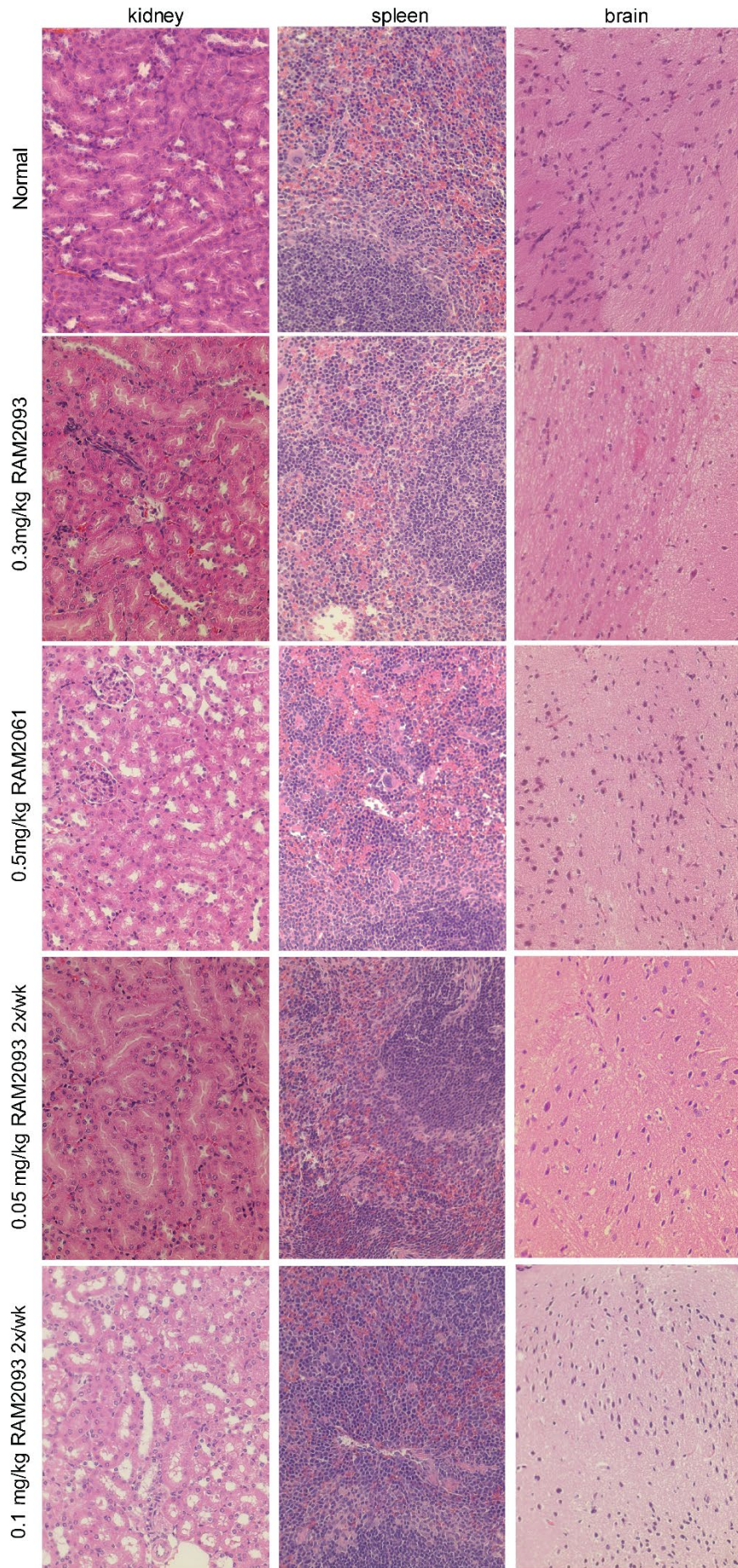


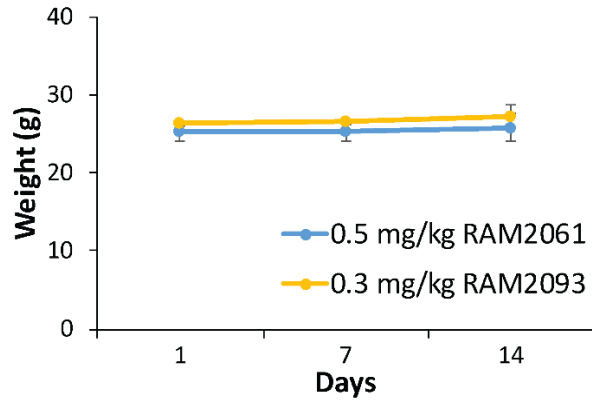
***In vivo* evaluation of isoprenoid triazole bisphosphonate inhibitors of geranylgeranyl diphosphate synthase: impact of olefin stereochemistry on toxicity and biodistribution**

Staci L. Haney, Yashpal S. Chhonker, Michelle L. Varney, Geoffrey Talmon, Lynette M. Smith, Daryl J. Murry, and Sarah A. Holstein

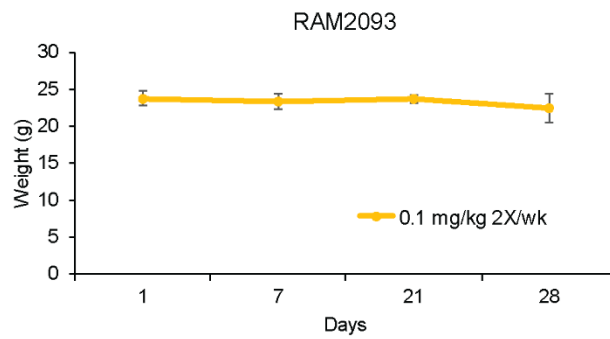
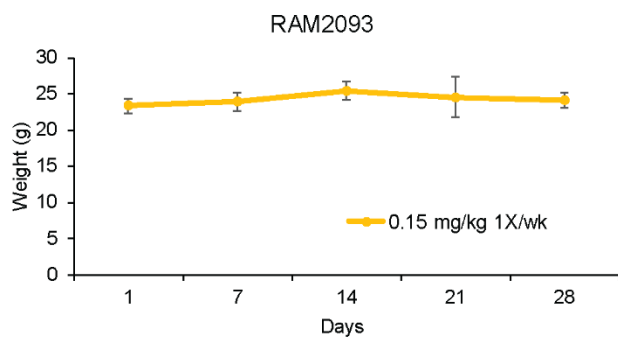
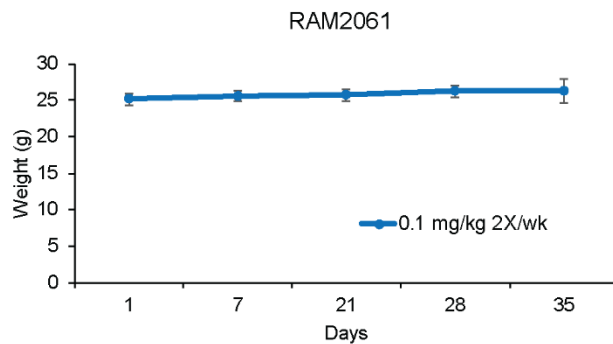
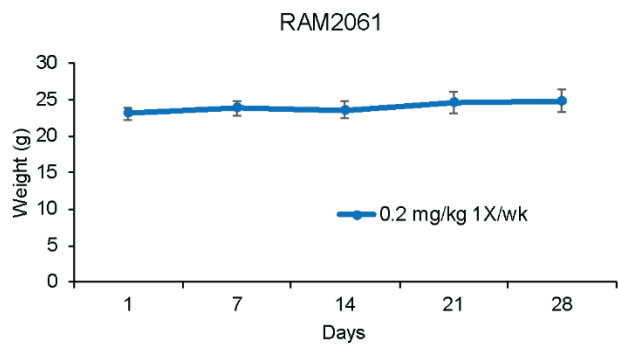
Journal of Pharmacology and Experimental Therapeutics



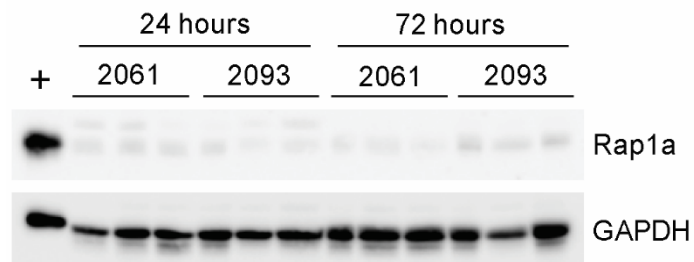
Supplemental Figure 1. The effects of RAM2061 and RAM2093 on tissue morphology. Kidney, spleen and brain H&E sections (200×) from mice following IV injection with 0.3 mg/kg RAM2093, 0.5 mg/kg RAM2093, 0.1 mg/kg 2x/wk RAM2061, and 0.05 mg/kg 2x/wk. RAM2093. Normal tissue sections are shown as controls. Single dose tissues were collected 14 days post injection and multi-dose tissue were collected 35 days post injection.



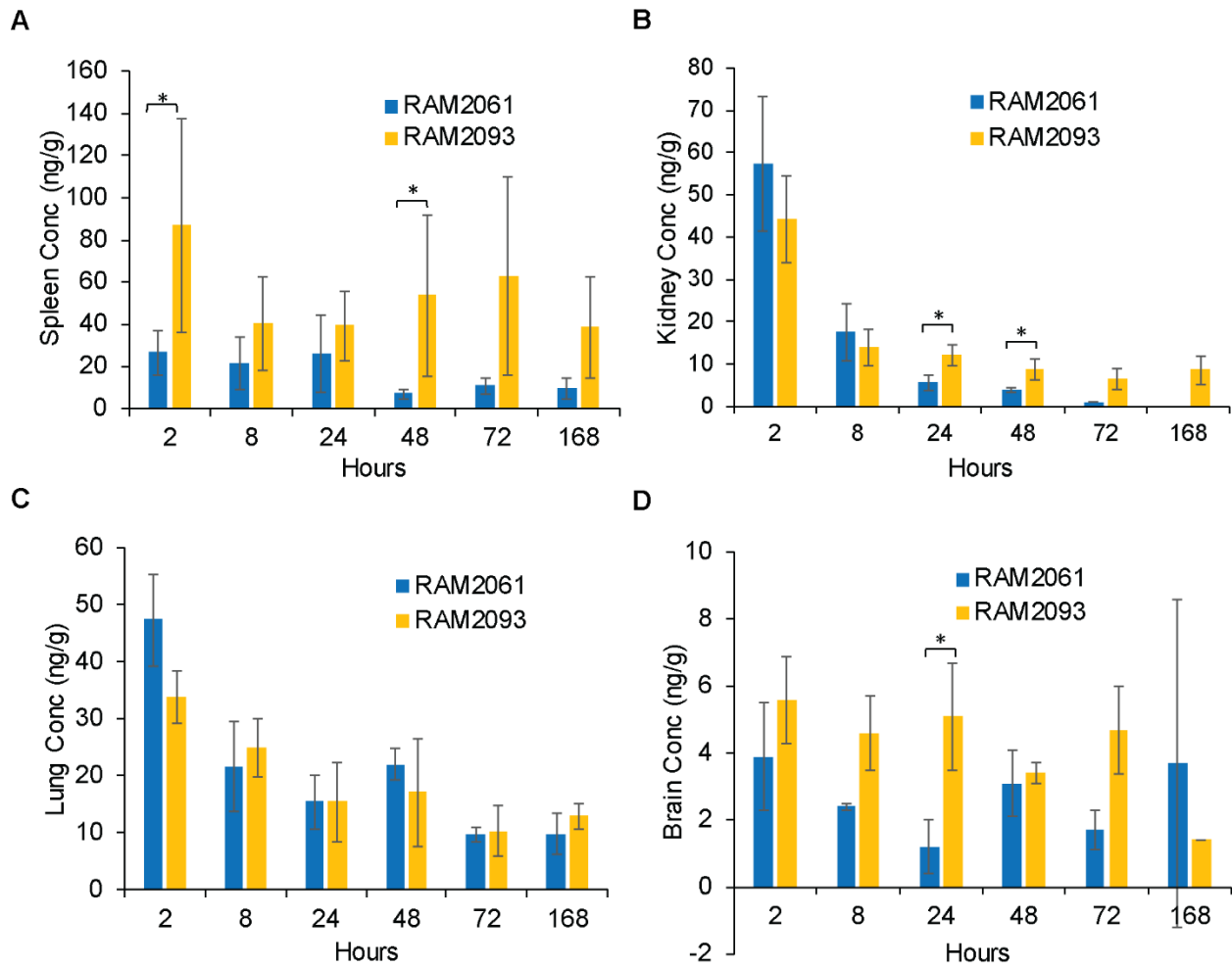
Supplemental Figure 2. RAM2061 and RAM2093 do not alter animal weight. Weight (grams) of mice were recorded at days 1, 7 and 14 following IV administration of 0.3 mg/kg RAM2093 or 0.5 mg/kg RAM2061. Error bars denote Stdev. N=5.



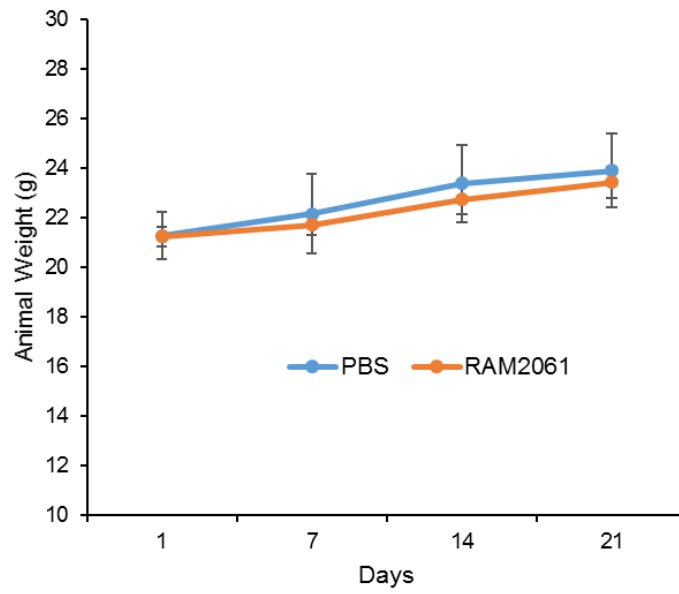
Supplemental Figure 3. Multiple doses of RAM2061 and RAM2093 do not alter animal weight. Weight (grams) of mice were recorded once weekly following IV administration of once or twice weekly regimens of RAM2093 and RAM2061. Error bars denote Stdev. N=5.



Supplemental Figure 4. Single dose of GGDPS inhibitor is not sufficient to disrupt protein geranylgeranylation in vivo. Immunoblot analysis of unmodified Rap1a in livers collected from mice treated with a single IV dose of 0.3mg/kg RAM2061 or RAM2093. Tissues were collected 24 and 72 hours post injection. Lovastatin treated MM.1S cells served as a positive control. GAPDH is shown as a loading control.



Supplemental Figure 5. Tissue distribution patterns. Tissue concentrations (spleen, kidney, lung, and brain) over time for RAM2061 and RAM2093 following a single dose (0.3 mg/kg IV). *P < 0.05, two tailed student t-test. n=5 (spleen) and n=3 (lung, kidney and brain).



Supplemental Figure 6. Multiple doses of RAM2061 do not alter animal weight in MM1.S tumor xenograft studies. Weight (grams) of mice were recorded once weekly during twice-weekly IV administration of RAM2061 or PSB. Error bars denote Stdev.

Supplemental Table 1: qRT-PCR primers used in these studies.

ATF4	
F	AAGCCTAGGTCTCTTAGATG
R	TTCCAGGTCATCTATACCCA
β-ACTIN	
F	ACGTTGCTATCCAGGCTGTGCTAT
R	TTAATGTCACGCACGATTTCCCGC
CHOP	
F	TCTTCACCACTCTTGACCCTGCTT
R	GTTCTTTCTCCTTCATGCGCTGCT
IRE1	
F	AGACTTTGTCATCGGCCTTTGCAG
R	ATTCACTGTCCACAGTCACCACCA
PERK	
F	GCAACAACGTTTATTGTGCGCAGG
R	AAACAACCTCAAAGCCACCACGTC

Supplemental Table 2: Western blot antibodies used in these studies

Antibody	Company	Cat. #	Dilution
ATF-4	Cell Signaling	11815	1:1000
Cleaved Caspase 3	Cell Signaling	9664	1:500
Cleaved Caspase 8	Cell Signaling	9496	1:500
eIF2 α	Cell Signaling	9722	1:1000
p-eIF2 α	Cell Signaling	3597	1:1000
β -Tubulin	Sigma	T5201	1:25,000
Rap1a	Santa Cruz	sc-373968	1:500

Supplemental Table 3: Blood Chemistry panel for single dose testing of 0.3mg/kg RAM2061

Blood was collected at 7 and 14 days post-injection (Mean \pm SD, n = 4 mice per group).

		0.3 mg/kg RAM2061	
		Day 7	Day 14
	Normal	Average	Average
BUN	9 to 33	18.5 \pm 2.9	20.3 \pm 1.5
CRE	0.2 to 0.9	0.3 \pm 0.06	0.3 \pm 0.2
ALT	17 to 77	363 \pm 62.8	62.8 \pm 2.2
ALP	35 to 222	78.5 \pm 73.8	73.8 \pm 5.9
AST	54 to 298	424.3 \pm 100	100.3 \pm 21.4
TBIL	0 to 0.9	0.3 \pm 0.05	0.3 \pm 0.1
GLU	140 to 263	128 \pm 11	145.8 \pm 15.1
CA	6 to 13	11.5 \pm 0.6	10.6 \pm 0.3
TP	3.9 to 6.4	5.7 \pm 0.06	5.5 \pm 0.2
ALB	2.5 to 4.6	4.7 \pm 0.2	4.4 \pm 0.1
GLOB	1.2 to 2.2	1 \pm 0.16	1 \pm 0.2
Na	110 to 195	149.3 \pm 2.2	150.3 \pm 0.5
K	4 to 10.5	7.9 \pm 0.5	8 \pm 0.7
Cl	NR	110.5 \pm 2.5	110.8 \pm 2.8
TCO2	NR	24.8 \pm 3	24.8 \pm 5

Abbreviations: blood urea nitrogen (BUN), creatinine (CRE), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin (TBIL), glucose (GLU), total protein (TP), albumin (ALB), globulin (GLOB), lactate dehydrogenase (LDH), creatine kinase (CK), NR (not reported),