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Farnesyl pyrophosphate synthase modulators: a patent review (2006 - 2010)

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Abstract

Introduction—Farnesyl pyrophosphosphate synthase (FPPS (also known as farnesyl diphosphate synthase, FDPS)) is one of the key enzymes involved in the mevalonate pathway and as such is widely expressed. FPPS modulators, specifically FPPS inhibitors, are useful in treating a number of diseases, including bone related disorders characterized by excessive bone resorption e.g. osteoporosis, cancer metastasis to bone and infectious diseases caused by certain parasites.

Areas covered—This review covers structures and applications of novel FPPS modulators described in the patent literature from 2006 to 2010. Patents disclosing new formulations and uses of existing FPPS inhibitors are also reviewed. Thirty-three patents retrieved from the USPTO, EP and WIPO databases are examined with the goal of defining current trends in drug discovery related to FPPS inhibition, and its therapeutic effects.

Expert opinion—Bisphosphonates continue to dominate in this area, although other types of modulator are making their appearance. Remarkable for their high bone mineral affinity, bisphosphonates are structural mimics of the dimethylallyl pyrophosphate (DMAPP) substrate of FPPS, and constitute the major type of FPPS inhibitor currently used in the clinic for treatment of bone-related diseases. Lipophilic bisphosphonates and new classes of non-bisphosphonate FPPS inhibitors (salicylic acid and quinoline derivatives) have been introduced as possible alternatives for treatment of soft tissue diseases, such as some cancers. Novel formulations, fluorescent diagnostic probes and new therapeutic applications of existing FPPS inhibitors are also areas of significant patent activity, demonstrating growing recognition of the versatility and underdeveloped potential of these drugs.

Keywords

farnesyl pyrophosphate synthase; FPPS; bisphosphonate; osteoporosis; cancer therapeutics; bone diseases; malaria; infectious diseases; fluorescent imaging probes

1. Introduction

Farnesyl pyrophosphate synthase (FPPS, also known as farnesyl diphosphate synthase, FDPS) is one of the key enzymes involved in the mevalonate pathway [1-3] (Figure 1). This enzyme catalyzes the two-step synthesis of the C15 isoprenoid farnesyl pyrophosphate (FPP): isoprenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are coupled to produce geranyl pyrophosphate (GPP), which is then condensed with an additional IPP to produce FPP. This sesquiterpene derivative is a crucial precursor in the synthesis of several classes of essential metabolites, including sterols, dolichols, ubiquinones and carotenoids. It is also used by farnesyl transferase (FT) to prenylate proteins that

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function as hormones, visual pigments, constituents of membranes, and components of signal transduction [4, 5].

FPPS belongs to the prenyltransferase group and FPPS proteins purified from different organisms have all proved to be homodimeric with subunit sizes from 32 to 44 kDa, typically displaying two aspartate-rich motifs with two substrate binding sites per monomer, one for an allylic diphosphate and one for IPP [1, 3]. Since the discovery of FPPS in 1959 [6], knowledge about the prenyltransferases has advanced significantly, providing an understanding of their reaction kinetics, catalytic mechanisms and conformational changes [2, 7]. Due to its importance in the isoprenoid biosynthesis pathway, many studies have evaluated the potential of FPPS as a drug target [8-11].

FPPS was recently identified as the apparent main biochemical target of nitrogen-containing bisphosphonate (N-BP) drugs [12, 13] (Figure 2) that are used to treat bone resorption diseases such as osteoporosis. Inhibition of FPPS by these N-BPs prevents the biosynthesis of FPP and downstream products, e.g. geranylgeranyl pyrophosphate (GGPP) (Figure 1). Besides being an intermediate for biosynthesis of important biomolecules ranging from retinoids and chlorophylls, GGPP is required for post-translational prenylation of small GTPases, such as the Ras, Rho, and Rab family proteins in osteoclasts [14]. Disruption of protein prenylation leads to loss of essential signaling processes that are necessary for osteoclast function and survival [13, 15-17].

Although these pathways are crucial in all cell types, the extraordinary ability of N-BPs to bind to bone mineral selectively results in the exposure of osteoclasts to high concentrations of the drug when they mediate resorption of surface bone in vivo [18].

The successful application of some N-BPs as FPPS inhibitors to treatment of bone diseases has underscored the importance of FPPS as a drug target, stimulating research groups in both industry and academia to focus on design of new FPPS inhibitors as well as new applications of existing drugs. This review surveys a total of 33 patents, filed or issued between 2006 to 2010 inclusively, that reference FPPS modulators and their applications in treatment of diseases. The patent documents were retrieved from the USPTO, EP and WIPO databases; where applications were filed at more than one patent office, or involve continuation patents, the latest version is reviewed, and related patent numbers are given as references.

2. Bisphosphonates

Methylenebisphosphonates are pyrophosphate analogues in which a carbon atom replaces the bridging oxygen atom between the two phosphate groups. Various substitutions on the bridging carbon with different side chains (R_1 , R_2 ; Figure 2) produce novel compounds, making possible large generation of compounds derived from this simple scaffold [18]. For convenience, as a class these compounds are commonly referred to as “bisphosphonates” (BPs), although strictly this term applies to any compound containing two phosphonate groups, and certain clinical drugs are actually formulated as bisphosphonic acids (5, 6). In vivo, they will be ionized to an extent determined by their physiological environment and particular structure. Bisphosphonates are currently the major class of drugs used for the treatment of osteoporosis and other diseases characterized by excessive bone resorption. Bisphosphonates were first used clinically in the late 1960s and early 1970s, but it was only in the early 2000s that their mechanism of action began to emerge [18-20]. During this period, it emerged that the modern generations of highly potent N-BP drugs inhibit FPPS [12, 13].

The patents of several clinical bisphosphonates have already expired or are due to expire relatively soon, which has stimulated the investigation of new FPPS inhibitor design and, to a certain extent, new synthetic methods during the last few years [21-23]. We will begin our review with a discussion of patents based on novel bisphosphonate structures as FPPS inhibitors; new formulations and applications will be discussed in sections 4 and 5, and patents related to non-bisphosphonate FPPS modulators are covered in section 3.

Even in the recent patent literature, use of the older “diphosphonic acid” nomenclature in place of the IUPAC-recommended “bisphosphonic acid” is still not infrequent: for the purposes of this review, we have reproduced the original compound names as given in each patent under discussion.,

2.1 (Pyridinium-1-yl)ethyl-1,1-diphosphonic acids, (quinolinium-1-yl)-1,1-diphosphonic acids and related compounds

Sanders *et al.* [24-27] claimed a series of (pyridinium-1-yl)ethyl-1,1-diphosphonic acids, (quinolinium-1-yl)-1,1-diphosphonic acids and related bisphosphonate structures (Figure 3, GPS I), which feature a permanent positive charge on a heterocyclic nitrogen (N^+) created by alkylation with a carbon spacer attached to the P-C-P backbone, potentially mimicking the carbocation intermediate that is proposed to form [28, 29] during isoprenoid synthesis. The general synthetic route is presented in Scheme 1. Several assays were applied to evaluate the biological activities of the compounds, including an FPPS inhibition assay, a *D. discoideum* growth inhibition assay, a T-cell activation assay and a bone resorption assay. The structures of some of the compounds identified as potent in the above assays are shown in Figure 3.

In the *L. major* FPPS inhibition assay, compound 278 was found to have a K_i of 18 nM and was thus similar in potency to two clinical bisphosphonates, zoledronate (5, K_i = 11 nM in this assay) and risedronate (4, K_i = 17 nM in this assay). In an effort to enhance activity further, a series of compounds with different *meta*-substituents on the pyridyl ring in compound 278 were synthesized and evaluated systematically (Figure 3). It was found that the *meta*-methyl analogue, compound 297 was not as active as 278 (K_i = 38 nM versus 18 nM), but substitution with a *meta*-phenyl group (300), resulted in a modest increase in potency (K_i = 9 nM). The *para*-phenoxy derivative of 278, compound 359, had lower activity (K_i = 75 nM), possibly due to unfavorable electrostatic or steric interactions of the -OH group within the FPPS active site. Analogues with larger substituents at the *meta*-position (e.g., 335, 364), or with *para*-substitution (e.g. 443) and isoquinoline and quinoline analogues (398, 447) were less active (Table 1).

Additional biological assays were carried out on several of the synthesized compounds in Figure 3, with the results summarized in Table 1. An electron withdrawing *meta*-substitution effect is suggested by compound 461, which was found to be very potent in inhibition of bone resorption (IC_{50} = 0.075 μ M). Interestingly, the desoxy analogues (472, 474, 480) have comparable or even higher activity than their corresponding hydroxy analogues (300, 297, 335) in certain biological assays (*cf.* the *D. discoideum* IC_{50} values of these compounds listed in Table 1).

2.2 Sulfonium- phosphonium-, arsonium- and other bisphosphonate derivatives

The role of the nitrogen in N-BPs has been explained in terms of an interaction between the positively charged nitrogen and residues of the FPPS active site that contribute to stabilizing a putative carbocation intermediate, e.g. threonine and lysine [29, 30]. However, Oldfield *et al.* [31, 32] has disclosed a group of compounds (Figure 4, BX1) which, although lacking a nitrogen-containing groups, contain aryl, substituted aryl, sulfonium and phosphonium

groups instead and show activity in several biological assays, including cytotoxicity towards cancer cells *in vitro*, inhibition of human and *Trypanosoma brucei* FPPS, and stimulation of $\gamma\delta$ T cells in the human immune system. Since the aryl- and substituted aryl-bisphosphonates (Figure 4, CX5) were not evaluated in FPPS inhibition assays but evaluated as undecaprenyl pyrophosphate synthase (UPPS) inhibitors, this group of novel bisphosphonates will not be discussed. The syntheses of the sulfonium and phosphonium-containing bisphosphonates are depicted in Scheme 2.

Some substituted sulfonium and phosphonium diphosphonic acids are exemplified in Figure 4. Several biological assays were performed to evaluate their potency as FPPS inhibitors, and the data are summarized in Table 2. Generally, in the human FPPS inhibition assay the potency of these compounds is not as great as risedronate and zoledronate, although some of them have only slightly less activity (human FPPS K_i values), e.g., compound 546, $K_i = 2.92$ nM, 2 - 3 times greater than the K_i values of risedronate (1.23 nM) and zoledronate (1.25 nM). In a *Trypanosoma brucei* FPPS inhibition assay, some of the compounds had subnanomolar IC_{50} values (e.g., compound 585, $IC_{50} = 0.18$ μ M), which indicated their potential as anti-parasitic reagents.

2.3 Lipophilic bisphosphonates as dual farnesyl/geranylgeranyl pyrophosphate synthase inhibitors

Oldfield *et al.* [33-35], proposed that cationic transition states could be relevant for both FPPS and geranylgeranyl pyrophosphate synthase (GGPPS), as well as for decaprenyl pyrophosphate synthase (DPPS), and thus bisphosphonates could be “polypharmaceuticals” capable of inhibiting multiple targets, which might correlate with enhanced potency against tumor cell growth and invasiveness.

Comparative molecular similarity analysis (CoMSIA)[36] and pharmacophore models for FPPS inhibition [37] have suggested that moving the positive charge closer to the bisphosphonate backbone would enhance activity; and earlier work [38, 39] on *Saccharomyces cerevisiae* and human GGPPS suggested the possible importance of having a large, hydrophobic “tail” in effective bisphosphonate drugs. Based on these considerations, a series of long-chain bisphosphonates has been disclosed [33-35].

One of these compounds, BPH-715 (Figure 5) [35], was found to be more active against tumor cell (e.g. MCF-7) growth than conventional bisphosphonate drugs such as zoledronate and pamidronate, with an IC_{50} value of ~ 50 nM, compared to values on the order of ~ 15 μ M for zoledronate and ~ 300 μ M for pamidronate. The observation that cell growth inhibition was not rescued by addition of either farnesol (FOH) or geranylgeraniol (GGOH) suggested to the authors that both FPPS and GGPPS are drug targets for BPH-715. FPPS and GGPPS inhibition assay results, as well as X-ray, NMR, and calorimetric investigations of bisphosphonate-target interactions further examined the dual-target model for inhibition by BPH-715. Differing pK_i values of 7.3 M ($K_i = 50.1$ nM) for FPPS and 8.1 M ($K_i = 7.94$ nM) for GGPPS, and also interaction studies, indicated that inhibition of tumor cell growth by BPH-715 is due primarily to GGPPS inhibition. The inhibitor can bind to the GGPP binding site in GGPPS as the uncharged side-chain species, whereas the cationic species also inhibits FPPS. BPH-715 is only weakly adsorbed on to bone *in vivo*, which was claimed to be desirable in the context of immunotherapy and treating infectious diseases and cancers [35].

2.4 Novel nitrogen-containing bisphosphonates

As previously mentioned, the current generation of highly active clinical bisphosphonates are all nitrogen-containing BPs (Figure 2) and inventive efforts to find improved N-BPs

have been evident during the last 5 years. Ebetino, *et al.* disclosed several novel nitrogen-containing bisphosphonates (Figure 6), e.g., 4-azaindole [40] and 5-azaindole bisphosphonates [41]. Two compounds are illustrated in Figure 6 as examples of these two groups of bisphosphonates (EX I and EX II). Human FPPS inhibition assays of the two compounds showed that EX II, (5-azaindol-3-yl)-ethyl-bisphosphonic acid ($IC_{50} = 6.6$ nM, $K_i = 0.6$ nM) is more potent than EX I, (4-azaindol-3-yl)-ethyl-bisphosphonic acid ($IC_{50} = 37.4$ nM, $K_i = 5.9$ nM); both compounds are somewhat less potent than risedronate and minodronate, but more active than alendronate.

Another example of a novel N-BP from the recent patent literature is 2-azabicyclo[4.3.0]nonane-8,8-diphosphonic acid [42, 43], which exists as two enantiomers: the (1R, 6S)-isomer (Figure 6, EX III) and the (1S, 6R)-isomer (Figure 6, EX IV). It is interesting that the (1R, 6S)-isomer shows at least an order of magnitude more activity ($IC_{50} = 15$ nM) than its (1S, 6R) enantiomer ($IC_{50} = 359$ nM) in the FPPS inhibition assay (the activity of the racemic mixture is approximately 42 nM). Each isomer could be prepared by chiral separation of the racemic mixture or stereoselective synthesis.

The existing drugs zoledronate and minodronate have provided scaffold for modification to create novel bisphosphonates. Ebetino, *et al.* disclosed a group of imidazo[1,2- α]-pyridinyl bisphosphonate derivatives [44] (Figure 7), of which the most active was compound M1 (FPPS $IC_{50} = 2.46$ nM, $K_i = 0.014$ nM, more potent than risedronate and zoledronate, and much more potent than alendronate (Table 3)). The fluoro analogue of risedronate developed by McKenna *et al.* [45] also inhibited FPPS at nM concentrations (IC_{50} value 16.4 nM compared with risedronate, ($IC_{50} = 5.7$ nM). It is noteworthy that despite the lack of an α -hydroxy group in these compounds (e.g., EX I, EX II in Figure 6; M1 in Figure 7), they still show good activity, which is unexpected in light of the usually common assumption that a large reduction of inhibition activity usually occurs when the α -hydroxy group in bisphosphonates is substituted.

Weiler, *et al.* [46], synthesized a series of C2-C5-alkyl-substituted [(imidazol-1-yl)-1-hydroxy-1-phosphono-ethyl]diphosphonic acids (Figure 8). The compounds are stated to exhibit IC_{50} values in the range of 0.8 to 10 nM in the FPPS inhibition assay, with the preferred compounds having IC_{50} values from 0.9 to 3.3 nM, including the 5'-ethyl zoledronate derivative (FM1 in Figure 8).

2.5 Phosphonocarboxylate analogues of bisphosphonates

Although as a class bisphosphonates have certain common properties, as can be seen from the preceding discussion, the chemical, biochemical, and pharmacological differences among the individual compounds can be profound. Early efforts to invent new bisphosphonates focused on changing the R_1 , R_2 substituents on the methylene carbon, but efforts to define clear structure-activity relationships (SAR) were impeded by lack of mechanistic information. This situation is evolving since the identification of FPPS as a major target of N-BPs [15, 47-49].

An alternative approach would be to modify the phosphonate group, or replace it with a different group. For example, replacement of one of the phosphonate groups in risedronate and minodronate by a carboxylate group leads to 3-PEHPC (NE10790) and 3-IPEHPC, respectively [50] (Figure 9). Although these PC analogues of the bisphosphonate drugs inhibit bone resorption *in vivo*, their anti-resorptive potency is significantly lower than that of the parent BPs. Coxon, *et al.*, demonstrated that these compounds are very poor FPPS inhibitors, and owe their anti-resorptive activity, at least in part, to inhibition of a different enzyme target, Rab geranylgeranyl transferase (RGGT), which geranylgeranylates small Rab GTPases [51-53].

McKenna, *et al.* synthesized a series of halogen-substituted and desoxy analogues of the PC compounds [45, 54] (Figure 9, 7a-d, 8a-d), which proved to be even poorer inhibitors of FPPS than the parent compound (halo-analogues: $IC_{50} > 600 \mu M$; 3-PEHPC: $IC_{50} = 254 \mu M$). In contrast, all of the halo-analogues 7b-d were 1-1.5 times as potent as 3-PEHPC ($IC_{50} = 24.1 \pm 5.7 \mu M$) for RGGT inhibition, with the fluoro-analogue 7b being most active ($IC_{50} = 16.3 \pm 0.3 \mu M$ for RGGT inhibition, $LED = 31 \mu M$ in a Rab11 prenylation inhibition assay; $IC_{50} = 1.8 \pm 0.3 mM$ in a reduction of J774 viability assay).

Modulator stereochemistry is only recently beginning to be addressed in the FPPS patent literature. An example of an N-BP invention comprising a chiral R_2 substituent whose stereochemistry influenced potency was given above (Figure 6). Unlike bisphosphonic acids, PCs can have a chiral bridging carbon, and it was recently shown that enantiomeric forms of a PC compound can have significantly different activities, leading to identification of the most active selective RGGT inhibitor to date [50]. Individual 3-PEHPC and 3-IPEHPC enantiomers, obtained by chiral HPLC ($> 98\%$ ee), were non-equivalent in an isolated RGGT inhibition assay but to different degree. For 3-PEHPC, (+)-7-E1 was about four times as potent as (-)-7-E2 for inhibition of Rab11 prenylation ($LED < 63$ vs. $250 \mu M$, respectively), and slightly more active than isomer (-)-7-E2 in RGGT inhibition assays (IC_{50} values $39 \pm 2.3 \mu M$ and $150 \pm 54 \mu M$, respectively). However, (+)-8-E1 was significantly more active than (-)-8-E2 in RGGT inhibition assays (IC_{50} : $1.1 \mu M$ for E1 vs. $67.7 \mu M$ for E2), ~17 times more potent than (-)-8-E2 in inhibiting Rab prenylation in J774 cells (LED : $3 \mu M$ for E1 vs. $50 \mu M$ for E2) and > 26 times more active in the cell viability assay (IC_{50} : $31 \mu M$ for E1 vs. $> 800 \mu M$ for E2). A detailed explanation of the FPPS vs. RGGT selectivity of these PC compounds has been impeded by the absence of an X-ray crystal structure for a PC-RGGT complex [55, 56].

3. Other classes of FPPS modulators

In the recent patent literature only a few inventions disclosing FPPS modulators structurally unrelated to BPs have appeared, in particular compounds that are derivatives of salicylic acid and quinoline.

3.1 Salicylic acid derivatives as FPPS modulators

Cotesta, *et al.* [57] disclosed that certain salicylic acid derivatives (Figure 10) inhibit FPPS, and claimed that they are appropriate for the treatment of diseases that depend on FPPS activity, especially cancers of soft and hard tissues, e.g., bone metastasis, or as cholesterol lowering agents. This series of compounds was shown in an FPPS Scintillation Proximity Assay (FPPS SPA) test system to have IC_{50} values in the range of $50 nM$ to $100 \mu M$ (zoledronate as a positive control in this test system had an IC_{50} of between $50 - 200 nM$). One of the most potent compounds disclosed, 13da (Figure 10), had an IC_{50} of $0.13 \mu M$.

3.2 Quinolines as FPPS inhibitors

Amstutz, *et al.* [58] disclosed another group of non-BP compounds with FPPS inhibition activity, namely a series of quinoline derivatives (Figure 11). Activity against cancer was claimed for these compounds.

For both of the new classes of non-bisphosphonate FPPS inhibitors discussed above, the binding sites require elucidation. A research group at Novartis recently published a paper [59], reporting two potent non-bisphosphonate FPPS inhibitors (Figure 12) with IC_{50} values of $200 nM$ and $80 nM$, respectively. These new inhibitors bind to a previously unknown allosteric site on FPPS, identified by fragment-based approaches using NMR and X-ray crystallography. The inhibitory potencies of these compounds were similar to or greater than those of zoledronate (5), risedronate (4), ibandronate (3), alendronate (1) and pamidronate

(2) (IC_{50} = 130 nM, 150 nM, 340 nM, 850 nM, 950 nM, respectively), when measured without preincubation.

4. New formulations of existing drugs

As the bisphosphonates still constitute the major group of FPPS inhibitors utilized for treatment of bone diseases, new formulations of existing drugs have continued to attract the interest of inventors during the past 5 years.

Typical BP administration methods include the oral, intravenous (IV) and subcutaneous (SC) routes; each method has both advantages and disadvantages [60] and BP formulations are usually designed to be used by a specific method.

Oral administration of BPs is convenient, especially for long-term use, but in order to improve tolerability, a low dose is usually prescribed and the oral administration process is rather strict and rigid, which may be bothersome for patients. Zanetti, *et al.* [61], disclosed an oral formulation composition which comprises at least one bisphosphonate; viscosity agents comprising carboxymethylcellulose and xanthan gum; at least one flavoring agent; and purified water, for prevention, treatment and/or diagnosis of metabolic diseases of bones to enable more comfortable administration of a bisphosphonate drug, promising strict as compliance with prescriptions and thereby, improved long-term clinical effects.

Liposomal clodronate is known as a potent anti-macrophage agent, both in vitro and in vivo [62, 63]; however, liposomal formulations have been found to cause hypersensitivity reactions in many patients, causing a variety of symptoms such as dyspnea, tachycardia, hypertension, and other signs of cardiopulmonary distress [64]. Johnson *et al.* [65], disclosed a new formulation for treating or preventing the growth, invasion and/or metastasis of tumors, comprising a bisphosphonate associated with a non-liposomal carrier. The non-liposomal carriers included hydroxyapatite nanoparticles, calcium carbonate nanoparticles, poly(lactic-co-glycolic acid) (PLG) nanospheres. The resulting non-liposomal bisphosphonate particles were claimed to advantageously target cancer-associated macrophages.

Another recent invention in this category is based on the argument that SC administration of BPs has several advantages compared with oral and IV infusions; it should reduce the incidence of gastrointestinal irritation associated with oral administration, and systemic reactions, improve the bioavailability of BPs, and avoid sometimes difficult IV infusion. SC administration of BPs is not currently prescribed due to difficulties with skin toxicity and poor absorption of the drug. Frost [60, 66] has disclosed a new method of SC BP administration that combines the drug in a soluble hyaluronidase composition, to reduce the injection site lesion. An implantable formulation for SC administration was disclosed by Ashton [67], in which the BP drug is disposed in a tube for implantation in the patient subcutaneously to provide sustained release of a therapeutically effective dose of a bisphosphonate drug. An implantable device administration was also adopted for an invented formulation comprising the poorly water-soluble bisphosphonate salts of C2-C5-alkyl-substituted [(imidazol-1-yl)-1-hydroxy-1-phosphono-ethyl]-phosphonic acids (Figure 8), together with one or more biocompatible polymers [68, 69].

Other new formulation inventions include combination of the bisphosphonate drug with other agents that have different enzyme targets, leading to synergetic or multifunction effects. For example, Levy *et al.* [70, 71] disclosed the use of a hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor together with a bisphosphonate to achieve a synergistic effect targeting the accumulation and/or persistence in cells of prenylated proteins. Okamura *et al.* [72] disclosed a composition to enhance V γ 9V δ 2 T cell

proliferation comprising a bisphosphonate, interleukin 2 (IL-2), and interleukin 18 (IL-18); IL-18 was asserted to act as a cofactor to increase the effects of the bisphosphonate and IL-2 on V γ 9V δ 2 T cell proliferation.

5. New applications of existing drugs

Although FPPS inhibitors are developed primarily for the treatment of bone-related disorders [10, 73, 74], several new inventions have focused on anti-bacterial or anti-parasitic applications [11, 75].

Recently, Cau *et al.* [76, 77] disclosed a composition comprising an anti-HIV treatment and a treatment for certain side effects associated with anti-HIV agents, such as early aging and lipodystrophy. The composition includes at least one HMG-CoA reductase inhibitor, at least one FPPS inhibitor, and at least one anti-HIV agent.

The same inventors [78] disclosed a composition for use in the treatment of skin and hair disorders based on a composition comprising an HMG-CoA reductase inhibitor, an FPPS inhibitor and a cosmetic and/or dermatological product. This invention is claimed to be advantageous for the treatment of early aging effects on the skin and hair.

Kim *et al.* [79, 80], have described an invention for modulating cellular processing of beta-amyloid precursor protein (APP), to prevent or treat diseases associated with abnormal APP processing, such as Alzheimer's disease (AD). FPPS inhibitors such as the bisphosphonates alendronate, etidronate, risedronate and zoledronate were said to reduce APP cleavage in a dose dependent manner, suggesting their potential application in the prevention or treatment of AD.

The availability of a sensor for the concentration and distribution of FPPS-inhibiting drugs in bone mineral and cells would greatly facilitate mechanistic and pharmacological study of the drugs. McKenna *et al.* [81-83], disclosed a series of novel fluorescent bisphosphonate conjugates based on heterocyclic N-BPs such as risedronate, including a general, facile synthetic method to link the drug to a suitable fluorescent dye molecule. These probes can be used to image the drug within osteoclasts and also to compare bone mineral penetration by different BP drugs [84]. Some of the probes, such as FAMRIS (11, Figure 13), exhibit an anti-prenylation effect in cell assays [82], suggesting the probes can mimic the parent drugs in drug mechanism studies. A different invention based on a bisphosphonate conjugate has been disclosed by Satchi-Fainaro *et al.* [85, 86], namely a conjugate of a polymer (e.g., N-(2-hydroxypropyl)-methacrylamide copolymer (HPMA)), an anti-angiogenesis agent and a bisphosphonate, for treatment of bone-related angiogenesis; the bisphosphonate in the conjugate functions mainly as a targeting moiety. The linkers that connect the polymer with the other moieties were designed to be enzyme degradable.

6. Conclusions

FPPS is a key enzyme in the isoprenoid biosynthesis pathway. Nitrogen-containing bisphosphonates, the major group of FPPS inhibitors, have been used in clinic for more than a decade in treatment of diseases characterized by excessive bone resorption, such as osteoporosis, Paget's disease, and more recently, metastatic bone-related tumors and cancer. Because of the prevalence of FPPS in diverse organisms, FPPS inhibitors also find application in treatment of some infectious diseases as anti-bacterial, or anti-parasitic agents. A range of new FPPS inhibitors have been disclosed during the last 5 years, including new bisphosphonates (Section 2) as well as other classes of non-BP inhibitors (Section 3; salicylic acid derivatives and quinoline derivatives). Some of these new inhibitors have comparable or better FPPS inhibition potency than current clinical drugs, and have desirable

properties such as low bone affinity, indicating their potential for development as the therapeutic agents in clinic of the future.

7. Expert opinion

The successful application of bisphosphonates as FPPS inhibitors for the treatment of bone-related diseases has established that FPPS is an important and valuable target for therapy; efforts from both academia and industry to develop new FPPS inhibitors are continuing apace, as the patents of existing clinical bisphosphonate drugs approach expiration. The majority of new patents in this area relate to the development of new bisphosphonates, which are structural mimics of the dimethylallyl pyrophosphate (DMAPP) substrate of FPPS, and have already proved successful as FPPS inhibitors. The high bone affinity of BPs is advantageous for the treatment of bone diseases but is a limitation in their use for soft tissue diseases such as some cancers, because most of the BP dose will go to bone once entering the circulation. Lipophilic bisphosphonates and non-BP FPPS inhibitors offer possible alternatives, but a final judgement on their merits awaits clinical evaluation for safety and efficacy.

New formulations and applications of existing FPPS inhibitors have produced a number of recent inventions. These patents focus on improving convenience and safety of administration and on expanding applications for treatment of various conditions. The invention of clinically important heterocyclic N-BP drugs conjugated with a fluorophore to create bone imaging molecules has made available an invaluable tool for better understanding of the action of this important class of FPPS modulators. X-ray crystallography together with computer modeling experiments will likely provide useful insights to design the next generation of FPPS modulators.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of interest

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highlights

- Inhibitors of FPPS are used to treat bone related disorders characterized by excessive bone resorption and infectious diseases caused by certain parasites.
- Bisphosphonates are the major class of FPPS inhibitors used successfully in the treatment of bone-related diseases, including osteoporosis, Paget's disease, bone-related tumors and metastatic cancers.
- Novel lipophilic bisphosphonates and new classes of non-bisphosphonate FPPS inhibitors (e.g., salicylic acid and quinoline derivatives) have been introduced that show FPPS inhibition activity with reduced bone affinity, which may be desirable for treatment of soft tissue diseases.
- Phosphonocarboxylate analogues of bisphosphonates have been invented that show anti-resorptive effects, but act on a different enzyme target: Rab geranylgeranyl transferase (RGGT). These compounds have reduced bone affinity and have been proposed to have potential utility in cancer treatment.
- New formulations and applications of existing FPPS inhibitors have also been invented. Work on new formulations has emphasized improvements in convenience and safety of administration.
- Newly disclosed applications for FPPS modulators demonstrate the increasing scope of existing drugs' utility, e.g., as agents for treatment of side effects of anti-HIV therapy, Alzheimer's disease, and skin/hair disorders. Bisphosphonate FPPS inhibitors have also been disclosed as useful bone targeting moieties for drug delivery and as components of fluorescent bone imaging probes.

Figure 1.
FPPS participation in isoprenoid biosynthetic pathways

Figure 2.

Examples of nitrogen-containing bisphosphonate drugs (N-BPs). For simplicity of illustration, all are depicted in bisphosphonic acid form, which strictly applies only to **5** (zoledronic acid) and **6** (minodronic acid).

Figure 3.
(Pyridinium-1-yl)ethyl-1,1-diphosphonic acid, (quinolinium-1-yl)-1,1-diphosphonic acid and derivatives (The original numbering has been retained); from refs. [24-27]

Figure 4.
 Substituted sulfonium and phosphonium bisphosphonic acids from refs. [31, 32]

Figure 5.
A lipophilic bisphosphonate: from ref. [35]

R₁ = H, OH, lower alkyl, methoxy and F
 R₂ = H, OH, lower alkyl and F
 R₃ = H and halogen

Compound	IC ₅₀ (nM)	Ki
EX I (4-azaindol-3-yl)-ethyl-bisphosphonic acid	37.4	5.9
EX II (5-azaindol-3-yl)-ethyl-bisphosphonic acid	6.6	0.6
6 Minodronate	1.9	0.0005
4 Risedronate	5.7	0.36
1 Alendronate	330.4	57

Compound	LED* (mg P/kg)	
	BMC*	BMD*
EX III (1R, 6S)-isomer	0.01	0.01
EX IV (1S, 6R)-isomer	> 0.1**	0.1
Racemic mixture	0.03	0.03

Figure 6.
 Novel nitrogen-containing bisphosphonates disclosed in refs. [40-43]

Figure 7.
 Imidazo[1,2- α]-pyridinyl bisphosphonate derivatives from ref. [44]

Figure 8.
C2-C5-alkyl-substituted [(imidazol-1-yl)-1-hydroxy-1-phosphono-ethyl]-phosphonic acids
from ref. [46]

7 $R_1 = \text{OH}$, **3-PEHPC**

7a $R_1 = \text{H}$

7b $R_1 = \text{F}$

7c $R_1 = \text{Cl}$

7d $R_1 = \text{Br}$

8 $R_1 = \text{OH}$, **3-IPEHPC**

8a $R_1 = \text{H}$

8b $R_1 = \text{F}$

8c $R_1 = \text{Cl}$

8d $R_1 = \text{Br}$

Figure 9.

Phosphonocarboxylate analogues of bisphosphonates, from ref. [45, 54]

X is CR₂;

Y is hydrogen, or with R₁, forms a bridge to complete an annealed benzo group;

Position **a** and **b** specify where the naphthyl moiety is bound to the rest of the molecule;

p is an integer from 0 to 4;

q is an integer from 0 to 3;

r is 1 or 2.

Figure 10.
Salicylic acid derivatives: from ref. [57]

Figure 11.
 Quinoline derivatives: from ref. [58]

9

FPPS inhibition
IC₅₀: 0.2 μM

10

FPPS inhibition
IC₅₀: 0.08 μM

Figure 12.

Other non-bisphosphonate FPPS inhibitors: from ref. [59]

Figure 13.
Fluorescent risedronate conjugate: from ref. [82]

Scheme 1. Synthesis of (pyridinium-1-yl)ethyl-1,1-diphosphonic acid, (quinolinium-1-yl)-1,1-diphosphonic acids and derivatives
 According to [24-27].

1) Synthesis of substituted sulfonium bisphosphonic acids

2) Synthesis of substituted phosphonium bisphosphonic acids

Scheme 2. Synthesis of substituted sulfonium and phosphonium bisphosphonates
 According to [31, 32].

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Table 1

Biological activity assay data for (pyridinium-1-yl)ethyl-1,1-diphosphonic acids, (quinolinium-1-yl)-1,1-diphosphonic acids and related analogues described in refs. from refs. [24-27]

Compound	<i>L. major</i> FPPS Ki (nM)	<i>D. discoideum</i> IC ₅₀ (μM)	human FPPS IC ₅₀ (μM)	γδT cell stimulation EC ₅₀	Bone resorption IC ₅₀ (μM)	Toxicity IC ₅₀ (μg/mL - human nasopharyngeal carcinoma cells)
1 Alendronate	95	32	NM	52	NM	NM
2 Pamidronate	190	167	NM	940	NM	NM
4 Risedronate	17	2.8	NM	6.2	NM	NM
5 Zoledronate	11	1.9	NM	7.3	NM	NM
278	18	2.1(2.6)*	1.6	5.1	0.67	300
297	38	1.5 (2.8)*	NT	4.6	0.22	5.3
300	9	2.8	1.7	3.7	0.41	5.4
335	160	8.5 (11)*	NT	430	NT	NT
359	75	2.9	NT	24	NT	NT
364	950	2.3	37	> 1000	NT	NT
443	110	12	NT	230	NT	NT
398	80	20	NT	41	NT	NT
447	380	72	NT	53	NT	NT
461	NM	3.7	NT	2.7	0.075	NT
472	NM	2.5	NT	NT	NT	NT
474	NM	4.4	NT	NT	NT	NT
480	NM	3	NT	NT	NT	NT

NT: not tested. NM: not mentioned in the patent.

* Both the data in and out the parenthesis are for the same compound in the patent, we include both data in the table.

Table 2

Biological activity assays of sulfonium and phosphonium bisphosphonic acids: from refs. [31, 32]

Compound	<i>T. brucei</i> FPPS IC ₅₀ (μM)	<i>D. discoideum</i> IC ₅₀ (μM)	human FPPS Ki (nM)	γδT cell stimulation EC ₅₀
1 Alendronate	NM	32	NM	52
2 Pamidronate	NM	167	NM	940
4 Risedronate	0.1	2.8	1.23	6.2
5 Zoledronate	0.32	1.9	1.25	7.3
527	0.4	4.78	NM	22.11
536	2	13.8	8.18	127.2
540	1.4	7.51	5.24	23.68
541	NM	207	22.63	1799
546	0.78	5.62	2.92	26.02
547	0.25	5.95	3.54	17.32
550	NM	101	9.84	375.6
564	1.1	8.30	4.34	260.9
572	0.24	8.87	3.44	217
575	0.59	6.58	3.42	75.52
585	0.18	1.85	5.68	NM
594	NM	7.15	13.94	413.8

NM: not mentioned in the patent.

Table 3Human FPPS inhibition assay data for Imidazo[1,2- α]-pyridinyl bisphosphonate derivatives

Compound	IC ₅₀ nM*	Ki
1-fluoro-2-(imidazo-[1,2- α]pyridin-3-yl)-ethyl-bisphosphonic acid (M1)	2.46	0.014
2-(imidazo-[1,2- α]pyridin-3-yl)-ethyl-bisphosphonic acid (M2)	2.59	0.013
[2-ethyl-imidazo-[1,2- α]pyridin-3-yl)-methyl-bisphosphonic acid (M3)	27	2.5
2-(7-methyl)-imidazo-[1,2- α]pyridin-3-yl-ethyl-bisphosphonic acid (M4)	39.7	6.1
1-fluoro-2-(7-methyl)-imidazo-[1,2- α]pyridin-3-yl-ethyl-bisphosphonic acid (M5)	60.3	9.6
2-(6-fluoro)-imidazo-[1,2- α]pyridin-3-yl-ethyl-bisphosphonic acid (M6)	106.4	17.5
2-(6-methyl)-imidazo-[1,2- α]pyridin-3-yl-ethyl-bisphosphonic acid (M7)	380	64
2-(8-hydroxy)-imidazo-[1,2- α]pyridin-3-yl-ethyl-bisphosphonic acid (M8)	893	151
6 Minodronate	1.9	0.0005
4 Risedronate	5.7	0.36
1 Alendronate	330.4	57
5 Zoledronate	4.1	0.07

* After pre-incubation. Data from ref. [44]