Bioorganic & Medicinal Chemistry 18 (2010) 7212–7220

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of a series of aromatic bisphosphonates

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ARTICLE INFO

Article history:
Received 4 June 2010
Revised 11 August 2010
Accepted 16 August 2010
Available online 19 August 2010

Keywords:
Bisphosphonate
GGDPS
Enzyme inhibitor
Isoprenoid biosynthesis
Protein prenylation
Geranylgeranyl diphosphate

ABSTRACT

Geminal bisphosphonates display varied biological activity depending on the nature of the substituents on the central carbon atom. For example, the nitrogenous bisphosphonates zoledronate and risedronate inhibit the enzyme farnesyl diphosphate synthase while digeranyl bisphosphonate has been shown to inhibit the enzyme geranylgeranyl diphosphate synthase. We now have synthesized isoprenoid bisphosphonates where an aromatic ring has been used to replace one of the isoprenoid olefins in an isoprenoid bisphosphonate and investigated the ability of these new compounds to impair protein geranylgeranylation within cells. Several of these new compounds are potent inhibitors of the enzyme geranylgeranyl diphosphate synthase.

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1. Introduction

Interest in geminal bisphosphonates (**1**) as structural analogs of pyrophosphates (**2**) is well established (Fig. 1).[1] These compounds are formed by formal replacement of the P–O–P linkage with the P–C–P bond, but the bisphosphonates are more stabile to metabolism and introduction of the methylene linker allows additional structural modifications that would be impossible in the pyrophosphate structure. Geminal bisphosphonates have demonstrated utility in a variety of applications. Historically, they have been used as chelating agents[2] and water softeners.[3] More recently bisphosphonates have found application in the clinic as drugs for the treatment of bone related disease. For example, zoledronate (**3**) has been used in the treatment of osteoporosis as well as multiple myeloma and a variety of cancers,[1] and risedronate (**4**) is used for treatment of osteoporosis.[4] In addition, bisphosphonates have shown great potential as cancer therapeutics[5] and as anti-parasitic agents.[6,7] Their ability to inhibit growth of malignant cell lines[8,9] as well as their ability to activate γδ-Tcells[10] has been documented.

There is conjecture that inhibitors can be designed for specific steps in the mevalonate pathway, and in fact bisphosphonates have served as inhibitors of squalene synthase,[11,12] isopentenyl pyrophosphate (IPP, **6**, Fig. 2) isomerase,[13,14] farnesyl diphosphate synthase (FDPS, EC 2.5.1.10),[15,16] and other enzymes involved in isoprenoid metabolism.[17] It is now recognized that the enzymatic target of the clinically used nitrogenous bisphosphonates (NBPs) is FDPS.[15,16] This enzyme is responsible for production of farnesyl diphosphate (FPP), the key branch point in the mevalonate pathway. However the mechanisms leading to the observed cellular effects of these bisphosphonates are less well understood. Through inhibition of FDPS, bisphosphonates such as zoledronate cause depletion of FPP which has a direct impact on protein farnesylation. Depletion of FPP levels also results in diminished cellular levels of geranylgeranyl diphosphate (GGPP), and the cellular effects observed may result from an impact on the downstream process of protein geranylgeranylation.[18–22] Many forms of cancer exhibit abnormalities in prenylated GTPases,[23] which encourages investigation into the potential of bisphosphonates as direct anti-cancer therapeutics.

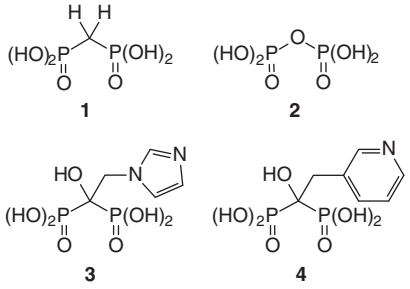

Figure 1. Pyrophosphate and some bisphosphonates.

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doi:10.1016/j.bmc.2010.08.036

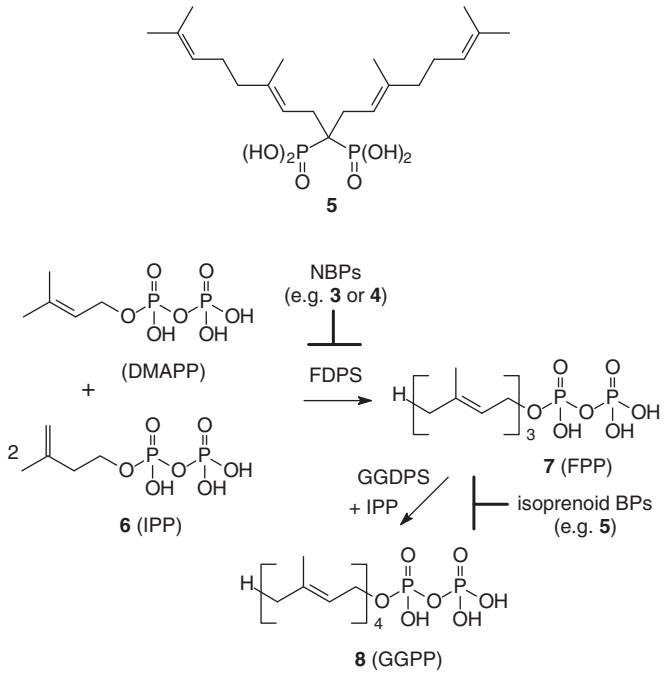

Figure 2. Some steps in isoprenoid biosynthesis and bisphosphonate inhibitors.

Our labs have reported the synthesis of mono- and dialkyl isoprenoid bisphosphonates (e.g., **5**, Fig. 2) that selectively target geranylgeranyl diphosphate synthase (GGDPS, EC 2.5.1.29) over FDPS.[24,25,26] The enzyme GGDPS catalyzes elaboration of FPP (**7**) to GGPP (**8**), which is the required precursor for all protein geranylgeranylation. To explore further this class of compounds, aromatic analogs of mono- and digeranyl bisphosphonate (**5**) have been designed, synthesized, and assayed. These analogues incorporate both a geranyl chain as found in our GGDPS inhibitors and an aromatic ring parallel to that of risedronate.

There are several reasons to include an aromatic moiety in the structure of potential GGDPS inhibitors. First, many of the most potent inhibitors of FDPS are nitrogenous bisphosphonates with an aromatic substructure, including zoledronate (**3**) and risedronate (**4**), but it is not yet known whether introduction of an aromatic moiety will enhance or diminish specificity for GGDPS inhibition in an isoprenoid bisphosphonate. It would be interesting to determine if the aromatic compounds will retain their activity as inhibitors of GGDPS despite their more sterically demanding profile. Second, there is evidence that the high charge to mass ratio in salts of bisphosphonic acids at physiological pH can present some problem with transversing the cell membrane.[27] Aromatic bisphosphonates are more lipophilic than the lead compound digeranyl bisphosphonate (**5**), which may result in more facile drug delivery to the cell and relieve the need for use of a prodrug.[28] Third, bisphosphonates that contain isoprenoid olefin isomers display differing biological activity, but the potential isomerization or transposition of an alkene in vivo would be eliminated with an aromatic substructure. Finally, the larger π system of an aromatic ring may lead to more favorable stacking interactions in the active site of GGDPS (e.g., with tyrosine 205 or phenylalanine 175).[29] For these reasons, we have pursued the chemical synthesis and biological evaluation of aromatic-isoprenoid bisphosphonates, and we report here these findings.

2. Chemical synthesis

The bisphosphonates described herein were synthesized using parallel reaction sequences. For example, both compounds **12** and **14** were synthesized from commercially available *m*-bromobenzyl alcohol (Fig. 3). The alcohol **8** was protected by treatment with TBSCl and imidazole to provide the silyl ether **9** in 90% yield. The resulting silyl ether undergoes halogen-metal exchange upon treatment with *n*-BuLi and subsequent reaction with prenyl bromide readily affords the alkylated product. Deprotection upon treatment with TBAF then gives the known alcohol **10** in 72% yield over three steps. Initial attempts at bromide formation via reaction of alcohol **10** with PBr_3 gave unacceptable results. Instead, bromide formation was achieved by preparation of the mesylate followed by treatment with LiBr to form the target benzylic bromide. Because it was assumed to be reactive, this material was not purified further but instead was moved forward in the synthesis as first isolated. Thus, tetraethyl methylenebisphosphonate was treated with NaH and the resulting anion was treated with the intermediate benzylic bromide to provide the desired bisphosphonate **11** in 47% yield over 3 steps. Hydrolysis of bisphosphonate **11** gave the tetrasodium salt **12** smoothly. Synthesis of the dialkyl bisphosphonate **13** was achieved by alkylation of compound **11** with geranyl bromide. Ester hydrolysis through reaction with TMSBr and final treatment with sodium hydroxide provided the bisphosphonate **14** salt in 88% yield over these two steps.

The isomeric *para*-substituted compounds were obtained through an analogous series of reactions as shown in Figure 4. These reactions proceeded in a fashion parallel to those described above, and gave the monoalkyl bisphosphonate **19** and the dialkyl bisphosphonate **21** in good yields.

Compound **24**, a bisphosphonate bearing an unsubstituted phenyl ring and one geranyl chain, was prepared from tetraethyl methylenebisphosphonate (Fig. 5). In this case it was attractive to add the benzyl substituent to the parent bisphosphonate first, followed by alkylation with geranyl bromide to obtain the dialkyl bisphosphonate **23**. To prepare the corresponding pyridyl compound, an analogue more similar to risedronate, it was found to be advantageous to add the geranyl chain first and then alkylate the geranyl bisphosphonate with bromide **25**. In both cases, the standard hydrolysis was employed to obtain the desired bisphosphonate salts **24** and **27**. Finally, the known bisphosphonate **28** was prepared to serve as a control.[30]

3. Biological results and discussion

The aromatic bisphosphonates described above were evaluated for activity in both enzyme and various whole cell assays. Our laboratory has previously identified numerous mono- and dialkyl bisphosphonates as inhibitors of GGDPS, and many of these compounds also have been shown to decrease protein geranylgeranylation in cellular assays.[25] Based on our previous work, we hypothesized that the aromatic bisphosphonates synthesized would inhibit GGDPS. Compounds were first screened in vitro against recombinantly purified human GGDPS enzyme (Fig. 6A). Compound **5** was used as a positive control because it was previously shown to inhibit GGDPS.[26] At 10 μM concentrations, the dialkyl bisphosphonates **24**, **14**, **21**, and **27** all displayed various degrees of GGDPS inhibition while the mono alkyl compounds **12**, **19**, and **28** displayed little or no activity. Concentration–response curves were then generated to further characterize the compounds active in the initial screen. Compounds **14** and **21** were found to display potent inhibitory activity with IC_{50} values (the concentration at which the enzyme is inhibited to 50% maximal activity) extrapolated to be 250 nM and 800 nM, respectively (Fig. 6B). As a comparison, the published IC_{50} value of compound **5** is 200 nM.[26] The structure of GGDPS has been solved when complexed with digeranyl bisphosphonate (**5**), which bound to the 'inhibitor' binding site[31] in a 'V-shaped' conformation occupying portions of both the FPP and GGPP binding sites.[29] Based on the

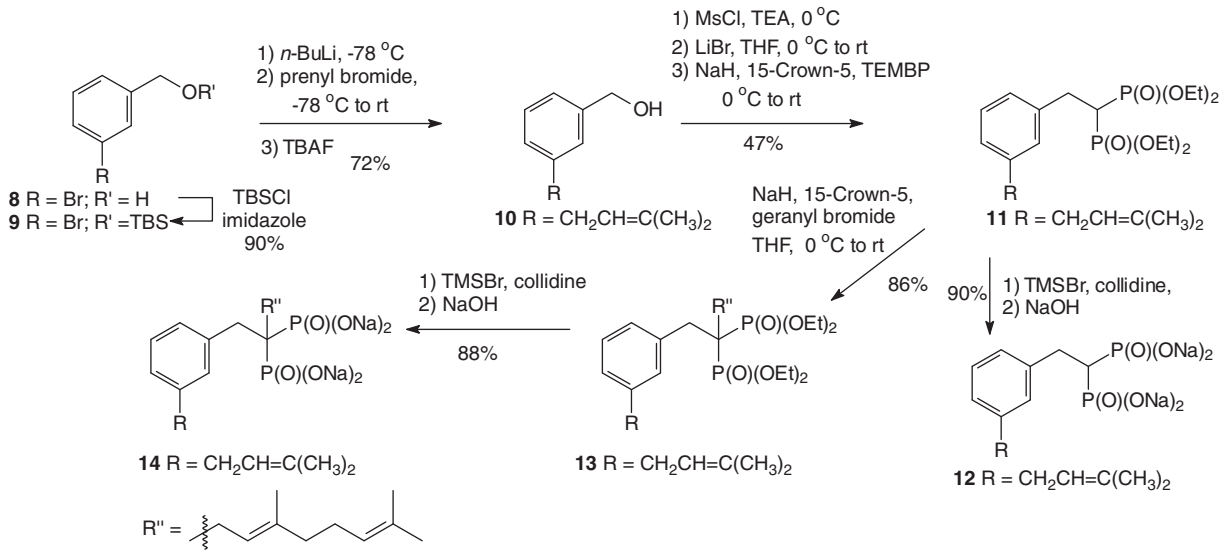

Figure 3. Synthesis of bisphosphonates **12** and **14**.

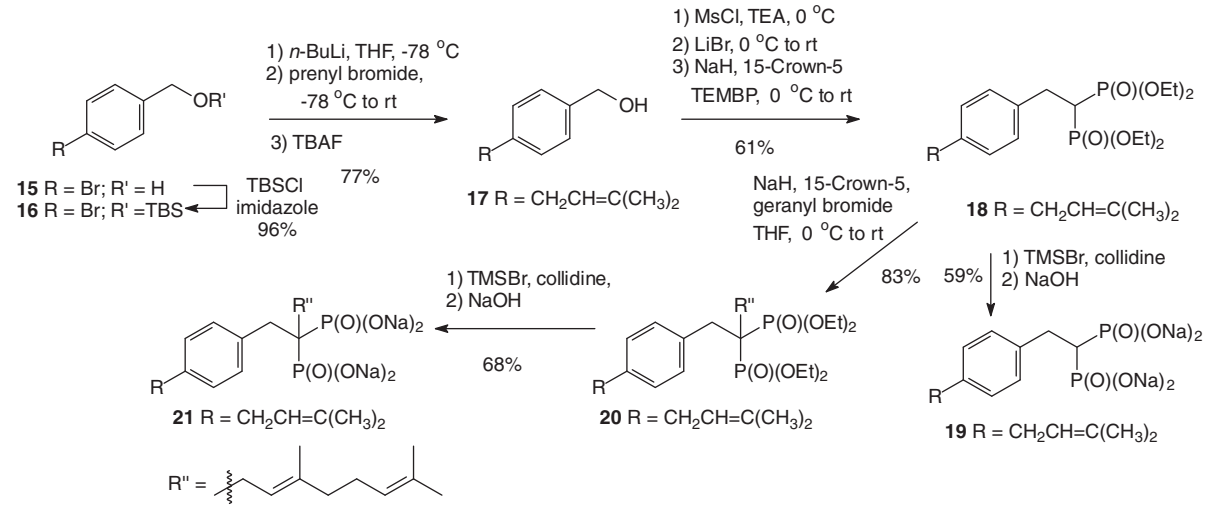

Figure 4. Synthesis of bisphosphonates **19** and **21**.

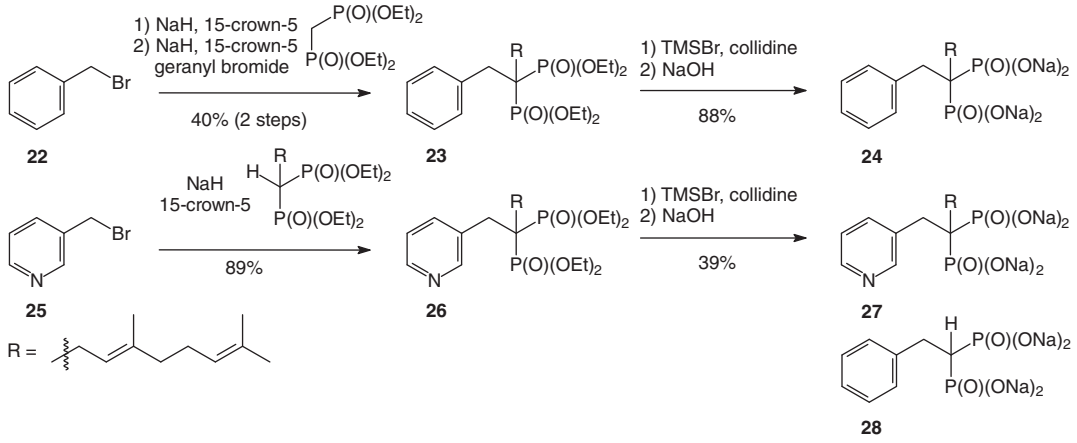

Figure 5. Synthesis of bisphosphonates **24** and **27**.

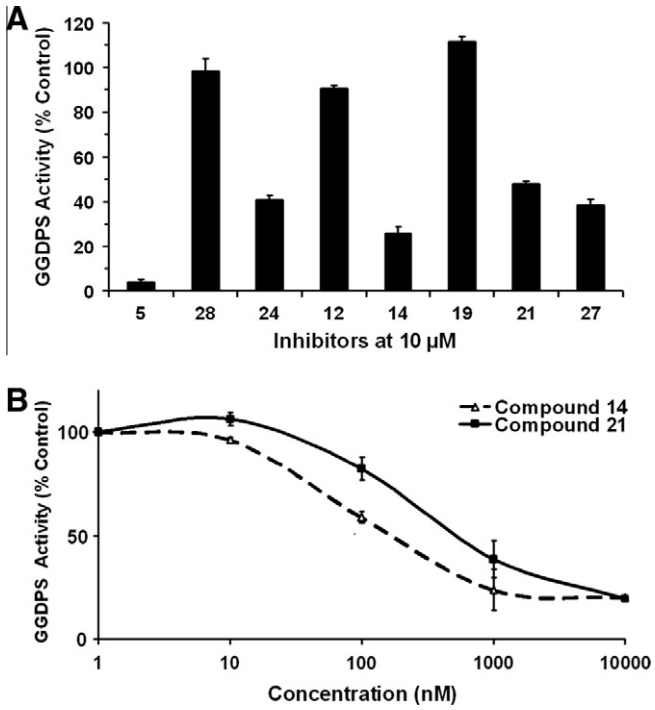

Figure 6. Inhibition of GGDPS in vitro by novel bisphosphonates. (A) Compound screen at 10 μM of each compound (mean ± SD, $n = 2$). (B) Concentration response of compounds **14** and **21** (mean ± SD, $n = 2$).

structure of the most potent compounds identified herein, it would be anticipated that these molecules bind GGDPS in a similar manner.

This set of compounds then was tested against the K562 human myelogenous leukemia cell line for inhibition of protein prenylation. Western blots were performed and prenylation status of a panel of proteins was determined (Fig. 7A). Because the antibodies available to monitor protein prenylation have different specificities for modified and unmodified forms, these analyses must be interpreted with special care. The Ras protein is farnesylated and reduction of farnesylation is made evident by the appearance of a more slowly migrating, unmodified band on the gel. In contrast, Rap1a is geranylgeranylated in a reaction catalyzed by the enzyme geranylgeranyl transferase I (GGTase I) and the antibody used here detects only the unmodified form of the protein; thus accumulation of a detectable band represents impairment of geranylgeranylation.

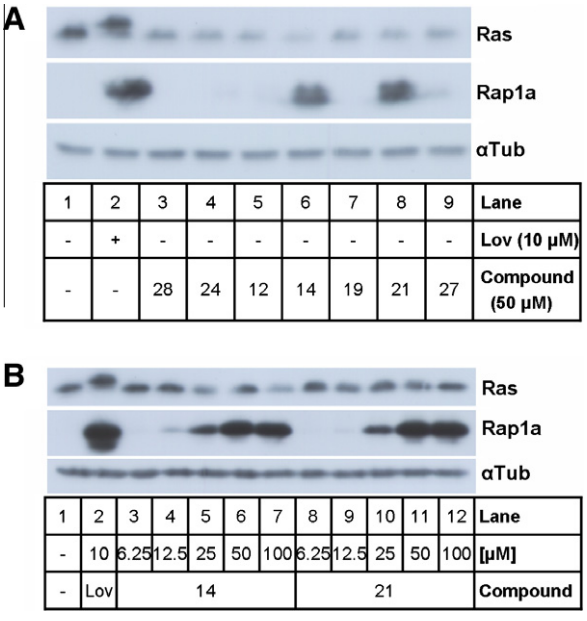

Figure 7. Impairment of protein prenylation in intact cells. (A) Compound screen. (B) Concentration response of compounds **14** and **21**. K562 cells were treated with lovastatin (lov) and compounds as indicated for 48 h.

At 48 h, lovastatin (Fig. 7A, lane 2), an inhibitor of HMG-CoA reductase, depletes mevalonate resulting in a reduction of protein farnesylation and geranylgeranylation. Compounds **14** and **21** diminish geranylgeranylation of Rap1a, while farnesylation of Ras appeared unaffected at this level of detection (Fig. 7A). These compounds also decreased geranylgeranylation of Rab6 (data not shown), a GGTase II substrate. The in vitro data correlated well with the cellular data, as the two potent in vitro GGDPS inhibitors were the only compounds to show significantly diminished protein geranylgeranylation. Compounds **24**, **14**, **21**, and **27**, all had similar enzyme inhibitory effects on GGDPS under initial screening conditions (Fig. 6A) while only compounds **14** and **21** impaired Rap1a modification in intact cells at 50 μM (Fig. 7A). This implies that intracellular levels of compounds **14** and **21** were higher than those of compounds **24** and **27**, which may be a consequence of greater cellular influx or diminished efflux. Were the former to be the case, then a prodrug approach might further enhance the potency of these molecules by increasing cellular entry.[27] Cellular concentration–response assays also were performed with both compounds **14** and **21** (Fig. 7B). Reduction of Rap1a geranylgeranylation was apparent at concentrations as low as 12.5 μM with bisphosphonate **14**, while both compounds appeared to display maximal effects at 50 μM.

To define further the specificity of the compounds for GGDPS in cells, 'add-back' experiments were performed wherein intermediates of the isoprenoid biosynthetic pathway were added in combination with the lead compounds (Fig. 8). Lovastatin again was used as a positive control, where the reduction of protein farnesylation and geranylgeranylation is prevented by addition of mevalonate. Furthermore, FPP addition prevents lovastatin-induced reduction of farnesylation, but not geranylgeranylation, and the converse is true for GGPP. For bisphosphonates **14** and **21**, no effects are noticed from the addition of mevalonate or FPP, while the addition of GGPP prevents the impairment of Rap1a and Rab6 geranylgeranylation (Rab6 data not shown). This data supports the conclusion that only GGDPS is inhibited in cells and not FDPS, GGTase I, or GGTase II.

Cell viability was determined in response to compound treatment by determination of the amount of DNA synthesis with a ^{3}H-thymidine incorporation assay (Fig. 9). Bisphosphonates **14** and **21** inhibited DNA synthesis both concentration and time dependently, with the *meta* isomer **14** being the more potent compound. These results suggest that the degree of impairment of geranylgeranylation correlates with cytotoxicity.

Figure 8. Impairment of protein geranylgeranylation by compounds **14** and **21** was prevented by exogenous GGPP. K562 cells were treated with lovastatin (10 μM) and novel bisphosphonates (**14** and **21** at 50 μM) in the presence or absence of exogenous mevalonate (Mev, 500 μM), FPP (F, 20 μM), and GGPP (GG, 20 μM) for 48 h.

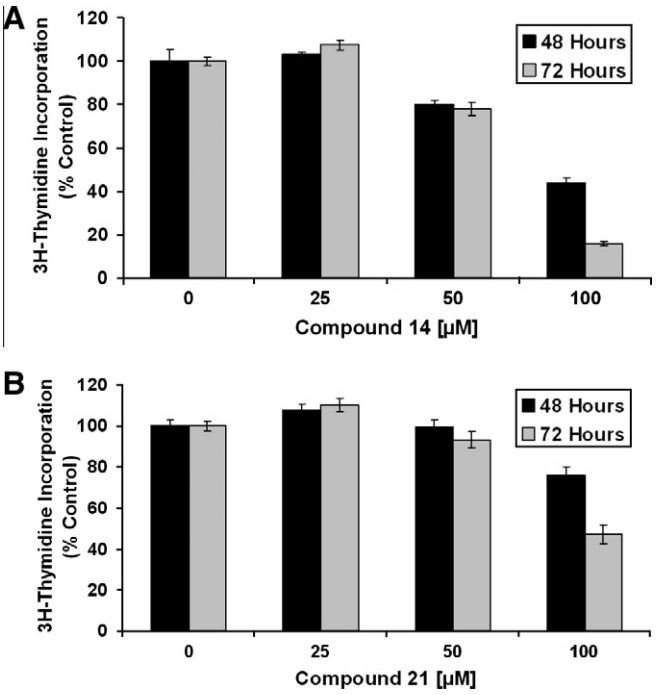

Figure 9. Compounds **14** and **21** inhibited K562 cell proliferation. Cell proliferation as a percentage of untreated cells at 48 and 72 h was evaluated by [^{3}H]thymidine incorporation (mean ± S.E., n = 4). (A) Compound **14**. (B) Compound **21**.

4. Conclusions

Although there has been some pursuit of multi-enzyme (i.e., FDPS and GGDPS) inhibitors within the mevalonate pathway as potential anti-cancer agents,[32] specificity remains the ideal for molecular intervention. Compounds with the ability to inhibit a single enzyme are very useful tools to study the interrelationships of this complex system, and may have use in anti-cancer applications in the clinic.[33] The aromatic bisphosphonates reported here, and especially compounds **14** and **21**, demonstrate selective inhibition of GGDPS over FDPS, and thus expand the list of tools available for manipulation of isoprenoid biosynthesis. Further studies in this vein will be reported in due course.

5. Experimental procedures and methods

5.1. General experimental conditions

Tetrahydrofuran was freshly distilled from sodium/benzophenone, while methylene chloride was distilled from calcium hydride prior to use. All other reagents and solvents were purchased from commercial sources and used without further purification. All reactions in nonaqueous solvents were conducted in flame-dried glassware under a positive pressure of argon and with magnetic stirring. All NMR spectra were obtained at 300 MHz for ^{1}H, and 75 MHz for ^{13}C with $CDCl_3$ as solvent, and $(CH_3)_4Si$ (1H, 0.00 ppm) or $CDCl_3$ (^{13}C, 77.0 ppm) as internal standards unless otherwise noted. The ^{31}P chemical shifts were reported in ppm relative to 85% H_3PO_4 (external standard). High resolution mass spectra were obtained at the University of Iowa Mass Spectrometry Facility. Silica gel (60 Å, 0.040–0.063 mm) was used for flash chromatography.

5.2. Silyl ether 9[34]

To a solution of 3-bromobenzyl alcohol (4.99 g, 26.7 mmol, 1.0 equiv) in CH_2Cl_2 at 0 °C was added imidazole (8.89 g, 130 mmol, 4.90 equiv) followed by TBSCl (5.24 g, 34.7 mmol, 1.30 equiv). The reaction mixture was allowed to warm to room temperature and left to stir overnight. After the solution was quenched by addition of H_2O, it was extracted with CH_2Cl_2, dried ($MgSO_4$), and concentrated in vacuo. Final purification by flash chromatography (8% EtOAc in hexanes) afforded the TBS protected alcohol **9** (7.26 g, 90%). Both the ^{1}H NMR and ^{13}C NMR data correlated to the literature values.[34]

5.3. Alcohol 10[35]

A stirred solution of silyl ether **9** (5.89 g, 19.5 mmol, 1.0 equiv) in THF was cooled to −78 °C in a dry-ice/acetone bath. Once cooling was complete, a solution of *n*-BuLi in hexanes (10.2 mL, 2.1 M, 21.5 mmol, 1.1 equiv) was added slowly via syringe, and the solution was allowed to stir for 15 min. Prenyl bromide (3.82 g, 25.6 mmol, 1.3 equiv) then was added dropwise via syringe. After the solution was held at −78 °C for 2 h, it was allowed to warm gradually to room temperature and stirred overnight. The resulting mixture was quenched by addition of H_2O, extracted with diethyl ether, dried ($MgSO_4$) and concentrated in vacuo. Without further purification, the initial product was dissolved in THF to make approximately a 2 M solution, cooled to 0 °C, and a 1 M solution of TBAF in THF (23.3 mmol, 1.2 equiv) was added dropwise. The reaction was allowed to stir for 4 h, and then quenched by addition of H_2O. The resulting mixture was extracted with diethyl ether, dried ($MgSO_4$), and concentrated in vacuo. Final purification by flash chromatography (10% EtOAc in hexanes) gave compound **10** (2.48 g, 72% over two steps): ^{1}H NMR δ 7.20–7.26 (m, 1H), 7.06–7.13 (m, 3H), 5.26–5.44 (m, 1H), 4.54 (s, 2H), 3.31 (d, J = 7.2 Hz, 2H), 2.57 (s, 1H), 1.73 (s, 3H),1.70 (s, 3H); ^{13}C NMR δ 142.0, 140.9, 132.4, 128.5, 127.4, 126.9, 124.3, 122.9, 65.0, 34.2, 25.6, 17.7; HRMS (ESI, m/z) calcd for $(M)^+$ $C_{12}H_{16}O$: 176.1201. Found 176.1204.

5.4. Tetraethyl bisphosphonate 11

A stirred solution of alcohol **10** (2.00 g, 11.4 mmol, 1.0 equiv) in CH_2Cl_2 was cooled to 0 °C in an ice bath, and triethylamine (2.01 mL, 14.5 mmol, 1.3 equiv) was added followed by dropwise addition of MsCl (1.05 mL, 13.6 mmol, 1.2 equiv). After the resulting solution was allowed to stir for 30 min, a solution of LiBr (2.52 g, 29.0 mmol, 2.6 equiv) in anhydrous THF was added via syringe. The reaction mixture was allowed to stir for 1.5 h then quenched by addition of H_2O followed by addition of saturated NaCl solution. The resulting solution was extracted with CH_2Cl_2, and the organic extract was dried (Na_2SO_4), filtered, and concentrated in vacuo. The resulting material was utilized directly in the following reaction without further purification.

To a stirred suspension of NaH (60% in oil, 437 mg, 10.9 mmol, 1.0 equiv) in THF was added 15-crown-5 (0.21 mL, 1.06 mmol, 0.1 equiv), and the resulting solution was cooled to 0 °C in an ice bath. Tetraethyl methylenebisphosphonate (3.46 g, 12.0 mmol, 1.1 equiv) was added slowly via syringe and the reaction mixture was allowed to stir for 30 min. After a solution of the benzylic bromide (prepared above) in THF was added slowly via syringe, the mixture was immediately removed from the ice bath and allowed to stir overnight. The resulting solution was filtered through a bed of florasil and concentrated in vacuo. Final purification by flash column chromatography (6% EtOH in hexanes) afforded the target bisphosphonate ester **11** (2.40 g, 47% overall yield from compound **10**): ^{1}H NMR δ 7.11 (dd, J = 8.1, 7.2 Hz, 1H), 7.04–7.00 (m, 2H), 6.94 (d, J = 7.2 Hz, 1H), 5.25–5.16 (m, 1H), 4.12–3.93 (m, 8H), 3.23 (d, J = 8.1 Hz, 2H), 3.15 (td, J_{PH} = 16.5, J = 6.0 Hz, 2H), 2.69–2.47 (m, 1H), 1.66 (s, 3H), 1.64 (s, 3H), 1.20 (t, J = 7.2, 6H), 1.18 (t, J = 7.1 Hz, 6H); ^{13}C NMR δ 141.3, 139.3 (t, J_{CP} = 7.3 Hz), 131.9, 128.5, 127.9, 126.1, 125.9, 122.9, 62.1 (dd, J_{CP} = 13.4, 6.5 Hz, 4C),

38.7 (t, J_{CP} = 131.5), 33.9, 30.8 (t, J_{CP} = 4.7 Hz), 25.4, 17.4, 15.9 (d, J_{CP} = 6.4 Hz, 4C); ^{31}P NMR δ 23.6; HRMS (ESI, *m*/*z*) calcd for (M)+$C_{21}H_{36}O_6P_2$: 446.1987. Found: 446.1991.

5.5. Bisphosphonate 12

A stirred solution of bisphosphonate ester **11** (0.767 g, 1.72 mmol, 1.0 equiv) in CH_2Cl_2 was cooled to 0 °C in an ice bath. After 2,4,6-collidine (1.14 mL, 8.60 mmol, 5.0 equiv) was added, TMSBr (1.11 mL, 8.58 mmol, 5.0 equiv) was added slowly and the reaction mixture was allowed to stir overnight. Toluene was added and removed in vacuo. This process was repeated three times. After drying the crude material, the residue was treated with NaOH solution (5 mL, 1.73 M, 8.65 mmol, 5.0 equiv) and allowed to stir at room temperature overnight. Acetone was added to the solution and the resulting material was allowed to cool at 3 °C for 72 h. The solution was filtered, the solid was washed with several portions of cold acetone, and then dried in vacuo to provide compound **12** (651 mg, 90%): ^{1}H NMR (D_20) δ 7.26–7.14 (m, 3H), 6.99 (d, *J* = 6.3 Hz, 1H), 5.39–5.34 (m, 1H), 3.29 (d, *J* = 7.2 Hz, 2H), 3.08 (td, J_{PH} = 15.3, *J* = 6.3 Hz, 2H), 2.07 (tt, J_{PH} = 21.9, *J* = 5.1 Hz, 1H), 1.68 (s, 3H), 1.67 (s, 3H); ^{13}C NMR 145.5 (t, J_{CP} = 6.6 Hz), 141.8, 134.2, 129.1, 128.6, 126.7, 125.1, 123.7, 42.8 (t, J_{CP} = 117.8 Hz), 33.9, 33.7–33.5 (m), 25.1, 17.3; ^{31}P NMR δ 20.8; HRMS (ESI, *m*/*z*) calcd for $(M-H)^-$ $C_{13}H_{19}O_6P_2$: 333.0657. Found: 333.0662.

5.6. Dialkylbisphosphonate 13

A suspension of NaH (60% in oil, 202 mg, 5.15 mmol, 2.4 equiv) in THF was cooled to 0 °C in an ice bath. Once the solution was thoroughly cooled, 15-crown-5 (0.05 mL, 0.3 mmol, 0.1 equiv) was added followed ester **11** (0.956 g, 2.14 mmol, 1.0 equiv) via syringe. The solution was allowed to stir for 30 min, then geranyl bromide (0.816 g, 3.76 mmol, 1.8 equiv) was added, and the solution was allowed to stir overnight. After the reaction mixture was filtered through a bed of florasil, the filtrate was concentrated in vacuo. Final purification via flash chromatography (4% EtOH in hexanes) afforded the desired compound **13** (1.08 g, 86% yield): ^{1}H NMR δ 7.15–7.12 (m, 3H), 7.03–6.97 (m, 1H), 5.69–5.64 (m, 1H), 5.34–5.26 (m, 1H), 5.17–5.12 (m, 1H), 4.18–4.02 (m, 8H), 3.32–3.21 (m, 4H), 2.64 (td, J_{PH} = 16.2, *J* = 5.7 Hz, 2H), 2.16–2.05 (m, 4H), 1.72 (s, 3H), 1.71 (s, 3H), 1.67 (s, 3H), 1.63 (s, 3H), 1.61 (s, 3H), 1.25 (t, *J* = 6.6 Hz, 6.9 Hz, 6H), 1.21 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR δ 140.7, 136.8, 136.3 (t, J_{CP} = 7.1 Hz), 132.0, 131.5, 131.3, 128.8, 127.4, 126.3, 124.2, 123.4, 119.2 (t, J_{CP} = 7.6 Hz), 62.4–62.1 (m, 4C), 47.4 (t, J_{CP} = 129.7 Hz), 39.9, 35.1–34.9 (m), 34.3, 28.2–28.0 (m), 26.5, 25.6, 25.5, 17.6, 17.5, 16.3, 16.2–15.8 (m, 4C); ^{31}P NMR δ 26.2; HRMS (ESI, *m*/*z*) calcd for $(M)^+$ $C_{31}H_{52}O_6P_2$: 582.3239. Found: 582.3235.

5.7. Bisphosphonate 14

A stirred solution of compound **13** (70 mg, 0.12 mmol, 1.0 equiv) in CH_2Cl_2 was cooled to 0 °C in an ice bath. After 2,4,6-collidine (0.12 mL, 0.91 mmol, 7.6 equiv) was added, slow addition of TMSBr (0.12 mL, 0.93 mmol, 7.8 equiv) was done and the resulting solution was allowed to stir overnight. Toluene was added and removed in vacuo, three times. The resulting residue was treated with NaOH solution (1.0 M, 0.9 mL, 0.90 mmol, 7.5 equiv) and allowed to stir at room temperature overnight. Acetone was added to the solution and the resulting material was stored at 3 °C for 72 h. The suspension was filtered and the solid material was washed with several portions of anhydrous acetone. The precipitate then was dissolved in H_2O, filtered, and the aqueous portion was concentrated in vacuo to give compound **14** as a fine white powder (59 mg, 88%): ^{1}H NMR δ 7.36 (d, *J* = 7.5 Hz, 1H), 7.30–7.12 (m, 2H), 7.02 (d, *J* = 7.5 Hz, 1H), 5.83–5.78 (m, 1H), 5.41–5.30 (m, 1H), 5.23–5.19 (m, 1H), 3.28 (d, *J* = 6.9 Hz, 2H), 3.11 (t, *J* = 14.1 Hz, 2H), 2.40 (td, J_{PH} = 15.6, *J* = 6.0 Hz, 2H), 2.15–1.96 (m, 4H), 1.69 (s, 3H), 1.67 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H), 1.14 (s, 3H); ^{13}C NMR 141.0, 140.8 (t, J_{CP} = 7.8 Hz), 135.0, 134.2, 133.6, 131.7, 129.8, 128.2, 125.6, 125.2, 124.0 (t, J_{CP} = 5.9 Hz), 123.8, 45.5 (t, J_{CP} = 109.8 Hz), 39.6, 37.4–37.2 (m), 34.0, 30.9–30.6 (m), 26.3, 25.2, 25.1, 17.4, 17.2, 15.7; ^{31}P NMR 22.7; HRMS (ESI, *m*/*z*) calcd for $(M-H)^-$ $C_{23}H_{35}O_6P_2$: 469.1909. Found: 469.1911.

5.8. Silyl ether 16[36]

Imidazole (4.39 g, 64 mmol, 2.5 equiv) was added to a solution of 4-bromobenzyl alcohol (4.73 g, 25.7 mmol, 1.0 equiv) in CH_2Cl_2. The solution was cooled to 0 °C, TBSCl (4.70 g, 31.2 mmol, 1.2 equiv) was added, and the reaction mixture was allowed to stir overnight. The solution was quenched by addition of H_2O, extracted with CH_2Cl_2, dried ($MgSO_4$), and concentrated in vacuo. Final purification by flash chromatography (15% EtOAc in hexanes) afforded the TBS protected alcohol **16** (7.40 g, 96%) with ^{1}H and ^{13}C NMR data corresponding to the literature values.[36]

5.9. Alcohol 17

A stirred solution of RJB-1-70 (6.61 g, 21.9 mmol, 1.0 equiv) in THF was cooled to −78 °C in a dry-ice/acetone bath. Once cooling was complete, *n*-BuLi (11.5 mL, 2.1 M in THF, 1.1 equiv) was added slowly via syringe, and the solution was allowed to stir for 15 min. Prenyl bromide (4.26 g, 28.6 mmol, 1.3 equiv) was added dropwise via syringe. The reaction solution was held at −78 °C for 2 h, and then allowed to gradually warm to room temperature and stir overnight. The resulting mixture was quenched by addition of H_2O, extracted with diethyl ether, dried ($MgSO_4$), and concentrated in vacuo. The crude mixture was used in the next reaction without additional purification.

A stirred solution of the above material in THF was cooled to 0 °C in an ice bath and a 1 M solution of TBAF in THF (27.1 mL, 27.1 mmol, 1.0 equiv) was added dropwise to the reaction vessel. The reaction was allowed to stir for 4 h, then the solution was diluted with diethyl ether, and finally H_2O was added. The resulting mixture was extracted with diethyl ether, dried ($MgSO_4$), and concentrated in vacuo. Final purification by column chromatography (10% EtOAc in hexanes) afforded alcohol **17** (2.99 g, 77% over two steps): ^{1}H NMR δ 7.20 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 5.25–5.33 (m, 1H), 4.51 (s, 2H), 3.31 (d, *J* = 7.5 Hz, 2H), 2.78 (s, 1H), 1.73 (s, 3H), 1.70 (s, 3H); ^{13}C NMR δ 141.0, 138.2, 132.4, 128.3 (2C), 127.1 (2C), 123.0, 64.7, 33.9, 25.6, 17.7; HRMS (ESI, *m*/*z*) calcd for $(M)^+$ $C_{12}H_{16}O$: 176.1201. Found: 176.1199.

5.10. Tetraethyl bisphosphonate 18

A stirred solution of alcohol **17** (4.46 g, 25.3 mmol, 1.0 equiv) in CH_2Cl_2 was cooled to 0 °C in an ice bath. To the cooled solution was added anhydrous triethylamine (4.58 mL, 40.0 mmol, 1.6 equiv) followed by dropwise addition of MsCl (2.36 mL, 30.5 mmol, 1.2 equiv), and the resulting solution was allowed to stir for 30 min. After LiBr (5.55 g, 63.9 mmol, 2.5 equiv) was dissolved in THF, the solution was transferred via syringe into the reaction vessel. The reaction mixture was allowed to stir for 1.5 h and then the solution was quenched by addition of H_2O followed by addition of saturated NaCl solution. The resulting solution was extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated in vacuo. Without additional purification, the product was utilized in the following reaction.

To a stirred suspension of NaH (60% in oil, 0.932 g, 23.3 mmol, 1.0 equiv) in THF was added 15-crown-5 (0.51 mL, 2.58 mmol,

0.1 equiv) and the resulting solution was cooled to 0 °C in an ice bath. Tetraethyl methylenebisphosphonate (7.38 g, 25.6 mmol, 1.1 equiv) was added slowly via syringe and the reaction was allowed to stir for 30 min to facilitate complete formation of the anion. After a solution of the benzylic bromide in THF was added slowly via syringe to the reaction vessel, the resulting mixture was immediately removed from the ice bath and allowed to stir overnight. After the solution was filtered through a bed of florasil and concentrated in vacuo, final purification by flash column chromatography (gradient 6–8% EtOH in hexanes) gave the target bisphosphonate **18** (6.89 g, 61% over two steps): ^{1}H NMR δ 7.11 (dd, J = 8.3, 2.1 Hz, 2H), 7.01 (dd, J = 7.8, 2.1 Hz, 2H), 5.23–5.16 (m, 1H), 4.10–3.94 (m, 8H), 3.22 (d, J = 7.2 Hz, 2H), 3.09 (td, J_{PH} = 16.5, J = 6.3 Hz, 2H), 2.56 (tt, J_{PH} = 23.7, J = 6.3 Hz, 1H), 1.65 (s, 3H), 1.63 (s, 3H), 1.20 (t, J = 7.2, 6H), 1.17 (t, J = 7.2 Hz, 6H), ^{13}C NMR δ 139.8, 136.7 (t, J_{CP} = 7.4 Hz), 132.1, 128.7 (2C), 127.9 (2C), 123.1, 62.2 (dd, J_{CP} = 13.8, 6.8 Hz, 4C), 38.9 (t, J_{CP} = 131.3 Hz), 33.7, 30.6 (t, J_{CP} = 4.9 Hz), 25.5, 17.6, 16.1 (d, J_{CP} = 7.2 Hz, 4C), ^{31}P NMR δ 23.6; HRMS (ESI, m/z) calcd for (M)+ $C_{21}H_{36}O_6P_2$: 446.1987. Found: 446.1989.

5.11. Bisphosphonate 19

A stirred solution of compound **18** (289 mg, 0.64 mmol, 1.0 equiv) in CH_2Cl_2 was cooled to 0 °C in an ice bath. After 2,4,6-collidine (0.70 mL, 5.28 mmol, 8.3 equiv) was added and slow addition of TMSBr (0.69 mL, 5.34 mmol, 8.2 equiv) the resulting was allowed to stir overnight. Toluene was added and removed in vacuo three times. The remaining residue was treated with NaOH solution (1 M, 3.2 mL, 3.20 mmol, 5.0 equiv) and allowed to stir at room temperature overnight. Acetone was added to the solution and the resulting suspension was stored at 3 °C for 72 h. The solution was filtered and the solid was washed with several portions of cold acetone and dried in vacuo giving compound **19** (162 mg, 59%) ^{1}H NMR (D_2O) δ 7.38 (d, J = 7.8 Hz, 2H), 7.21 (d, J = 7.8 Hz, 2H), 5.46–5.37 (m, 1H), 3.35 (d, J = 7.2 Hz, 2H), 3.11 (td, J_{PH} = 15.6, J = 6.3 Hz, 2H), 2.18 (tt, J_{PH} = 21.6, J = 6.3 Hz, 1H), 1.76 (s, 6H); ^{13}C NMR 143.6 (t, J_{CP} = 7.3 Hz), 142.0, 136.8, 132.0 (2C), 130.7 (2C), 126.1, 44.6 (t, J_{CP} = 112.4 Hz), 36.0, 34.0–34.7 (m), 27.6, 19.8; ^{31}P NMR δ 20.6; HRMS (ESI, m/z) calcd for $(M-H)^-$ $C_{13}H_{19}O_6P_2$: 333.0657. Found: 333.0677.

5.12. Dialkylbisphosphonate 20

A suspension of NaH (60% in oil, 215 mg, 5.37 mmol, 2.4 equiv) in THF was cooled to 0 °C in an ice bath. Once the solution was thoroughly cooled, 15-crown-5 (0.05 mL, 0.3 mmol, 0.1 equiv) was added followed by the addition of compound **18** (1.02 g, 2.27 mmol, 1.0 equiv) via syringe. The resulting solution was allowed to stir for 30 min, geranyl bromide (849 mg, 3.91 mmol, 1.7 equiv) was added, and the solution was allowed to stir overnight. The reaction mixture was filtered through a bed of florasil and the filtrate was concentrated in vacuo. Final purification via flash chromatography (4% EtOH in hexanes) afforded the desired compound (1.09 g, 83%): ^{1}H NMR δ 7.23 (d, J = 6.9 Hz, 2H), 7.03 (d, J = 7.2 Hz, 2H), 5.68–5.61 (m, 1H), 5.33–5.24 (m, 1H), 5.18–5.10 (m, 1H), 4.15–4.02 (m, 8H), 3.30–3.21 (m, 4H), 2.62 (td, J_{HP} = 15.9, J = 6.3 Hz, 2H), 2.18–2.01 (m, 4H), 1.72 (s, 3H), 1.70 (s, 3H), 1.67 (s, 3H), 1.62 (s, 6H), 1.24 (t, J = 6.9 Hz, 6H), 1.20 (t, J = 7.2 Hz, 6H); ^{13}C NMR δ 139.7, 136.6, 133.4 (t, J_{CP} = 7.5 Hz), 132.0, 131.3 (2C), 131.1, 127.2 (2C), 124.2, 123.3, 119.1 (t, J_{CP} = 7.7 Hz), 62.3–62.0 (m, 4C), 47.4 (J_{CP} = 129.6 Hz), 39.9, 34.7–34.5 (m), 33.8, 28.2–27.9 (m), 26.4, 25.6 (2C), 17.6, 17.5, 16.3, 16.2–16.0, (m, 4C); ^{31}P NMR δ 25.6; HRMS (ESI, m/z) calcd for $(M)^+$ $C_{31}H_{52}O_6P_2$: 582.3239. Found: 582.3238.

5.13. Dialkylbisphosphonate 21

A stirred solution of ester **20** (0.915 g, 1.57 mmol, 1.00 equiv) in CH_2Cl_2 was cooled to 0 °C in an ice bath. After 2,4,6-collidine (1.06 mL, 7.99 mmol, 5.09 equiv) was added and slow addition of TMSBr (1.01 mL, 7.81 mmol, 4.97 equiv) the solution was allowed to stir overnight. Toluene was added and removed in vacuo. The resulting residue was treated with NaOH (1.04 M, 6.4 mL, 6.66 mmol, 4.2 equiv) and allowed to stir at room temperature overnight. Acetone was added to the solution and the resulting suspension was stored at 3 °C for 72 h. The suspension was filtered, and the filtrand was washed with several aliquots of anhydrous acetone. The resulting solid was dissolved in H_2O, filtered, and concentrated in vacuo to provide compound **21** (598 mg, 68%): ^{1}H NMR δ 7.47 (d, J = 7.8 Hz, 2H), 7.14 (d, J = 7.8 Hz, 2H), 5.88–5.82 (m, 1H), 5.47–5.40 (m, 1H), 5.32–5.25 (m, 1H), 3.35 (d, J = 7.5 Hz, 2H), 3.17 (t, J_{PH} = 14.4 Hz, 2H), 2.47 (td, J_{PH} = 15.9, J = 6.6 Hz, 2H), 2.22–2.03 (m, 4H), 1.76 (s, 6H), 1.69 (s, 3H), 1.67 (s, 3H), 1.54 (s, 3H); ^{13}C NMR 141.8, 140.7 (t, J_{CP} = 7.3 Hz), 137.4, 136.7, 136.0, 134.9 (2C), 130.0 (2C), 127.8, 126.9 (t, J_{CP} = 6.8 Hz), 126.2, 48.3 (t, J_{CP} = 110.6 Hz), 42.1, 39.7–39.5 (m, 1C), 36.0, 33.6–33.4 (m, 1C), 28.7, 27.7, 27.6, 19.8 (2C), 18.0; ^{31}P NMR 23.4; HRMS (ESI, m/z) calcd for $(M-H)^-$ $C_{23}H_{35}O_6P_2$: 469.1909. Found: 469.1912.

5.14. Dialkylbisphosphonate 23

To a stirred suspension of NaH (16.7 mmol, 1.4 equiv) in THF at 0 °C was added 15-crown-5 (0.91 mmol, 0.1 equiv) followed by dropwise addition of the known tetraethyl benzyl bisphosphonate.[30] The resulting solution was allowed to stir for 30 min and geranyl bromide (12.1 mmol, 1.0 equiv) was added dropwise as a neat liquid. The resulting solution was allowed to stir overnight, dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. Final purification by flash column chromatography (gradient 2–10% MeOH in diethyl ether) gave compound **23** (1.97 g, 40% over two steps): ^{1}H NMR δ 7.31 (dd, J = 7.8, 1.8 Hz, 2H), 7.25–7.17 (m, 3H), 5.67–5.60 (m, 1H), 5.15–5.08 (m, 1H), 4.17–3.99 (m, 8H), 3.27 (dd, J_{PH} = 16.5, 12.9 Hz, 2H), 2.61 (td, J_{PH} = 15.3, J = 6.0 Hz, 2H), 2.15–2.03 (m, 4H), 1.66 (s, 3H), 1.60 (s, 6H), 1.23 (t, J = 6.9, 6H), 1.19 (t, J = 6.9 Hz, 6H); ^{13}C NMR δ 136.9, 136.4 (t, J_{CP} = 8.6 Hz), 131.5 (2C), 131.4, 127.4 (2C), 126.4, 124.3, 119.2 (t, J_{CP} = 7.7 Hz), 62.5–62.1 (4C, m), 47.4 (t, J_{CP} = 129.6 Hz), 40.0, 35.3–35.1 (m), 28.4–28.2 (m), 26.5, 25.7, 17.7, 16.4, 16.3–16.1 (m, 4C); ^{31}P NMR δ 26.2; HRMS $(M+Na)^+$ calcd $C_{26}H_{44}O_6P_2$: 537.2511. Found: 537.2515.

5.15. Bisphosphonate 24

To a solution of **23** (1.66 mmol, 1.0 equiv) in CH_2Cl_2 at 0 °C, were added collidine (13.4 mmol, 8.09 equiv) and TMSBr (13.3 mmol, 8.1 equiv) as neat liquids. After 1 h the solution was allowed to warm gradually to room temperature and stirred overnight. After the solvent was removed in vacuo, toluene (15 mL) was added and removed in vacuo. An aqueous solution of NaOH (7.4 mL, 0.9 M, 6.66 mmol, 4.0 equiv) was added, and the mixture was stirred for 1.75 h. The resulting mixture was then poured into acetone and stored at 3 °C for 72 h. The resulting suspension was filtered and the filtrand was washed with several portions of anhydrous acetone. The remaining solid was dissolved in H_2O, filtered, and the filtrate was concentrated in vacuo to provide the desired compound **24** (746 mg, 88% yield): ^{1}H NMR (D_2O) δ 7.48 (dd, J = 8.1, 1.2, 2H), 7.31–7.16 (m, 3H), 5.79–5.69 (m, 1H), 5.27- 5.18 (m, 1H), 3.15 (t, J_{PH} = 15.0 Hz, 2H), 2.46 (td, J_{PH} = 15.6, J = 6.9 Hz, 2H), 2.15–2.01 (m, 4H), 1.64 (s, 3H), 1.61 (s, 3H), 1.55 (s, 3H) ^{13}C NMR 139.0 (t, J_{CP} = 7.2 Hz), 137.0, 133.7, 131.8 (2C), 127.8 (2C), 126.3, 125.1, 121.8 (t, J_{CP} = 7.4 Hz), 46.0 (t, J_{CP} = 111.4 Hz), 39.6,

36.5 (br s, 1C), 29.9 (br s, 1C), 26.0, 25.2, 17.2, 15.6; ^{31}P NMR δ 23.3; HRMS $(M-H)^-$ calcd $C_{18}H_{27}O_6P_2$: 401.1283. Found: 401.1288.

5.16. Bisphosphonate 26

Sodium hydride (60% in oil, 588 mg, 14.7 mmol, 2.5 equiv) was placed in a 3-neck flask fitted with two septa and a solid addition tube filled with 3-(bromomethyl)pyridine HBr (1.9 g, 7.76 mmol, 1.3 equiv), and the apparatus was purged with argon. Anhydrous THF was added and the solution was cooled to 0 °C in an ice bath. After 15-crown-5 (0.31 mL, 1.57 mmol, 0.3 equiv) was added, monogeranyl bisphosphonate RJB-1–51A was added dropwise as a neat liquid. The solution was allowed to stir for 30 min, the pyridine salt was added via the solid addition tube, and the reaction was allowed to stir overnight. After H_2O was added, the aqueous fraction was extracted with diethyl ether, dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo. Final purification by flash column chromatography (8% MeOH in Et_2O) afforded the desired bisphosphonate **26** (3.01 g, 89%): ^{1}H NMR δ 8.46 (s, 1H), 8.36 (d, J = 3.9 Hz, 1H), 7.59 (d, J = 7.5 Hz, 1H), 7.10 (dd, J = 7.5, 5.1 Hz, 1H), 5.59–5.51 (m, 1H), 5.08–5.01 (m, 1H), 4.13–3.95 (m, 8H), 3.18 (t, J_{PH} = 15.3, 2H), 2.54 (td, J_{PH} = 15.6, J = 6.0 Hz, 2H), 2.09–1.89 (m, 4H), 1.57 (s, 3H), 1.54 (s, 3H), 1.52 (s, 3H), 1.20 (t, J = 7.2 Hz, 6H), 1.15 (t, J = 7.2 Hz, 6H); ^{13}C NMR δ 152.2, 147.5, 138.6, 137.5, 132.2 (t, J_{CP} = 7.7 Hz), 131.3, 124.1, 122.3, 118.6 (t, J_{CP} = 7.6 Hz), 62.6–62.3 (m, 4C), 47.0 (t, J_{CP} = 130.2 Hz), 39.9, 32.8 (t, J_{CP} = 4.1 Hz), 28.4 (t, J_{CP} = 4.6 Hz), 26.4, 25.6, 17.5, 16.3, 16.2–16.0 (m, 4C); ^{31}P NMR δ 25.7; HRMS $(M+Na)^+$ calcd $C_{25}H_{43}NO_6P_2$: 538.2463. Found: 538.2468.

5.17. Bisphosphonate 27

A solution of compound **26** (329 mg, 0.64 mmol, 1.0 equiv) in CH_2Cl_2 was cooled to 0 °C in an ice bath. After 2,4,6-collidine (0.63 mL, 4.75 mmol, 7.4 equiv) and TMSBr (0.62 mL, 4.79 mmol, 7.5 equiv) was added, the reaction mixture was stirred overnight. Toluene was added and removed in vacuo, three times. The residue was treated with NaOH solution (1.56 M, 2.45 mL, 6.66 mmol, 6.0 equiv) and allowed to stir at room temperature overnight. Acetone was added to the solution and the resulting material was allowed to stand at 3 °C for 72 h. The resulting suspension was filtered, and the filtrand was washed with several aliquots of anhydrous acetone. The solids were then dissolved in water, filtered, and the filtrate was concentrated in vacuo to provide the target compound (121 mg, 39%): ^{1}H NMR (D_2O) δ 8.44 (d, J = 2.1 Hz, 1H), 8.18 (dd, J = 5.1, 1.5 Hz, 1H), 7.94 (ddd, J = 8.4, 1.8, 1.5 Hz, 1H), 7.21 (dd, J = 7.8, 5.1 Hz, 1H), 5.75–5.70 (m, 1H), 5.20–5.14 (m, 1H), 3.07 (t, J_{PH} = 15.0, Hz, 2H), 2.39 (td, J_{PH} = 14.1, J = 7.5 Hz, 2H), 2.09–1.92 (m, 4H), 1.56 (s, 6H), 1.44 (s, 3H); ^{13}C NMR 153.7, 147.9, 143.0, 139.4 (t, J_{CP} = 6.9 Hz), 138.0, 135.9, 127.5, 126.6 (t, J_{CP} = 6.7 Hz), 125.6, 48.3 (t, J_{CP} = 112.3 Hz), 42.0, 37.5–37.3 (m), 33.4–33.2 (m), 28.5, 27.5, 19.6, 17.8; ^{31}P NMR δ 22.7; HRSM (ESI, m/z), $(M-H)^-$ calcd $C_{17}H_{26}NO_6P_2$: 402.1235. Found: 402.1244.

5.18. Cell culture

K562 leukemia cells were obtained from American Type Culture Collection (Manassas, VA). Cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum at 37 °C and 5% CO_2.

5.19. GGDPS in vitro assay

The GGDPS in vitro assay was performed as described previously.[26] Briefly, plasmids containing GST-tagged recombinant human GGDPS were expressed in BL21 gold bacteria by induction with IPTG. Proteins were purified by batch centrifugation with glutathione agarose. The GGDPS reaction mixtures contained 20 μM FPP and 40 μM ^{14}C-IPP in 20 μL buffer (50 mM imidazole pH 7.5, 0.5 mM $MgCl_2$, 0.5 mM $ZnCl_2$). Following a 10-min pre-incubation with the indicated compounds, reactions were initiated by simultaneous addition of ^{14}C-IPP and FPP. Reactions proceeded for 1 h at 37 °C, and then the longer isoprenoids were extracted with 1 mL saturated butanol and the extracts were washed twice with 300 μL saturated water. The amount of radioactivity in the butanol extracts were detected by liquid scintillation counting.

5.20. Western blot analysis

The K562 cells were diluted to a final concentration of 5×10^5 cells/mL. After 5 mL of cell suspension was added to 6-well plates in the presence of compounds, the plates were incubated for 48 h. Cells were harvested by centrifugation and lysed (2% SDS in 66 mM Tris) by passing cells several times through a 27G needle. Lysates were cleared by centrifugation and protein concentration determined by the bicinchoninic acid (BCA) assay. Proteins were resolved by electrophoresis on 12% and 15% gels and transferred to PVDF membrane. Membranes were blocked in 5% non-fat dry milk for 40 min at 37 °C. Primary and secondary antibodies were added sequentially for 1 h at 37 °C and proteins were visualized with ECL reagents. Anti pan-Ras antibody was obtained from InterBiotechnology (Tokyo, Japan). The Rap1a (sc-1482), Rab6 (sc-310), and α-tubulin (sc-8025) antibodies were obtained from Santa Cruz biotechnology, Inc. (Santa Cruz, CA). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit were obtained from GE Healthcare (Buckinghamshire, UK), and horseradish peroxidase-conjugated anti-goat was obtained from Santa Cruz Biotechnology, Inc.

For the add-back experiments, isoprenoid pathway intermediates (mevalonate, FPP, or GGPP) were added at indicated concentrations simultaneously with isoprenoid pathway inhibitors (lovastatin, compound **14**, or compound **21**).

5.21. DNA synthesis assay

Two hundred microliters of K562 cells were incubated in 96-well plates and treated with compounds as described previously.[25] The 48 h experiments required 2×10^5 cells/mL while 72 h experiments required 1×10^5 cells/mL. After 44 or 68 h, 20 μL of [^{3}H]thymidine (0.1385 TBq/mmol; 3.75 Ci/mmol in media) was added to each well. At 48 or 72 h, cells were filtered through glass microfiber paper using a Brandel (Gaithersburg, MD) cell harvester. [^{3}H]Thymidine incorporated into cellular DNA was quantified by scintillation counting.

Acknowledgment

This project was supported by the Roy. J. Carver Charitable Trust as a Research Program of Excellence.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.08.036.

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