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REVIEW ARTICLE

Inhibition of farnesyl pyrophosphate (FPP) and/or geranylgeranyl pyrophosphate (GGPP) biosynthesis and its implication in the treatment of cancers

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ABSTRACT

Dysregulation of isoprenoid biosynthesis is implicated in numerous biochemical disorders that play a role in the onset and/or progression of age-related diseases, such as hypercholesterolemia, osteoporosis, various cancers, and neurodegeneration. The mevalonate metabolic pathway is responsible for the biosynthesis of the two key isoprenoid metabolites, farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Post-translational prenylation of various proteins, including the small GTP-binding proteins (GTPases), with either FPP or GGPP is vital for proper localization and activation of these proteins. Prenylated GTPases play a critical role in cell signaling, proliferation, cellular plasticity, oncogenesis, and cancer metastasis. Pre-clinical and clinical studies strongly suggest that inhibition of protein prenylation can be an effective treatment for non-skeletal cancers. In this review, we summarize the most recent drug discovery efforts focusing on blocking protein farnesylation and/or geranylgeranylation and the biochemical and structural data available in guiding the current on-going studies in drug discovery. Furthermore, we provide a summary on the biochemical association between disruption of protein prenylation, endoplasmic reticulum (ER) stress, unfolded protein response (UPR) signaling, and cancer.

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Introduction

The mevalonate pathway is responsible for the biosynthesis of all mammalian isoprenoids (Figure 1). These metabolites serve as the starting material for numerous other essential biomolecules, including steroids, bile acids, lipoproteins, vitamin D, heme A, ubiquinone, dolichol, and isopentenyladenine (Goldstein and Brown 1990). Consequently, this pathway is crucial in a plethora of biological processes that maintain the integrity of cell membranes (e.g. cholesterol), the balance of reproductive hormones (e.g. estradiol, progesterone, testosterone), electron transport mechanisms (e.g. ubiquinone), glycoprotein biosynthesis (dolichol), and modifications of tRNAs (e.g. biosynthesis of isopentenyladenine). Additionally, isoprenoids are essential for the post-translational prenylation and activation of many proteins that are important to human health.

The rate-limiting step of the mevalonate pathway is catalyzed by hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, leading to the formation of mevalonic acid. This metabolite is the immediate precursor of the 5-carbon isoprenoid units, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP; Figure 1). In humans, the first branching point of the pathway is occupied by the enzyme farnesyl pyrophosphate synthase (hFPPS), which is responsible for the catalytic elongation of DMAPP first to the C-10 metabolite geranyl pyrophosphate (GPP) and then to the C-15 isoprenoid farnesyl pyrophosphate (FPP). The immediate downstream enzyme, the human geranylgeranyl pyrophosphate synthase (hGGPPS) catalyzes the extension of the FPP substrate to the C-20 isoprenoid geranylgeranyl pyrophosphate (GGPP).

Post-translational modification of proteins with either FPP or GGPP is estimated to account for approximately 2% of all mammalian proteins (Nguyen et al. 2009). Known farnesylated proteins include many of the small GTP-binding proteins (GTPases), such as the Ras superfamily (e.g. H/K/N-Ras) (Kho et al. 2004), but also others proteins, such as the DnaJ chaperone proteins

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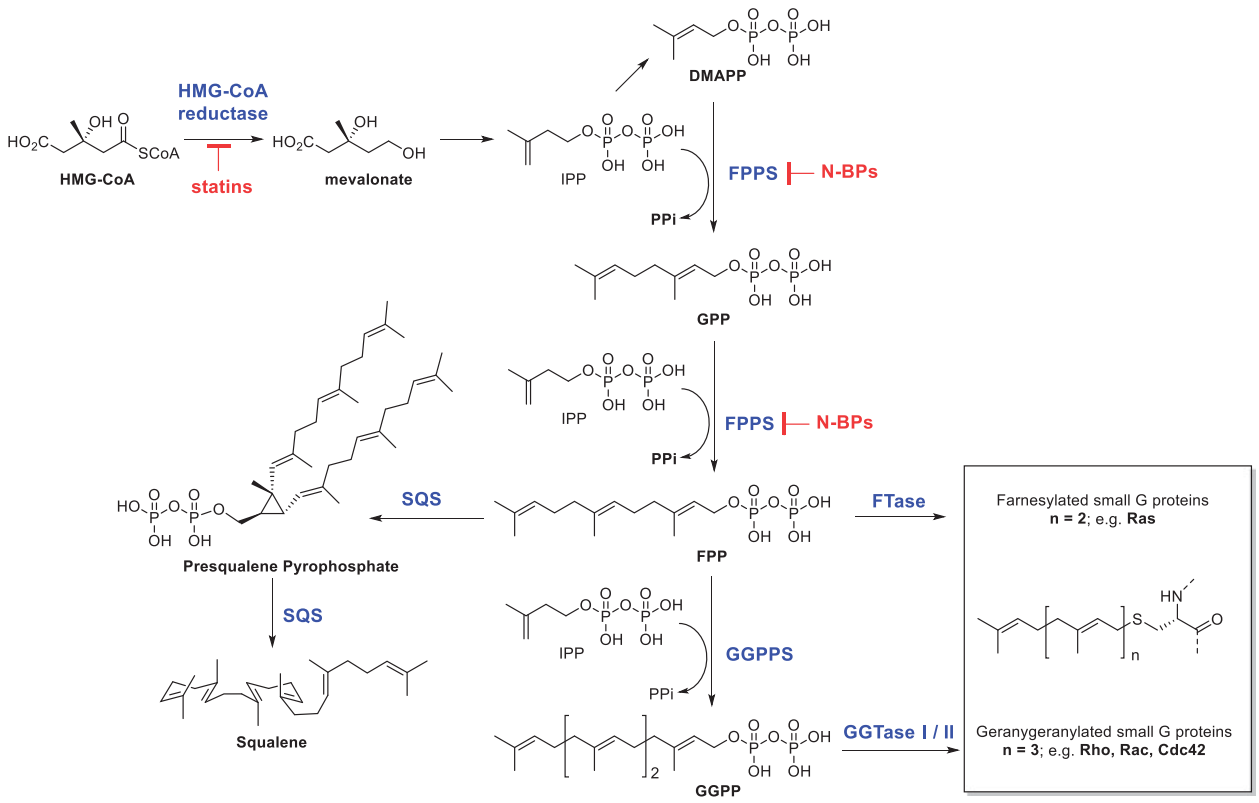

Figure 1. Schematic representation of the mevalonate pathway, indicating the major biochemical steps in isoprenoid biosynthesis. Important classes of clinically validated inhibitors of key enzymes in the pathway are indicated (e.g. statins and bisphosphonates) (see colour version of this figure at www.tandfonline.com/ibmg).

(Kampinga and Craig 2010; Stark et al. 2014) and the precursor peptide of the nuclear lamin A (Young et al. 2005; Chang et al. 2012). Geranylgeranylated GTPases include the Rho family of proteins (e.g. RhoA/B/C), the Ras-related proteins Rap1A, Rac-1 and Rab GTPases, and Cdc42. Post-translational prenylation of these proteins provides them with the ability to associate specifically with cellular membranes and participate in a plethora of biochemical mechanisms that are essential to cell survival, cell signaling, and proliferation (Takai et al. 2001), biological events that play a critical role in oncogenesis and cancer metastasis. It is noteworthy that there is also a strong association between prenylation and the synaptic plasticity of neurons (Hottman and Li 2014), as well as neurodegeneration and Alzheimer's disease (Eckert et al. 2009; Hooff et al. 2010; Hooff et al. 2012; De Schutter et al. 2014; Pelleieux et al. 2018).

In the past, drug discovery efforts targeting different steps of the mevalonate pathway focused mainly on inhibition of HMG-CoA reductase and hFPPS for the purpose of treating hypercholesterolemia and lytic bone disease, respectively. The statins are the best example of highly successful drugs targeting HMG-CoA reductase and widely used to reduce the risk of cardiovascular diseases. Statins represent an excellent example of a clinically validated class of prophylactic drugs that targets an essential metabolic pathway with minimal or negligible adverse effects.

Bisphosphonate drugs (BPs) are effective antiresorptive agents for the treatment of osteoporosis that were initially reported in the 1960s (Russell 2011). Although all BP drugs bind to bone and block osteoclastic activity, the molecular mechanism of action of the earlier analogs (i.e. the non-nitrogen containing analogs), such as clodronate (**1a**) and etidronate (**1b**), is different from that of the more potent (second generation analogs) nitrogen-containing BPs (N-BPs) which inhibit hFPPS with significant selectivity. The N-BP family of compounds includes hydrocarbon-based analogs, such as pamidronate (**2a**), alendronate (**2b**) and ibandronatoe (**3**), as well as heteroaromatic compounds, such as zoledronic acid (**4a**), risedronic acid (**5a**) and minodronic acid; some structures shown in Figure 2. Several excellent review articles have been written specifically on the therapeutic value of N-BPs and their osteoclast-mediated inhibition of bone resorption (Dunford et al. 2001; Dunford 2010; Fournier et al. 2010; Ebetino et al. 2011; Russell, 2011). Due to the high charge density of the bisphosphonate moiety, which exists as the trianion

under physiological conditions, BPs and N-BPs suffer from poor drug-like properties (in the classical sense). Nonetheless, N-BPs are important human therapeutics that improve the quality of life for patients with lytic bone diseases. For example, approximately 50% of all post-menopausal women suffer from osteoporosis and usually treated with N-BPs (Eastell et al. 2011) and approximately 70–80% of breast and prostate cancers ultimately metastasize to bone. In multiple myeloma (MM) patients, osteolytic lesions are one of several hallmark clinical features and more than 90% of these patients will develop bone lesions at some stage of their disease (Bianch and Munshi 2015). Skeletal manifestations of the aforementioned cancers are a major cause of morbidity that can be characterized by severe pain, impaired mobility, bone fractures, spinal cord compression, and hypercalcemia.

In addition to the hFPPS-mediated antiresorptive properties of N-BPs, numerous biochemical studies have suggested a strong association between the inhibition of protein prenylation and cancer cell survival (Clendening et al. 2010; Sorrentino et al. 2014; Mullen et al. 2016) or metastasis (Dudakovic et al. 2011). Although, inhibition of the mevalonate pathway at the HMG-CoA reductase step with statins has also been implicated in better survival of patients with various cancers (Nielsen et al. 2012; Kubatka et al. 2014), including breast cancer (Garwood et al. 2010) and MM (Sanfilippo et al. 2016), drug discovery in oncology has focused mainly on the downstream enzymes, hFPPS, hGGPPS, and their corresponding prenyl transferase enzymes, farnesyl transferase (FTase), as well as geranylgeranyl transferases (GGTase) I and II (Figure 1). This review will focus mainly on recent efforts aiming at downregulating the intracellular levels of FPP and/or GGPP biosynthesis, and consequently, the prenylation of proteins implicated in cancer.

The structure and function of the human farnesyl pyrophosphate synthase (hFPPS)

The mechanism of action of the early BP compounds (e.g. Figure 2; **1a**) does not involve inhibition of any particular enzyme of the isoprenoid pathway. Instead, metabolic incorporation of these compounds into stable ATP derivatives is believed to interfere with ATP-dependent cellular pathways (Rogers et al. 1992). In contrast, the main biological target of the N-BP drugs is the hFPPS enzyme, blocking the biosynthesis of FPP and modulating a large biochemical cascade that impacts both upstream and downstream events in the mevalonate pathway (Figure 1). The details of the

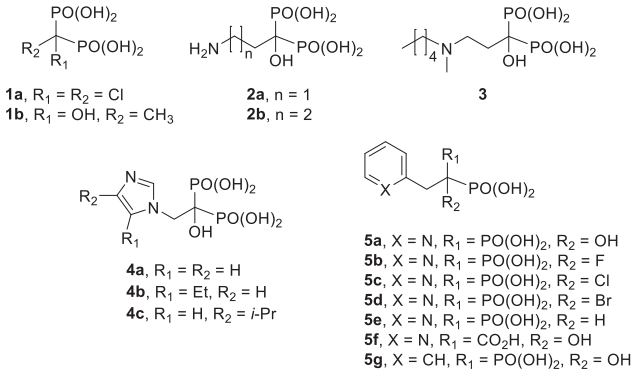

Figure 2. Examples of clinically validated N-BP inhibitors of the human FPPS and some of their derivatives.

interactions between N-BP drugs and hFPPS were first reported in 2006, independently by scientists from Novartis (Rondeau et al. 2006) and the Structural Genomic Consortium at the University of Oxford (Kavanagh, Guo et al. 2006). Their crystallographic studies revealed that hFPPS consists of a large cavity, having two charged surfaces on opposite walls of the cavity and only a small, partly lipophilic region (Figure 3). One of these surfaces is composed of two conserved aspartate-rich motifs (^{117}DDIMD^{121}, ^{257}DDYLD^{261}) that bind the pyrophosphate moiety of the substrates DMAPP/GPP via metal-mediated interactions with three magnesium cations (Mg^{2+}; Figure 3(b)). Adjacent to this surface is the small lipophilic region, which is lined with the side chains of Phe98 and Phe99 (commonly referred to as the "capping" phenyls), which binds the lipophilic tail of the enzyme's catalytic product (i.e. the lipophilic tail of GPP and FPP). The capping phenyls define the size of this lipophilic pocket and control the extent of isoprenoid polymerization to a maximum chain length of C-15 (Tarshis et al. 1996). Mutation of these phenyls has been shown to result in errors in the final length of the product's hydrophobic side chain.

The IPP binding sub-pocket is at the opposite wall of the DMAP/GPP binding cavity and lined with the positively-charged side chains of Arg and Lys residues, which interact directly with the IPP's pyrophosphate moiety (Figure 3(b)). The lipophilic side chains of the substrates lie against each other within van der Waals distance. Each condensation step in the polymerization reaction is driven by the dissociation of the pyrophosphate moiety of DMAP (or GPP in the second step) to give an allylic carbocation intermediate (this sub-pocket is also known as the allylic sub-pocket), which is subsequently captured by the double bond of an IPP unit with concerted deprotonation of IPP (Figure 4) (Poulter et al. 1978).

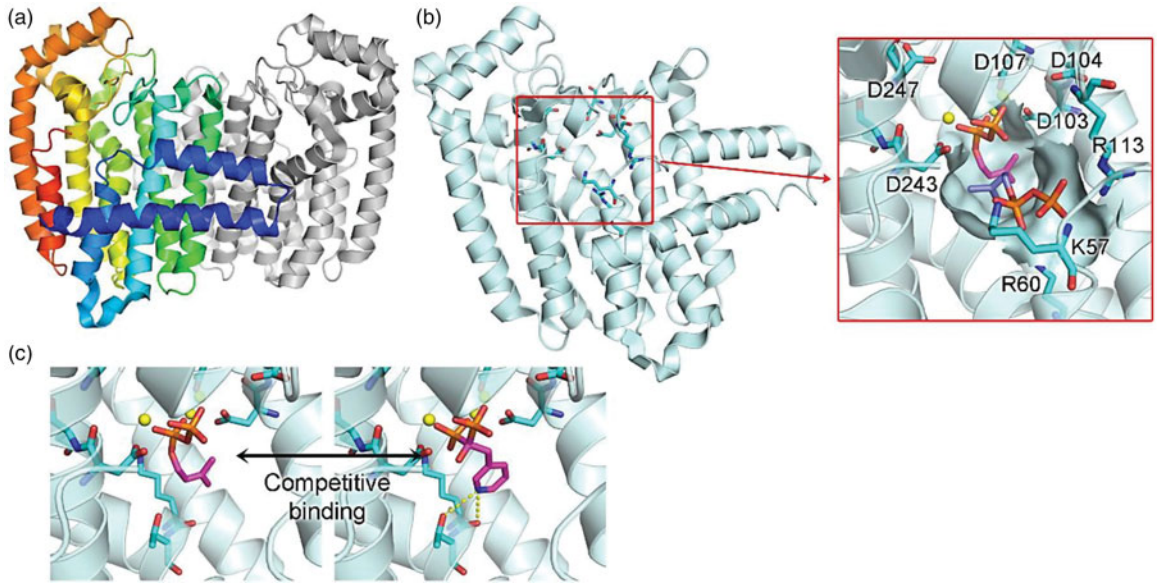

Figure 3. The structure of human FPPS (hFPPS). (a) The homodimeric full structure. One subunit is represented in a rainbow color scheme to indicate the N-terminal (blue) and C-terminal (red) ends. (b) The monomeric structure showing the active site cavity. The binding of the substrates is illustrated in the inset (DMAPP in magenta; IPP in purple; Mg^{2+} ions in yellow). The protein residues and surface of the key binding region are highlighted. (c) The competitive binding of risedronic acid (right panel) against DMAPP (left). Yellow dashes indicate the bifurcated H-bond formed between the N-BP drug and Lys-200/Thr-201 of the protein (see colour version of this figure at www.tandfonline.com/ibmg).

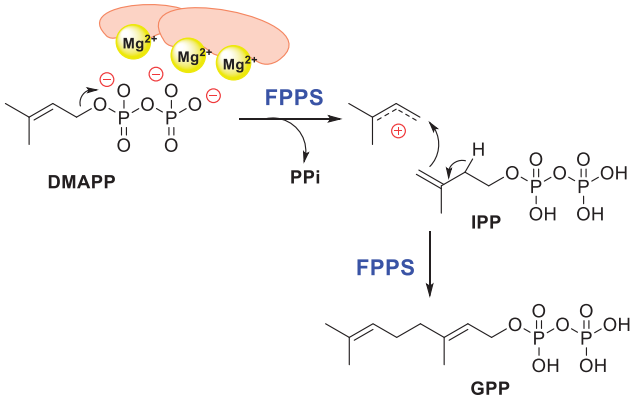

Figure 4. Schematic representation of the mechanism associated with the first condensation step in the biosynthesis of FPP (i.e. the condensation of DMAPP with IPP to give GPP) catalyzed by the human FPPS (see colour version of this figure at www.tandfonline.com/ibmg).

Inhibition of hFPPS

Clinically validated N-BP inhibitors of hFPPS, such as zoledronic acid (**4a**) and risedronic acid (**5a**), are bioisosteres of DMAPP and GPP, thus they compete for binding with these substrates in the allylic sub-pocket of the active site (Figure 3(c)). At the molecular level, formation of the hFPPS/N-BP complex, at least for small N-BP drugs, is driven primarily by the bisphosphonate moiety. However (under physiological conditions), their nitrogen-containing side chains are protonated and contribute to binding via the formation of a bifurcated hydrogen-bond with the carbonyl of Lys-200 and the hydroxyl of Thr-201 (Figure 3(c)). It has been proposed that this latter interaction mimics the putative allylic carbocation transition-state that forms during catalysis and removal of the nitrogen atom leads to dramatic loss in potency (Martin et al. 1999); for example, the in vitro potency (i.e. IC_{50}) of the phenyl analog **5 g** is 250-fold lower than the potency of risedronic acid (**5a**) in inhibiting hFPPS (Figure 2; Table 1) (Dunford et al. 2008). The Cα-hydroxyl moiety that characterizes the most potent clinical drugs is also involved in interactions with the enzyme and affects potency (e.g. IC_{50} values for **5a** and **5e** are approximately 6 nM and 33 nM, respectively; Table 1), in addition to maximizing the affinity of these compounds for the bone mineral hydroxyapatite (Marma et al. 2007; Dunford et al. 2008).

Interestingly, large time-dependent shifts in the intrinsic potency (IC_{50} values) of the most potent *N*-BP inhibitors (e.g. **4a**, **5a**, **7a**) (Dunford et al. 2008; Lin et al. 2012) has been shown to correlate with their binding

Table 1. Relative inhibition data of risedronic acid analogs.

Compound	hFPPS Final IC_{50} (nM)
5a	5.7[a]
5b	34[b]
5c	16[b]
5d	95[b]
5e	32.6[b]
5f	>200,000[a,b]

[a]IC_{50} values reported (Kavanagh, Guo et al. 2006).
[b]IC_{50} values reported (Marma et al. 2007).

mechanism and the conformational changes they induce to the protein structure of hFPPS (Rondeau et al. 2006; Dunford et al. 2008; Räikkönen et al. 2011). The initial binding of an N-BP inhibitor to the DMAPP/GPP sub-pocket occurs in the fully "open" and solvent-exposed active site of the enzyme. This step is followed by a rigid body movement of the C-terminal subdomain of the protein that closes the entrance to the allylic sub-pocket and shapes the IPP binding sub-pocket simultaneously. These events are followed by the binding of IPP and folding of the previously disordered ^{350}KRRK^{353} C-terminal tail over the IPP sub-pocket, thus completely shielding the entire active site cavity. The basic residues of the C-terminal tail are essential for catalysis (Song and Poulter 1994) and upon closing, they secure all substrates into position and prevent quenching of the allylic carbocation intermediates by bulk water during the catalytic cycle (Figure 4). Interestingly, binding of N-BP inhibitors to hFPPS in the presence of inorganic pyrophosphate (PPi) also leads to the "fully closed" state of the enzyme (Park et al. 2014), thus preventing replacement of the deeply buried inhibitor by a competing substrate. This mechanistic model may explain why the binding of potent N-BP drugs is deemed nearly irreversible and responsible for the excellent in vivo efficacy of these drugs as antiresorptive agent (Rondeau et al. 2006; Park et al. 2012).

However, exploratory N-BP inhibitors with much larger and conformationally rigid side chains, such as inhibitor **6a** (Figure 5), suggest a somewhat different mechanism (Lin et al. 2012). The co-crystal structure of **6a** bound to the allylic sub-pocket (PDB code 4DEM) revealed that the ^{350}KRRK^{353} tail is mostly closed even in the absence of co-bound IPP or PPi, although perhaps less rigidified than in the ternary complex of hFPPS/**4a**/IPP (PDB code 1ZW5). These differences may be due to the conformational changes in the protein caused by the expansion of the allylic sub-pocket upon binding of these larger molecules, and possibly some crystallographic variation. It is noteworthy that larger inhibitors with flexible side chains, such as the pyridinium-based inhibitor **8b** (Zhang et al. 2009, 2010) do not distort the conformation of the allylic sub-pocket (in the way analogs **6** and **7** do). Instead, it has been shown that the flexible side chain of **8b** adopts a "folded" conformation within the *Saccharomyces cerevisiae* FPPS active site, thus avoiding steric clashes with the capping phenyls. As expected, the target specificity of analogs with general structure **8** is highly dependent on the length of their side chain. For example, the short chain analog **8a** exhibits greater potency in inhibiting hFPPS than hGGPPS, whereas analog **8b** that has a

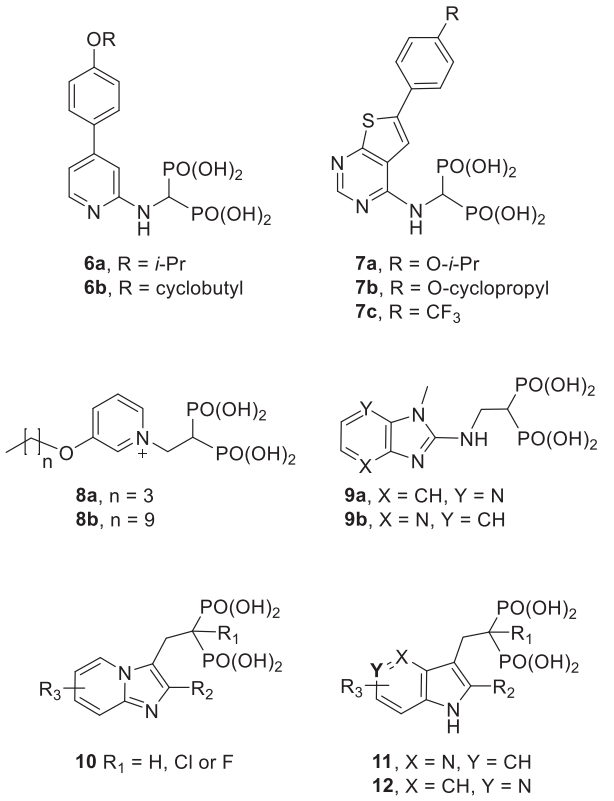

Figure 5. Examples of exploratory N-BP inhibitors of the human FPPS.

longer side chain exhibits virtually no selectivity between hFPPS and its functional/structural homolog hGGPPS (IC_{50} values of 100 nM and 280 nM in inhibiting hFPPS and hGGPPS, respectively) (Zhang et al. 2010); consequently, compound **8b** has been described as a dual hFPPS/hGGPPS inhibitor. Numerous other structurally diverse bisphosphonate inhibitors of hFPPS have been reported and explored as potential therapeutic agents; some representative examples are shown in Figures 2 and 5.

Since small N-BP molecules are chemically stable bioisosteres of pyrophosphates (diphosphates), their physicochemical properties are very similar to those of small isoprenoids (i.e. existing as trianions at physiological pH), thus limiting their utility to bone-related disease. In fact, drugs such as zoledronic acid (**4a**), bind so avidly to bone that their half-life (in bone) can be months to years, depending on the specific drug used and the type of disease affecting the rate of bone turnover (Grey et al. 2009; Frost et al. 2012). In chronic diseases (e.g. osteoporosis), concerns that prolonged use of high-dose N-BPs can cause side effects, such as osteonecrosis of the jaw (ONJ) and atypical femoral fractures (Rizzoli et al. 2008), have led to the recommendation by some physicians for patients to take a "drug holiday". However, this treatment can lead to uncertainty with

respect to the type of drug and dose used, as well as the duration of treatment for different patients (Eastell et al. 2011). Although ONJ is fairly uncommon in patients treated for osteoporosis, it is a long-lasting disorder that can occur in patients treated for bone cancer metastasis with high doses of intravenous bisphosphonates (Hoff et al. 2008; Dimopoulos et al. 2009; Ripamonti et al. 2009). The systemic half-life of current N-BP drugs is extremely low; for example, after i.v. administration of zoledronic acid (**4a)**, 50% of the dose gets bound to bone and the rest is rapidly cleared by the kidneys. Consequently, the dose-limiting toxicity of zoledronic acid is based on nephrotoxicity (Skerjanec et al. 2003; Weiss et al. 2008).

In order to increase the systemic exposure of N-BP drugs to non-skeletal tissues, POM esters (Zhang et al. 2006) and peptide pro-drugs (Ezra et al. 2000), as well as formulation with liposomes (Shmeeda et al. 2010), have been investigated, unfortunately with limited success. Past efforts also focused on replacing the Cα-hydroxyl moiety, in order to reduce the pKa of the bisphosphonate and decrease the affinity of these drugs for bone (Marma et al. 2007; Jahnke and Henry 2010). For example, the Cα-deoxy analog of risedronic acid, analog **5b**, the Cα-halogenated derivatives **5c**–**5e**, as well as the less polar phosphonocarboxylate **5f** have been investigated (Marma et al. 2007). A clear drop in intrinsic potency was observed that correlates with decreased charge density on the bisphosphonate pharmacophore and potentially a steric clash with the protein surface in the case of the larger halide atoms (Table 1). Replacement of one phosphonate moiety with the less charged carboxylic acid (e.g. analog **5f**), leads to essentially an inactive compound against hFPPS (an IC_{50} value greater than 200 μM was reported) (Marma et al. 2007). It is noteworthy that the phosphonocarboxylate analog **5f** was found to be a weak inhibitor of the prenyl transferase enzyme GGTase II (also known as Rab geranylgeranyl transferase; RGGT) with IC_{50} vales in the double digit micromolar range (IC_{50} of ~24 μM). The ability of this compound to reduce the viability of J774 cells was also explored and an EC_{50} value of 2.6 mM was reported. However, at such high concentrations of a compound (i.e. mM concentrations), there is significant concern that reduction in cell viability may be (at least in part) due to non-selective toxicity.

Numerous other structurally diverse N-BP inhibitors of hFPPS with much larger lipophilic side chains have also been explored as selective inhibitors of hFPPS (Figure 5) (examples include: Dunford et al. 2001; Simoni et al. 2008; De Schutter et al. 2010; Lolli et al. 2010; Ebetino et al. 2010a; Ebetino et al. 2010b; Ebetino et al. 2010c; De Schutter et al. 2012; Leung, Langille et al. 2013; Leung, Park et al. 2013; De Schutter et al. 2014; Gritzalis et al. 2015). Unfortunately, none of these compounds exhibit the ability to block cancer cell proliferation at a therapeutically relevant, low nanomolar potency range. In contrast, the dual hFPPS/hGGPPS inhibitor pyridinium **8b** was shown to block the viability of MCF-7 breast cancer cells with an EC_{50} of ~100–200 nM (Zhang et al. 2009). Some insight into whether a compound is selectively binding to its intended biological target in cells can be gained by co-treating the cells with a toxic concentration of an inhibitor and farnesol (FOH) or geranylgeraniol (GGOH), to circumvent the effects of the inhibitor. The prenyl alcohols (FOH and GGOH) are metabolically converted to their corresponding pyrophosphate isoprenoids in cells (Crick et al. 1995; Fliesler and Keller 1995) and therefore, can rescue cells from growth inhibition or apoptosis, assuming these are caused by intracellular FPP or GGPP depletion. For example, rescue of cell growth inhibition (e.g. MCF-7 breast and PC-3 prostate cancer cells) and protein prenylation impairment when induced by zoledronic acid (**4a**) has been observed with both FOH and GGOH (Jagdev et al. 2001), although the effect was more pronounced with GGOH (Goffinet et al. 2006). However, neither FOH nor GGOH was able to revert the growth inhibition of MCF-7 cells caused by inhibitor **8b** (Zhang et al. 2009), perhaps suggesting more complex intracellular effects associated with the mechanism of action of this compound.

In spite of the numerous biochemical studies indicating some (weak) activity in blocking the proliferation of various types of cancer cells, including prostate (Iguchi et al. 2010; Mani et al. 2012), breast (Räikkönen et al. 2010; Dedes et al. 2012), and colorectal (Notarnicola et al. 2004) cancers, human glioblastoma (Cimini et al. 2011), and MM, clinical validation of an hFPPS inhibitor as a bona fide anti-tumor agent is still elusive. Higher expression of hFPPS has been observed in human prostate cancer tissues (as compared to controls), suggesting an association between abnormally high levels of prenylation and disease progression (Todenhöfer et al. 2013). Similarly, whole genome sequencing of MM tumors from 38 MM patients demonstrated that 50% of these patients harbored either K-Ras or N-Ras coding mutations, underscoring the importance of prenylation/farnesylation of these oncogenes in MM (Chapman et al. 2011). More importantly, a randomized clinical trial (>1700 patients) has shown that when standard chemotherapy is supplemented with zoledronic acid (**4a**) it leads to a statistically significant increase in the

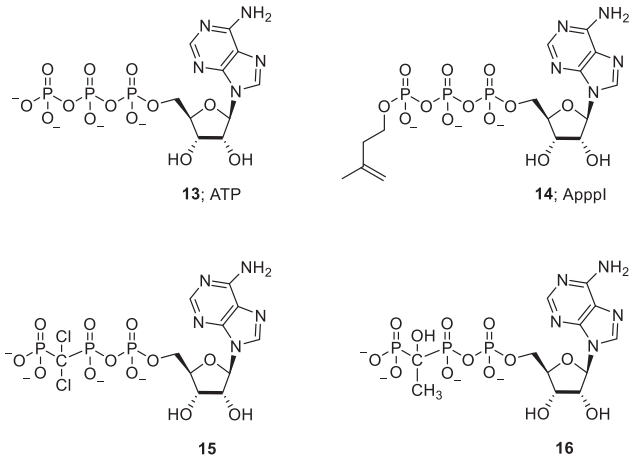

Figure 6. Structures of the ATP (**19**) metabolites of IPP (**20**), clodronate (**21**), and etidronate (**22**).

disease progression-free survival and overall survival of MM patients, as compared to patients treated with chemotherapy plus clodronic acid (**1a**); the latter compound is not an inhibitor of hFPPS (Morgan et al. 2010; Morgan et al. 2012). Analogous observations were also made in a randomized clinical trial (1803 patients) involving premenopausal breast cancer patients treated with standard adjuvant chemotherapy plus zoledronic acid (Gnant et al. 2009); however, these findings seemed to be inconsistent for different age groups of breast cancer patients (Coleman et al. 2011).

Although most of the above studies attributed the anti-tumor effects observed (albeit minimal) to the decrease in prenylation of various oncogenic GTPases, as a consequence of inhibiting hFPPS, the upstream levels of isoprenoids in the mevalonate pathway are also simultaneously affected. For example, intracellular accumulation of IPP has been shown to cause an increase in the concentration of an ATP-derivative adduct, known as ApppI (**14**; Figure 6). ApppI inhibits the mitochondrial adenine nucleotide translocase (ANT) enzyme, inducing cell apoptosis (Mönkkönen et al. 2006; Mitrofan et al. 2009). IPP is also a natural antigen that directly stimulates γδ T cells expressing Vγ2Vδ2 T cell receptors and is strongly implicated in the human innate immune response against tumors (Morita et al. 2007). As previously mentioned, ATP derivatives **15** and **16** of the non-nitrogen-containing BP, clodronate (**1a**) and etidronate (**1b**), respectively (Figure 6), have been identified and proposed to be the molecular mediators of the osteoclast apoptosis observed with these compounds (Rogers et al. 2011).

Additionally, short hairpin RNA-mediated knockdown of hFPPS in hematopoietic and non-hematopoietic tumor cell lines has been shown to activate Vγ2Vδ2 T cells and induce IFN-γ secretion (Li et al. 2009; Wang et al. 2011). Immunostimulation and increased Vγ9Vδ2 T cell-mediated cytotoxicity has been observed in animal models of human breast cancer after treatment with N-BPs, suggesting an adjuvant immunosurveillance role induced by N-BPs in cancer chemotherapy (Benzaïd et al. 2012). Evidence for the stimulation of Vγ2Vδ2-bearing T cells by N-BPs has also been observed in MM patients treated with pamidronic acid (**2a**) (Kunzmann et al. 1999) and prostate cancer patients treated with zoledronic acid (**4a**) (Naoe et al. 2010). In the case of prostate cancer, the observed T cell effects coincided with reduction in serum prostate-specific antigen (PSA), providing further support of the hypothesis that N-BPs can contribute to an anti-tumor immune response *in vivo* (Naoe et al. 2010). Activation of γδ T cells in vitro correlates specifically with inhibition of hFPPS and has not been observed with structurally related N-BPs that target other downstream prenyl synthase enzymes, such as hGGPPS, hSQS, or decaprenyl pyrophosphate synthase (hDPPS) (Zhang et al. 2010).

Allosteric inhibition of the hFPPS

To date, all inhibitors of hFPPS reported that bind to the active site of the enzyme are characterized by a bisphosphonate pharmacophore. For many years, it has been assumed that the chemical nature of such molecules limits their cell membrane permeability and distribution to non-skeletal tissues, thus compromising their clinical validation as true anti-neoplastic agents. This reasonable hypothesis, in addition to the inability to discover selective active site inhibitors of hFPPS that exhibit low nanomolar potency in cell-based anti-tumor assays, has fueled efforts towards the identification of allosteric inhibitors for this target. Initially, use of fragment-based screening by NMR and X-ray crystallography allowed the identification of such compounds and showed that they bind to an allosteric pocket, near the IPP binding site (Jahnke et al. 2010). More recently, the biological role of this allosteric pocket was shown to bind the FPP catalytic product of the enzyme, locking its conformation in an inactive form and consequently, providing a feed-back mechanism for controlling the intracellular levels of isoprenoid biosynthesis in vivo (Park, Zielinski et al. 2017). To date, many structurally diverse non-bisphosphonate inhibitors have been reported that bind to this allosteric pocket with high affinity, including analogs **17**–**22** (Figure 7) (Cotesta et al. 2010; Jahnke et al. 2010; Marzinzik et al. 2015; Park, Leung et al. 2017). In vitro potencies in the low nanomolar range have been observed with some

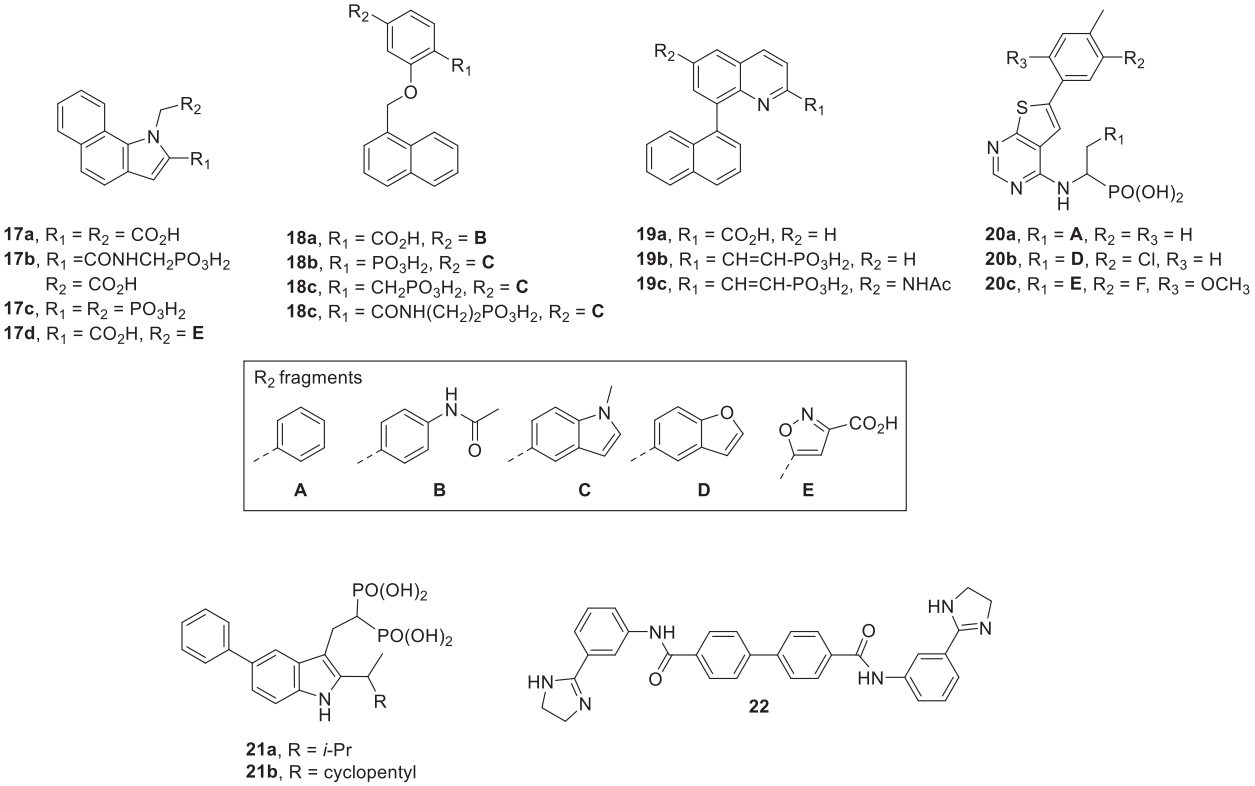

Figure 7. Structures of non-bisphosphonate inhibitors that bind to the allosteric pocket of hFPPS (**17**–**21**) or another pocket on the protein away from the active site (**22**).

of these compounds using either a traditional enzymatic assay (Kavanagh, Dunford et al. 2006; Park, Leung et al. 2017) or an inhibition assay based on LC/MS/MS (Glickman and Schmid 2007; Jahnke et al. 2010; Marzinzik et al. 2015). Efforts by a number of research groups have contributed to this field (Liu et al. 2014, 2015), although some of the compounds reported do not actually bind inside the hFPPS allosteric pocket (e.g. compound **22**) (Liu et al. 2015). Unfortunately, despite the fact that many of these non-bisphosphonate compounds possess superior "drug-like" properties (as defined by Lipinski's rules; Lipinski et al. 2001), as compared to the N-BPs, none of them exhibit any significant anti-tumor activity in cell-based assays, perhaps suggesting that the challenges impeding drug discovery efforts may be more complex than the currently understood/expected biochemical consequences of hFPPS inhibition.

The structure and function of the human geranylgeranyl pyrophosphate synthase (hGGPPS)

In contrast to hFPPS, much less is known about the structure, function, and potential therapeutic value of the immediate downstream enzyme in the mevalonate pathway, the human GGPPS (hGGPPS). Although there are over 50 co-crystal structures reported of inhibitors bound to the human FPPS (both active site and allosteric pocket inhibitors), only two structures of the human GGPPS have been reported so far (Kavanagh, Dunford et al. 2006; Lacbay et al. 2018). Consequently, most of our current assumptions regarding the structure of hGGPPS and its interactions with substrates and inhibitors are based primarily on analogies with hFPPS and the structures of GGPPSs from other sources (Guo et al. 2007), such as *S. cerevisiae* (PDB code 2DH4) (Chen CK-M et al. 2008), and *Plasmodium vivax* (PDB codes: 3MAV, 3PH7, 3LDW) (Artz et al. 2011). Other crystal structures of GGPPSs include those from *Geoglobus acetivorans* (archaeon; PDB code 5JFQ), *Sinapis alba* (mustard; PDB code 2J1P), *Pyrococcus horikoshii* (PDB code 1WY0), and *Thermus thermophilus* (PDB code 1WMW).

Despite low sequence identity (17%), the tertiary structure of hGGPPS is remarkably similar to that of hFPPS (Figure 8(a) vs. Figure 3(b)). The conserved aspartate-rich (^{64}DDIED^{68} and ^{188}DDYAN^{192}) motifs are also found at locations equivalent to those in hFPPS, facing the central catalytic cavity from opposite sides of the active site (Figure 8(a)). However, hGGPPS displays a different quaternary structure: unlike hFPPS, which exists as a homodimer, dimers of hGGPPS associate further into a three-blade propeller-shaped homohexameric complex (Figure 8(b)). Biochemical investigations have

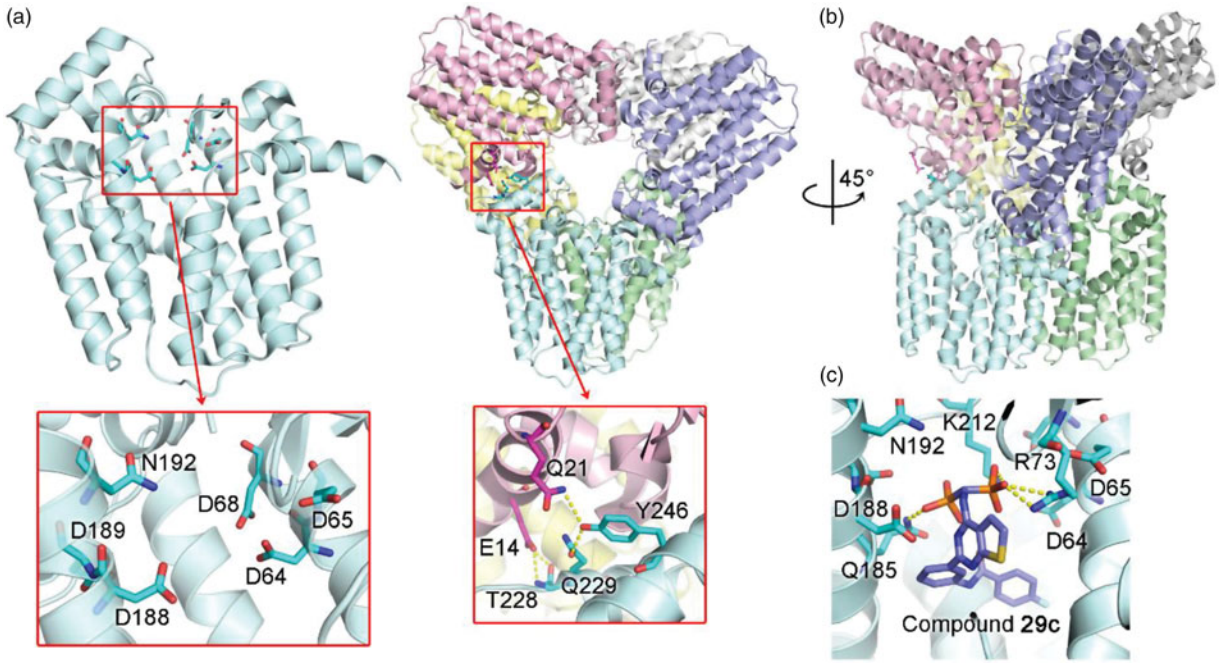

Figure 8. The structure of hGGPS. (a) The tertiary structure showing the conserved DDXXD/N motifs. (b) The homohexameric complex of wild-type human GGPPS. Monomeric subunits are indicated by different colors. The inset shows residues forming inter-dimer H-bonds (yellow dashes). (c) Compound **29c** bound to the active site of a dimeric mutant form of hGGPPS (Y246D; PDB code 6C57) (see colour version of this figure at www.tandfonline.com/ibmg).

even reported the formation of octameric complexes in solution (Miyagi et al. 2007). The core of the dimer-dimer interface is composed of the hydrophobic surface formed by the N-terminal residues Tyr18, Phe76, Pro77, Ile82, and Tyr83 of one subunit and the C-terminal residues Ile233, Ile243, and Tyr246 of the contacting subunit. This contact is further stabilized by the H-bond interactions between Glu14 and Thr228, and Gln21 and Tyr246 (Figure 8(b), inset). Interestingly, the hexameric quaternary structure is unique only to mammalian and insect GGPPS. A sequence alignment analysis indicated that the residues forming the inter-dimer region in hGGPPS are not conserved in plant, fungal, archaeal, or bacterial orthologs (Kavanagh, Dunford et al. 2006); the dimeric nature of these orthologs have been confirmed crystallographically over the years.

The catalytic mechanism of hGGPPS is also presumed to be virtually identical to that of hFPPS (Figure 4). The pyrophosphate moiety of the allylic substrate (i.e. FPP) binds against a highly conserved patch of negatively charged side chains via three Mg^{2+}-mediated interactions. However, the capping phenyls (Phe98/99) observed in the hFPPS structure that bind the allylic substrates' isoprenyl tail are replaced by the smaller side chains of Ala59 and Ser60 in the hGGPPS, thus allowing space for the C-20 isoprenoid product of this enzyme. The IPP binding sub-pocket of hGGPPS is also lined with basic residues (as in hFPPS), which interact directly with the pyrophosphate of IPP (i.e. Arg28, His57, Arg73, and Arg74). During the catalytic reaction, the pyrophosphate of FPP dissociates to produce the allylic carbocation, which is then captured by the IPP double bond (Figure 4). Consistent with this hypothesis, the three amino acid residues presumed to stabilize the allylic carbocation intermediate formed in the hFPPS active site (i.e. from DMAPP or GPP) are all conserved in hGGPPS active site (i.e. Lys151, Thr152, and Gln185).

Inhibitor design of hGGPPS

To date, very few selective inhibitors of hGGPPS have been reported (Figure 9) and although none of these inhibitors have yet advanced to clinical development a couple have been evaluated in vivo. It is noteworthy that the value of developing selective inhibitors of hGGPPS as potential anti-tumor agents has been debated in the literacture (Zhang et al. 2009). A reasonable assumption is that inhibitors of hFPPS can directly block the farnesylation of mutated, oncogenic Ras proteins, and indirectly downregulate the intracellular levels of GGPP (via depletion of the FPP substrate of hGGPPS), thus will also block the prenylation of GGPP-dependent GTPases (Figure 1). Based on this assumption, compounds that inhibit exclusively hGGPPS were presumed to be less effective as anti-tumor agents than

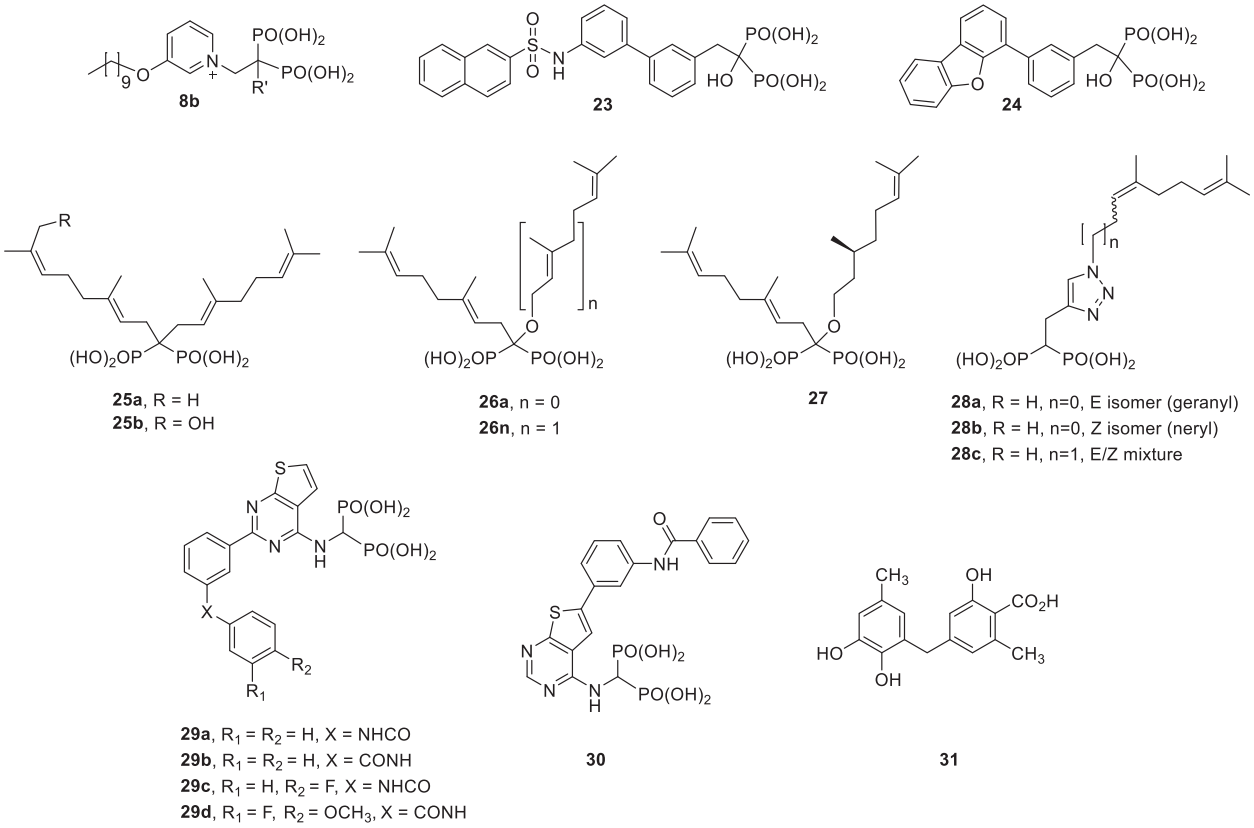

Figure 9. Structures of selective hGGPPS inhibitors or dual hFPPS/hGGPPS inhibitors.

those inhibiting hFPPS, or dual hFPPS/hGGPPS inhibitors, such as the pyridinium bisphosphonate inhibitor **8b** (Figure 9). As mentioned earlier, compound **8 b** has been described as a dual hFPPS/hGGPPS inhibitors and is ~100-fold more potent than zoledronic acid (**4a**; Figure 3) in blocking tumor cell growth; the N-BP drug zoledronic acid is the most potent and selective hFPPS inhibitor. Both compounds were tested in MCF-7 breast cancer cells and EC_{50} values of ~100–200 nM and ~15 μM were reported for **8b** and **4a**, respectively (Zhang et al. 2009). Interestingly, the anti-tumor effects of **4a** in MM has been attributed (at least in part) to its ability to indirectly block geranylgeranylation of GTPases (e.g. Rap1A), by inhibiting hFPPS and causing intracellular depletion of FPP (Guenther et al. 2010).

Examples of hGGPPS inhibitors reported, include the polyaromatic bisphosphonates **23** and **24** (Guo et al. 2007; Zhang et al. 2009), the isoprenoid derivatives **25**–**28** (Shull et al. 2006; Wiemer et al. 2007; Barney et al. 2010), and the C-2 substituted thienopyrimidine bisphosphonates (C^2ThP-BPs) **29** (Lacbay et al. 2018). Although the natural product gerfelin (**31**) has also been reported to inhibit hGGPPS (IC_{50} of 12 μM) (Zenitani et al. 2003; Kanoh et al. 2013), its catechol-based structure is a known pan-assay interference structural motif that binds metals and interferes with redox cellular functions (Baell and Walters 2014). Bisphosphonate analogs **23**–**29** are all more selective in inhibiting hGGPPS than hFPPS.

Some insight on the enzyme-ligand interactions has been provided by the co-crystal structure of bacterial GGPP bound to the human GGPPS (Kavanagh, Dunford et al. 2006). This structure revealed that the pyrophosphate of the GGPP ligand was bound to the DDXX(D/N) motifs in the allylic site, and its hydrocarbon tail extended into a deep mostly lipophilic channel, which is lined with aliphatic and aromatic side chains and located below the active site. The GGPP ligand in this structure was an unintentional purification artifact, derived from the heterologous bacterial expression of the human recombinant GGPPS enzyme. This hGGPPS-GGPP complex is thought to represent a feedback inhibitory state and is consistent with the observation that both GGPP and 3-azaGGPP can act as competitive inhibitor of this enzyme with respect to its FPP substrate (Kavanagh, Guo et al. 2006; Kavanagh, Dunford et al. 2006). Given that hGGPPS and other GGPPSs have two large hydrophobic sub-pockets, one within and another near the active site cavity (the latter is referred to as the GGPP inhibitor channel), it is not surprising that larger lipophilic bisphosphonates can bind to these enzymes in multiple binding modes (Guo et al. 2007).

For example, diverse binding modes of compounds **23** and **24** have been observed in the co-crystal structures of the yeast GGPPS (Guo et al. 2007; Cao et al. 2008; Chen CK-M et al. 2008; Zhang et al. 2009). Although partial binding in the allylic substrate site has been observed involving the bisphosphonate moiety of these inhibitors, their side chains often extended into the tail elongation site (e.g. PDB entries 2Z4X and 2Z52), whereas for others, their binding mode is analogous to that of the *Escherichia coli* GGPP bound to human GGPPS (PDB code 2Q80), where their lipophilic side chains occupy the presumed GGPP inhibitory channel (e.g. PDB codes 2Z7H and 2ZEV). Furthermore, some compounds have been found to bind at two separate sites, where one molecule binds at the allylic substrate site, and the other at the IPP binding site but with its bulky side chain extending into the GGPP inhibitory channel (PDB IDs: 2E93 and 2Z4Y). Recently, a co-crystal structure of inhibitor C^2BhP-BP **29c** bound to the active site of a dimeric and catalytically competent hGGPPS Y246D mutant was reported. This mutant was created by disrupting the interdimer contacts mediated by Tyr 246 in the wild-type hGGPPS, which forms a hexameric complex in solution. The Y246D hGGPPS mutant was created based on the presumption that a dimeric form of the enzyme may be less challenging that the wild type protein for crystallographic studies. In spite low resolution of the co-crystal structure of hGGPPS Y246D-**29c** (PDB code 6C57; 3.50 Å resolution), the electron density for the inhibitor indicated binding between the aspartate-rich motifs, which suggests that it competes against FPP (Figure 8(c)). Additionally, the *p*-fluorophenyl tail of **29c** appeared to insert into the hydrophobic cavity formed between α_D and α_F, which typically accommodates the isoprenyl tail of FPP in the catalytic cycle. The thienopyrimidine core of **29c** appeared to extend into the second substrate site and consequently, this inhibitor may also interfere with IPP binding.

It is generally agreed upon by scientists involved in drug discovery that the hallmark of medicinal chemistry is the establishment of a reliable structure-activity relationship (SAR) model. Unfortunately, the collective structural information currently available on GGPPSs (albeit mostly from the yeast enzyme), does not provide a clear pharmacophore model that can be used to guide the design of potent and highly selective inhibitors of the human enzyme that also possess good drug-like properties. Examples of apparent SAR discrepancies include lack of structural evidence to account for the potency difference observed for different compounds. For example, although the intrinsic potency of the highly flexible bisphosphonate **8b** is approximately 10-fold better than that of the conformationally more ridged polyaromatic compound **23** (IC_{50} values of 280 nM and 2.7 μM) in inhibiting hGGPPS, they both bind in the same GGPP inhibitory channel of the enzyme (~25 Å in length) with mostly hydrophobic interactions (Guo et al. 2007). It was reported that the distinct pyridinium cation of compound **8b** is not required for binding in this channel (PDB code 2zeu), whereas the sulfonamide moiety of compound **23** forms a bifurcated H-bond with His68 (PDB code 2E95) and its side chain is approximately the same length as the GGPP metabolite (16.2 Å vs. 17.1 Å) (Guo et al. 2007). Consequently, the structural information for these two compounds does not provide any obvious explanation regarding their potency difference.

Interestingly, the V-shaped digeranyl pyrophosphate **25a** has equivalent potency to **8b** in inhibiting hGGPPS, but has a much larger window of selectivity against hFPPS (IC_{50} values of 410 nM vs >10 μM in inhibiting hGGPPS vs. hFPPS). This branched isoprenyl bisphosphonate (i.e. **25a)** has been shown to bind with one of its aliphatic chain in the allylic substrate binding site (i.e. similar binding to FPP) and the other in the GGPP product binding inhibition channel (e.g. PDB: 2Z4W and 2Z4Z) of the yeast GGPPS (Chen CK-M et al. 2008). A similar binding mode has been proposed for the triazole derivatives **28** (Wills et al. 2015). Surprisingly, whereas the homoneryl analog **28b** (Z isomer) is approximately 40-fold more potent than its corresponding homogeranyl derivative **28a** (E isomer) (IC_{50} values of 17 μM and 380 nM, respectively) (Zhou et al. 2014), the *E/Z* mixture of the slightly longer compound **28c** exhibits an inhibition potency (IC_{50}) of 45 nM and is more portent than either one of its single isomers (Wills et al. 2017). The bishomoisoprenoid triazole *E/Z*-**28c** is currently one of the most potent and selective inhibitors of hGGPPS reported (Wills et al. 2015). Its excellent potency has been attributed to a plausible synergistic co-binding in both the FPP substrate and GGPP product inhibitory channel (Chen CK-M et al. 2008; Wills et al. 2017); however, confirmation of this hypothesis is pending crystallographic evidence.

Recently, medicinal chemistry efforts focusing on the C^2ThP-BP inhibitors of hGGPPS with general structure **29** revealed that some analogs of this chemotype exhibit selectivity against hFPPS (Lacbay et al. 2018). For example, inhibitor **29a** was found to exhibit in vitro potencies (IC_{50}) of 64 nM and 2.0 μM in hGGPPS and hFPPS, respectively. It is noteworthy that C-6 substituted thienopyrimidine-based bisphosphonates (C^6ThP-BPs) were previously identified to be more potent in inhibiting hFPPS (e.g. analogs **7**; Figure 5). However,

synthesis of the C^6ThP-BP derivative **30**, having the exact same sidechain as the C^2ThP-BP analog **29a** revealed that **30** was totally inactive in both enzymatic assays at concentrations up to 10 μM. These results strongly suggest that very subtle differences in the molecular recognition elements involved in protein–ligand interactions are critical for both potency and target selectivity.

The exquisite precision with which hFPPS and hGGPPS differentiate between their respective substrates, in spite the fact that these substrates have very little structural diversity and significant conformational flexibility (i.e. C-5, C-10, or C-15 olefinic side chain), surpasses our current understanding of the molecular recognition elements dictating ligand selectivity for hGGPPS versus hFPPS. Therefore, the notion that SAR studies focusing on hFPPS and hGGPPS can simply be guided by the presence of a bisphosphonate pharmacophore and the size and length of the side chain are inaccurate.

Preclinical in vivo evaluation of hGGPPS inhibitors

Collectively, and in spite of all the challenges, past efforts have generated a number of molecular tools (i.e. several compounds shown in Figure 9) that can provide some insight into the plausible clinical relevance of hGGPPS inhibitors. For example, in vivo treatment with the digeranyl bisphosphonate **25a** (IC_{50} value of 200 nM in inhibiting hGGPPS) in a mouse model produced a decrease in the progression of pulmonary fibrosis after lung injury (Osborn-Heaford et al. 2015). Similarly, the hydroxylated derivative **25b** was reported to decelerate prostate cancer metastasis in mice injected with luciferase-expressing PC-3 prostate cancer cells (Reilly et al. 2015, 2017). However, the potency of **25b** in inhibiting hGGPPS in vitro (IC_{50}) is only 0.8 μM, and this compound is not toxic to luciferase-expressing PC-3 prostate cells in a MTT assay (an EC_{50} value of approximately 100 μM was estimated), thus casting some uncertainty as to its intracellular selectivity in target engagement and exact biochemical mechanism. In contrast, inhibitor *E/Z*-**28c** blocks the proliferation of MM cells with significant potency (EC_{50} of 190 ± 58 nM in RPMI-8226 cells) (Wills et al. 2015). Therefore, its in vivo properties are of interest and under investigation; preliminary metabolic stability, toxicology, and pharmacokinetics studies were recently reported (Haney et al. 2018). Inhibitor *E/Z*-**28c** was reported to be metabolically stable in human and mouse liver microsomes and to disrupt geranylgeranylation of Rap1A in CD-1 mice liver, kidney, and spleen, thus confirming systemic distribution and target engagement in vivo. However, single i.v. dosing in CD-1 mice revealed a maximum tolerated dose of 0.5 mg/kg, while doses higher than 1 mg/kg resulted in liver toxicity.

Similarly, the C^2ThP-BP inhibitor **29d** (EC_{50} value of 100–150 nM) was shown to be approximately equipotent to *E/Z*-**28c** and to doxorubicin in blocking the proliferation of RPMI-8226 cells. However, the hGGPPS inhibitor **29d** is significantly less toxic to normal bronchial cells (NHBE) than doxorubicin (Lacbay et al. 2018). Pre-clinical evaluation of this compound revealed that it is metabolically stable in male CD-1 mouse (MLM), Sprague-Dawley rat (RLM) and human (HLM) liver microsomes, with a half-life clearance greater than 2 h in all species. The anti-myeloma properties of **29d** were evaluated in aged Vk*MYC transgenic mice (average 50 weeks old), which faithfully recapitulates the characteristics of the human MM disease and mimic the therapeutic responses of MM patients to clinically validated drugs (Chesi et al. 2012; Gomez-Palou et al. 2013). Mice with disease burden measurable by serum protein electrophoresis (i.e. M-protein levels higher than 15% of total serum proteins, a biomarker of MM disease burden) were treated with 12 doses of compound **29d** at 3 mg/kg/d or vehicle (phosphate buffered saline; PBS) by intraperitoneal injection over a period of 14 d. At the end of treatment, Western blot analysis of peripheral blood mononuclear cell (PBMC) lysates clearly showed inhibition of Rap 1 A geranylgeranylation, confirming systemic exposure and target engagement in vivo. Additionally, proof of anti-myeloma efficacy was also confirmed, as evidenced by a significant decrease in serum M-protein seen in the mice treated with **29d** as compared to an increase in animals treated with vehicle. It is noteworthy that the half-life of mouse immunoglobulins (i.e. M-protein) is approximately 7 d (Vieira and Rajewsky 1988). Therefore, the observed decrease in M-protein after less than 2 weeks of treatment is an exciting result that clearly proves the in vivo anti-myeloma efficacy of inhibitor **29d**. Although assessment of the blood chemistry of plasma samples from the treated mice revealed some liver toxicity, the observations were highly variable between animals. Furthermore, there was no correlation between the increase of alanine transaminase (ALT) and aspartate transaminase (AST) levels and decrease of M-protein levels that could potentially imply a mechanism-based toxicity. However, more in-depth investigations are required to confirm the safety of this compound.

Biochemical association between disruption of protein prenylation, endoplasmic reticulum stress, unfolded protein response (UPR) signaling, and cancer

Continued proliferation and immune evasion within harsh tumor microenvironments (e.g. hypoxic, nutrient-limited, etc.) requires malignant cells to co-opt cellular homeostatic mechanisms. One important adaptive mechanism utilized by cancer cells is the unfolded protein response (UPR), which comprises a set of signaling pathways that are initiated to overcome accumulation of unfolded protein within the endoplasmic reticulum, a situation referred to as ER stress (Walter and Ron 2011). Evidence for ER stress and corresponding activation of UPR signaling has been broadly reported across many human cancer types (Wang and Kaufman 2014). The mammalian UPR is initiated by three ER transmembrane sensor proteins: inositol requiring enzyme 1 alpha (IRE1α), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6). The concerted downstream effects of these three ER stress transducers is to expand the size and protein folding capacity of the ER, to remove misfolded protein via increased ER-associated degradation (ERAD), and to transiently decrease ER protein folding demand by two means: Ire1-regulated mRNA decay (RIDD) and global repression of protein translation by PERK through phosphorylation of eIF2α. If these various UPR adaptive mechanisms fail to reduce the burden of unfolded protein in the ER in a timely fashion, UPR signaling transitions from adaptive to pro-apoptotic responses (Tabas and Ron 2011).

Evidence implicating the UPR in tumorigenesis, inflammation, tissue invasion, angiogenesis, immune evasion, and resistance to cancer therapy have been the subject of numerous excellent reviews (Urra et al. 2016; Avril et al. 2017; Cubillos-Ruiz et al. 2017), so these important topics will not be discussed in detail here. Instead this section focuses on how inhibitors of isoprenoid biosynthesis impact upon ER proteostasis and the UPR homeostatic mechanisms that are employed by cancer cells to help tolerate elevated secretory protein demands and to sustain proliferation within harsh tumor microenvironments.

Over the last decade, it has been appreciated that in vitro statin exposure can result in apoptosis of cancer cell lines from various tumor types, including: glioma (Jones et al. 1994), promyelocytic leukemia (Perez-Sala and Mollinedo 1994), mesothelioma (Rubins et al. 1998), acute myeloid leukemia (AML) (Xia et al. 2001), and MM (van de Donk et al. 2002). The principal cause of statin-induced apoptosis in AML, MM, and lymphoma cells was shown to result primarily from indirect impairment of protein geranylgeranylation (Xia et al. 2001; van de Donk, Kamphuis, et al. 2003; van de Donk, Schotte, et al. 2003), occurring via depletion of cellular reserves of the substrates required for both isoprenoid pyrophosphate synthase enzymes, hFPPS and hGGPPS. Similarly, hFPPS-targeting bisphosphonates have been shown to induce apoptosis in cancer cell lines, albeit typically at relatively high concentrations (20–100 μM) (Aparicio et al. 1998; Fromigue et al. 2000; Lee et al. 2001). As with the apoptosis caused by statin-mediated HMG-CoA reductase inhibition, it has been reported that apoptotic responses to N-BPs that are selective inhibitors of hFPPS appear to be predominantly due to impaired geranylgeranylation (Goffinet et al. 2006; Okamoto et al. 2014). Despite several decades of research on the topic, a complete and detailed mechanism for the observed (direct) anti-tumor activities of statins and N-BPs are still lacking. There is pressing need for further studies to delineate the complex cell biology that is elicited during inhibition of isoprenoid biosynthesis and so this remains an area of great interest.

Regarding the mechanism of apoptosis induced by impaired isoprenoid biosynthesis, it has been shown that treatment of human cells with either statins or hFPPS-targeting N-BPs, results in elevated ER stress and corresponding activation of UPR signaling (Chen JC et al. 2008; Ghavami et al. 2012; Lan et al. 2013). For example, it was shown that statin treatment can activate all three branches of the mammalian UPR in cultured human airway cells (Ghavami et al. 2012). Autophagy was also shown to be induced following statin exposure and, more importantly, both chemical (bafilomycin A1) or genetic (ATG3 or ATG5 knockout cells, or ATG7 knockdown) impairment of this autophagic response was shown to augment the apoptosis seen in response to statin treatment (Ghavami et al. 2012, 2014). It is well established that UPR activation leads to upregulation of autophagy (Bernales et al. 2006), thus providing yet another layer of complexity to the sophisticated cellular adaptive responses that are instigated when ER proteostasis is disrupted by inhibition of isoprenoid biosynthesis.

Given that inhibition of isoprenoid biosynthesis results in increased ER stress, autophagy, and ultimately apoptosis, it is evident that cancers with high secretory protein burdens, such as immunoglobulin-secreting MM cells, should be ideally suited for evaluating the therapeutic potential of novel hGGPPS inhibitors. Indeed, it has been shown that depletion of cellular GGPP by lovastatin, zoledronic acid (**4a**), or direct inhibition of hGGPPS by the digeranyl inhibitor **25a** leads to disrupted intracellular light chain trafficking and a concomitant activation of UPR signaling in MM cells (Holstein and Hohl 2011). The disruption of ER-to-Golgi

trafficking of MM light chains is thought to result from impaired geranylgeranylation of a variety of Rab GTPases that are known to be important for intracellular vesicular trafficking (Hutagalung and Novick 2011).

Inhibition of prenyl transferase enzymes

Previously, in addition to direct inhibition of the human enzymes involved in the biosynthesis of the isoprenoids FPPS and GGPPS, the prenyl transferase enzymes, FTase, GGTase I and II (Figure 1), also received considerable attention as potential therapeutic targets in oncology. Efforts towards the discovery of anti-tumor agents that specifically target the prenyl transferase enzyme FTase led to a number of disappointing clinical trials, before it was realized that a biochemical redundancy mechanism allows K-Ras activation by geranylgeranylation (catalyzed by GGTase I), which takes over the task of Ras prenylation, when FTase is inhibited (Yokoyama et al. 1997; Rowinsky 2006). This redundancy mechanism has been blamed for the failure of FTase inhibitors (e.g. tipifarnib) to demonstrate significant clinical efficacy in the intended treatments of pancreatic (90% K-Ras mutations), lung and colon carcinomas (~30% K-Ras mutations) (Sparano et al. 2009). Nonetheless, clinical development of some FTase inhibitors is still ongoing and a phase III registration-directed trial of tipifarnib in H-RAS mutant head and neck squamous cell carcinomas (HNSCC) was recently initiated.

Investigations in support of GGTase I as a valid therapeutic target have also been reported. Genetic studies have shown that conditional deletion of the gene encoding the β-subunit of GGTase I in myeloid and lung cancer, almost completely eliminates proliferation and tumor formation in mice, leading to markedly improved survival (Sjogren et al. 2007). This study and others have suggested that inhibition of geranylgeranylation may be a useful strategy for treating K-Ras-induced malignancies, in addition to other human diseases that are driven by GGPP specific prenylation of proteins. For greater anti-tumor efficacy, dual inhibitors of the prenyl transferase enzymes FTase and GGTase I have also been evaluated (Lerner et al. 1997); examples include the Merck compound L-778,123, a dual inhibitor of FTase and GGTase I with in vitro inhibition potencies (IC_{50} values) of 2 nM and 98 nM, respectively (Lobell et al. 2002). This compound was purposely designed as a dual FTase/GGTase I inhibitor in order to overcome the biochemical redundancy mechanism described above and consequently, completely block K-Ras prenylation (Whyte et al. 1997). L-778,123 was advanced to phase I clinical trials in patients with pancreatic cancer, but it was eventually withdrawn from clinical development due to its toxicity (Martin et al. 2004).

In summary, although some interest remains in potentially treating cancer by downregulating protein prenylation at the prenyl transferase enzymes (i.e. FTase, GGTase I and II), due to the apparent lack of stringent substrate selectivity of these enzymes and the above mentioned redundancy mechanism, interest in this approach has markedly declined in recent years.

Conclusions and future directions

The cumulative knowledge gained from the above body of literature is that blocking protein prenylation by inhibiting hFPPS and/or hGGPPS, thus decreasing the intracellular levels of FPP and GGPP isoprenoids and consequently, protein prenylation results in ER stress, UPR signaling, autophagy, and (ultimately) apoptosis across many different cancer types; these effects appear to be caused predominantly by impaired protein geranylgeranylation. One plausible reason for the higher efficacy of hGGPPS inhibitors could be related to the lower levels of expression of this enzyme in cancer cells as compared to hFPPS (Lacbay et al. 2018). High intracellular levels of a biological target can be a major challenge for achieving clinical efficacy, since a high dosing regimen would likely be required that could compromise the therapeutic window.

Therefore, given the central importance of GGPP depletion in inducing cancer cell apoptosis, a number of research groups have directed their more recent efforts towards the identification of selective inhibitors of hGGPPS. This direct targeting of hGGPPS has already yielded compounds with markedly improved cell-based anti-tumor potency (e.g. **28c**, **29d**) when compared to established hFPPS targeting N-BP drugs (Wiemer et al. 2007; Zhou et al. 2014; Wills et al. 2015; Wills et al. 2017; Lacbay et al. 2018). In addition, direct inhibition of the downstream hGGPPS enzyme may avoid some of the unintended consequences that occur when more upstream enzymes in the mevalonate pathway, specifically hFPPS or HMG-CoA reductase, are inhibited. To date, a very limited number of groups have reported hGGPPS inhibitors that are sufficiently potent and have reasonable biopharmaceutical properties to allow preclinical assessment in animal models (Reilly et al. 2017; Haney et al. 2018; Lacbay et al. 2018).

In spite of all the challenges and past disappointments, recent studies clearly suggest that there is a strong biochemical connection between prenylation and cancer thus, strongly suggesting that this field of research is worthy of further investigation. The relationship between

isoprenoids and cancer is likely more complex than initially presumed and skepticism about the design of drugs targeting human metabolic pathways (that are essential to both heathy and malignant cells) should not be ignored. Currently, an estimated 15% of the world's population is over the age of 60 and this group is expected to increase to approximately 25% by 2050. For many individuals, a longer life will also mean more years of suffering due to age-related diseases, particularly cancer, which accounts for approximately 23% of the leading causes of death in the US (De Magalhães et al. 2017). Oncology is clearly an area of major unmet therapeutic needs and changes in metabolic pathways are implicated in both cancer progression and malignancies of the ageing population, such as MM, breast, and prostate cancer. Furthermore, cellular senescence (a state of cell cycle arrest) is induced by cellular stress and believed to play a central role in ageing and age-related diseases, such as cancer and Alzheimer's disease (Childs et al. 2017). In addition to the strong association between intracellular levels of prenylated GTPases and cancer, other prenylated proteins, such as the farnesylated precursors of nuclear lamins (prelamin A) play a critical role in cellular senescence. The latter knowledge may suggest that hFPPS inhibitors may also find new applications in medicine, assuming compounds with good drug-like properties can be identified. Induction of cellular senescence has been proposed as a potent tumor-suppressive mechanism that arrests cell proliferation (Freund et al. 2012). Inhibition of processing of the farnesylated prelamin A to the mature nuclear lamina has been shown to induce senescence and block cancer cell migration, a required biological process for cancer metastasis (Matralis et al. 2018). Therefore, multidisciplinary investigations that can lead to better understanding of the role of protein prenylation in cancer could provide new therapeutic agents and uncover insights in biomedical sciences that can address the prevention of many cancers in the aging population.

Disclosure statement

No potential conflict of interest was reported by the authors.

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