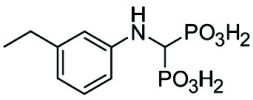
Journal of Medicinal Chemistry

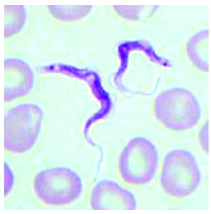
Article

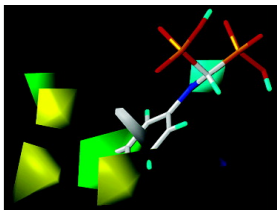
Bisphosphonate Inhibition of the Exopolyphosphatase Activity of the *Trypanosoma brucei* Soluble Vacuolar Pyrophosphatase

Evangelia Kotsikorou, Yongcheng Song, Julian M. W. Chan, Stephanie Faelens, Zev Tovian, Erin Broderick, Norbert Bakalara, Roberto Docampo, and Eric Oldfield

J. Med. Chem., **2005**, 48 (19), 6128-6139• DOI: 10.1021/jm058220g • Publication Date (Web): 25 August 2005







More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Bisphosphonate Inhibition of the Exopolyphosphatase Activity of the *Trypanosoma brucei* Soluble Vacuolar Pyrophosphatase

Evangelia Kotsikorou,[†,§] Yongcheng Song,[†,§] Julian M. W. Chan,[†] Stephanie Faelens,[‡] Zev Tovian,[†] Erin Broderick,[†] Norbert Bakalara,[‡,#] Roberto Docampo,[∥] and Eric Oldfield*[,†]

Department of Chemistry, University of Illinois at Urbana−Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801, Laboratoire de Genomique Fonctionnelle des Trypanosomatides, UMF-CNRS 5162, Université Bordeaux 2, 146, Rue Léo Saignat, 33000 Bordeaux, France, and Center for Tropical and Global Emerging Diseases and Department of Cellular Biology, University of Georgia, Athens, Georgia 30602

Received April 11, 2005

Trypanosoma brucei, the causative agent of African trypanosomiasis, contains a soluble, vacuolar pyrophosphatase, *Tb*VSP1, not present in humans, which is essential for the growth of bloodstream forms in their mammalian host. Here, we report the inhibition of a recombinant *Tb*VSP1 expressed in *Escherichia coli* by a panel of 81 bisphosphonates. The IC_{50} values were found to vary from ∼2 to 850 μM. We then used 3D QSAR (comparative molecular field and comparative molecular similarity index; CoMFA and CoMSIA) methods to analyze the enzyme inhibition results. The R^2 values for the experimental versus the QSAR-predicted activities were 0.78 or 0.61 for CoMFA and 0.79 or 0.68 for CoMSIA, for two different alignments. The root-mean-square (rms) pIC_{50} error for the best CoMFA model was 0.41 for five test sets of five activity predictions, which translates to a factor of ∼2.6 error in IC_{50} prediction. For CoMSIA, the rms pIC_{50} error and error factors were 0.35 and 2.2, respectively. In general, the most active compounds contained both a single aromatic ring and a hydrogen bond donor feature. Thirteen of the more potent compounds were then tested in vivo in a mouse model of *T. brucei* infection. The most active compound in vivo provided a 40% protection from death with no apparent side effects, suggesting that further development of such compounds may be of interest.

Introduction

Trypanosoma brucei is the protozoan parasite that causes human African trypanosomiasis (HAT) or sleeping sickness, a disease that is transmitted by the bite of the tsetse fly and is fatal if not treated.[1] In East Africa, the causative agent is *T. brucei rhodesiense*, while in West Africa, it is *T. brucei gambiense*. The actual number of cases each year are thought to be between 300 000 and 500 000,[2] with some 60 million individuals at risk of infection.[1] There are two drugs currently used to treat the initial phase of the disease: suramin and pentamidine, employed in the treatment of trypanosomiasis caused by *T. brucei rhodesiense* and *T. brucei gambiense*, respectively.[3] For the treatment of the second or neurological phase, melarsoprol, an arsenical drug, is used for the treatment of both *T. brucei rhodesiense* and *T. brucei gambiense* infections.[4] However, this drug causes severe side effects and can sometimes be lethal. A newer but much more expensive drug, eflornithine, has recently been introduced but is effective only for the treatment of *T. brucei gambiense* infections.[5] The need for other, less toxic, inexpensive drugs active against infections caused by both species is therefore pressing.

Recently, bisphosphonate drugs such as risedronate (Actonel) and pamidronate (Aredia), used to treat bone resorption diseases, have also been found to have activity against the in vitro proliferation of several protozoan parasites, including *T. brucei*, *T. cruzi*, *Leishmania donovani*, *Leishmania mexicana*, *Toxoplasma gondii*, *Entamoeba histolytica*, and *Plasmodium falciparum*.[6−14] These compounds affect the mevalonate/isoprene biosynthesis pathway enzyme farnesyl pyrophosphate (FPP) synthase[15−18] in humans, and some bisphosphonates have been shown to be potent inhibitors of FPP synthases from *T. brucei* and *T. cruzi* as well.[14,19] In *T. cruzi*, bisphosphonates such as pamidronate and risedronate have been found to be quite effective both in vitro and in vivo,[8−10] but such highly potent bisphosphonates are less effective against *T. brucei* in vivo.[14] Additional possible targets for bisphosphonates, which chemically are analogues of pyrophosphate (or diphosphate), are the *T. brucei* pyrophosphatase (*Tb*VSP1) and the PPX1 exopolyphosphatase.[20,21] In *T. brucei*, the *Tb*VSP1 enzyme contains 415 amino acids (M_r = 47.3 kDa) and appears to contain two domains, a polyphosphatase domain and an EF-hand domain,[20] and is located in the acidocalcisome, an organelle found in all trypanosomatids and several apicomplexan parasites.[22] Depending on the nature of its divalent metal ion cofactors, this soluble, vacuolar pyrophosphatase (*Tb*VSP1) can act either as a pyrophosphatase (+Mg^{2+}) or as a short-chain polyphosphatase (+Zn^{2+}). In initial experiments, we found that the polyphosphatase (tripolyphosphatase) activity (in the presence of Zn^{2+}, which is abundant in acido-

* To whom correspondence should be addressed. Phone: 217-333-3374. Fax: 217-244-0997. E-mail: eo@chad.scs.uiuc.edu.
† University of Illinois at Urbana−Champaign.
§ These authors contributed equally to this paper.
‡ Université Bordeaux 2.
# Present address: Centre de Pharmacologie et Biotechnologie pour La Santé, CNRS-UMR 5160, Faculté de Pharmacie, 15, Avenue Charles Flahault, 34093 Montpellier Cedex 5, France.
∥ University of Georgia.

10.1021/jm058220g CCC: $30.25
Published on Web 08/25/2005

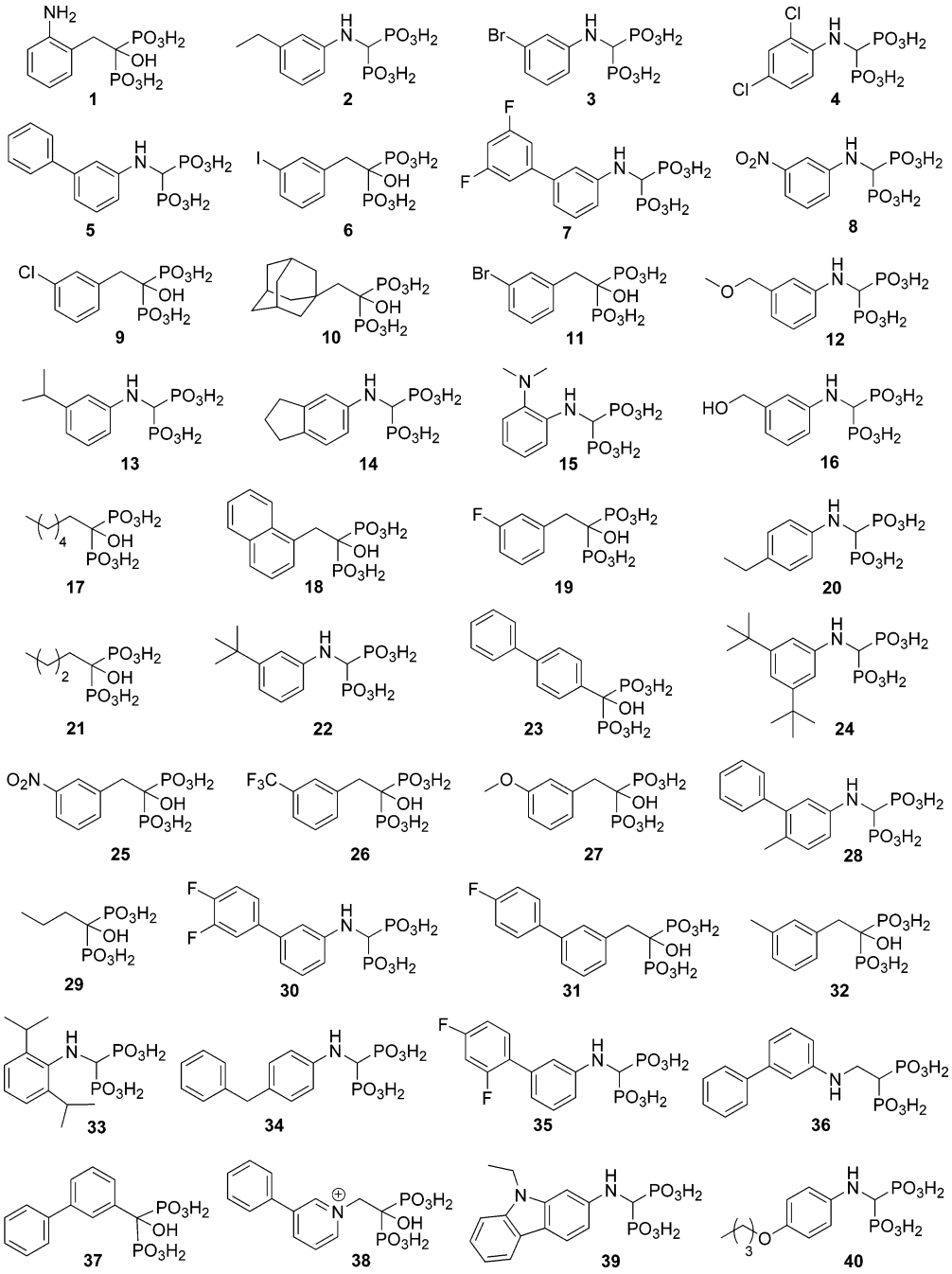
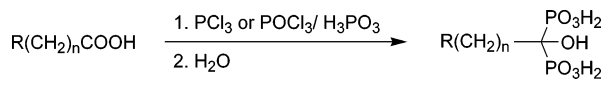


Figure 1. Structures of bisphosphonates (**1**−**40**) investigated, ordered by decreasing activity.

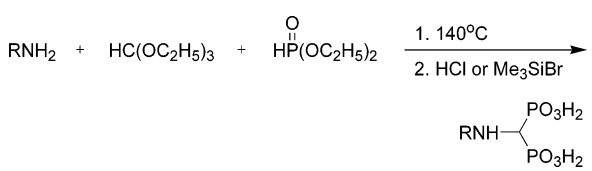
calcisomes) of *Tb*VSP1 was inhibited by bisphosphonates. Since *Tb*VSP1 has been shown to be essential for the growth of bloodstream forms of *T. brucei* in a mammalian host,[20] where it is involved in osmoregulation and virulence, we have now investigated its inhibition by a chemically diverse series of bisphosphonates and used three-dimensional quantitative structure−activity relationship (3D QSAR) techniques to analyze the inhibition results. We have also investigated the activity of some of the more potent bisphosphonates against *T. brucei brucei* infected mice, finding that some of the bisphosphonates that have high activity in vitro are also active in vivo.

Experimental Section

Synthetic Aspects. The structures of the 81 bisphosphonates investigated (**1**−**81**) are shown in Figures 1 and 2. The basic synthetic methods used have been described elsewhere[6−7,13,23−25] and, in brief, involved either (1) phosphonylation of a carboxylic acid,[26,27]



(2) condensation of an amine with ethylorthoformate and diethyl phosphite,[28]



or (3) Michael addition of an aromatic amine to tetraethyl-

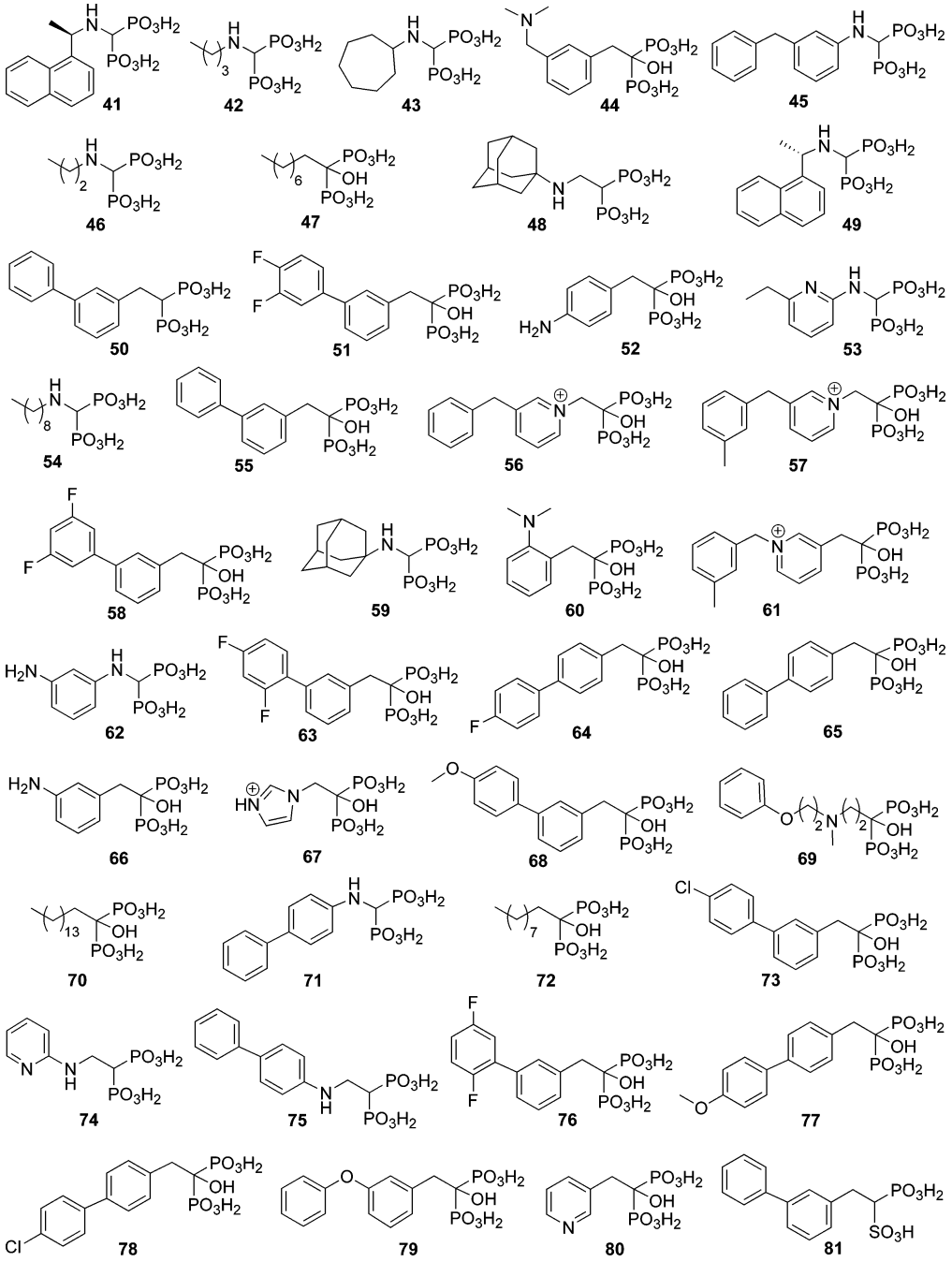
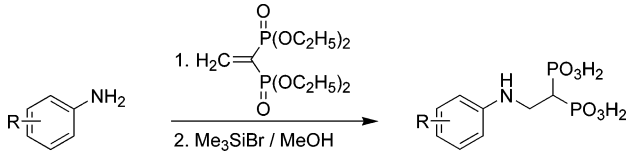


Figure 2. Structures of bisphosphonates (**41**−**81**) investigated, ordered by decreasing activity.

vinylidene bisphosphonate, followed by TMSBr cleavage,[29]



The syntheses and characterization of **2**, **5**, **14**, **17**, **21**, **24**, **29**, **38**−**40**, **42**, **43**, **46**, **47**, **52**, **54**, **56**, **57**, **61**, **67**, **69**−**72**, **74**, and **80** have been reported before.[6−7,13,23] Microchemical analysis results for all new compounds, **1**, **3**, **4**, **6**−**13**, **15**, **16**, **18**−**20**, **22**, **23**, **25**−**28**, **30**−**37**, **41**, **44**, **45**, **48**−**51**, **53**, **55**, **58**−**60**, **62**−**66**, **68**, **73**, **75**−**79**, and **81**, were satisfactory. All compounds were also routinely characterized by using ^{1}H and ^{31}P NMR spectroscopy at 500 MHz (Varian Unity spectrometer). All reagents were purchased from Aldrich.

The following five general procedures illustrate the synthetic aspects in more detail.

General Procedure 1. An amine (3 mmol), triethyl orthoformate (3.6 mmol), and diethyl phosphite (12 mmol) were heated at 140 °C under N_2 for 12 h.[28] The mixture was subjected to column chromatography to give the tetraethyl ester, which was then treated with TMSBr (6 equiv) in acetonitrile for 8 h at room temperature. Upon removal of the solvent, the residue was dissolved in EtOH/H_2O (5:1), after which a substituted aminomethylene bisphosphonate precipitated as a white powder.

General Procedure 2. To a carboxylic acid (3 mmol) in benzene (10 mL) was added oxalyl chloride (6 mmol) followed by 1 drop of DMF. The solution was stirred for 1 h, then the solvent was removed in vacuo. The residue was dissolved in THF, and tris(trimethylsilyl)phosphite (6 mmol) was added dropwise.[30] After 2 h, the solvent was evaporated and the residue was dissolved in MeOH/H_2O (4:1). Upon removal of

MeOH in vacuo, the pH of the solution was adjusted to ~4 by addition of NaOH (50%), upon which a white precipitate appeared that was collected by filtration to give a 1-hydroxymethylene bisphosphonate as a monosodium salt.

General Procedure 3. A mixture of a carboxylic acid (3 mmol), H_3PO_3 (15 mmol), and toluene (8 mL) was heated to 80 °C with stirring. After all solids melted, $POCl_3$ (15 mmol) was added slowly and the reaction mixture was vigorously stirred at 80 °C for 5 h.[27] When the mixture was cooled, toluene was decanted and 6 N HCl (3 mL) was added to the residue. The resulting solution was refluxed for 1 h, then most of the solvent was removed in vacuo. 2-Propanol (25 mL) was added to precipitate a 1-hydroxymethylene bisphosphonate as a white powder, which was filtered, washed with 2-propanol (5 × 5 mL), dried, and could be further purified by recrystallization in H_2O/*i*-PrOH. In some cases, the bisphosphonate was neutralized with NaOH and crystallized as its sodium salt from H_2O/*i*-PrOH.

General Procedure 4. To tetraethylvinylidene bisphosphonate[31] (1 mmol) in THF was added an amine (1 mmol), and the solution was stirred at room temperature overnight.[29] Upon removal of solvent, the residue was subjected to column chromatography to give the tetraethyl ester of 2-aminoethylidene 1,1-bisphosphonate, which was then treated with TMSBr (6 equiv) in acetonitrile for 8 h at room temperature. Upon removal of the solvent, the residue was dissolved in EtOH/H_2O (5:1), upon which a 2-aminoethylidene-1,1-bisphosphonic acid precipitated as a white powder.

General Procedure 5. All of the biphenyl starting compounds (if not commercially available) were prepared by Suzuki coupling.[32] In brief, a substituted phenylboronic acid (3.3 mmol), a substituted bromobenzene (3 mmol), $Pd(PPh_3)_4$ (0.075 mmol), K_2CO_3 (9 mmol) in toluene (10 mL), and water (3 mL) were refluxed under N_2 with vigorous stirring for 10 h. The product was extracted with diethyl ether and purified by column chromatography.

1-Hydroxy-2-(2−aminophenyl)ethylidene-1,1-bisphosphonic Acid (1). 1 was prepared from 2-nitrophenylacetic acid (540 mg, 3 mmol), following general procedure 3, followed by hydrogenation overnight (10% Pd/C, H_2, 60 psi) (580 mg, 62% yield). Anal. ($C_8H_{11}NNa_2O_7P_2 \cdot 1.5H_2O$) C, H, N.

(3-Bromophenylamino)methylenebisphosphonic Acid (3). 3 was prepared from 3-bromoaniline (516 mg, 3 mmol), following general procedure 1 (468 mg, 40% yield). Anal. ($C_7H_7NBrNa_3O_6P_2 \cdot 0.5H_2O \cdot 0.5EtOH$) C, H, N.

(2,4-Dichlorophenylamino)methylenebisphosphonic Acid (4). 4 was prepared from 2,4-dichloroaniline (490 mg, 3 mmol), following general procedure 1 (420 mg, 34% yield). Anal. ($C_7H_8NNaO_6P_2 \cdot 3H_2O$) C, H, N.

1-Hydroxy-2-(3-iodophenyl)ethylidene-1,1-bisphosphonic Acid (6). 6 was prepared from 3-iodophenylacetic acid (786 mg, 3 mmol), following general procedure 2 (470 mg, 35% yield). Anal. ($C_8H_{10}INaO_7P_2 \cdot H_2O$) C, H.

[3-(3,5-Difluorophenyl)phenylamino]methylene-1,1-bisphosphonic Acid (7). 3-(3,5-Difluorophenyl)aniline was prepared from *N*-acetyl-3-bromoaniline (642 mg, 3 mmol) and 3,5-difluorophenylboronic acid (474 mg, 3 mmol) following general procedure 5, followed by acid hydrolysis (3 N HCl, reflux). Compound **7** was made from the fluoroaniline, following general procedure 1 (349 mg, 30% yield). Anal. ($C_{13}H_{13}NF_2O_6P_2 \cdot H_2O$) C, H, N.

(3-Nitrophenylamino)methylenebisphosphonic Acid (8). 8 was prepared from 3-nitroaniline (414 mg, 3 mmol), following general procedure 1 (651 mg, 46% yield). Anal. ($C_7H_6N_2Na_4O_8P_2 \cdot 4.5H_2O$) C, H, N.

1-Hydroxy-2-(3-chlorophenyl)ethylidene-1,1-bisphosphonic Acid (9). 9 was prepared from 3-chlorophenylacetic acid (512 mg, 3 mmol), following general procedure 2 (457 mg, 41% yield). Anal. ($C_8H_9ClNaO_7P_2 \cdot 0.25H_2O$) C. H: calcd, 3.09; found, 2.47.

1-Hydroxy-2-adamantanylethylidene-1,1-bisphosphonic Acid (10). 10 was prepared from adamantane acetic acid (583 mg, 3 mmol), following general procedure 2 (457 mg, 41% yield). Anal. ($C_{12}H_{21}NaO_7P_2 \cdot 0.5H_2O$) C, H.

1-Hydroxy-2-(3-bromophenyl)ethylidene-1,1-bisphosphonic Acid (11). 11 was prepared from 3-bromophenylacetic acid (645 mg, 3 mmol), following general procedure 2 (597 mg, 52% yield). Anal. ($C_8H_{10}BrNaO_7P_2$) C. H: calcd, 2.63; found, 2.12.

(3-Methoxymethylphenylamino)methylenebisphosphonic Acid (12). To NaH (132 mg, 3.3 mmol, 60% in oil) in THF (10 mL) was added successively 3-aminobenzyl alcohol (370 mg, 3 mmol) and MeI (426 mg, 3 mmol). After 1 h, the reaction was quenched by addition of NaCl(aq) and 3-methoxymethylaniline was extracted with diethyl ether and purified by column chromatography (silica gel; ether/CH_2Cl_2 1:5). Compound **12** was prepared from the aniline obtained, following general procedure 1 (315 mg, 34% overall yield). Anal. ($C_9H_{15}NO_7P_2$) C, H, N.

(3-Isopropylphenylamino)methylenebisphosphonic Acid (13). 13 was prepared from 3-isopropylaniline (406 mg, 3 mmol), following general procedure 1 (455 mg, 39% yield). Anal. ($C_{10}H_{15}NNaO_6P_2 \cdot 2H_2O$) C, H, N.

(2-Dimethylaminophenylamino)methylenebisphosphonic Acid (15). 2-Dimethylaminoaniline was prepared by monoprotection of 1,2-diaminobenzene (650 mg, 6 mmol) with benzyl chloroformate, followed by alkylation with MeI and hydrogenation (5% Pd/C). Compound **15** was prepared from the aniline obtained, following general procedure 1 (400 mg, 21% overall yield). Anal. ($C_9H_{16}N_2O_6P_2 \cdot 0.5H_2O$) C, H, N.

(3-Hydroxymethylphenylamino)methylenebisphosphonic Acid (16). To NaH (132 mg, 3.3 mmol, 60% in oil) in THF (10 mL) was added successively 3-aminobenzyl alcohol (370 mg, 3 mmol) and TBSCl (450 mg, 3 mmol). After 1 h, the reaction was quenched by addition of NaCl(aq), and *O*-TBS-protected 3-hydroxymethylaniline was extracted with diethyl ether and purified by column chromatography (silica gel, ether/CH_2Cl_2 1:10). Compound **16** was prepared from the aniline obtained following general procedure 1 (370 mg, 40% overall yield). Anal. ($C_8H_{13}NO_7P_2 \cdot 0.5H_2O$) C, H, N.

1-Hydroxy-2-(1-naphthyl)ethylidene-1,1-bisphosphonic Acid (18). 18 was prepared from 1-naphthylacetic acid (559 mg, 3 mmol), following general procedure 2 (520 mg, 49% yield). Anal. ($C_{12}H_{13}NaO_7P_2 \cdot 0.25H_2O$) C, H.

1-Hydroxy-2-(3-fluorophenyl)ethylidene-1,1-bisphosphonic Acid (19). 19 was prepared from 3-fluorophenylacetic acid (462 mg, 3 mmol), following general procedure 2 (652 mg, 60% yield). Anal. ($C_8H_9FNa_2O_7P_2 \cdot H_2O$) C, H.

(4-Ethylphenylamino)methylenebisphosphonic Acid (20). 20 was prepared from 4-ethylaniline (360 mg, 3 mmol), following general procedure 1 (336 mg, 38% yield). Anal. ($C_9H_{15}NO_6P_2$) C, H, N.

(3-*tert*-Butylphenylamino)methylenebisphosphonic Acid (22). 22 was prepared from 3-*tert*-butylaniline (448 mg, 3 mmol), following general procedure 1 (532 mg, 52% yield). Anal. ($C_{11}H_{19}NO_6P_2 \cdot H_2O$) C, H, N.

Hydroxy-[(4-phenyl)phenyl]methylenebisphosphonic Acid (23). 23 was prepared from 4-phenylbenzoic acid (595 mg, 3 mmol), following general procedure 2 (817 mg, 54% yield). Anal. ($C_{13}H_{10}Na_4O_7P_2 \cdot 4H_2O$) C, H.

1-Hydroxy-2-(3-nitrophenyl)ethylidene-1,1-bisphosphonic Acid (25). 25 was prepared from 3-nitrophenylacetic acid (540 mg, 3 mmol), following general procedure 2 (430 mg, 40% yield). Anal. ($C_8H_{10}NNaO_9P_2 \cdot 0.5H_2O$) C, H, N.

1-Hydroxy-2-(3-trifluoromethylphenyl)ethylidene-1,1-bisphosphonic Acid (26). 26 was prepared from 3-trifluoromethylphenylacetic acid (612 mg, 3 mmol), following general procedure 2 (705 mg, 57% yield). Anal. ($C_9H_9NF_3Na_2O_7P_2 \cdot H_2O$) C, H, N.

1-Hydroxy-2-(3-methoxyphenyl)ethylidene-1,1-bisphosphonic Acid (27). 27 was prepared from 3-methoxyphenylacetic acid (512 mg, 3 mmol), following general procedure 2 (564 mg, 44% yield). Anal. ($C_9H_{10}Na_4O_8P_2 \cdot 1.5H_2O$) C, H.

(3-Phenyl-4-methylphenylamino)methylenebisphosphonic Acid (28). 3-Phenyl-4-methylaniline was prepared from phenylboronic acid (366 mg, 3 mmol) and *N*-acetyl-3-bromo-4-methylaniline (684 mg, 3 mmol), following general procedure 5, followed by acid hydrolysis (3 N HCl, reflux).

Compound **28** was made from the aniline obtained, following general procedure 1 (420 mg, 36% overall yield). Anal. ($C_{14}H_{16}NNaO_6P_2 \cdot 0.25H_2O$) C, H, N.

[3-(3,4-Difluorophenyl)phenylamino]methylenebisphosphonic Acid (30). 3(3,4-Difluorophenyl)aniline was prepared from 3,4-difluorophenylboronic acid (474 mg, 3 mmol) and *N*-acetyl-3-bromoaniline (642 mg, 3 mmol), following general procedure 5, followed by acid hydrolysis (3 N HCl, reflux). Compound **30** was made from the aniline obtained, following general procedure 1 (353 mg, 31% overall yield). Anal. ($C_{13}H_{13}NF_2O_6P_2$) C, H, N.

1-Hydroxy-2-[3-(4-fluorophenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (31). 31 was prepared from 3-(4-fluorophenyl)phenylacetic acid (691 mg, 3 mmol), following general procedure 2 (690 mg, 53% yield). Anal. ($C_{14}H_{14}FNaO_7P_2 \cdot 2H_2O$) C, H.

1-Hydroxy-2-(3-methylphenyl)ethylidene-1,1-bisphosphonic Acid (32). 32 was prepared from 3-methylphenylacetic acid (450 mg, 3 mmol), following general procedure 2 (410 mg, 43% yield). Anal. ($C_9H_{13}NaO_7P_2 \cdot 0.25H_2O$) C, H.

(2,6-Diisopropylphenylamino)methylenebisphosphonic Acid (33). 33 was prepared from 2,6-diisopropylaniline (532 mg, 3 mmol), following general procedure 1 (443 mg, 40% yield). Anal. ($C_{13}H_{23}NO_6P_2 \cdot 1.25H_2O$) C, H, N.

(4-Benzylphenylamino)methylenebisphosphonic Acid (34). 34 was prepared from 4-benzylaniline (550 mg, 3 mmol), following general procedure 1 (417 mg, 35% yield). Anal. ($C_{14}H_{16}NNaO_6P_2 \cdot H_2O$) C, H, N.

[3-(2,4-Difluorophenyl)phenylamino]methylenebisphosphonic Acid (35). 3-(2,4-Difluorophenyl)aniline was prepared from 2,4-difluorophenylboronic acid (470 mg, 3 mmol) and *N*-acetyl-3-bromoaniline (640 mg, 3 mmol), following general procedure 5, followed by acid hydrolysis (3 N HCl, reflux). Compound **35** was made from the aniline obtained, following general procedure 1 (410 mg, 35% overall yield). Anal. ($C_{13}H_{13}NF_2O_6P_2 \cdot 0.5H_2O$) C, H, N.

2-[(3-Phenyl)phenylamino]ethylidene-1,1-bisphosphonic Acid (36). 36 was prepared from 3-aminobiphenyl (508 mg, 3 mmol), following general procedure 4 (771 mg, 48% yield). Anal. ($C_{14}H_{13}NNa_4O_6P_2 \cdot 5H_2O$) C, H, N.

Hydroxy-[(3-phenyl)phenyl]methylenebisphosphonic Acid (37). 37 was prepared from 3-phenylbenzoic acid (595 mg, 3 mmol), following general procedure 2 (552 mg, 49% yield). Anal. ($C_{13}H_{13}NaO_7P_2 \cdot 0.5H_2O$) C, H.

[(*R*)-1-(1-Naphthyl)ethylamino]methylenebisphosphonic Acid (41). 41 was prepared from (*R*)-1-(1-naphthyl)-ethylamine (171.24 mg, 1 mmol), following general procedure 1 (132 mg, 37% yield). Anal. ($C_{13}H_{17}NO_6P_2 \cdot 0.25H_2O$) C, H, N.

1-Hydroxy-2-(3-dimethylaminomethylphenyl)ethylidene-1,1-bisphosphonic Acid (44). Ethyl 3-formylphenylacetate (384 mg, 2 mmol)[33] was reductively aminated with dimethylamine hydrochloride (326 mg, 4 mmol) and $NaBH_4$ (150 mg, 4 mmol) in the presence of Ti(*i*-PrO)$_4$ (1.18 mL, 4 mmol).[34] The product was then refluxed in 2 N HCl to give 3-dimethylaminomethylphenylacetic acid. Compound **44** was prepared from the acid obtained, following general procedure 3 (285 mg, 31% yield). Anal. ($C_{11}H_{16}NNa_3O_7P_2 \cdot 3H_2O$) C, H, N.

(3-Benzylphenylamino)methylenebisphosphonic Acid (45). 45 was prepared from 3-benzylaniline (550 mg, 3 mmol), following general procedure 1 (494 mg, 45% yield). Anal. ($C_{14}H_{17}NO_6P_2 \cdot 0.5H_2O$) C, H, N.

2-(Adamantanylamino)ethylidene-1,1-bisphosphonic Acid (48). 48 was prepared from adamantanamine (454 mg, 3 mmol), following general procedure 4 (440 mg, 40% yield). Anal. ($C_{12}H_{23}NO_6P_2 \cdot 1.5H_2O$) C, H, N.

[(*S*)-1-(1-naphthylethyl)amino]methylenebisphosphonic Acid (49). 49 was prepared from (*S*)-1-(1−naphthyl)-ethylamine (171 mg, 1 mmol), following general procedure 1 (142 mg, 41% yield). Anal. ($C_{13}H_{17}NO_6P_2$) C, H, N.

2-[(3-Phenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (50). Tetraethylmethylene bisphosphonate (288 mg, 1 mmol) in THF (5 mL) was treated successively with NaH (44 mg, 1.1 mmol, 60% in oil) and 3-bromomethylbiphenyl (248 mg, 1 mmol) at room temperature for 3 h. The resulting tetraethyl ester of **50** was purified by column chromatography (silica gel; ethyl acetate/MeOH 20:1) and was then treated with TMSBr (6 equiv), followed by hydrolysis (MeOH/H_2O) to give compound **50** as a white powder (200 mg, 45% overall yield). Anal. ($C_{14}H_{12}Na_4O_6P_2 \cdot 0.75H_2O$) C, H.

1-Hydroxy-2-[3-(3,4-difluorophenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (51). 3-(3,4-Difluorophenyl)phenylacetic acid was prepared from 3,4-difluorophenylboronic acid (470 mg, 3 mmol) and methyl 3-bromophenylacetate (690 mg, 3 mmol), following general procedure 5, followed by hydrolysis (1 N NaOH). Compound **51** was prepared from the acid obtained, following general procedure 2 (536 mg, 42% overall yield). Anal. ($C_{14}H_{13}F_2NaO_7P_2 \cdot 0.5H_2O$) C, H.

(6-Ethylpyridin-2-ylamino)methylenebisphosphonic Acid (53). 53 was prepared from 2-amino-6-ethylpyridine (367 mg, 3 mmol), following general procedure 1 (374 mg, 32% yield). Anal. ($C_8H_{13}N_2NaO_6P_2 \cdot 2H_2O$) C, H, N.

1-Hydroxy-2-[(3-phenylphenyl]ethylidene-1,1-bisphosphonic Acid (55). 3-Bromomethylbiphenyl (248 mg, 1 mmol) in DMSO (5 mL) was treated with NaCN (54 mg, 1.1 mmol) at room temperature for 6 h. The product was purified by column chromatography (silica gel; CH_2Cl_2/ether 10:1) and was then refluxed in 3 N NaOH in EtOH/H_2O for 1 h to give (3-phenyl)phenylacetic acid. Compound **55** was prepared from the acid obtained, following general procedure 2 (170 mg, 36% overall yield). Anal. ($C_{14}H_{12}Na_4O_7P_2 \cdot 1.75H_2O$) C, H.

1-Hydroxy-2-[3-(3,5-difluorophenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (58). 3-(3,5-Difluorophenyl)phenylacetic acid was prepared from 3,5-difluorophenylboronic acid (470 mg, 3 mmol) and methyl 3-bromophenylacetate (690 mg, 3 mmol), following general procedure 5, followed by hydrolysis (1 N NaOH). Compound **58** was prepared from the acid obtained, following general procedure 2 (560 mg, 42% overall yield). Anal. ($C_{14}H_{13}F_2NaO_7P_2 \cdot 1.5H_2O$) C, H.

(Adamantanylamino)methylenebisphosphonic Acid (59). 59 was prepared from adamantanamine (454 mg, 3 mmol), following general procedure 1 (480 mg, 47% yield). Anal. ($C_{11}H_{21}NO_6P_2 \cdot H_2O$) C, H, N.

1-Hydroxy-2-(2-dimethylaminophenyl)ethylidene-1,1-bisphosphonic Acid (60). 2-Dimethylaminophenylacetic acid was prepared by methylation (MeI) of 2-aminophenylacetonitrile (396 mg, 3 mmol), followed by hydrolysis (2 N NaOH, reflux). Compound **60** was prepared from the acid obtained, following general procedure 3 (285 mg, 26% overall yield). Anal. ($C_{10}H_{16}NNaO_7P_2 \cdot H_2O$) C, H, N.

1-Hydroxy-2-[*N*-(3-methylbenzyl)pyridinium-3-yl]ethylidene-1,1-bisphosphonic Acid (61). Ethyl 3-pyridylacetate (495 mg, 3 mmol) was reacted with 3-methylbenzyl bromide (555 mg, 3 mmol) in MeOH at room temperature overnight. Upon removal of solvent, the white powder was refluxed in 1 N HCl for 1 h to give *N*-(3-methylbenzyl)pyridinium-3-ylacetic acid. Compound **61** was prepared from the acid, following general procedure 3 (640 mg, 48% overall yield). Anal. ($C_{15}H_{18}NNaO_7P_2 \cdot 0.75H_2O \cdot 0.5$ *i*-PrOH) C, H, N.

(3-Aminophenylamino)methylenebisphosphonic Acid (62). 1,3-Diaminobenzene (650 mg, 6 mmol) was monoprotected with acetyl anhydride. Compound **62** was prepared from the aniline obtained following general procedure 1, followed by acidic hydrolysis (3 N HCl, reflux) (670 mg, 35% overall yield). Anal. ($C_7H_{11}N_2NaO_6P_2 \cdot H_2O$) C, H, N.

1-Hydroxy-2-[3-(2,4-difluorophenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (63). 3-(2,4-Difluorophenyl)phenylacetic acid was prepared from 2,4-difluorophenylboronic acid (470 mg, 3 mmol) and methyl 3-bromophenylacetate (690 mg, 3 mmol) following general procedure 5, followed by hydrolysis (1 N NaOH). Compound **63** was prepared from the acid obtained, following general procedure 2 (490 mg, 40% overall yield). Anal. ($C_{14}H_{14}F_2O_7P_2 \cdot H_2O$) C, H.

1-Hydroxy-2-[4-(4-fluorophenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (64). 64 was prepared from 4-(4-fluorophenyl)phenylacetic acid (691 mg, 3 mmol), following general procedure 2 (633 mg, 53% yield). Anal. ($C_{14}H_{14}FNaO_7P_2$) C, H.

1-Hydroxy-2-[(4-phenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (65). 65 was prepared from 4-phenylphenylacetic acid (637 mg, 3 mmol), following general procedure 2 (676 mg, 56% yield). Anal. ($C_{14}H_{14}Na_2O_7P_2$) C, H.

1-Hydroxy-2-(3-aminophenyl)ethylidene-1,1-bisphosphonic Acid (66). 66 was prepared from 3-aminophenylacetic acid (450 mg, 3 mmol), following general procedure 3 (450 mg, 40% yield). Anal. ($C_8H_{11}NNa_2O_7P_2 \cdot 2H_2O$) C, N. H: calcd, 4.00; found, 3.49.

1-Hydroxy-2-[3-(4-methoxyphenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (68). 68 was prepared from 3-(4-methoxyphenyl)phenylacetic acid (727 mg, 3 mmol), following general procedure 2 (604 mg, 47% yield). Anal. ($C_{15}H_{17}NaO_8P_2 \cdot H_2O$) C, H.

1-Hydroxy-2-[3-(4-(chlorophenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (73). 73 was prepared from 3-(4-chlorophenylphenylacetic acid (740 mg, 3 mmol), following general procedure 2 (649 mg, 48% yield). Anal. ($C_{14}H_{14}ClNaO_7P_2 \cdot 2H_2O$) C, H.

2-(4-Phenylphenylamino)ethylidene-1,1-bisphosphonic Acid (75). 75 was prepared from 4-aminobiphenyl (508 mg, 3 mmol), following general procedure 4 (384 mg, 33% yield). Anal. ($C_{14}H_{16}NNaO_6P_2 \cdot 0.5H_2O$) C, N. H: calcd, 4.41; found, 4.98.

1-Hydroxy-2-[3-(2,5-difluorophenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (76). 3-(2,5-Difluorophenyl)phenyl acetic acid was prepared from 2,5-difluorophenylboronic acid (470 mg, 3 mmol) and methyl 3-bromophenylacetate (690 mg, 3 mmol), following general procedure 5, followed by hydrolysis (1 N NaOH). Compound **76** was prepared from the acid obtained, following general procedure 2 (510 mg, 38% overall yield). Anal. ($C_{14}H_{13}F_2NaO_7P_2 \cdot 2H_2O$) C, H.

1-Hydroxy-2-[4-(4-methoxyphenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (77). 77 was prepared from 4-(4-methoxyphenyl)phenylacetic acid (727 mg, 3 mmol), following general procedure 2 (591 mg, 46% yield). Anal. ($C_{15}H_{17}NaO_8P_2 \cdot H_2O$) C, H.

1-Hydroxy-2-[4-(4-chlorophenyl)phenyl]ethylidene-1,1-bisphosphonic Acid (78). 78 was prepared from 4-(4-chlorophenyl)phenylacetic acid (740 mg, 3 mmol), following general procedure 2 (577 mg, 49% yield). Anal. ($C_{14}H_{15}ClNaO_7P_2$) C, H.

1-Hydroxy-2-(3−phenoxyphenyl)ethylidene-1,1-bisphosphonic Acid (79). 79 was prepared from 3-phenoxyphenylacetic acid (685 mg, 3 mmol), following general procedure 2 (415 mg, 32% yield). Anal. ($C_{14}H_{15}NaO_8P_2 \cdot 2H_2O$) C, H.

[2-(3-Phenyl)phenyl]-1-phosphonoethylidene-1-sulfonic Acid (81). Cyclohexyl diethylphosphonomethylsulfonate (315 mg, 1 mmol)[35] was treated successively with NaH (44 mg, 1.1 mmol, 60% in oil) and 3-bromomethylbiphenyl (248 mg, 1 mmol) in THF at room temperature overnight. The triester of compound **81** was purified by column chromatography (silica gel, ethyl acetate) and was then hydrolyzed successively with excess ammonia in MeOH at 50 °C for 5 h, followed by TMSBr, to give compound **81** (175 mg, 42% overall yield). Anal. ($C_{14}H_{12}Na_3O_6PS \cdot 0.5H_2O$) C, H.

Computational Aspects. To relate the 3D structures of the bisphosphonates to their activity, we used two computational methods: comparative molecular field analysis (CoMFA[36]) and comparative molecular similarity indices analysis (CoMSIA[37]). Compounds were built by using the Cerius[2], version 4.6,[38] program and geometry-optimized using a three-step protocol consisting of steepest-decent,[39] conjugate-gradient,[40] and Newton−Raphson[41] minimizations, with no constraints on the internal geometries of the molecules, using the minimizer function of the OFF methods module in Cerius[2].[38] We used a universal molecular mechanics force field with a convergence criterion requiring a minimum energy change of 0.001 kcal/mol. Since there is no crystallographic or other three-dimensional structure of *Tb*VSP1 with a bound inhibitor, we first used the extended or all-trans form of the larger alkyl substituents to provide the basis for one alignment, while in a second alignment, we chose to use the structure of farnesyl diphosphate bound to farnesyl diphosphate synthase,[42] in which there is a pronounced curvature of the side chain. This provided at least one measure of the sensitivity of the QSAR analysis to side chain conformation. The bisphosphonate backbone conformations were based on the crystallographic structure of risedronate (**80**[43]). The CoMFA and CoMSIA analyses were performed by using the Sybyl, version 6.9, program[44] with default settings. To calculate charges, we used the Gasteiger−Marsili[45] method. We then used default settings in Sybyl, version 6.9, to automatically build a 3D rectangular grid with a 2.00 Å spacing and used a partial least-squares (PLS) analysis[46] approach to deduce the 3D QSAR relationships. The optimal number of components for each equation was determined by using standard error of prediction values and SAMPLS[47] leave-one-out cross-validation statistics.

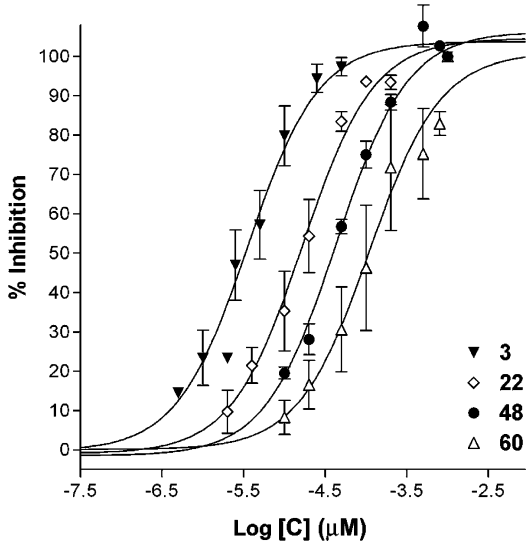


Figure 3. Dose response curve for four representative compounds: **3**, **22**, **48**, and **60**.

Additional data analyses were carried out using an in-house implementation of the JChem software package[48] from ChemAxon, Ltd.[49]

Enzyme Inhibition by Bisphosphonates. An amount of 2 μg of desalted *Tb*VSP1 was incubated for 2 min at 20 °C in a buffer containing 50 mM Tris-HCl, pH 7.5, 1 mM $ZnCl_2$, 100 μM sodium tripolyphosphate, and a variable concentration of the bisphosphonate of interest. Released Pi was quantitated as described previously.[50]

Animal Testing. For the in vivo assays, the parasite strain used was *T. brucei brucei* 427.[20] Female Swiss−Webster mice, 20 g, were injected intraperitoneally with 500 bloodstream form parasites that had been cultivated at 37 °C in minimum essential medium containing 10% fetal calf serum. Mice were sorted randomly into groups of five and given 5 mg/kg of bisphosphonate twice per day ip for 5 days.

Results and Discussion

We show in Figures 1 and 2 the structures of the 81 bisphosphonates investigated, shown in order of decreasing activity. The compounds shown in Figure 1 have IC_{50} values less than 40 μM; those in Figure 2 have IC_{50} values greater than 40 μM. In Figure 3, we show four representative dose response curves, for compounds **3**, **22**, **48**, and **60**. The IC_{50} values for TbVSP1 tripolyphosphatase inhibition were fitted to a rectangular hyperbola,

$$I = \frac{I_{max} C}{IC_{50} + C} \quad (1)$$

where I is the percent inhibition, I_{max} is 100% inhibition,

Table 1. Experimental (IC_{50} and pIC_{50}) and CoMFA Predicted (pIC_{50}) Values for Bisphosphonates against *T. brucei* Soluble Pyrophosphatase *Tb*VSP1 and Statistical Results of 3D QSAR CoMFA Models

	exptl activity		predicted pIC_{50}							exptl activity		predicted pIC_{50}					
				five-compound test sets									five-compound test sets				
compd	IC_{50} (μM)	pIC_{50}	training set						compd	IC_{50} (μM)	pIC_{50}	training set					
1	2.1	5.68	4.80	4.82	4.82	4.77	4.72	4.71	**45**	46.7	4.33	4.46	**4.52**	4.54	4.47	4.46	4.47
2	2.4	5.62	5.19	5.20	5.25	5.24	4.97	5.02	**46**	47.7	4.32	4.39	4.38	4.35	4.29	4.27	4.22
3	3.8	5.42	5.16	5.11	5.15	5.17	5.01	4.98	**47**	47.7	4.32	4.40	4.42	4.46	4.32	**4.15**	4.25
4	4.3	5.37	4.89	4.91	4.90	**4.54**	4.85	4.87	**48**	48.0	4.32	4.64	**5.05**	4.63	4.57	4.73	4.78
5	4.3	5.37	5.18	5.17	5.27	5.16	5.23	5.26	**49**	49.7	4.30	4.20	4.02	4.20	4.30	3.92	3.96
6	5.5	5.26	4.91	4.94	4.98	4.91	4.82	4.78	**50**	52.3	4.28	4.20	4.18	4.29	4.16	4.50	**4.45**
7	6.2	5.21	4.92	4.89	5.04	4.88	5.13	5.16	**51**	53.3	4.27	3.72	3.70	3.72	3.72	3.94	3.87
8	6.5	5.19	5.10	5.07	5.11	5.11	5.00	4.99	**52**	55.0	4.26	4.61	4.60	4.60	4.57	**4.50**	4.46
9	7.5	5.12	4.94	4.97	4.98	4.94	4.87	4.83	**53**	55.0	4.26	3.88	**3.67**	3.88	3.76	3.91	3.83
10	8.0	5.10	4.86	4.86	4.90	4.90	4.72	4.74	**54**	56.7	4.25	4.00	4.00	4.02	**3.53**	3.58	3.64
11	9.0	5.05	4.94	4.97	4.98	4.94	4.86	4.83	**55**	65.7	4.18	4.29	4.26	4.42	4.30	4.44	4.44
12	9.7	5.01	5.07	5.06	**5.05**	5.11	4.97	4.95	**56**	70.0	4.15	3.95	4.04	3.95	4.07	4.04	4.00
13	9.7	5.01	5.08	5.13	5.05	5.13	5.07	5.09	**57**	78.0	4.11	3.93	4.02	3.97	3.92	3.94	3.91
14	9.7	5.01	5.08	5.02	5.04	5.05	4.81	4.82	**58**	82.7	4.08	3.76	3.74	3.76	**3.78**	3.96	3.91
15	10.0	5.00	4.83	4.89	4.84	4.73	4.97	4.96	**59**	98.3	4.01	3.87	3.80	3.88	3.86	4.05	4.12
16	10.2	4.99	5.20	5.19	5.20	5.22	5.03	5.02	**60**	108.3	3.97	4.23	4.20	4.25	4.23	4.38	**4.52**
17	10.3	4.99	4.92	4.91	4.91	4.88	**4.55**	4.64	**61**	130.0	3.89	3.88	3.97	3.95	3.92	3.95	3.95
18	11.3	4.95	4.91	4.92	4.93	4.92	4.85	4.81	**62**	133.3	3.88	4.49	4.34	4.50	4.46	4.60	4.56
19	11.3	4.95	4.84	4.86	4.88	4.81	4.78	4.74	**63**	138.3	3.86	3.69	3.70	3.69	3.71	3.98	3.92
20	12.3	4.91	4.70	4.58	4.65	4.61	4.40	4.41	**64**	191.7	3.72	3.58	3.60	3.59	3.66	3.44	3.43
21	12.7	4.90	5.04	5.03	5.04	5.01	4.84	4.82	**65**	211.7	3.67	3.65	3.66	3.65	3.72	3.50	3.49
22	13.3	4.88	4.97	5.10	4.93	5.00	5.04	5.07	**66**	215.0	3.67	4.32	4.29	4.33	4.31	**4.51**	4.39
23	14.7	4.83	4.77	**5.28**	4.78	4.68	5.00	5.07	**67**	226.7	3.64	3.44	3.47	3.42	3.45	3.76	3.72
24	16.3	4.79	4.46	4.49	4.50	**4.55**	4.85	4.95	**68**	300.0	3.52	3.68	3.65	3.68	3.67	3.98	3.91
25	16.7	4.78	5.07	5.11	5.09	5.06	4.88	4.85	**69**	325	3.49	3.72	3.68	3.72	3.69	3.39	3.41
26	17.3	4.76	4.97	5.00	5.01	5.00	4.81	4.80	**70**	340.0	3.47	3.41	3.44	**3.78**	3.31	3.45	3.53
27	18.7	4.73	5.04	5.05	5.09	5.02	4.98	4.94	**71**	355.0	3.45	3.78	3.64	3.82	3.72	3.95	3.99
28	19.0	4.72	4.61	4.63	4.62	4.52	4.79	4.88	**72**	356.7	3.45	4.01	4.03	4.10	3.90	3.99	4.04
29	21.5	4.67	4.92	4.94	4.92	4.87	4.80	**4.81**	**73**	366.7	3.44	3.51	3.49	3.56	3.54	**3.89**	3.80
30	24.3	4.61	4.94	4.91	**5.06**	4.88	5.14	5.16	**74**	383.3	3.42	3.61	3.61	3.53	3.54	3.86	3.86
31	25.0	4.60	3.76	3.74	3.76	3.78	3.99	3.92	**75**	420.0	3.38	3.54	3.53	3.66	3.41	3.62	3.62
32	25.3	4.60	4.93	4.96	**4.95**	4.94	4.84	4.81	**76**	430.0	3.37	3.69	3.70	3.68	3.72	3.95	3.91
33	25.3	4.60	4.43	4.49	4.42	4.39	4.72	4.88	**77**	435.0	3.36	3.25	3.29	3.23	3.28	3.13	3.13
34	28.3	4.55	4.57	4.61	4.62	4.56	4.53	**4.65**	**78**	446.7	3.35	3.46	3.48	3.46	**3.57**	3.36	3.34
35	28.7	4.54	4.90	4.89	5.01	4.85	5.15	5.17	**79**	540.0	3.27	3.98	4.02	**4.51**	3.96	4.13	4.13
36	29.7	4.53	4.49	4.49	4.46	4.55	4.60	4.62	**80**	656.7	3.18	3.50	3.52	3.48	3.49	3.79	3.74
37	31.0	4.51	4.65	4.87	4.59	4.55	4.43	4.50	**81**	841.7	3.08	3.43	3.38	3.40	3.36	3.61	3.52
38	35.3	4.45	3.94	3.90	4.03	3.96	3.98	3.98									
39	38.0	4.42	4.16	4.26	4.17	4.12	4.63	**4.81**	q^2			0.47	0.49	0.48	0.48	0.46	0.47
40	39.7	4.40	4.22	4.13	4.30	4.15	4.01	4.05	R^2			0.78	0.79	0.79	0.79	0.70	0.70
41	42.7	4.37	4.51	**4.29**	4.52	4.61	4.04	4.07	N [a]			81	76	76	76	76	76
42	43.3	4.36	4.66	4.65	4.61	4.45	4.38	4.40	% S [b]			0.770	0.775	0.762	0.774	0.779	0.779
43	44.3	4.35	4.47	4.47	4.48	4.42	4.33	4.35	% E [c]			0.230	0.225	0.238	0.226	0.221	0.221
44	44.7	4.35	4.36	4.36	4.34	4.47	4.46	4.44									

[a] N denotes the number of compounds used to generate the model. [b] %S denotes the steric contribution to the model. [c] %E denotes the electrostatic contribution to the model.

C is the concentration of the inhibitor, and IC_{50} is the concentration for 50% enzyme inhibition. A regression analysis was performed by using Sigma Plot 5.0 (SPSS Inc., Chicago, IL), and all IC_{50} values have errors of ~20%. The IC_{50} values for all compounds investigated are given in Table 1 (and again, for convenience, in Table 2) as are the pIC_{50} values, defined as

$$pIC_{50} = -\log_{10} IC_{50} \quad (2)$$

with IC_{50} in units of M. As may be seen in Table 1, the IC_{50} values vary considerably, from ~2 μM (compound **1**) to ~1 mM (compound **81**). For the more potent compounds (**1**−**40**), it can be seen that bisphosphonates containing α-NH groups are relatively common (20/40 compounds), while for the less active compounds (**41**−**81**), such species are less prominent (only 11/41). In addition, there is a slightly larger number of more bulky compounds (containing two, as opposed to one, aromatic rings) in the less active set (23 versus 15 compounds). However, it should be possible to improve upon these empirical observations by using QSAR techniques such as CoMFA and CoMSIA.

To begin with, we built two sets of alignments. In both cases, we used the crystallographic structure of **80** (risedronate) as a shape reference compound for the bisphosphonate moiety and used singly protonated phosphonate groups in all cases, basically as described previously for our studies of FPPS inhibition, bone resorption, $\gamma\delta$ T cell activation, and *Dictyostelium discoideum* and *T. brucei* growth inhibition by bisphosphonates.[7,23,24,51,52] Since the side chain conformations are not known, we used both an all-trans (alignment I) and a bent (alignment II) conformation, as used previously in our investigation of bone resorption,[51] to assess the sensitivity of the QSAR results to side chain conformation. Aniline or substituted aniline side chains were not protonated, while more basic species such as

Table 2. Experimental (IC_{50} and pIC_{50}) and CoMSIA Predicted (pIC_{50}) Values for Bisphosphonates against *T. brucei* Soluble Pyrophosphatase *Tb*VSP1 and Statistical Results of 3D QSAR CoMSIA Models

	exptl activity		predicted pIC_{50}							exptl activity		predicted pIC_{50}					
				five-compound test sets									five-compound test sets				
compd	IC_{50} (μM)	pIC_{50}	training set						compd	IC_{50} (μM)	pIC_{50}	training set					
1	2.1	5.68	5.43	5.44	5.44	5.35	5.22	5.59	**45**	46.7	4.33	4.54	**4.64**	4.51	4.54	4.59	4.53
2	2.4	5.62	5.09	5.12	5.09	5.12	5.03	5.07	**46**	47.7	4.32	4.50	4.44	4.48	4.43	4.50	4.43
3	3.8	5.42	5.42	5.41	5.49	5.42	5.25	5.38	**47**	47.7	4.32	4.17	4.20	4.16	4.17	**3.99**	4.12
4	4.3	5.37	5.17	5.16	5.19	**4.96**	5.16	5.22	**48**	48.0	4.32	4.06	**4.12**	4.05	4.07	4.20	4.05
5	4.3	5.37	4.99	5.01	5.01	5.03	5.04	4.94	**49**	49.7	4.30	4.22	4.09	4.24	4.27	4.00	4.20
6	5.5	5.26	5.34	5.34	5.28	5.33	5.27	5.34	**50**	52.3	4.28	3.89	3.87	3.93	3.89	4.06	**3.75**
7	6.2	5.21	4.62	4.63	4.72	4.63	4.77	4.62	**51**	53.3	4.27	3.78	3.77	3.84	3.76	3.87	3.78
8	6.5	5.19	5.14	5.15	5.11	5.13	5.14	5.12	**52**	55.0	4.26	3.98	3.99	4.07	3.95	**3.71**	3.96
9	7.5	5.12	5.00	4.97	5.03	4.99	4.95	5.00	**53**	55.0	4.26	4.19	**4.15**	4.22	4.15	4.12	4.17
10	8.0	5.10	5.11	5.12	5.11	5.15	4.83	5.14	**54**	56.7	4.25	4.14	4.22	4.15	**3.91**	4.00	4.10
11	9.0	5.05	5.12	5.10	5.12	5.12	5.07	5.13	**55**	65.7	4.18	4.31	4.31	4.37	4.32	4.28	4.30
12	9.7	5.01	4.84	4.88	**4.76**	4.85	4.93	4.81	**56**	70.0	4.15	4.28	4.29	4.29	4.28	4.40	4.29
13	9.7	5.01	5.19	5.22	5.13	5.22	5.14	5.15	**57**	78.0	4.11	4.06	4.08	4.07	4.03	4.21	4.07
14	9.7	5.01	5.14	5.16	5.13	5.14	5.01	5.14	**58**	82.7	4.08	3.78	3.77	3.83	**3.76**	3.88	3.78
15	10.0	5.00	5.17	5.16	5.18	5.14	5.37	5.16	**59**	98.3	4.01	4.02	3.92	4.01	4.03	4.03	4.10
16	10.2	4.99	4.98	5.00	4.95	4.98	5.01	4.95	**60**	108.3	3.97	4.38	4.34	4.35	4.34	4.45	**4.66**
17	10.3	4.99	4.47	4.46	4.45	4.49	**4.30**	4.44	**61**	130.0	3.89	3.70	3.72	3.78	3.69	3.80	3.67
18	11.3	4.95	4.96	4.93	5.00	4.97	5.00	4.93	**62**	133.3	3.88	3.95	3.91	3.97	3.88	3.83	4.00
19	11.3	4.95	4.81	4.78	4.89	4.81	4.79	4.82	**63**	138.3	3.86	3.85	3.84	3.88	3.83	3.98	3.84
20	12.3	4.91	4.92	4.93	4.89	4.91	4.79	4.92	**64**	191.7	3.72	3.43	3.43	3.44	3.52	3.46	3.44
21	12.7	4.90	4.51	4.48	4.49	4.55	4.50	4.43	**65**	211.7	3.67	3.64	3.62	3.63	3.69	3.62	3.65
22	13.3	4.88	5.20	5.22	5.14	5.25	5.17	5.17	**66**	215.0	3.67	3.94	3.91	3.97	3.89	**3.94**	3.95
23	14.7	4.83	4.64	**4.40**	4.69	4.66	4.62	4.61	**67**	226.7	3.64	3.98	3.95	3.89	3.98	4.12	3.99
24	16.3	4.79	4.69	4.70	4.65	**4.68**	4.72	4.76	**68**	300.0	3.52	3.96	3.95	3.97	3.96	4.03	3.97
25	16.7	4.78	4.71	4.66	4.82	4.70	4.69	4.71	**69**	325	3.49	3.25	3.26	3.32	3.23	3.07	3.37
26	17.3	4.76	4.76	4.76	4.70	4.77	4.78	4.77	**70**	340.0	3.47	3.52	3.59	**3.57**	3.50	3.44	3.49
27	18.7	4.73	4.65	4.59	4.82	4.64	4.63	4.62	**71**	355.0	3.45	4.23	4.24	4.19	4.23	4.22	4.24
28	19.0	4.72	4.56	4.59	4.49	4.49	4.69	4.60	**72**	356.7	3.45	3.98	4.01	3.98	3.97	3.87	3.92
29	21.5	4.67	4.41	4.36	4.40	4.44	4.43	**4.29**	**73**	366.7	3.44	3.91	3.90	3.91	3.90	**4.02**	3.89
30	24.3	4.61	4.69	4.72	**4.76**	4.70	4.86	4.70	**74**	383.3	3.42	3.27	3.26	3.22	3.25	3.52	3.21
31	25.0	4.60	3.98	3.97	3.99	3.98	4.05	3.98	**75**	420.0	3.38	3.34	3.38	3.38	3.24	3.43	3.30
32	25.3	4.60	4.71	4.69	**4.72**	4.72	4.71	4.71	**76**	430.0	3.37	3.79	3.77	3.85	3.77	3.87	3.76
33	25.3	4.60	4.50	4.49	4.45	4.45	4.74	4.60	**77**	435.0	3.36	3.32	3.31	3.34	3.34	3.37	3.32
34	28.3	4.55	4.67	4.70	4.66	4.69	4.68	**4.78**	**78**	446.7	3.35	3.37	3.37	3.38	**3.50**	3.40	3.37
35	28.7	4.54	4.71	4.73	4.77	4.72	4.87	4.70	**79**	540.0	3.27	3.92	3.90	**4.27**	3.89	3.91	3.89
36	29.7	4.53	4.55	4.53	4.51	4.54	4.61	4.58	**80**	656.7	3.18	4.14	4.11	4.12	4.14	4.22	4.15
37	31.0	4.51	4.50	4.46	4.47	4.48	4.37	4.51	**81**	841.7	3.08	3.07	3.07	3.04	3.05	3.36	2.97
38	35.3	4.45	4.43	4.43	4.49	4.42	4.42	4.49									
39	38.0	4.42	4.28	4.29	4.25	4.22	4.45	**4.36**	q^2			0.50	0.51	0.50	0.47	0.50	0.52
40	39.7	4.40	4.17	4.23	4.20	4.13	4.07	4.17	R^2			0.79	0.80	0.80	0.79	0.77	0.80
41	42.7	4.37	4.36	**4.21**	4.40	4.40	4.05	4.35	N [a]			81	76	76	76	76	76
42	43.3	4.36	4.83	4.85	4.81	4.65	4.76	4.77	% S [b]			0.203	0.197	0.195	0.200	0.212	0.193
43	44.3	4.35	4.65	4.63	4.65	4.53	4.57	4.63	% H [c]			0.453	0.453	0.463	0.434	0.456	0.461
44	44.7	4.35	4.59	4.59	4.63	4.61	4.52	4.59	% D [d]			0.344	0.350	0.341	0.366	0.333	0.346

[a] N denotes the number of compounds used to generate the model. [b] %S denotes the steric contribution to the model. [c] %H denotes the hydrophobic contribution to the model. [d] %D denotes the donor contribution to the model.

ibandronate (**43**) and risedronate (**80**) were protonated on their basic nitrogen sites. Results with protonated anilines were poor (data not shown) and are not discussed in the text. The results of these CoMFA analyses showed slightly improved results for the curved side chain conformations. Using a partial least-squares[46] method, we found the optimum number of components for both models to be 4, based on the q^2 values obtained by the SAMPLS[47] leave-one-out cross-validation method implemented in Sybyl, version 6.9.[44] The CoMFA models yielded correlation coefficients $R^2 = 0.78$ and 0.61, $q^2 =$ 0.47 and 0.30, and F-test results of 68.0 and 39.4 for the curved and linear geometries, respectively. To test the validity of these models, we then used a data randomization method in which SAMPLS cross-validation was performed using randomly generated pIC_{50} values instead of the experimentally determined ones. The average q^2 value for 100 randomized sets was less than zero, and even the highest values were substantially lower than the q^2 of the experimental data set, indicating that the models are not the result of a chance correlation.

To further test the validity of the CoMFA models, as well as their predictive utility, we randomly deleted five compounds from the training sets and recalculated the CoMFA models. We then used the new CoMFA models to predict the activities of the omitted compounds. The process was repeated four more times, predicting a total of 25 pIC_{50} values, shown in Table 1. The predicted pIC_{50} values are in relatively good agreement with experiment (Figure 4A), the average error of prediction being ~3.0 (alignment I) or ~2.6 (alignment II). For alignment II (Table 1), the steric terms contribute ~77% to the interaction energy while electrostatic interactions contribute ~23%.

As an alternative to the CoMFA technique, we next used the CoMSIA method,[37] which differs from CoMFA in the use of Gaussian functions, as opposed to Lennard-

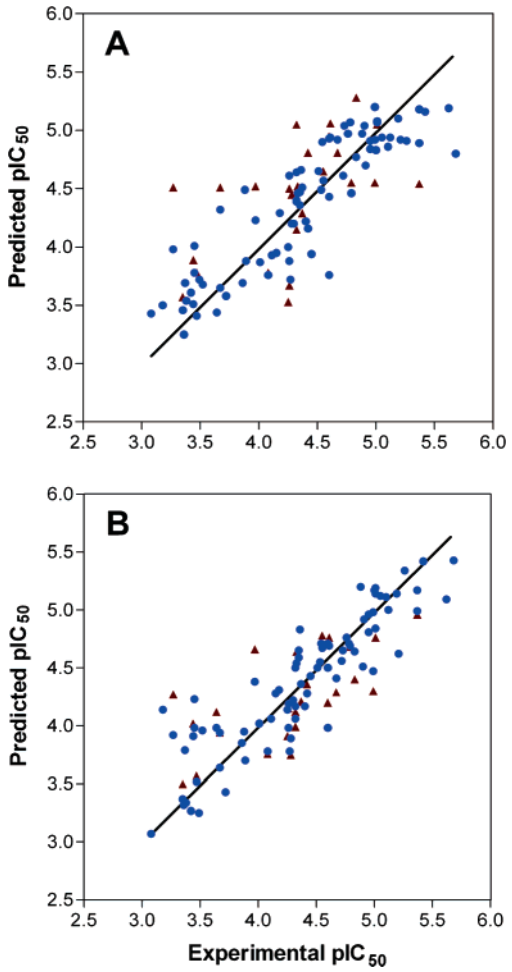


Figure 4. Plots of experimental pIC_{50} versus predicted pIC_{50} values for the CoMFA (A) and CoMSIA (B) models of bisphosphonates inhibiting *T. brucei* soluble pyrophosphatase *Tb*VSP1: (blue) training set; (red) test sets.

Jones and Coulombic terms, to calculate interaction energies at grid points. The CoMSIA method is, in general, less sensitive to alignment deficiencies (i.e., when grid points are close to the surface of a molecule). We used the same setup and procedures as described for the CoMFA analysis, but this time we used steric, hydrophobic, and donor CoMSIA fields instead of just steric and electrostatic probes, as used in the CoMFA analyses. Training and test set results are presented in Table 2 (alignment II) and Figure 4B, and the CoMSIA field maps are shown in Figure 5.

The resulting CoMSIA models for all 81 compounds had $R^2 = 0.79$ and 0.72, $q^2 = 0.5$ and 0.35, and *F*-test results of 72.7 and 65.3 using four components for alignments II and I, respectively. We then tested the validity of the CoMSIA model, as we did for CoMFA, by randomizing the pIC_{50} values and performing SAMPLS cross-validation. We obtained very low average q^2 for 100 runs, which supports the validity of the analysis. We also followed the method described above to test the predictive utility of the models, predicting the activity of five sets of five compounds using reduced training sets. The average error in activity for the 25 compounds was approximately a factor of 2.2 for alignment II and ~2.6 for alignment I, consistent with the lower q^2 and *F*-test results for alignment I. For alignment II, the steric plus hydrophobic field contribution was 65.6% (20.3% steric, 45.3% hydrophobic) while the remaining 34.4% was attributable to donor interactions. Clearly, in both the CoMFA and CoMSIA models, electrostatic or donor interactions make a relatively minor contribution to the overall range in activity, with the steric or steric-plus-hydrophobic interactions, dominating.

In Figure 5A−C we show the steric (Figure 5A), hydrophobic (Figure 5B), and donor (Figure 5C) field features superimposed on the structure of an active compound (**3**, one of the most active compounds), while in Figure 5D−F the same features are shown superimposed on the least active compound **81**. The color codes are as follows: steric favored (green), steric disfavored (yellow), hydrophobic favored (yellow), hydrophobic disfavored (white), donor favored (cyan), and donor disfavored (purple). On inspection, the CoMSIA field results confirm the qualitative observations made above, in particular, that the presence of α-NH containing side chains generally favors activity (donor favored) while the presence of large (biphenyl or biphenylmethane) side chains is associated with low activity due to steric repulsions.

In Vivo Results. After obtaining the activity results shown in Table 1, we selected a series of compounds for in vivo testing in *T. brucei brucei* infected Swiss−Webster mice, where *T. brucei* infections are fatal. We investigated compounds **1**−**5**, **7**−**11**, **13**, **15**, and **22**, which were chosen primarily for their activity against *Tb*VSP1 and availability and, for **22**, known high activity versus *T. cruzi* in vitro. Most compounds provided little protection against death. However, three of the four compounds most active in the in vitro *Tb*VSP1 assay, **1**, **2**, and **4**, did provide a moderate increase in long-term survival (Figure 6A), with a 40% survival rate for **2**, 30 days postinfection. But why is **2** the most effective compound?

For 34 of the compounds investigated as inhibitors of *Tb*VSP1, their in vitro activity against a human (KB) cell line has been reported previously,[13] which enables an estimate of a "therapeutic index" ratio (TI) defined as

$$\mathrm{TI} = \frac{\mathrm{LD}_{50}(\text{KB cell line})}{\mathrm{IC}_{50}(\text{TbVSP1})} \qquad (3)$$

in which LD_{50} refers to that dose which produces a 50% inhibition of the growth of a human (KB) cell line and IC_{50} is the concentration required to produce a 50% inhibition of the exopolyphosphatase activity of *Tb*VSP1. These IC_{50}, LD_{50}, and TI ratios are presented in Table 3 and are shown graphically in Figure 6B. As may be seen in Figure 6B (and Table 3), the compound found to be the one most effective in prolonging survival, compound **2**, also had a rather large therapeutic index value (>400), implying low toxicity.

At present, we cannot be certain that these bisphosphonates inhibit the *Tb*VSP1 enzyme in *T. brucei* infected animals, and it is possible that other enzymes might also be involved, although the results shown in Figure 6 (and the IC_{50} values) certainly support a *Tb*VSP1 target. The most plausible alternative targets would presumably be FPP synthase, since FPPS is known to be potently inhibited by many different

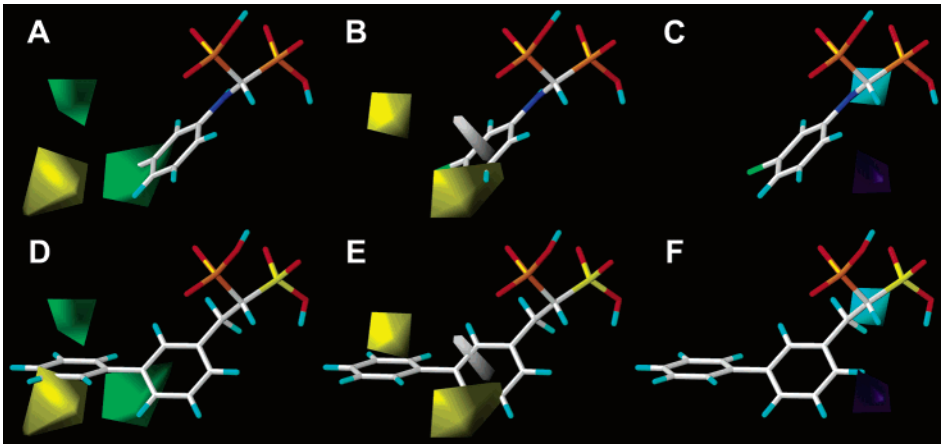


Figure 5. CoMSIA fields for an active compound (**3**) (A−C) and for an inactive compound (**81**) (D−F): (A, D) steric field (green favored, yellow disfavored); (B, E) hydrophobic field (yellow favored, white disfavored); (C, F) acceptor field (cyan favored, purple disfavored).

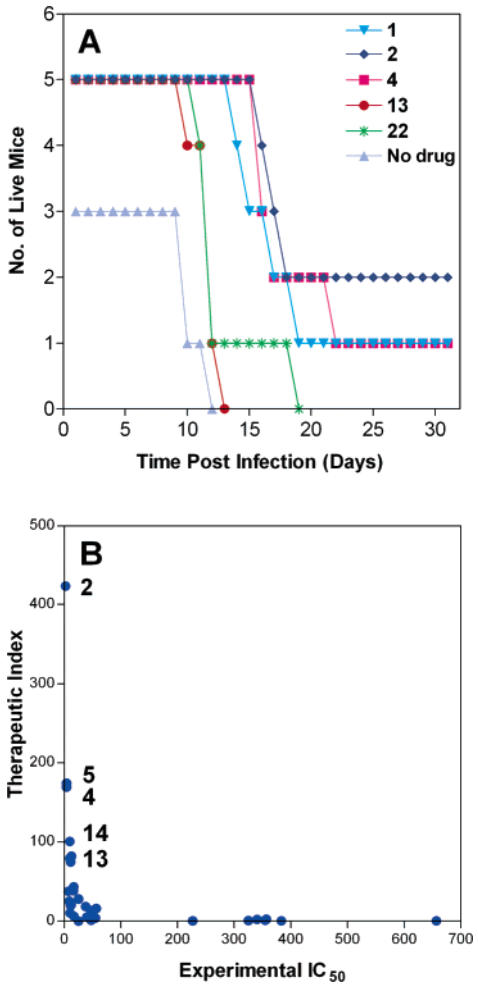


Figure 6. (A) Cumulative survival results for *T. brucei brucei* infected mice after administration of selected drugs in vivo. (B) Therapeutic index results for selected bisphosphonates.

bisphosphonates. In addition, it could be that pyrophosphatase activity could be involved. To investigate these possibilities in more detail, we first investigated the activity of a diverse subset of our bisphosphonates (**2**−**11**, **13**, **20**, **22**, **29**, **45**, **57**, **61**, **67**) for their ability to inhibit the pyrophosphatase activity of *Tb*VSP1 (PPi → 2Pi, in the presence of Mg^{2+}) and a yeast pyrophosphatase. With the exception of **3** (IC_{50} = 25 μM), the IC_{50} values were all >200 μM, making inhibition of *Tb*VSP1 pyrophosphatase activity an unlikely target. Second, we investigated the activity of another subset of the bisphosphonates (**1**, **2**, **4**, **6**, **9**, **11**, **15**, **38**, **46**, **47**, **50**, **54**−**57**, **60**−**62**, **67**, **70**, and **74**) for their ability to inhibit the FPPS of another trypanosomatid, *Leishmania major*, where numerous FPPS inhibition results have already been obtained[23] and analyzed using QSAR techniques. There is a 60% identity and 72% similarity between the *L. major* and *T. brucei* FPPS enzymes, sufficient to represent a good model for *T. brucei* FPPS inhibition. Of 33 compounds tested against both the

Table 3. Experimental Activity Values for Bisphosphonates against *T. brucei* Soluble Pyrophosphatase *Tb*VSP1 and a Human (KB) Cell Line and the Estimated Therapeutic Index

compd	exptl IC_{50} (μM)	toxicity ED_{50} (μM)	therapeutic index (TI)
2	2.4	1016	423
5	4.3	749	174
4	4.3	728	169
14	9.7	976	101
21	12.7	1041	82
13	9.7	771	79
18	11.3	847	75
25	16.7	727	44
24	16.3	634	39
9	7.5	283	38
33	25.3	713	28
11	9	228	25
20	12.3	257	21
19	11.3	225	20
39	38	710	19
54	56.7	919	16
53	55	847	15
45	46.7	645	14
46	47.7	499	10
17	10.3	107	10
26	17.3	109	6.3
40	39.7	210	5.3
42	43.3	212	4.9
52	55	218	4.0
43	44.3	166	3.7
72	356.7	892	2.5
70	340	729	2.1
71	355	495	1.4
47	47.7	64	1.3
69	325	312	1.0
80	656.7	255	0.4
74	383.3	144	0.4
67	226.7	63.7	0.3
32	25.3	0.9	0.04

*Tb*VSP1 and *L. major* FPPS enzymes, only 19 had measurable activity in both assays, but the overall correlation between the two data sets was only $R^2 = 0.095$. More significantly, of the three compounds (**1**, **2**, **4**) providing some protection against death, the compound most active in vivo (**2**) has no effect on FPPS inhibition ($IC_{50} > 100\ \mu M$), as also found with compound **4**. In the case of **1**, this compound has been reported to be an inhibitor of a human FPPS,[53] so it is certainly possible that in this case, both FPPS and the *Tb*VSP1 are inhibited in vivo, but this is clearly not the case for **2** (the most active compound in vivo) or for **4**. Of the 13 compounds investigated in vivo, the three providing protection against death are among the top four inhibitors of *Tb*VSP1. The compound most active in vivo (**2**) also has a very high therapeutic index in vitro and is not an inhibitor of FPPS. These results therefore support the idea that the most active compounds in vivo act, at least in part, by inhibiting *Tb*VSP1, an enzyme that is critical for osmoregulation and virulence in *T. brucei*. Since *Tb*VSP1 is absent in humans, this work opens up a potential route to the development of novel trypanocides.

Acknowledgment. This work was supported by the U.S. Public Health Service (National Institutes of Health Grant GM65307).

Supporting Information Available: Microanalytical data for compounds **1**, **3**, **4**, **6**−**13**, **15**, **16**, **18**−**20**, **22**, **23**, **25**−**28**, **30**−**37**, **41**, **44**, **45**, **48**−**51**, **53**, **55**, **58**−**60**, **62**−**66**, **68**, **73**, **75**−**79**, and **81** and ^1H and ^{31}P NMR spectra for compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) African trypanosomiasis or sleeping sickness. World Health Organization, Fact Sheet No. 259., http://www.who.int/mediacentre/factsheets/fs259/en/.

(2) East African Trypanosomiasis. Department of Health and Human Services, Center for Disease Control and Prevention. http://www.cdc.gov/ncidod/dpd/parasites/trypanosomiasis/factsht_ea_trypanosomiasis.htm.

(3) Kirchhoff, L. V.; Bacchi, C. J.; Wittner, M.; Tanowitz, H. B. African trypanosomiasis (sleeping sickness). *Curr. Treat. Options Infect. Dis.* **2000**, *2*, 66−69.

(4) Kennedy, P. G. Human African trypanosomiasis of the CNS: current issues and challenges. *J. Clin. Invest.* **2004**, *113*, 496−504.

(5) Docampo, R.; Moreno, S. N. J. Current chemotherapy of human African trypanosomiasis. *Parasitol. Res.* **2003**, S10−S13.

(6) Martin, M. B.; Grimley, J. S.; Lewis, J. C.; Heath, H. T., III; Bailey, B. N.; Kendrick, H.; Yardley, V.; Caldera, A.; Lira, R.; Urbina, J. A.; Moreno, S. N. J.; Docampo, R.; Croft, S. L.; Oldfield, E. Bisphosphonates inhibit the growth of *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondii* and *Plasmodium falciparum*: A potential route to chemotherapy. *J. Med. Chem.* **2001**, *44*, 909−916.

(7) Martin, M. B.; Sanders, J. M.; Kendrick, H.; de Luca-Fradley, K.; Lewis, J. C.; Grimley, J. S.; van Brussel, E. M.; Olsen, J. R.; Meints, G. A.; Burzynska, A.; Kafarski, P.; Croft, S. L.; Oldfield, E. Activity of bisphosphonates against *Trypanosoma brucei rhodesiense*. *J. Med. Chem.* **2002**, *45*, 2904−2914.

(8) Urbina, J. A.; Moreno, B.; Vierkotter, S.; Oldfield, E.; Payares, G.; Sanoja, C.; Bailey, B. N.; Yan, W.; Scott, D. A.; Moreno, S. N. J.; Docampo, R. *Trypanosoma cruzi* contains major pyrophosphate stores, and its growth in vitro and in vivo is blocked by pyrophosphate analogs. *J. Biol. Chem.* **1999**, *274*, 33609−33615.

(9) Garzoni, L. R.; Caldera, A.; Meirelles, M. N. L.; de Castro, S. L.; Docampo, R.; Meints, G. A.; Oldfield, E.; Urbina, J. A. Selective in vitro effects of the farnesyl-pyrophosphate synthase inhibitor risedronate on *Trypanosoma cruzi*. *Int. J. Antimicrob. Agents* **2004**, *23*, 273−285.

(10) Garzoni, L. R.; Waghabi, M. C.; Baptista, M. M.; de Castro, S. L.; Meirelles, M. N. L.; Britto, C. C.; Docampo, R.; Oldfield, E.; Urbina, J. A. Antiparasitic activity of risedronate in a murine model of acute Chagas disease. *Int. J. Antimicrob. Agents* **2004**, *23*, 286−290.

(11) Yardley, V.; Khan, A. A.; Martin, M. B.; Slifer, T. R.; Araujo, F. G.; Moreno, S. N. J.; Docampo, R.; Croft, S.; Oldfield, E. In vivo activity of the farnesyl pyrophosphate synthase inhibitors against *Leishmania donovani* and *Toxoplasma gondii*. *Antimicrob. Agents Chemother.* **2002**, *46*, 929−931.

(12) Rodriguez, N.; Bailey, B. N.; Martin, M. B.; Oldfield, E.; Urbina, J. A.; Docampo, R. Radical cure of experimental cutaneous leishmaniasis using pamidronate. *J. Infect. Dis.* **2002**, *186*, 138−140.

(13) Ghosh, S.; Chan, J. M.; Lea, C. R.; Meints, G. A.; Lewis, J. C.; Tovian, Z. S.; Flessner, R. M.; Loftus, T. C.; Bruchhaus, I.; Kendrick, M. H.; Croft, S. L.; Kemp, R. G.; Kobayashi, S.; Nozaki, T.; Oldfield, E. Effects of bisphosphonates on the growth of *Entamoeba* and *Plasmodium* species in vitro and in vivo. *J. Med. Chem.* **2004**, *47*, 175−187.

(14) Montalvetti, A.; Fernandez, A.; Sanders, J. M.; Ghosh, S.; Van Brussel, E.; Oldfield, E.; Docampo, R. Farnesyl pyrophosphate synthase is an essential enzyme in *Trypanosoma brucei*: in vitro RNA interference and in vivo inhibition studies. *J. Biol. Chem.* **2003**, *278*, 17075−17083.

(15) van Beek, E.; Pieterman, E.; Cohen, L.; Lowik, C.; Papapoulos, S. Farnesyl pyrophosphate synthase is the molecular target of nitrogen-containing bisphosphonates. *Biochem. Biophys. Res. Commun.* **1999**, *264*, 108−111.

(16) Keller, R. K.; Fliesler, S. J. Mechanism of aminobisphosphonate action: characterization of alendronate inhibition of the isoprenoid pathway. *Biochem. Biophys. Res. Commun.* **1999**, *266*, 560−563.

(17) Grove, J. E.; Brown, R. J.; Watts, D. J. The intracellular target for the antiresorptive aminobisphosphonate drugs in *Dictyostelium discoideum* is the enzyme farnesyl diphosphate synthase. *J. Bone Miner. Res.* **2000**, *15*, 971−981.

(18) Dunford, J. E.; Thompson, K.; Coxon, F. P.; Luckman, S. P.; Hahn, F. M.; Poulter, C. D.; Ebetino, F. H.; Rogers, M. J. Structure−activity relationships for inhibition of farnesyl diphosphate synthase in vitro and inhibition of bone resorption in vivo by nitrogen-containing bisphosphonates. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 235−242.

(19) Montalvetti, A.; Bailey, B. N.; Martin, M. B.; Severin, G. W.; Oldfield, E.; Docampo, R. Bisphosphonates are potent inhibitors of *Trypanosoma cruzi* farnesyl pyrophosphate synthase. *J. Biol. Chem.* **2001**, *276*, 33930−33937.

(20) Lemercier, G.; Espiau, B.; Ruiz, F. A.; Vieira, M.; Luo, S.; Baltz, T.; Docampo, R.; Bakalara, N. A pyrophosphatase regulating polyphosphate metabolism in acidocalcisomes is essential for *Trypanosoma brucei* virulence in mice. *J. Biol. Chem.* **2004**, *279*, 3420−3425.

(21) Rodrigues, C. O.; Ruiz, F. A.; Vieira, M.; Hill, J. E.; Docampo, R. An acidocalcisomal exopolyphosphatase from *Leishmania major* with high affinity for short chain polyphosphate. *J. Biol. Chem.* **2002**, *277*, 50899−50906.

(22) Docampo, R.; Moreno, S. N. Acidocalcisome: A novel Ca^{2+} storage compartment in trypanosomatids and apicomplexan parasites. *Parasitol. Today* **1999**, *15*, 443−448.

(23) Sanders, J. M.; Gomez, A. O.; Mao, J.; Meints, G. A.; Van Brussel, E. M.; Burzynska, A.; Kafarski, P.; Gonzalez-Pacanowska, D.; Oldfield, E. 3-D QSAR investigations of the inhibition of *Leishmania major* farnesyl pyrophosphate synthase by bisphosphonates. *J. Med. Chem.* **2003**, *46*, 5171−5183.

(24) Sanders, J. M.; Ghosh, S.; Chan, J. M.; Meints, G.; Wang, H.; Raker, A. M.; Song, Y.; Colantino, A.; Burzynska, A.; Kafarski, P.; Morita, C. T.; Oldfield, E. Quantitative structure−activity relationships for $\gamma\delta$ T cell activation by bisphosphonates. *J. Med. Chem.* **2004**, *47*, 375−384.

(25) Sanders, J. M.; Song, Y.; Chan, J. M. W.; Zhang, Y.; Jennings, S.; Kosztowski, T.; Odeh, S.; Flessner, R.; Schwerdtfeger, C.; Kotsikorou, E.; Meints, G. A.; Gómez, A. O.; González-Pacanowska, D.; Raker, A. M.; Wang, H.; Morita, C. T.; Oldfield, E. Pyridinium-1-yl bisphosphonates are potent inhibitors of farnesyl diphosphate synthase. *J. Med. Chem.* **2005**, *48*, 2957−2963.

(26) Kieczykowski, G. R.; Jobson, R. B.; Melillo, D. G.; Reinhold, D. F.; Grenda, V. J.; Shinkai, I. Preparation of (4-amino-1-hydroxybutylidene) bisphosphonic and sodium salt, MK-217 (alendronate sodium). An improved procedure for the preparation of 1-hydroxy-1,1-bisphosphonic acids. *J. Org. Chem.* **1995**, *60*, 8310−8312.

(27) Harel, Z.; Kovalevski-Liron, E.; Lidor-Hadas, R.; Lifshitz-Liron, R. Use of certain diluents for making bisphosphonic acids. World Patent WO03097655, November 27, 2003.

(28) Soloducho, J.; Gancarz, R.; Wieczorek, P.; Korf, J.; Hafner, J.; et al. Preparation of novel derivatives of (aminomethylene)bis-(phosphonic acid) as herbicides. Patent PL93-298436, 1997.

(29) Hutchinson, D. W.; Thornton, D. M. Michael addition reactions of ethenylidene bisphosphonates. *J. Organomet. Chem.* **1988**, *346*, 341−348.
(30) Lecouvey, M.; Mallard, I.; Bailly, T.; Burgada, R.; Leroux, Y. A mild and efficient one-pot synthesis of 1-hydroxymethylene-1,1-bisphosphonic acids. Preparation of new tripod ligands. *Tetrahedron Lett.* **2001**, *42*, 8475−8478.
(31) Degenhardt, C. R.; Burdsall, D. C. Synthesis of ethenylidenebis-(phosphonic acid) and its tetraalkyl esters. *J. Org. Chem.* **1986**, *51*, 3488−3490.
(32) Miyaura, N.; Yanagi, T.; Suzuki, A. The palladium-catalyzed cross-coupling reaction of phenylboronic acid with haloarenes in the presence of bases. *Synth. Commun*. **1981**, *11*, 513−519.
(33) Kasibhatla, S. R.; Bookser, B. C.; Probst, G.; Appleman, J. R.; Erion, M. D. AMP deaminase inhibitors. 3. SAR of 3-(Carboxyarylalkyl)coformycin aglycon analogs. *J. Med. Chem.* **2000**, *43* (8), 1508−1518.
(34) Bhattacharyya, S. Titanium(IV) isopropoxide and sodium borohydride: a reagent of choice for reductive amination. *Tetrahedron Lett.* **1994**, *35* (15), 2401−2404.
(35) Magnin, D. R.; Biller, S. A.; Dickson, J. K., Jr.; Lawrence, M. R.; Sulsky, R. B. α-Phosphorous substituted sulfonate derivatives as squalene synthetase inhibitors. Eur. Pat. Appl. EPXXDW EP 595635, 1994.
(36) Cramer, R. D., III; Patterson, D. E.; Bunce, J. D. Recent advances in comparative molecular field analysis (CoMFA). *Prog. Clin. Biol. Res*. **1989**, *291*, 161−165.
(37) Klebe, G.; Abraham, U.; Mietzner, T. Molecular similarity indices in a comparative analysis (CoMSIA) of drug molecules to correlate and predict their biological activity. *J. Med. Chem.* **1994**, *37*, 4130−4146.
(38) *Cerius*^{2} *Modeling Environment*, version 4.6; Accelrys, Inc.: San Diego, CA, 2001.
(39) Arfken, G. B.; Weber, H. J. *Mathematical Methods for Physicists*, 4th ed.; Academic Press: New York, 1995; p 446.
(40) Fletcher, R.; Reeves, C. M. Functional minimization by conjugate gradients. *Comput. J*. **1964**, *7*, 149−154.
(41) Acton, F. S. *Numerical Methods That Work*; Harper and Row: New York, 1970; Chapter 2.
(42) Tarshis, L. C.; Proteau, P. J.; Kellogg, B. A.; Sacchettini, J. C.; Poulter, C. D. Regulation of product chain length by isoprenyl diphosphate synthase. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 15018−15023.
(43) Gossman, W. L.; Wilson, S. R.; Oldfield, E. Three hydrates of the bisphosphonate risedronate, consisting of one molecular and two ionic structures. *Acta Crystallogr. C* **2003**, *59*, m33−m36.
(44) *Sybyl*, version 6.9; Tripos Inc. (1699 South Hanley Road, St. Louis, MO, 63144).
(45) Gasteiger, J.; Marsili, M. Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges. *Tetrahedron* **1980**, *36*, 3219−3228.
(46) Wold, H. In *Encyclopedia of Statistical Sciences*; Kotz, S., Johnson, N., Eds.; Wiley: New York, 1985; Volume 6, p 581.
(47) Bush, B. L.; Nachbar, R. B. Sample-distance partial least squares: PLS optimized for many variables, with application to CoMFA. *J. Comput.-Aided Mol. Des.* **1993**, *7*, 587−619.
(48) Csimadia, F. JChem: Java Applets and Modules Supporting Chemical Database Handling from Web Browsers. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 323−324.
(49) ChemAxon Ltd., Máramaros köz 3/a, Budapest, 1037 Hungary. http://www.chemaxon.com.
(50) Shatton, J. B.; Ward, C.; Williams, A.; Weinhouse, S. A microcolorimetric assay of inorganic pyrophosphatase. *Anal. Biochem*. **1983**, *130*, 114−119.
(51) Kotsikorou, E.; Oldfield, E. A quantitative structure−activity relationship and pharmacophore modeling investigation of aryl-X and heterocyclic bisphosphonates as bone resorption agents. *J. Med. Chem.* **2003**, *46*, 2932−2944.
(52) Szabo, C. M.; Martin, M. B.; Oldfield, E. An investigation of bone resorption and Dictyostelium discoideum growth inhibition by bisphosphonate drugs. *J. Med. Chem.* **2002**, *45*, 2894−2903.
(53) Thompson, K.; Dunford, J. E.; Ebetino, F. H.; Rogers, M. J. Identification of a bisphosphonate that inhibits isopentenyl diphosphate isomerase and farnesyl diphosphate synthase. *Biochem. Biophys. Res. Commun.* **2002**, *290*, 869−873.

JM058220G