Communications to the Editor

α-Phosphonosulfonic Acids: Potent and Selective Inhibitors of Squalene Synthase

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Inhibitors of squalene synthase have recently received considerable attention in the search for new antihypercholesterolemic agents.[1] We disclose herein the discovery of a new family of *in vivo* active squalene synthase inhibitors: the α-phosphonosulfonic acids. We previously reported that lipophilic 1,1-bisphosphonates were potent inhibitors of squalene synthase and effective cholesterol-lowering agents in animal models.[2] 1,1-Bisphosphonate inhibitors were designed as mimetics of the putative ion pair intermediate formed from farnesyl diphosphate (FPP) in the initial step of the squalene synthase reaction.[3] The bisphosphonate function served as a stable surrogate for the diphosphate moiety. Although the bisphosphonates were potent cholesterol-lowering agents, they possessed several pharmacological properties which warranted further improvement. The α-phosphonosulfonic acid (α-PSA) inhibitors were developed to address these issues.

Prototype 1,1-bisphosphonate **2** elevated plasma transaminase levels (ALT and AST) in mice when administered at a single iv dose of 20 mg/kg (see Figure 1), an indication of hepatotoxicity.[4] Disposition studies with [^{14}C]-**2**[5] in rats revealed that there was significant retention of radioactivity in bone and liver tissue 72 h after an intravenous dose.[6] The retention in bone was consistent with what is known about the bisphosphonate inhibitors of bone resorption.[7] Accumulation of inhibitor and metabolites in the liver may have contributed to the hepatotoxicity observed for **2.** In addition, **2** has low oral absorption in rats (1.3%).[8] We proposed that both the tissue retention and low oral absorption were due to the highly charged nature of the bisphosphonate moiety (a tetraacid), which would make it difficult for the inhibitor to traverse cellular membranes. Accordingly, we embarked on a search for a diphosphate surrogate with fewer acidic groups.[9]

A sulfonic acid can be considered to be an isostere of a phosphonic acid. The tetrahedral geometry and the interatomic distances of an alkyl sulfonic acid are very close to that of a phosphonic acid.[10−13] However, a sulfonate is a monoacid whereas a phosphonate is a diacid. In order to determine whether a sulfonate is a suitable replacement for a phosphonate, a series of three α-phosphonosulfonic acid analogs (**4**, **5**, and **6**) of potent bisphosphonates (**1**, **2**, and **3**) were prepared and evaluated as inhibitors of rat liver microsomal squalene synthase (Table 1).[14] The farnesylethyl-substituted α-PSA **4** exhibits comparable activity to the corresponding bisphosphonate **1**, whereas the shorter-chain analogs (**5** and **6**) are much less potent than the corresponding bisphosphonates (**2** and **3**). This result is consistent with our earlier observations with other triacid diphosphate surrogates, where only the longer-chain, farnesyl-length inhibitors displayed good inhibitory activity.[9] Compound **4** is also a potent inhibitor of cholesterol biosynthesis in rats[15] (iv ED_{50} = 0.09 mg/kg), comparable to the bisphosphonates, and also exhibits modest oral activity. For comparison, the iv ED_{50} value for the HMG-CoA reductase inhibitor pravastatin is 0.05 mg/kg in this assay.[2]

Further evaluation of **4** revealed several important improvements relative to bisphosphonate **2**. α-PSA **4**

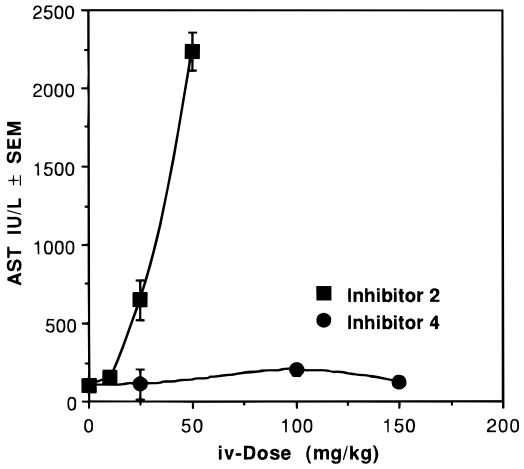
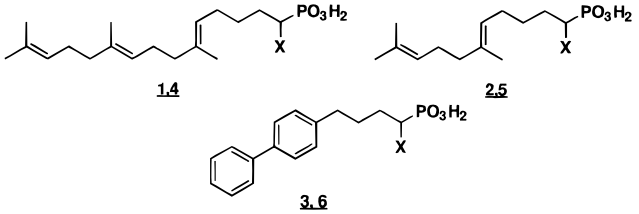


Figure 1. The effect of iv administration of bisphosphonate **2** and phosphonosulfonate **4** on AST levels in female mice.[4]

Table 1. Biological Evaluation of Prototype 1,1-Bisphosphonates and α-Phosphonosulfonates



compd	X	squal. syn. IC_{50} (nM) [95% confidence interval]	rat model ED_{50} (mg/kg) iv [SEM]	po [SEM]
1[a]	PO_3H_2	5.7 [4.7, 6.9]	0.04 [0.01]	inactive at 30
2[b]	PO_3H_2	1.0 [0.5, 1.7]	0.05 [0.003]	0.43 [0.19]
3[c]	PO_3H_2	0.7 [0.5, 0.9]	0.03 [0.005]	1.0 [0.15]
4[d]	SO_3H	15 [11.4, 19.6]	0.09 [0.01]	10.3 [0.95]
5[a]	SO_3H	740 [627, 875]		
6[a]	SO_3H	1980 [1600, 2400]		

[a] Trisodium salt. [b] Tetrasodium salt. [c] Free acid. [d] Tripotassium salt.

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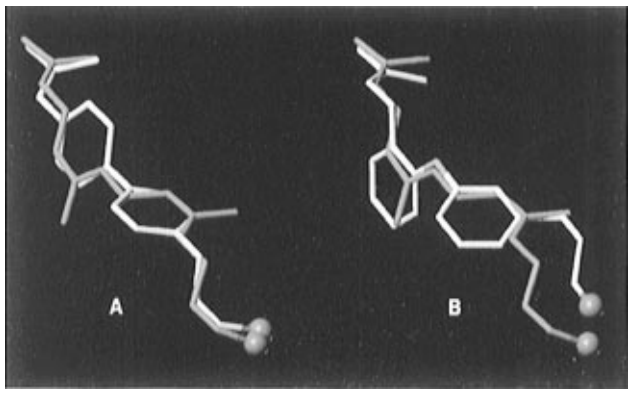
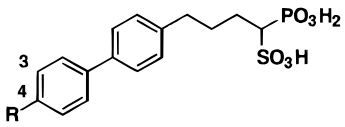


Figure 2. Molecular overlays of biphenyl α-PSA **13** (yellow, Figure 2A) and diphenyl ether α-PSA **24** (white with red oxygen, Figure 2B) with farnesyl α-PSA **4** (turquoise). The purple sphere indicates the point of attachment of the phosphonate and sulfonate functions. The conformation utilized for the farnesyl portion of **4** was that found in the crystal structure of squalene.[20−22] The coordinates for squalene were obtained from the Cambridge Crystallographic Database (Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.). The molecular overlays were performed using Sybyl 6.04 molecular modeling package, employing the Multifit program.

exhibited a greatly reduced acute effect on plasma transaminase levels in mice (Figure 1). Bisphosphonate **2** caused a >5-fold elevation in plasma AST levels at an iv dose of 20 mg/kg, whereas α-PSA **4** had a minimal effect on AST levels at 150 mg/kg. A disposition study of [^{14}C]-**4**[5] on iv dosing to rats indicated that neither **4** nor its metabolites accumulated in bone, but high concentrations (134 micromolar equiv) of radiolabel were present in the liver at 12 h. In contrast to **2**, 90% of the radiolabel was excreted in feces and urine within 72 h.[16] Therefore, two attributes of bisphosphonates, acute hepatotoxicity and tissue retention, were successfully addressed by α-PSA **4**. As with bisphosphonate **2**, the oral absorption of **4** in rats was minimal (<3%).[17] In addition, the farnesyl moiety of **4** suffered from extensive metabolism in rats. Two metabolites isolated from rat urine were identified,[18] and their structures suggest that the farnesyl moiety of **4** is subject to the known metabolic pathway for farnesyl diphosphate.[19]

In order to circumvent the metabolic pathways intended for the natural isoprene chain, inhibitors containing non-natural isoprene surrogates were sought. The results with compounds **4**, **5**, and **6** indicate the α-PSA must be paired with a full-length farnesyl chain to express good potency. We therefore investigated adding substituents to the biphenyl nucleus of **6** to better approximate the farnesyl chain. A molecular overlay between **6** and a conformational model of the farnesyl portion of **4**[20−22] (see Figure 2A) suggested that a lipophilic substituent in the 4-position of the biphenyl nucleus would occupy the same space as the terminal dimethylvinyl substituent of the farnesyl chain. In fact, the potency of the biphenyl inhibitors were significantly improved by incorporating a lipophilic 4-substituent of the approximate volume of dimethylvinyl (Table 2, compounds **9, 11, 13**−**15**). Smaller (**7** and **8**) or larger (**12**) 4-substituents led to dramatic losses in potency, as did substituting at the 3-position (**10**). These observations provide circumstantial evidence for the proposed conformational model and indicate that the binding

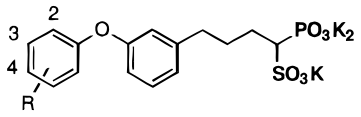
Table 2. 4-Substituted Biphenyl Phosphonosulfonates



compd[a]	R	squal. syn. IC_{50} (nM) [95% confidence interval]
6	H	1980 [1600, 2400]
7	Me	4600 [3900, 5500]
8	Et	2000 [1800, 2300]
9	*n*-Pr	144 [106, 196]
10	3-*n*-Pr[b]	8200 [6500, 10400]
11	*n*-Bu	192 [179, 206]
12	*n*-pentyl	6200 [4500, 8500]
13	$Me_2C{=}CH$	76 [64, 91]
14	phenyl	35 [29, 42]
15	2-pyridyl	13 [8.2, 19.2]

[a] All compounds in Table 2 are tripotassium salts, except for **6**, which is a trisodium salt. [b] The 3-*n*-propyl isomer as depicted in the structure above.

Table 3. Substituted Biaryl Ether Phosphonosulfonates



compd	R	squal. syn. IC_{50} (nM) [95% confidence interval]
16	H	169 [155, 184]
17	2-*n*-Pr	268 [221, 324]
18	3-*n*-Pr	6900 [5100, 9300]
19	4-*n*-Pr	14200 [12400, 16200]
20	2-Me	200 [174, 231]
21	2-*n*-Bu	35 [28.1, 43.4]
22	2-$PhCH_2$	18 [15.1, 22.1]
23	2-$Me_2CHCH_2CH_2$	17 [12.5, 24.2]
24	2-$Me_2C{=}CHCH_2$	19 [15.3, 23.4]
25	2-PhO	286 [241, 340]

pocket for the terminal isoprene subunit has very specific requirements.

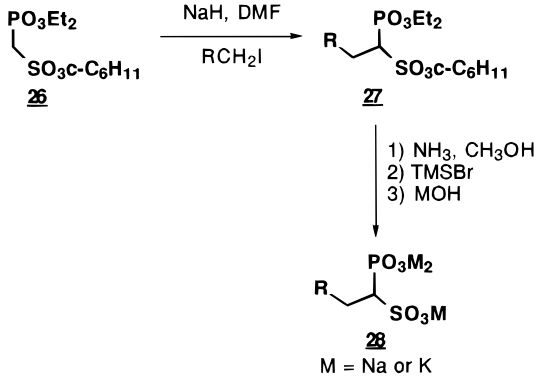
A related series of *m*-diphenyl ethers were synthesized and evaluated. The parent diphenyl ether **16** was 10-fold more potent than the corresponding parent biphenyl **6** (Table 3). In contrast to the biphenyl series, it was more difficult to predict where the appropriate substituent should be positioned to better mimic the full-length farnesyl chain. As a probe for the optimal substitution pattern, the 2-, 3-, and 4-propyl derivatives were synthesized. The 3- and 4-propyl analogs **18** and **19** were 30- and 64-fold less potent than the parent **16**. In contrast, the 2-propyl analog **17** retained good squalene synthase inhibitory activity. Potency improved further with the extension of the 2-substituent to butyl (**21**), and the most potent inhibitors closely corresponded to the natural dimethylallyl moiety (**23** and **24**). Additionally, the benzyl derivative **22** (IC_{50} = 18 nM) was found to be a very potent inhibitor of the enzyme. The results with the biphenyl and diphenyl ether inhibitors are consistent with the molecular overlay with **4** illustrated in Figure 2. The diphenyl ethers can adopt a conformation where the substituent on the 2-position projects into the site occupied by the terminal isoprene subunit of **4**. Thus, the 4-substituent of the biphenyls (Figure 2A) and the 2-substituent of the diphenyl ethers (Figure 2B) would occupy a similar region of the active site.

A number of the biphenyl and diphenyl ether α-PSAs were evaluated as inhibitors of cholesterol biosynthesis

Table 4. *In Vivo* Effects of α-Phosphonosulfonates on Cholesterol Biosynthesis in Rats

compd	rat model ED_{50} (mg/kg) iv [SEM]	po [SEM]
9	0.90 [0.05]	
13	0.42 [0.06]	Inactive at 20
14	0.10 [0.07]	Inactive at 20
15	0.10 [0.03]	
16	0.28 [0.04]	10 [2.8]
21	0.49 [0.05]	43% inhibition at 30
22	0.21 [0.06]	Inactive at 20
24	0.20 [0.08]	

Scheme 1. Synthesis of α-Phosphonosulfonates



in rats and proved to have good potency on intravenous dosing (Table 4). Parent diphenyl ether **16** exhibited superior *in vivo* activity on both iv and po dosing relative to its activity against rat liver squalene synthase. When administered to hamsters ip at a dose of 30 mg/kg/day for 5 days, **16** lowered plasma cholesterol levels by 45%.[23,24]

The α-PSAs in Tables 1−3 were prepared by the general method outlined in Scheme 1. Triester **26**[25] was coupled to primary alkyl iodides (NaH, DMF) to give **27**. The sulfonate cyclohexyl ester was deprotected under mild solvolytic conditions (methanol, NH_3, 50 °C).[26,27] The remaining phosphonate esters were cleaved with excess TMSBr,[28] generally in the presence of an acid scavenger (2,4,6-collidine or TMS_2NH). Saponification of the silyl esters with either NaOH or KOH provided the alkali metal salts, **28.**

With the discovery of the α-phosphonosulfonates, we have made significant advancements relative to the bisphosphonates. As illustrated above for inhibitor **4**, α-PSAs have a reduced tendency to elevate plasma transaminase levels and to be retained in bone and liver. In addition, the biphenyl and diphenyl ether inhibitors are not expected to be subject to the metabolic pathway intended for FPP.[29] At this juncture, the poor oral bioavailability of the α-PSAs remained a serious challenge (see Table 4). A successful prodrug strategy is described in the following paper.

Acknowledgment. We would like to thank Drs. Joseph A. Tino and Mark A. Hermsmeier for assistance with the molecular overlays in Figure 2, as well as Dr. John E. Swigor and George M. Luke for the synthesis of [^{14}C]-**2.**

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(3) The reader is referred to the discussions in ref 2, as well as the following: Biller, S. A.; Sofia, M. J.; DeLange, B.; Forster, C.; Gordon, E. M.; Harrity, T.; Rich, L. C.; Ciosek, C. P., Jr. The First Potent Inhibitor of Squalene Synthase: A Profound Contribution of an Ether Oxygen to Inhibitor-Enzyme Interaction. *J. Am. Chem. Soc.* **1991**, *113*, 8522−8524 and references therein.

(4) Compounds dissolved in saline were dosed iv at the indicated doses to female mice ($N = 5$). The control vehicle was saline. Twenty-four hours after injection, the mice were bled and plasma AST levels were determined using a Roche Cobas blood chemistry autoanalyzer.

(5) The ^{14}C-label was introduced at the carbon γ to the sulfur atom.

(6) At 72 h after single 13.3 μmol/kg (5.7 mg/kg) intravenous doses of [^{14}C]-**2** to two rats, the radioactivity in plasma, liver, and bone (femur) averaged 0.135, 17.7, and 17.6 micromolar equiv (0.046, 6.02, and 5.98 μg equiv/mL or g), respectively. The concentrations of radioactivity in liver and bone were both 130-fold higher than that of the plasma. At this time point, 28% and 17% of the total radioactivity was recovered in the urine and feces, respectively, while an average of 55% of the total radioactivity was retained in the carcass, presumably in the bone.

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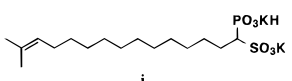
(8) In 12 h after single 13.3 μmol/kg oral doses of [^{14}C]-**2** to three bile-duct cannulated (BDC) rats, averages of 0.07%, 0.37%, 0.86%, and 99.76% of the dose were recovered in bile, urine, carcass (minus the GI tract), and the GI tract, respectively. The oral absorption averaged 1.3%.

(9) For earlier work, see: Magnin, D. R.; Biller, S. A; Dickson, J. K., Jr.; Logan, J. V.; Lawrence, R. M.; Chen, Y.; Sulsky, R. B.; Ciosek, C. P., Jr.; Harrity, T. W.; Jolibois, K. G.; Kunselman, L. K.; Rich, L. C.; Slusarchyk, D. A. 1,1-Bisphosphonate Squalene Synthase Inhibitors: Interplay Between the Isoprenoid Subunit and the Diphosphosphate Surrogate *J. Med. Chem.* **1995**, *38*, 2596−2605.

(10) The dibasic phosphonate group and the monobasic sulfonate group have considerable structural similarity. Both are tetrahedral, second row functions with a C_{3v} display of three negatively charged oxygen atoms. In addition, they show significant similarity, but not identity, in their interaction with Lewis acids.[11] Certain proteins can discriminate between binding phosphonyl and sulfonyl groups,[12] whereas others bind both functions well.[13] Bond angle and bond length data from the X-ray crystal structure of **i** illustrates the close isosteric relationship between the phosphonate and sulfonate groups (Malley, M. F.; DiMarco, J.; Gougoutas, J. Z., Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ; unpublished results). Average bond distances for **i** (Å): S−C, 1.80, P−C, 1.82; S−O, 1.46, P−O, 1.50. Average bond angles for **i** (degrees): C−S−O, 107.3, C−P−O, 110.1; O−S−O, 111.6; O−P−O, 115.7. These results are consistent with bond lengths and angles of a variety of sulfonates and phosphonates in crystal structures found in the Cambridge Crystallographic Database (Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.).



i

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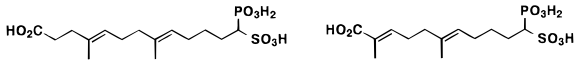
(14) The assay quantitates the conversion of [^{3}H]-FPP to squalene by rat liver microsomal squalene synthase, as detailed in ref 2.
(15) The assay quantitates the conversion of ip-dosed [^{14}C]-acetate to [^{14}C]-sterols in the intact rat, as detailed in ref 2.
(16) In 72 h after single 15 μmol/kg (7.8 mg/kg) intravenous doses of [^{14}C]-**4** to three rats, the percent of total radioactivity recovered in urine and feces averaged 90% . At 12 h after single 15 μmol/kg intravenous doses of [^{14}C]-**4** to three BDC rats, the radioactivity in plasma, liver and bone (femur) averaged 2.9, 134, and 2.3 micromolar equiv (1.2, 54.7, and 0.9 μg equiv/mL or g), respectively. The concentration of radioactivity in liver was 46-fold higher than that of the plasma.
(17) On the basis of the ratios of areas under the plasma concentration of total radioactivity vs time curves after intravenous and oral doses of [^{14}C]-**4**, the absorption of **4** in rats averaged 2.4%. On the basis of the sum of radioactivity recovered in bile, urine, and carcass (minus the GI tract) after an oral dose of [^{14}C]-**4**, the absorption averaged 2.9%.
(18) After an intravenous dose of [^{14}C]-**4** to rats, at least three metabolites were found in urine. Two of the metabolites were assigned, on the basis of LC/MS data, as **ii** and **iii:**



ii iii

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(20) The conformational model for the farnesyl group used for compound **4** in **Figure 2** is described in ref 21, and is based on the X-ray crystal structure of squalene.[22] The repeating local conformation about each isoprene subunit in the X-ray structure is a low-energy conformation.[21]
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(24) Male Syrian golden hamsters (120−140 g) were acclimated to a reverse light cycle for 2 weeks. The compound was solublized in saline and injected intraperitoneally. Plasma samples were obtained after the end of the treatment period and analyzed for lipids as previously reported.[2]
(25) The preparation of **22** was carried out in a similar manner to that described by Carretero et al. for the corresponding triethyl ester: Carretero, J. C.; Demillequand, M.; Ghosez, L. Synthesis of α,β-Unsaturated Sulfonates *Via* The Wittig-Horner Reaction. *Tetrahedron* **1987**, *43*, 5125−5134.
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(27) The ethyl sulfonate corresponding to **26** undergoes iodide-promoted displacement of the ethyl ester during the attempted conversion to **27**. The cyclohexyl ester was chosen as the sulfonate protecting group because it was stable enough to withstand the alkylation reaction, but could be removed under mild, solvolytic reaction conditions. Typical relative rates for displacement reactions at ethyl, isopropyl, and cyclohexyl centers are 1, 0.025, and 0.000 25, respectively: A. Streitwieser, Jr. *Solvolytic Displacement Reactions*; McGraw-Hill: New York, 1962.
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(29) When the (*S*)-enantiomer of **16** was administered to rats iv, >80% of the dose was excreted intact. The synthesis and biological evaluation of the enantiomers of **16** will be the subject of a future report.

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