ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Potent Triazole Bisphosphonate Inhibitor of Geranylgeranyl Diphosphate Synthase

Veronica S. Wills,[†] Cheryl Allen,[‡] Sarah A. Holstein,[‡] and David F. Wiemer*[,†,§]

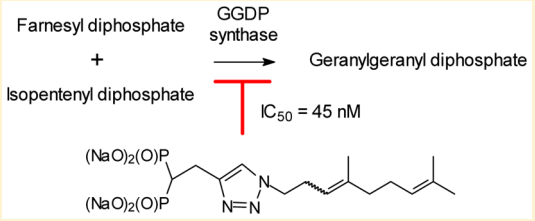
[†]Department of Chemistry, University of Iowa, Iowa City, Iowa 52242-1294, United States

[‡]Department of Medicine, Roswell Park Cancer Institute, Buffalo, New York 14263, United States

[§]Department of Pharmacology, University of Iowa, Iowa City, Iowa 52242-1109, United States

Supporting Information

ABSTRACT: Studies of triazole bisphosphonates have resulted in identification of a potent inhibitor of geranylgeranyl diphosphate synthase (IC_{50} = 45 nM) with very good selectivity for this enzyme over farnesyl diphosphate synthase (IC_{50} = 28 μM). This compound also potently disrupts geranylgeranylation and induces cytotoxicity in human myeloma cells at submicromolar levels, suggesting that it may serve as a lead compound for treatment of malignancies characterized by excessive protein secretion.



KEYWORDS: *Geranylgeranyl diphosphate synthase, GGDPS, bisphosphonate, farnesyl diphosphate synthase, myeloma*

Enzymes of the isoprenoid biosynthetic pathway are the targets of a number of important drugs.[1] One prominent therapeutic objective has been inhibition of the enzyme farnesyl diphosphate synthase (FDPS), which is addressed by the nitrogenous bisphosphonates that are used for treatment of osteoporosis and other diseases of the bone including zoledronate (**1**, Figure 1), risedronate (**2**), and pamidronate (**3**). These compounds all lead to reduced levels of the C_{15} isoprenoid farnesyl diphosphate (FPP),[2] but there is increasing evidence that the effect of these drugs may be expressed through their impact on cellular levels of the downstream C_{20} isoprenoid geranylgeranyl diphosphate (GGDP).[3] This C_{20} isoprenoid is produced from FPP and isopentenyl diphosphate by the enzyme geranylgeranyl diphosphate synthase (GGDPS), and among other functions, it is used as a substrate for the geranylgeranyl transferases that mediate protein geranylgeranylation.[4] Thus, it can be argued that inhibitors of GGDPS may be a more direct means to the biological effects desired from FDPS inhibitors and that they might have clinical value.[3]

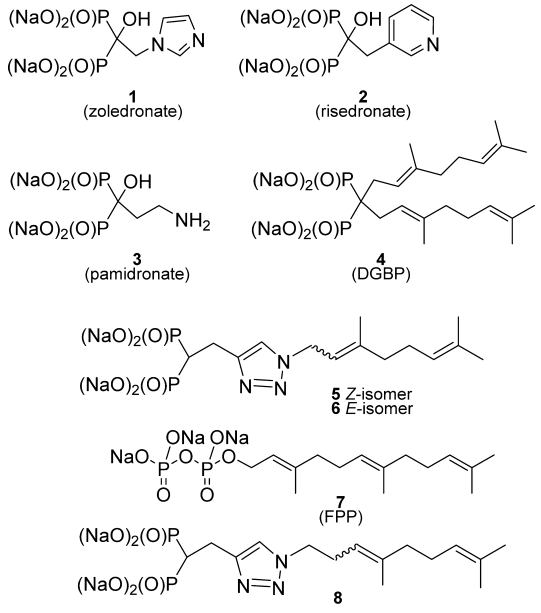


Figure 1. Bisphosphonates that inhibit isoprenoid biosynthesis.

Some years ago we reported the preparation of digeranyl bisphosphonate (DGBP, **4**)[5] and its identification as a selective[6] and reasonably potent inhibitor of GGDPS ($IC_{50} \approx 0.2$ μM).[7] While substantially higher concentrations were required to observe cellular effects, presumably because the high degree of negative charge limits diffusion across a cell membrane, this can be addressed in part by preparation of a prodrug.[8] For example, the pivaloyloxymethyl (POM) derivative[9] is much more hydrophobic in terms of a calculated ClogP and also demonstrates cellular activity at lower concentrations.[10] More recently, we have prepared some triazole bisphosphonates through click chemistry[11] of an acetylene bisphosphonate[12] and various isoprenoid azides. While some of these triazole bisphosphonates inhibit GGDPS, none has yet shown an IC_{50} below that of DGBP. Thus far, the most active GGDPS inhibitor in the triazole family is the nerol derivative **5**, which has an IC_{50} of ~380 nM versus the isolated enzyme.[13] Perhaps surprisingly, the isomeric geraniol derivative **6** is ~40-fold less active (IC_{50} of ~17 μM). However, the activity of compound **5** is sufficiently close to the potency of the original lead

Received: August 14, 2015
Accepted: October 28, 2015
Published: October 28, 2015

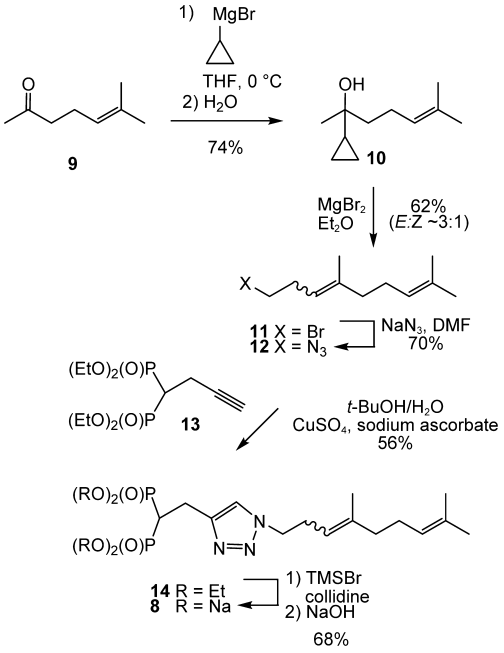
DOI: 10.1021/acsmedchemlett.5b00334
ACS Med. Chem. Lett. 2015, 6, 1195−1198

compound DGBP to be intriguing, and thus, it encouraged exploration of related compounds for more potent inhibitors of this enzyme.

The larger substrate for GGDPS is farnesyl diphosphate (**7**, FPP). As a first approximation, one might view the triazole moiety of compounds **5** and **6** as nearly an isosteric replacement for the first isoprenoid unit of FPP because it spaces the hydrophobic tail from the bisphosphonate headgroup by four atoms. From this perspective the triazoles **5** and **6** could access a conformation nearly the same length as FPP. However, such an analysis overlooks the facts that the heteroaromatic system would display three sp^2 hybridized atoms rather than the two found in an isoprenoid olefin and that bond angles will be constrained by the five-membered ring. We hypothesized that it might be possible to compensate for the shorter bonds of the triazole by incorporation of an additional methylene group on the distal side and thereby achieve an analogue closer in length to an extended conformation of FPP. Thus, our next target became the triazoles **8**, which would be a one carbon homologation of triazoles we have reported earlier (**5** and **6**).[13] In this letter, we report a new compound that is readily available, highly selective for inhibition of GGDPS over FDPS, and the most potent inhibitor of GGDPS reported to date.

The key compound was prepared by the short reaction sequence summarized in Scheme 1. The commercially available

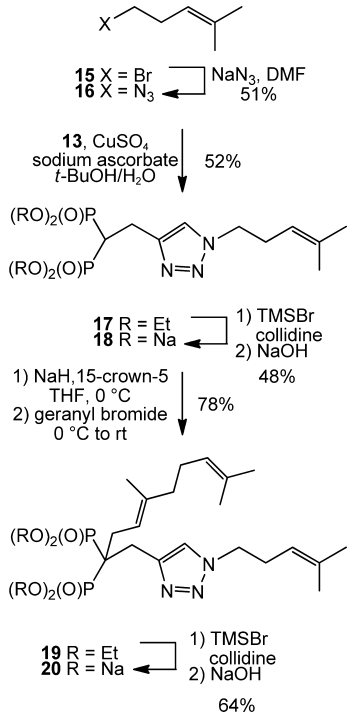
Scheme 1. Synthesis of Triazole 8 as a ~3:1 Mixture of *E*- and *Z*-Olefin Isomers



ketone **9** was treated with cyclopropyl magnesium bromide to obtain the tertiary alcohol **10**. When treated with magnesium bromide in ether, this alcohol undergoes a ring opening rearrangement to afford the homoallylic bromide **11** in good yield as a 3:1 mixture of *E*- and *Z*-isomers.[14] These bromides were not readily separable by column chromatography, and a study of various lower reaction temperatures only modestly impacted the ratio but did significantly lower the conversion. Therefore, bromide **11** was carried forward as a mixture of isomers, first to the azide **12**, and then to reaction with the acetylene **13** to give the expected cycloadducts **14**. Finally, hydrolysis of the phosphonate esters through standard procedures[15] gave the desired triazole bisphosphonate **8**, as a mixture of olefin isomers in a ratio of ~3:1 (*E*/*Z*).

Based on crystallographic studies of several enzyme−inhibitor or pseudosubstrate complexes, the biological activity of DGBP has been attributed in part to its ability to adopt a V-shape.[16] This shape allows placement of one hydrophobic chain in the channel through which FPP enters the active site and the other into the tunnel which allows departure of the GGDP product.[16] To obtain a V-shaped compound that incorporates a triazole moiety, we pursued the reaction sequence shown in Scheme 2. In this case, commercial homoprenyl bromide **15**

Scheme 2. Synthesis of a Dialkyl Bisphosphonate



was converted to the corresponding azide, and that azide was allowed to react with the acetylene bisphosphonate **13** under standard click conditions. The resulting triazole **17** readily undergoes hydrolysis under McKenna conditions[15] to give the desired salt **18**. Prior to hydrolysis, compound **17** can be treated with strong base and geranyl bromide to obtain the dialkyl bisphosphonate **19**. Hydrolysis of compound **19** gives the triazole **20**, which can be viewed as a triazole analogue of DGBP (**4**).

As shown in Table 1, the three triazole bisphosphonates **8**, **18**, and **20** were tested for their activity in a variety of bioassays. Notably, triazole bisphosphonate **8** was found to inhibit GGDPS in an *in vitro* enzyme assay with an IC_{50} of 45 nM. This compound was selective for GGDPS given that it was demonstrated to have only weak activity against the related enzyme FDPS. Furthermore, triazole **8** displayed potent activity in intact human myeloma cells (RPMI-8226 cells) with disruption of protein geranylgeranylation observed at concentrations as low as 50 nM (Figure 2). The homoprenyl triazole bisphosphonate **18** was found to be about 10-fold less active against GGDPS and 100-fold less active in cells compared with compound **8**. Interestingly, the dialkylated triazole bisphos-

DOI: 10.1021/acsmedchemlett.5b00334
ACS Med. Chem. Lett. 2015, 6, 1195−1198

Table 1. Activity of Novel Triazole Bisphosphonates

compd	GGDPS IC_{50} (μM)	FDPS IC_{50} (μM)	enzyme selectivity (FDPS/GGDPS)	disruption of protein geranylgeranylation in cell culture[a] (μM)	cytotoxicity EC_{50} (μM)
8	0.045 (±0.016)	28 (±5.0)	620	0.05	0.19 (±0.058)
18	0.43 (±0.21)	6.6 (±0.95)	15	5	15 (±2.2)
20	0.38 (±0.23)	13 (±6.1)	34	0.5	1.0 (±0.17)

[a]Cellular activity refers to the lowest concentration at which disruption of protein geranylgeranylation was observed after 48 h incubation in RPMI-8226 human myeloma cells as determined by immunoblot analysis of unmodified Rap1a (a marker of disruption of geranylgeranylation of a GGTase I substrate) and ELISA analysis of intracellular lambda light chain levels (a marker for disruption of Rab geranylgeranylation, cf. Figure 2).[17] MTT cytotoxicity assays were performed in RPMI-8226 cells, which were incubated in the presence or absence of the novel compounds for 48 h.

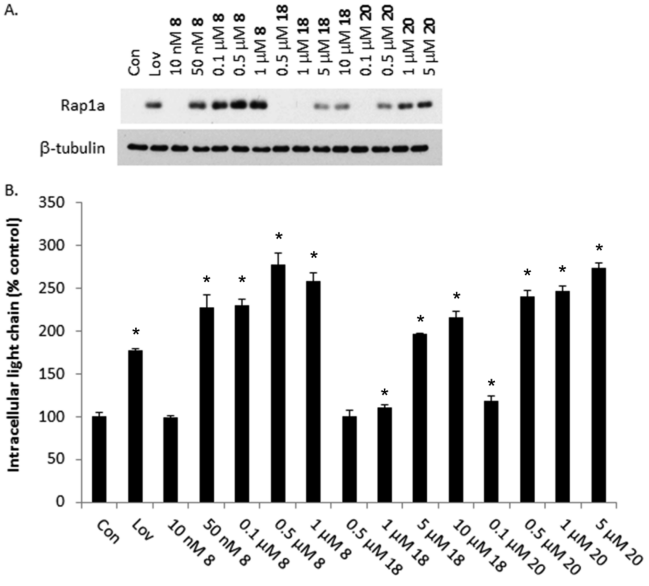


Figure 2. Effects of novel triazole bisphosphonates in myeloma cells. RPMI-8226 cells were incubated for 48 h in the presence or absence of the triazole bisphosphonates **8**, **18**, or **20**. Cells were also incubated with lovastatin (*Lov*, 10 μM) as a positive control. (A) Cells were lysed using RIPA buffer to generate whole cell lysate. The Rap1a antibody detects only unmodified protein. β-Tubulin was used as a loading control. The gels are representative of two independent studies. (B) Intracellular lambda light chain concentrations were determined via ELISA. Data are expressed as percentage of control (mean ± SD, n = 3). The * denotes $p < 0.05$ per unpaired two-tailed t-test and compares treated cells to untreated control cells.

phonate **20** displayed activity similar to compound **18** against GGDPS but was 10-fold more potent in cell culture studies. Finally, the ability of the novel inhibitors to disrupt protein geranylgeranylation in cells correlated with the cytotoxic effects of the compounds, with compound **8** demonstrating the greatest potency in the cytotoxicity experiments.

Our previous work with geranyl and neryl triazoles demonstrated that the olefin stereochemistry has a significant impact on inhibitory activity against GGDPS.[13] Based on this work, we would predict that the homoneryl component of the olefin mixture **8** would more potently inhibit GGDPS than the homogeranyl component, and efforts are underway to prepare pure isomers to test this hypothesis. However, the impact of chain length already is evident when triazole bisphosphonates **8** and **18** are compared. Although the V-shaped analogue **20** was prepared with the hope that it would display increased potency, it was less active than compound **8**. That this compound had similar inhibitory activity in the *in vitro* enzyme assay but was more active in intact cells compared with **18** suggests differences in cellular membrane permeability.

In conclusion, with an $IC_{50} \approx 45$ nM, compound **8** as a ~ 3:1 mixture of *E*- and *Z*-olefin isomers is the most potent GGDPS inhibitor prepared to date, while still maintaining high selectivity for GGDPS over FDPS. Even as a mixture of olefin isomers, this material is ~8-fold more potent than the triazole **5** ($IC_{50} \approx 380$ nM). Perhaps of greater importance, compound **8** potently disrupts protein geranylgeranylation in cells at concentrations as low as 50 nM and induces cytotoxic effects at submicromolar concentrations, suggesting potential clinical utility. The V-shaped compound **20** and the monoalkyl bisphosphonate **18** show nearly equal potency, and both are roughly 10-fold less active than compound **8**. This suggests that a triazole such as compound **8** may complex in the active site of GGDPS in a manner that obviates the benefit of a V-shape. It also suggests that additional structure–activity studies may further improve the potency of GGDPS inhibitors and increase the possibility of clinical application.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00334.

Representative experimental procedures, NMR spectra, and bioassay protocols (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: david-wiemer@uiowa.edu.

Funding

We thank the UI Graduate College for a Dean's Graduate Fellowship and an AGEP Fellowship (to V.S.W.), and the Center for Biocatalysis and Bioprocessing for a fellowship (to V.S.W.) through the Predoctoral Training Program in Biotechnology (T32 GM008365). Financial support from the NIH (R01CA-172070), the American Society of Hematology (a Scholar Award to S.A.H.), and the Roy J. Carver Charitable Trust through its Research Program of Excellence (to D.F.W.) is gratefully acknowledged.

Notes

The authors declare the following competing financial interest(s): D.F.W. is a named inventor of intellectual property related to digeranyl bisphosphonate that is owned by the University of Iowa Research Foundation. He is a founder of Terpenoid Therapeutics, Inc., which has licensed this property.

■ ACKNOWLEDGMENTS

We thank Dr. Lynn Teesch for her assistance with the UHPLC MS experiments.

DOI: 10.1021/acsmedchemlett.5b00334
ACS Med. Chem. Lett. 2015, 6, 1195–1198

REFERENCES

(1) Park, J.; Matralis, A. N.; Berghuis, A. M.; Tsantrizos, Y. S. Human isoprenoid synthase enzymes as therapeutic targets. *Front. Chem.* **2014**, *2*, 1−21.

(2) Ebetino, F. H.; Hogan, A. M. L.; Sun, S.; Tsoumpra, M. K.; Duan, X.; Triffitt, J. T.; Kwaasi, A. A.; Dunford, J. E.; Barnett, B. L.; Oppermann, U.; Lundy, M. W.; Boyde, A.; Kashemirov, B. A.; McKenna, C. E.; Russell, R. G. G. The Relationship between the chemistry and biological activity of the bisphosphonates. *Bone* **2011**, *49*, 20−33.

(3) Wiemer, A. J.; Wiemer, D. F.; Hohl, R. J. Geranylgeranyl diphosphate synthase: An emerging therapeutic target. *Clin. Pharmacol. Ther.* **2011**, *90*, 804−812.

(4) Sorrentino, G.; Ruggeri, N.; Specchia, V.; Cordenonsi, M.; Mano, M.; Dupont, S.; Manfrin, A.; Ingallina, E.; Sommaggio, R.; Piazza, S.; Rosato, A.; Piccolo, S.; Del Sal, G. Metabolic control of Yap and Taz by the mevalonate pathway. *Nat. Cell Biol.* **2014**, *16*, 357−366.

(5) Shull, L. W.; Wiemer, A. J.; Hohl, R. J.; Wiemer, D. F. Synthesis and biological activity of isoprenoid bisphosphonates. *Bioorg. Med. Chem.* **2006**, *14*, 4130−4136.

(6) Wiemer, A. J.; Tong, H. X.; Swanson, K. M.; Hohl, R. J. Digeranyl bisphosphonate inhibits geranylgeranyl pyrophosphate synthase. *Biochem. Biophys. Res. Commun.* **2007**, *353*, 921−925.

(7) Wiemer, A. J.; Yu, J. S.; Lamb, K. M.; Hohl, R. J.; Wiemer, D. F. Mono- and dialkyl isoprenoid bisphosphonates as geranylgeranyl diphosphate synthase inhibitors. *Bioorg. Med. Chem.* **2008**, *16*, 390−399.

(8) Wiemer, A. J.; Wiemer, D. F. Prodrugs of phosphonates and phosphates: Crossing the membrane barrier. *Top. Curr. Chem.* **2014**, *360*, 115−160.

(9) Rasmussen, M.; Leonard, N. J. Synthesis of 3-(2′-deoxy-D-ribofuranosyl)adenine. Application of a new protecting group, pivaloyloxymethyl (Pom). *J. Am. Chem. Soc.* **1967**, *89*, 5439−5445.

(10) Wiemer, A. J.; Yu, J. S.; Shull, L. W.; Barney, R. J.; Wasko, B. M.; Lamb, K. M.; Hohl, R. J.; Wiemer, D. F. Pivaloyloxymethyl-modified isoprenoid bisphosphonates display enhanced inhibition of cellular geranylgeranylation. *Bioorg. Med. Chem.* **2008**, *16*, 3652−3660.

(11) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective ″ligation″ of azides and terminal alkynes. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599.

(12) Skarpos, H.; Osipov, S. N.; Vorob'eva, D. V.; Odinets, I. L.; Lork, E.; Roschenthaler, G. V. Synthesis of functionalized bisphosphonates via click chemistry. *Org. Biomol. Chem.* **2007**, *5*, 2361−2367.

(13) Zhou, X.; Ferree, S. D.; Wills, V. S.; Born, E. J.; Tong, H.; Wiemer, D. F.; Holstein, S. A. Geranyl and neryl triazole bisphosphonates as inhibitors of geranylgeranyl diphosphate synthase. *Bioorg. Med. Chem.* **2014**, *22*, 2791−2798.

(14) McCormick, J. P.; Barton, D. L. Synthetic applications of metal halides - Conversion of cyclopropylmethanols into homoallylic halides. *J. Org. Chem.* **1980**, *45*, 2566−2570.

(15) McKenna, C. E.; Higa, M. T.; Cheung, N. H.; McKenna, M. C. Facile dealkylation of phosphonic acid dialkyl esters by bromotrimethylsilane. *Tetrahedron Lett.* **1977**, *18*, 155−158.

(16) Chen, C. K.-M.; Hudock, M. P.; Zhang, Y. H.; Guo, R. T.; Cao, R.; No, J. H.; Liang, P. H.; Ko, T. P.; Chang, T. H.; Chang, S.; Song, Y. C.; Axelson, J.; Kumar, A.; Wang, A. H. J.; Oldfield, E. Inhibition of geranylgeranyl diphosphate synthase by bisphosphonates: A crystallographic and computational investigation. *J. Med. Chem.* **2008**, *51*, 5594−5607.

(17) Holstein, S. A.; Hohl, R. J. Isoprenoid biosynthetic pathway inhibition disrupts monoclonal protein secretion and induces the unfolded protein response pathway in multiple myeloma cells. *Leuk. Res.* **2011**, *35*, 551−559.

DOI: 10.1021/acsmedchemlett.5b00334
ACS Med. Chem. Lett. 2015, 6, 1195−1198