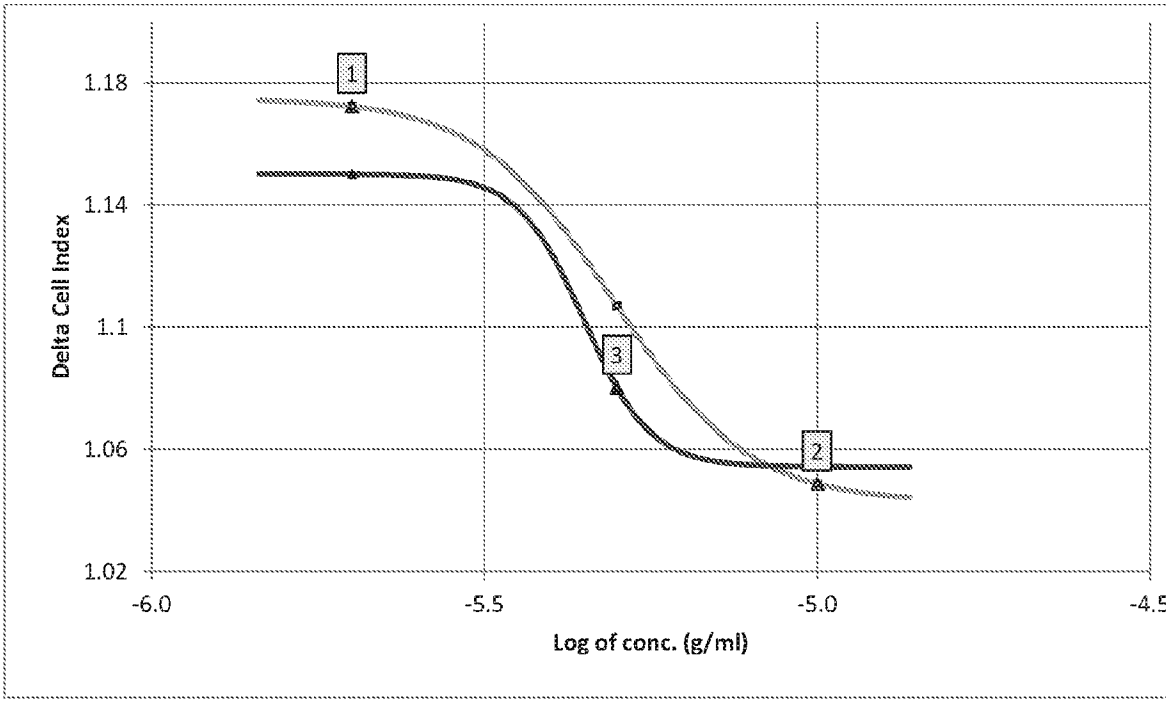

1.18
1.14
1.1
1.06
1.02
Delta Cell Index
-6.0
-5.5
-5.0
-4.5
Log of conc. (g/ml)
1
2
3

FIG. 27

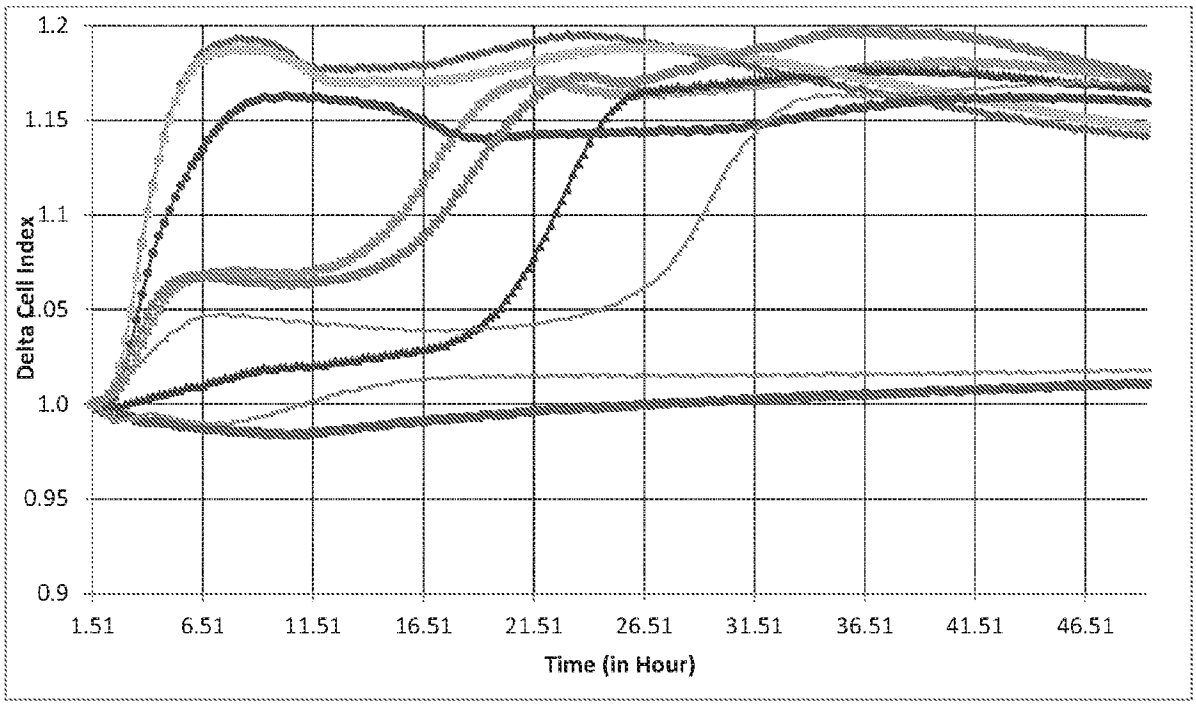

1.2
1.15
1.1
1.05
1.0
0.95
0.9
Delta Cell Index
1.51
6.51
11.51
16.51
21.51
26.51
31.51
36.51
41.51
46.51
Time (in Hour)

FIG. 28

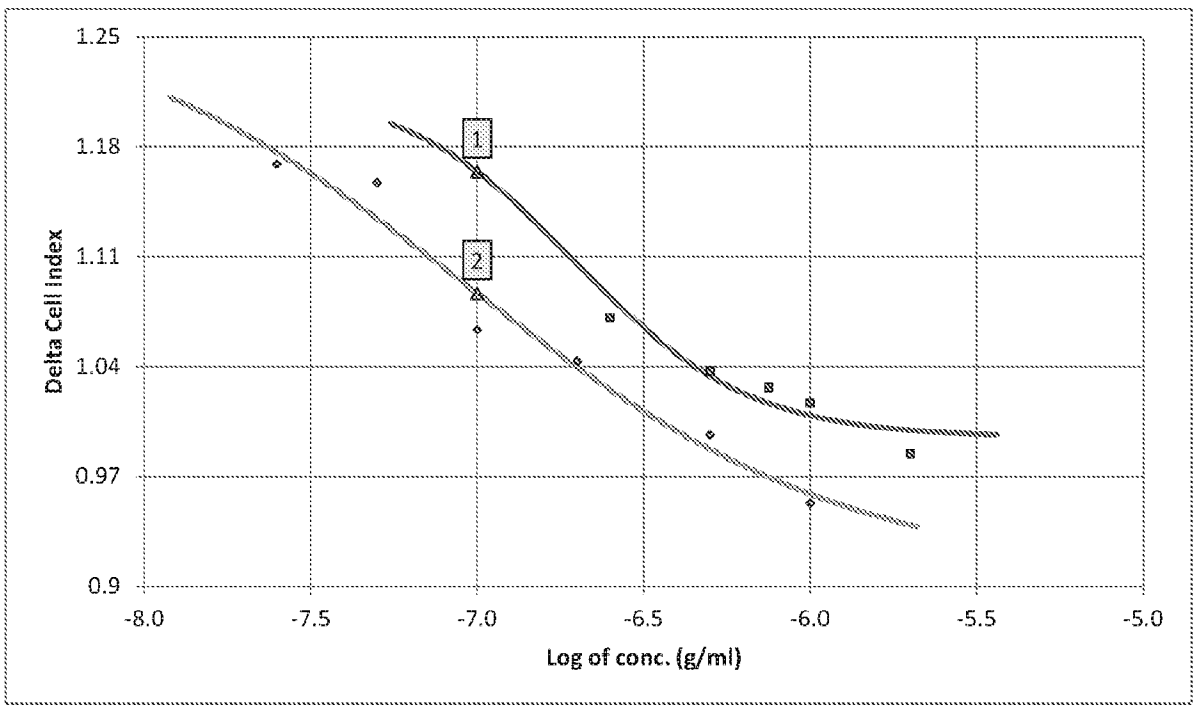

1.25
1.18
1.11
1.04
0.97
0.9
Delta Cell Index
1
2
-8.0
-7.5
-7.0
-6.5
-6.0
-5.5
-5.0
Log of conc. (g/ml)

FIG. 29

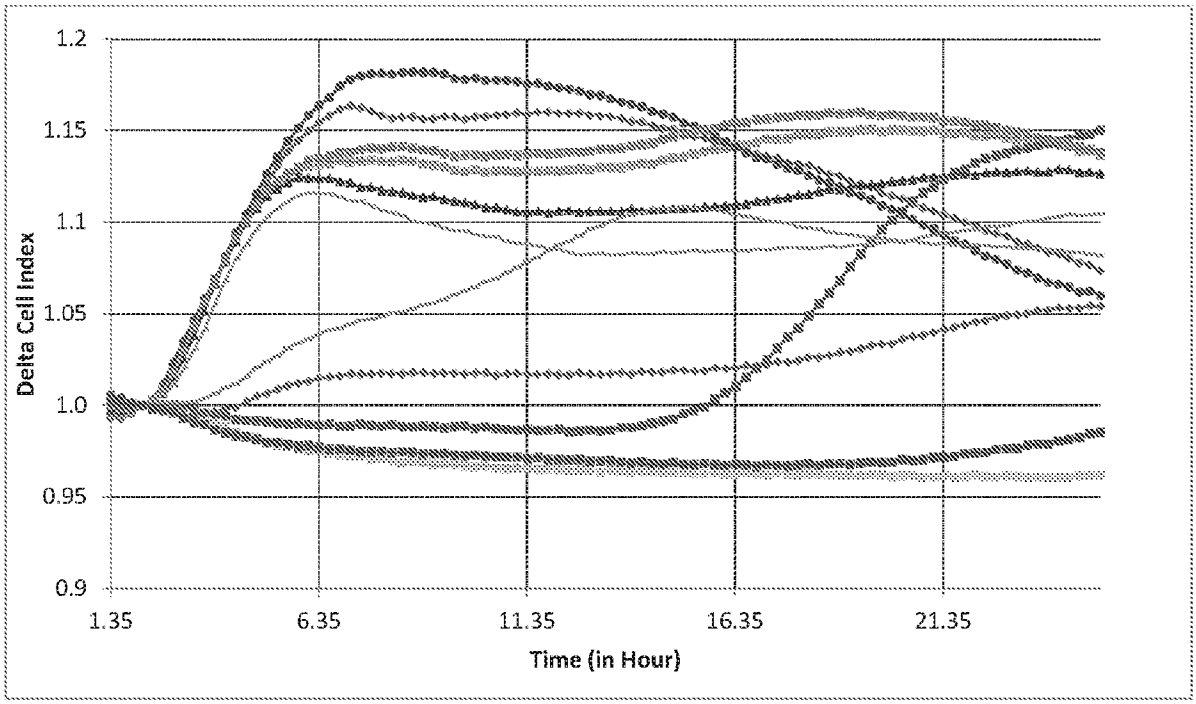

1.2
1.15
1.1
1.05
1.0
0.95
0.9
Delta Cell Index
1.35
6.35
11.35
16.35
21.35
Time (in Hour)

FIG. 30

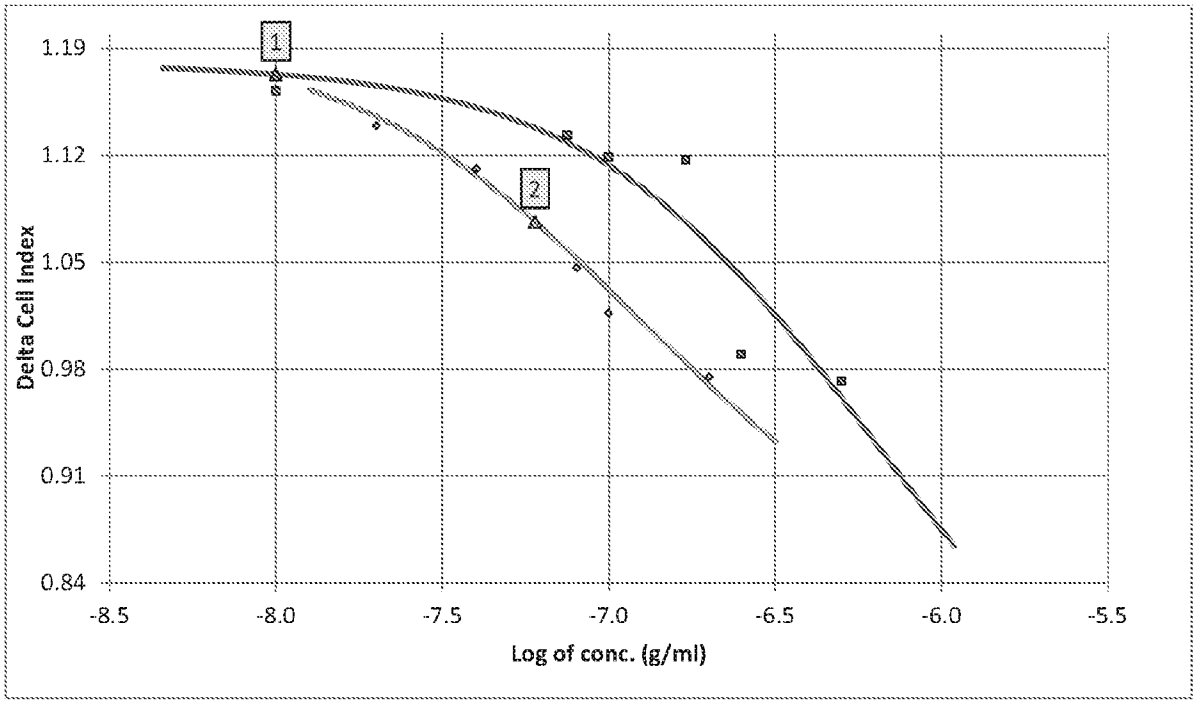

1.19
1.12
1.05
0.98
0.91
0.84
Delta Cell Index
1
2
-8.5
-8.0
-7.5
-7.0
-6.5
-6.0
-5.5
Log of conc. (g/ml)

FIG. 31

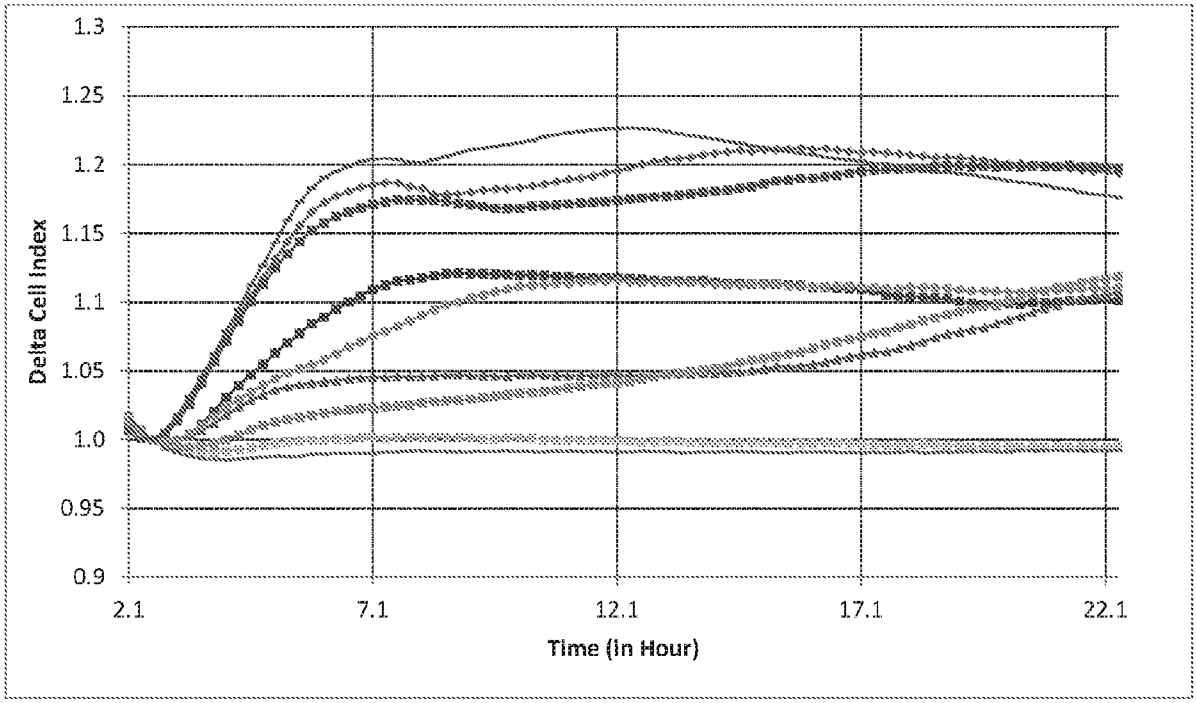

1.3
1.25
1.2
1.15
1.1
1.05
1.0
0.95
0.9
Delta Cell Index
2.1
7.1
12.1
17.1
22.1
Time (in Hour)

FIG. 32

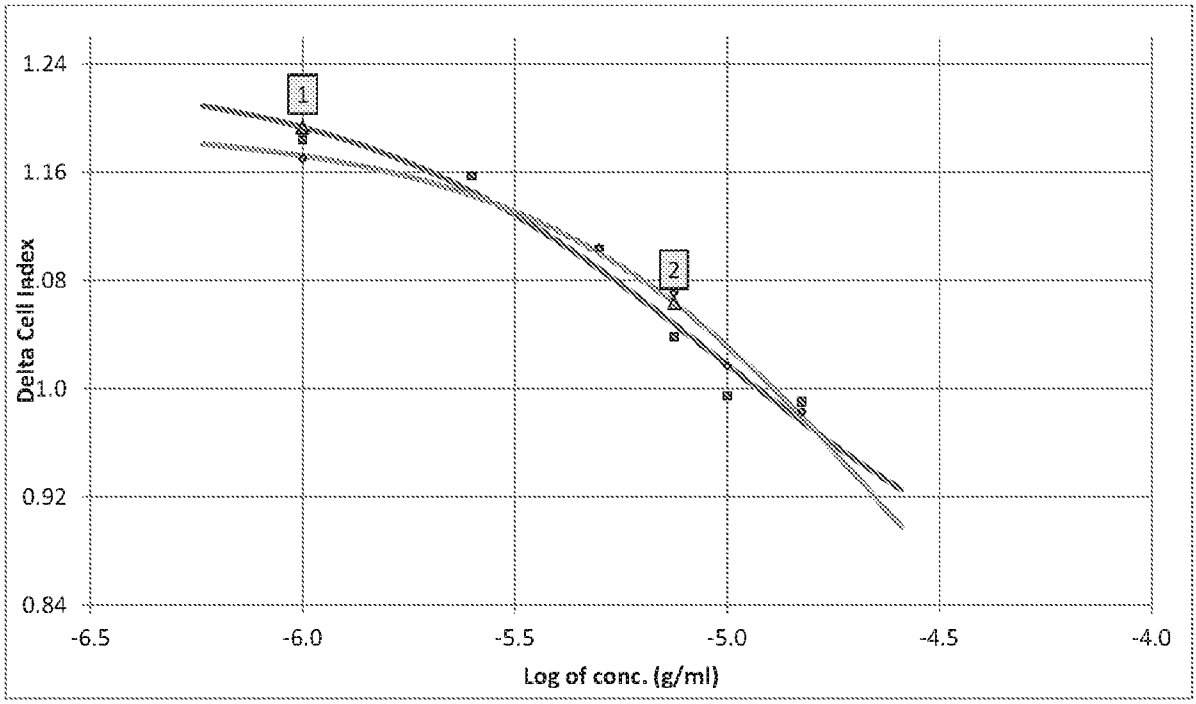

1.24
1.16
1.08
1.0
0.92
0.84
Delta Cell Index
1
2
-6.5
-6.0
-5.5
-5.0
-4.5
-4.0
Log of conc. (g/ml)

FIG. 33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/41063

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 19/10; A61P 31/04 (2019.01)
CPC - A61K 47/548; A61K 47/55; A61K 47/552

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2008/0287396 A1 (Delorme et al.) 20 November 2008 (20.11.2008); p58	1-11
A	Pubmed Compound Summary for CID 59845484, '[1-[[7-(1,2,3,4,4a,5,7,7a-Octahydropyrrolo[3,4-b]pyridin-6-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxoquinoline-3-carbonyl]oxymethoxycarbonyloxy]-1-phosphono-2-pyridin-3-ylethyl]phosphonic acid', U.S. National Library of Medicine, 20 August 2012 (20.08.2012), page1-15; p2 (https://pubchem.ncbi.nlm.nih.gov/compound/59845484)	1-11
A	Houghton et al. 'Linking Bisphosphonates to the Free Amino Groups in Fluoroquinolones: Preparation of Osteotropic Prodrugs for the Prevention of Osteomyelitis', Journal of Medicinal Chemistry, 04 October 2008 (04.10.2008), Vol.51, pages6955-6969; p6956	1-3, 6-9
A	Farrell et al. 'Bisphosphonate conjugation for bone specific drug targeting', Bone Reports, 03 July 2018 (03.07.2018), Vol.9, pages47-60; p53	1-11
A	Tanaka et al. 'Bisphosphonated fluoroquinolone esters as osteotropic prodrugs for the prevention of osteomyelitis', Bioorganic & Medicinal Chemistry, 0 September 2008 (09.09.2008), Vol.16, pages9217-9229; entire document	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 October 2019	18 NOV 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/41063

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 12-34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
Group I : Claims 1, 2(in part), 3, 6-8(in part), and 9
Group II : Claims 1, 2 (in part), 3, 4 (in part), 5, 6-8 (in part), 9, 10 (in part) and 11

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/41063

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-11 directed to a compound comprising a bisphosphonate (BP) and a quinolone in claim 1. The compound comprising a bisphosphonate (BP) and a quinolone will be searched to the extent that it encompasses the compound comprising a bisphosphonate (BP) and a quinolone wherein the BP has an alpha substituent and the alpha substituent is a hydroxyl group and wherein the quinolone is directly conjugated to the BP at the germinal carbon alpha substituent (X) of the BP of the following formula conjugates between alpha -X containing BP and quinolone, X = O, wherein R can be H, and wherein the quinolone is a fluoroquinolone. It is believed that claims 1-2(in part), 3, 6-8(in part), and 9 read on this first named invention, and thus these claims will be searched without fee. Applicant is invited to elect additional compounds of claim 1, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be the compound comprising a bisphosphonate (BP) and a quinolone in claim 1, wherein the BP has an alpha substituent and the alpha substituent is a hydroxyl group and wherein the quinolone is indirectly conjugated to the BP at the germinal carbon alpha substituent (X) of the BP of the following formula conjugates between alpha-X containing BP and quinolone, X = O, wherein R can be H, and wherein the quinolone is a sitafloxacin (i.e., claims 1-2(in part), 4-5(in part), 8(in part), and 10-11(in part)).

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique compound comprising a bisphosphonate (BP) and a quinolone, containing the same, which is not required by any other invention of Group I+.

Common technical features:

The inventions of Group I+ share the technical feature of a compound comprising a bisphosphonate (BP) and a quinolone containing the same.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by US 2008/0287396 A1 to Delorme et al. (hereinafter 'Delorme'). Delorme discloses a compound comprising a bisphosphonate (BP) and a quinolone, wherein the BP has an alpha substituent and the alpha substituent is a amino group and wherein the quinolone is indirectly conjugated to the BP at the germinal carbon alpha substituent (X) of the BP of the following formula conjugates between alpha-X containing BP and quinolone, X = NH, wherein R can be H, and wherein the quinolone is a moxifloxacin (para[0088] "2nd compound").

As said compound and compositions were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions of Groups I+. The inventions of Group I+ thus lack unity under PCT Rule 13.

Note Re: Item 4: claims 12-34 are determined unsearchable because they are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Applicants indicate the Group I contains claims 1, 2 (in part), 3, 4 (in part), 6-8 (in part), 9, 10 and 11, which is different from the first elected species of group I+ in the original form 206. Therefore, claims 1-2(in part), 3, 6-8(in part), and 9 have been searched for Group 1 as stated in Form 206.