FIG. 1

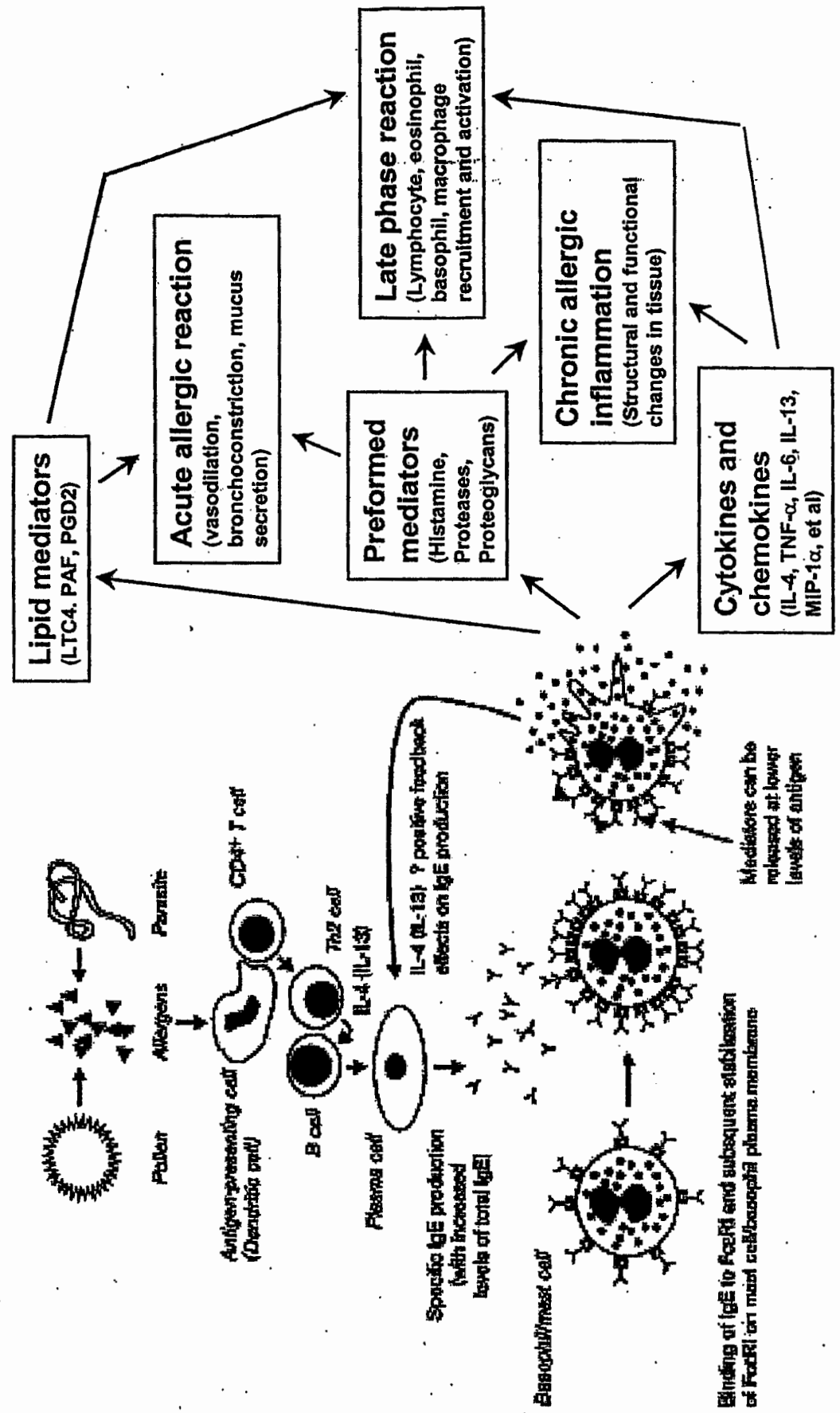

Lipid mediators
(LTC4, PAF, PGD2)
Acute allergic reaction
(vasodilation, bronchoconstriction, mucus secretion)
Preformed mediators
(Histamine, Proteases, Proteoglycans)
Late phase reaction
(Lymphocyte, eosinophil, basophil, macrophage recruitment and activation)
Chronic allergic inflammation
(Structural and functional changes in tissue)
Cytokines and chemokines
(IL-4, TNF-α, IL-6, IL-13, MIP-1α, et al)
Pollen
Allergens
Parasite
Antigen-processing cell (Dendritic cell)
CD4+ T cell
Th2 cell
IL-4 (IL-13)
B cell
Plasma cell
IL-4 (IL-13): ? positive feedback effects on IgE production
Specific IgE production (with increased levels of total IgE)
Basophil/mast cell
Binding of IgE to FcεRI and subsequent stabilization of FcεRI on mast cell/basophil plasma membrane
Mediators can be released at lower levels of antigen

FIG. 2

Mast Cell FceR1 Signaling Pathway

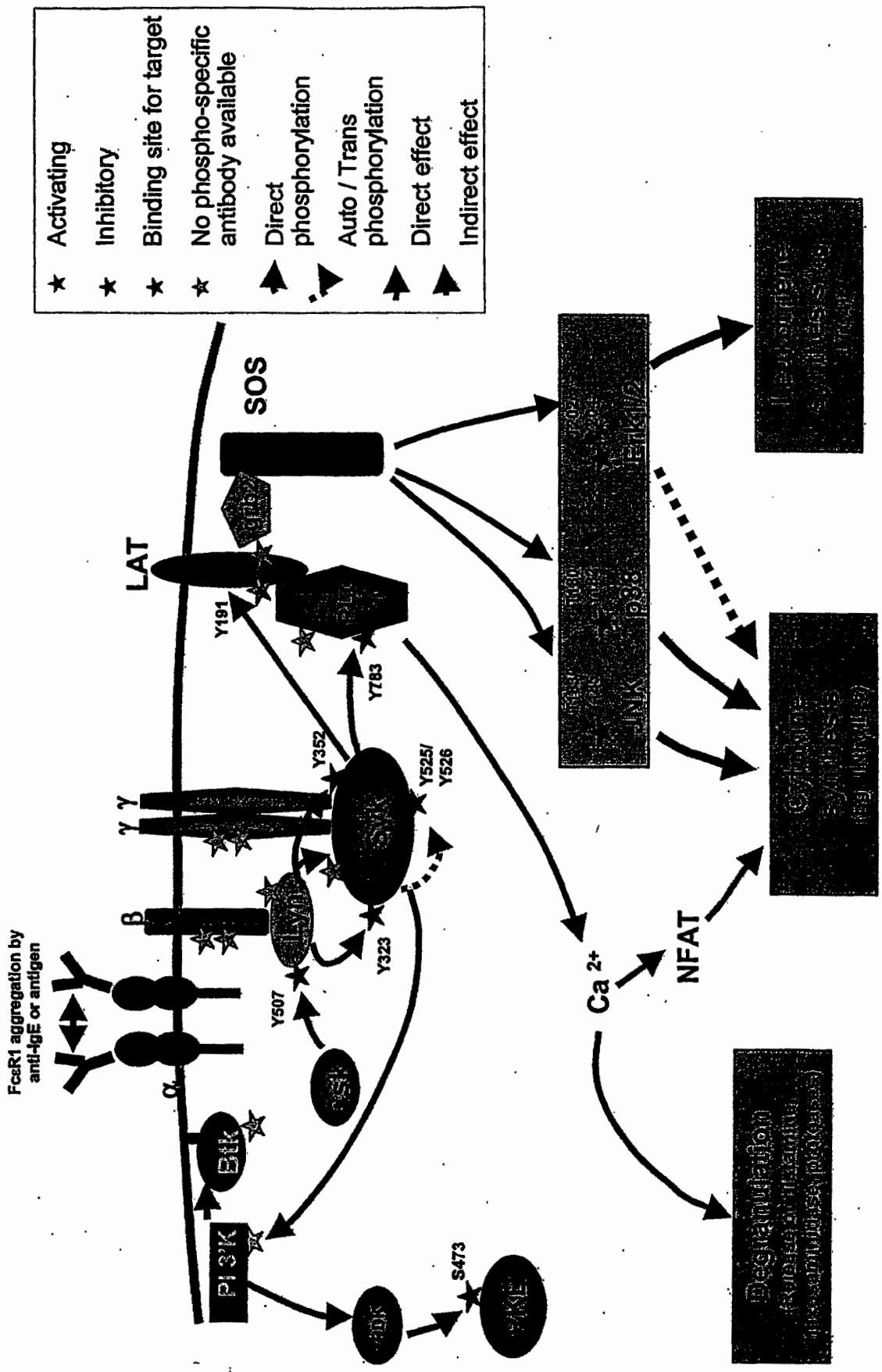


FIG. 3

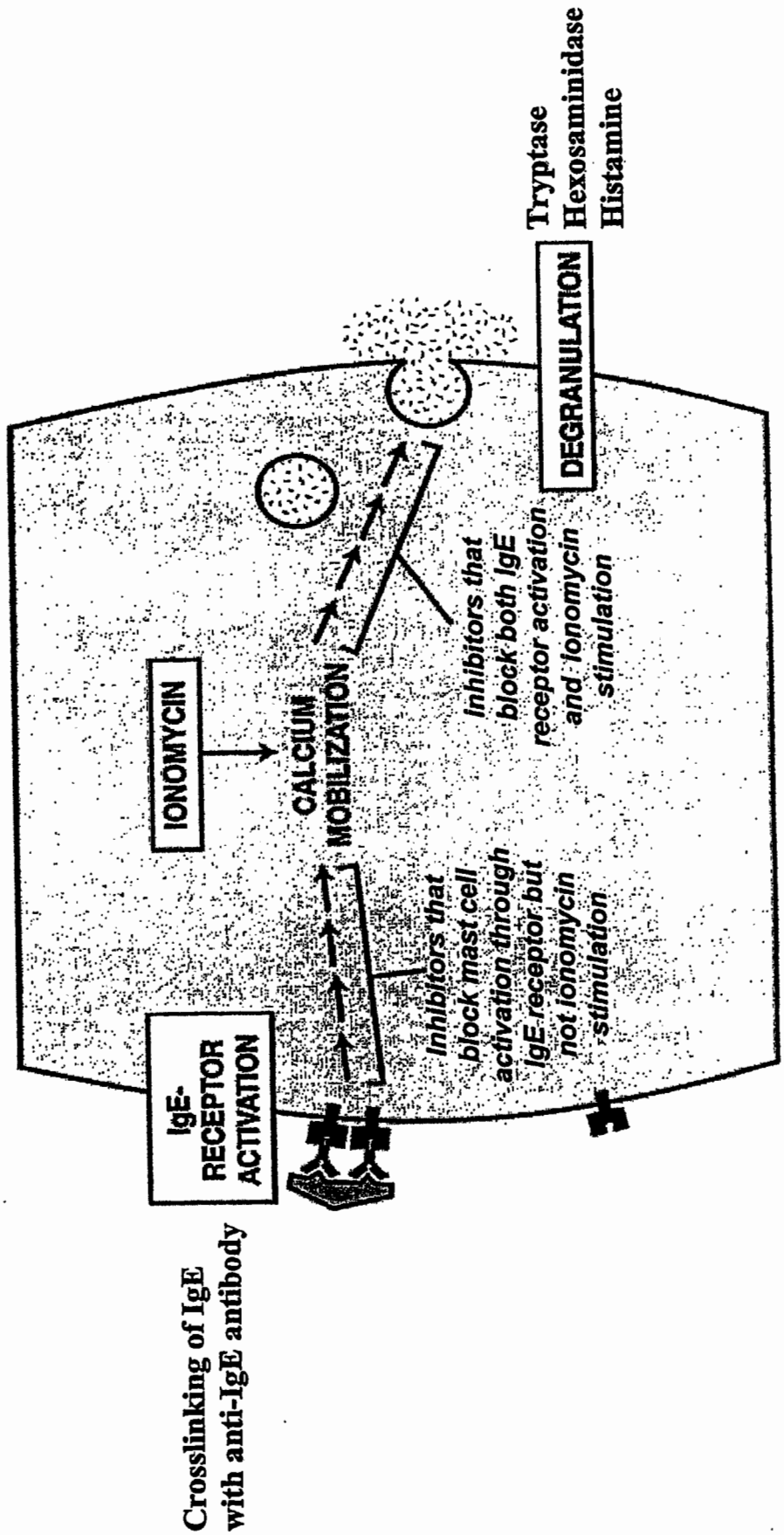

Crosslinking of IgE with anti-IgE antibody
IgE-RECEPTOR ACTIVATION
IONOMYCIN
CALCIUM MOBILIZATION
DEGRANULATION
Tryptase
Hexosaminidase
Histamine
Inhibitors that block mast cell activation through IgE receptor but not ionomycin stimulation
Inhibitors that block both IgE receptor activation and ionomycin stimulation

FIG. 4

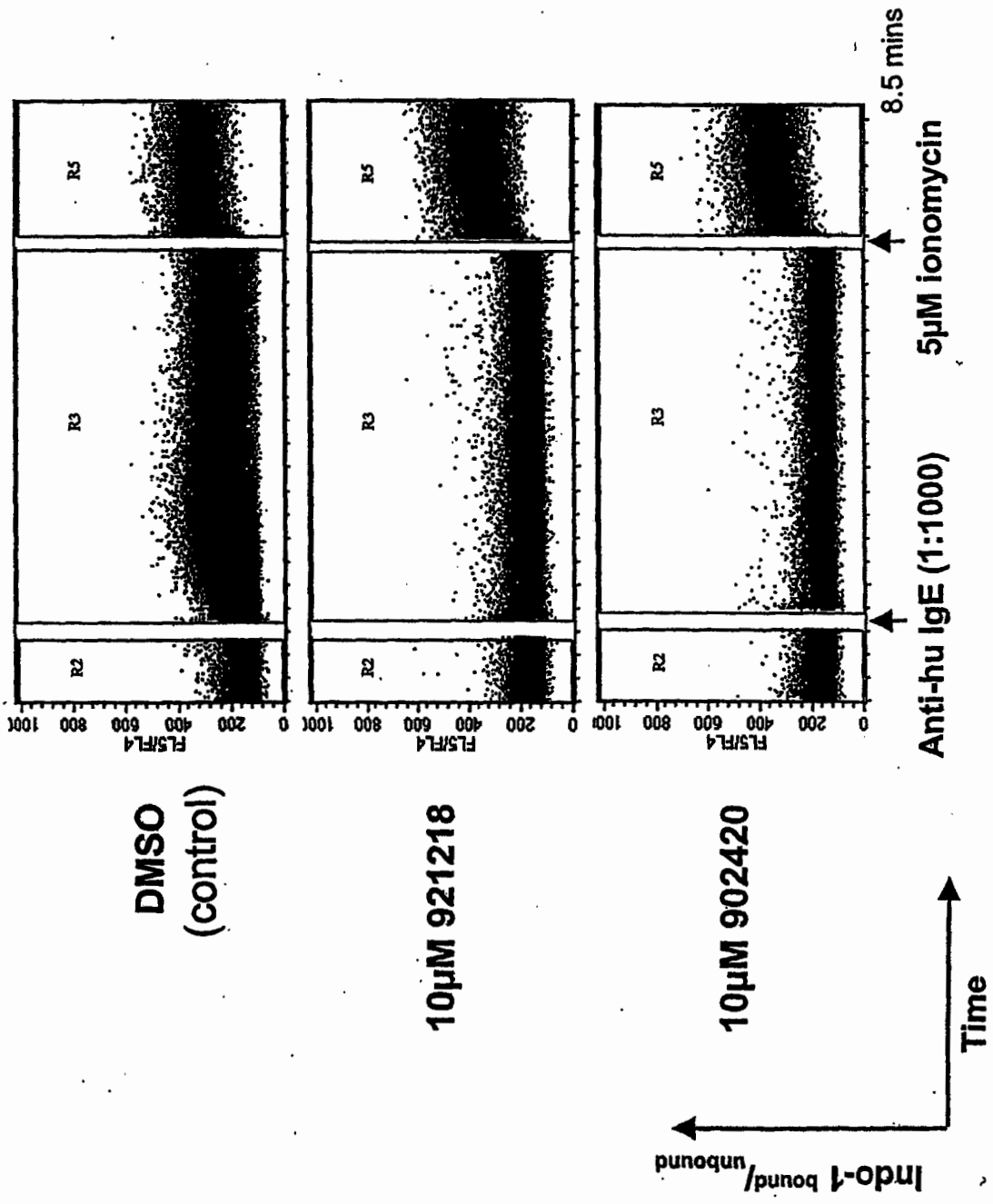

DMSO (control)
10µM 921218
10µM 902420
R2
R3
R5
FL5/FL4
0
200
400
600
800
1000
Anti-hu IgE (1:1000)
5µM ionomycin
8.5 mins
Time
Indo-1 bound/unbound

FIG. 5

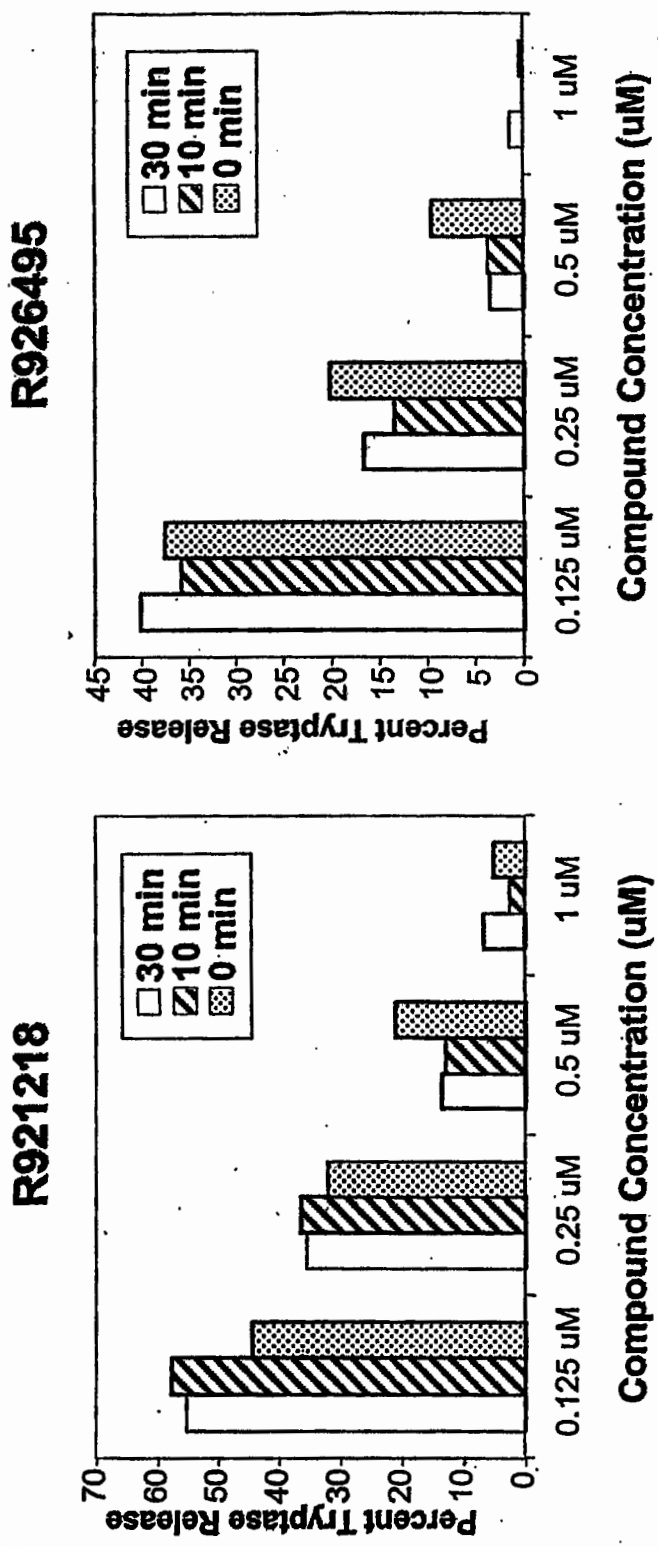

R926495
30 min
10 min
0 min
Percent Tryptase Release
0
5
10
15
20
25
30
35
40
45
0.125 uM
0.25 uM
0.5 uM
1 uM
Compound Concentration (uM)
R921218
30 min
10 min
0 min
Percent Tryptase Release
0
10
20
30
40
50
60
70
0.125 uM
0.25 uM
0.5 uM
1 uM
Compound Concentration (uM)

FIG. 6

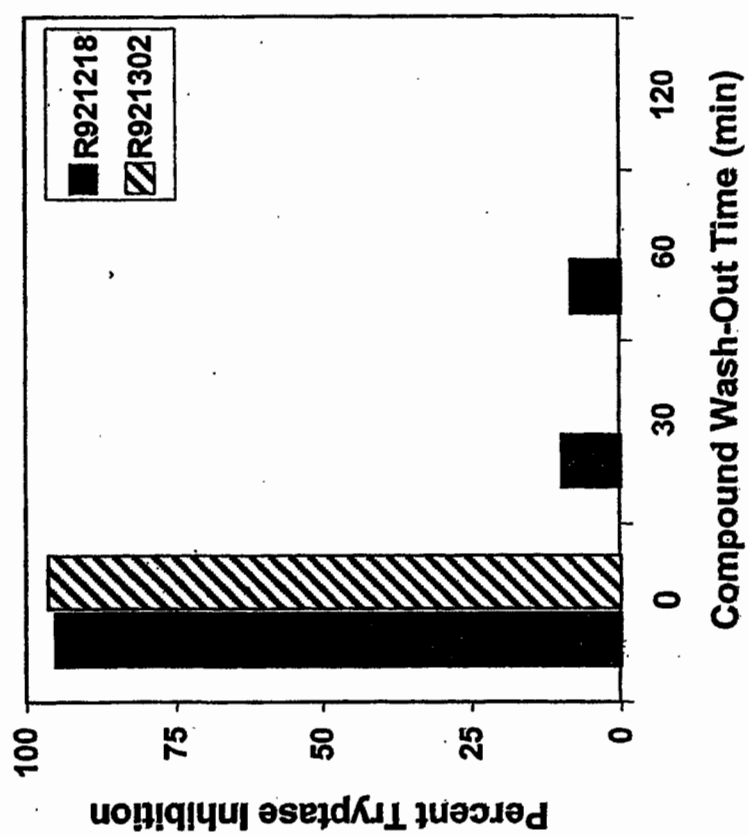

R921218
R921302
Percent Tryptase Inhibition
100
75
50
25
0
0
30
60
120
Compound Wash-Out Time (min)

FIG. 7

Inhibition of Phosphorylation of Proteins Downstream of Syk Kinase in Fce Receptor Activated BMMC Cells

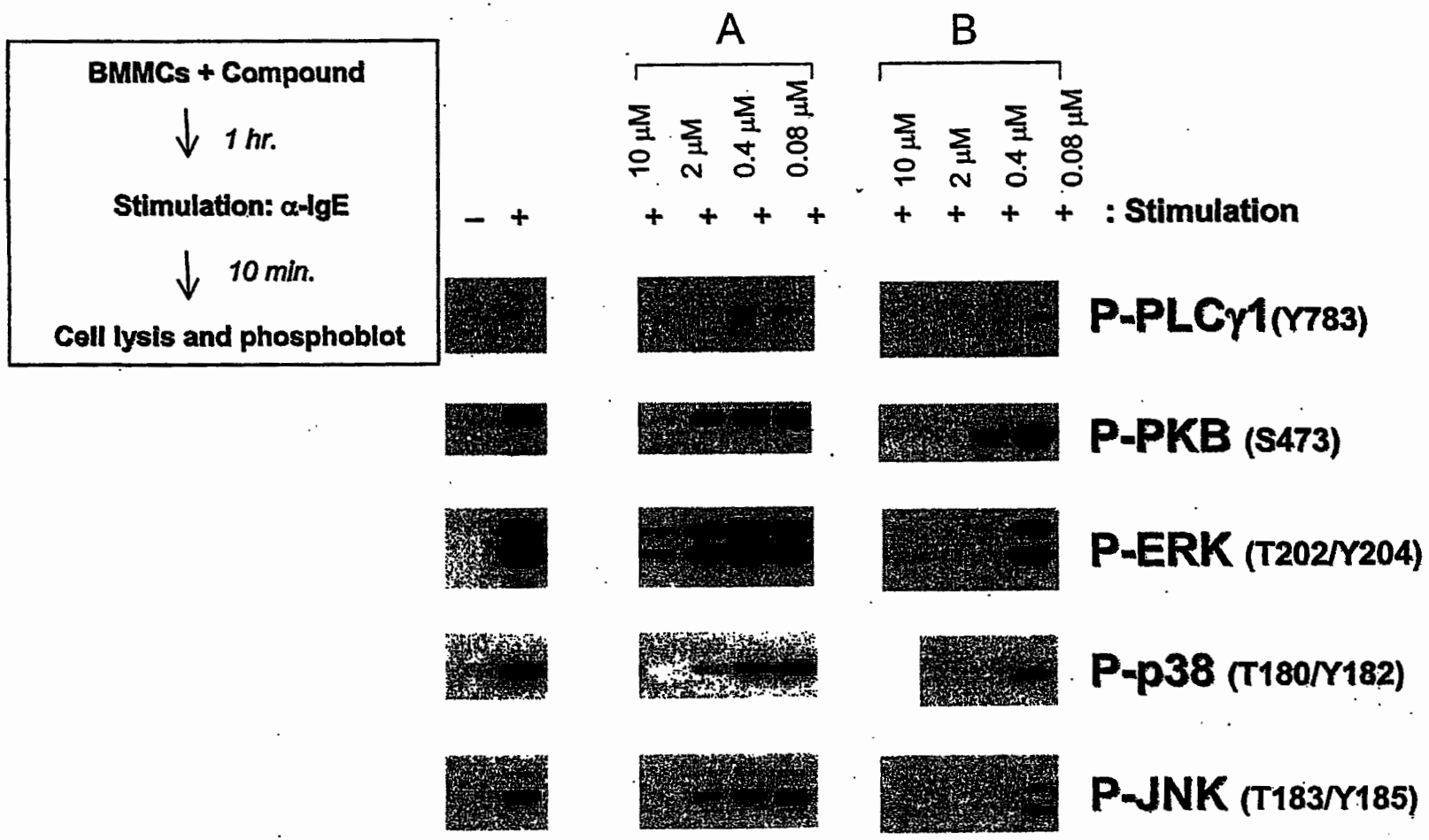
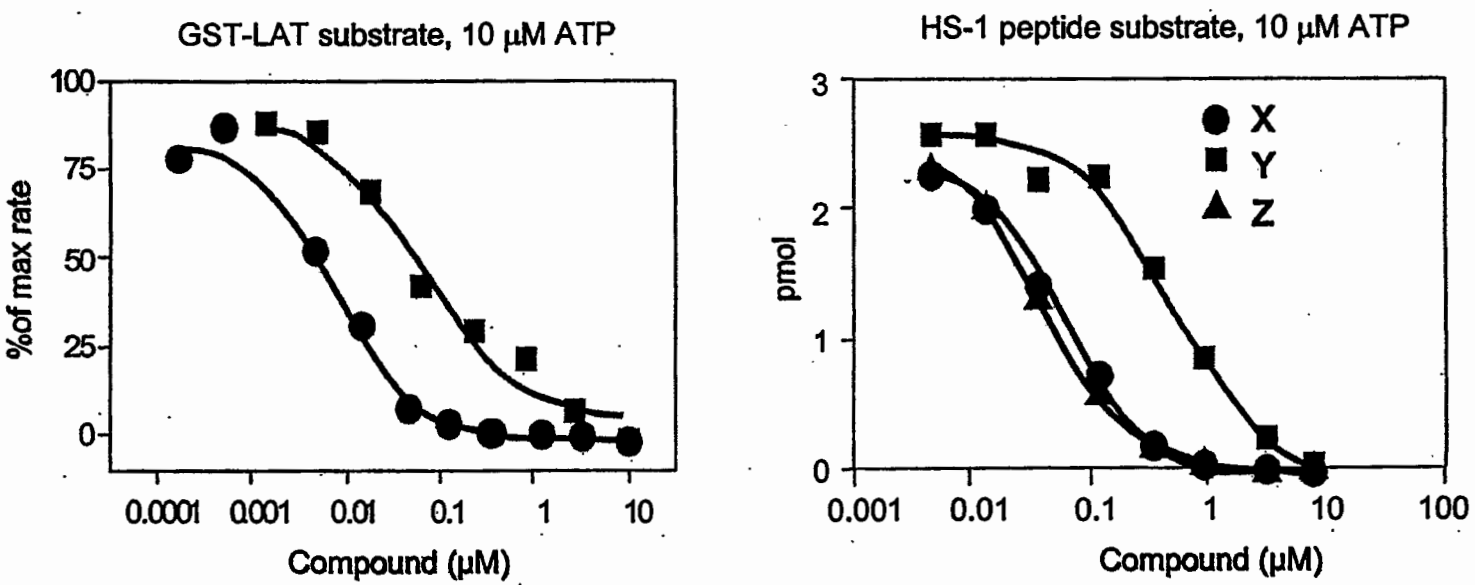


FIG. 8

The Disclosed Compounds Potently Inhibit the Activity of Syk Kinase

Human Syk kinase
***In vitro* Fluorescence Polarization Kinase Assay**

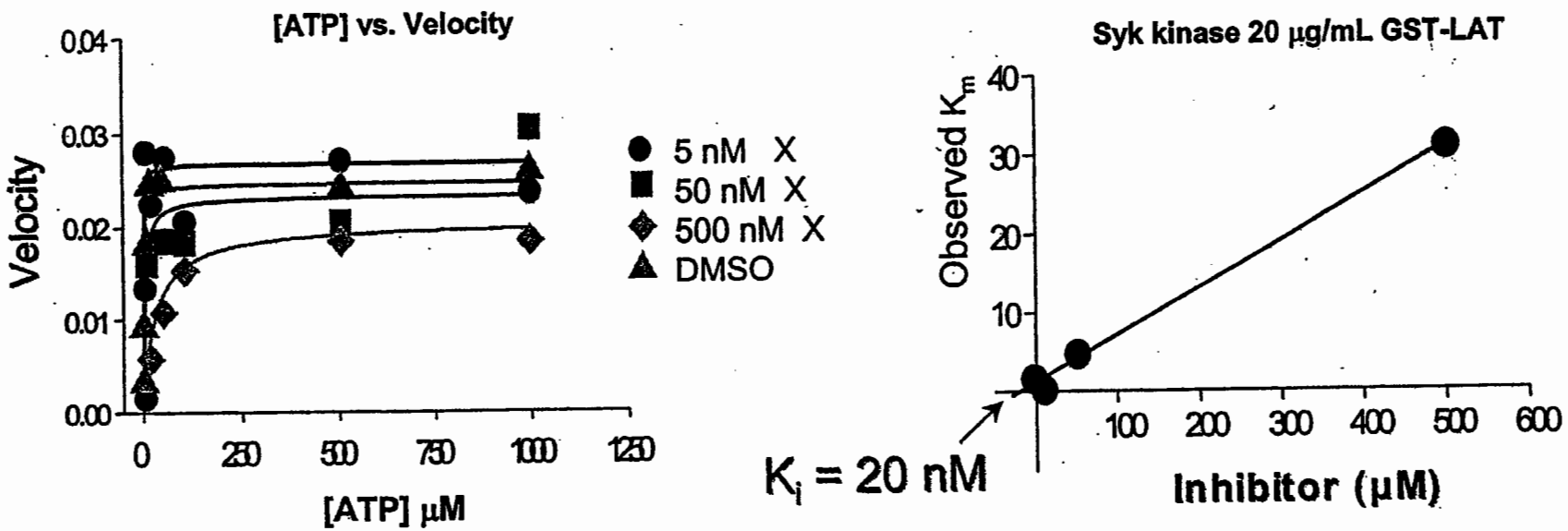


IC_{50} (nM)

	GST-LAT	HS-1
Y	200	570
X	10	62
Z	ND	43

FIG. 9

Compound Inhibition of Syk is ATP Competitive



	DMSO	5 nM X	50 nM X	500 nM X
V_{max}	.025	0.027	.023	0.020
K_m	1.54	0.79	4.5	31

FIG. 10

CHMC: Cultured human mast cells
Compound
(1 hour preincubation)
Stimulation: α-IgE
-
+
PP2
+ 10 μM
+ 2 μM
+ 0.4 μM
A
+ 2 μM
+ 0.4 μM
+ 0.08 μM
B
+ 10 μM
+ 2 μM
+ 0.4 μM
Lyn Targets
P-Cbl (Y774)
P-Syk (Y352)
Syk Targets
P-LAT (Y191)
P-PLCγ1(Y783)
Indirect Targets
P-PKB (S473)
P-ERK (T202/Y204)

FIG. 11A

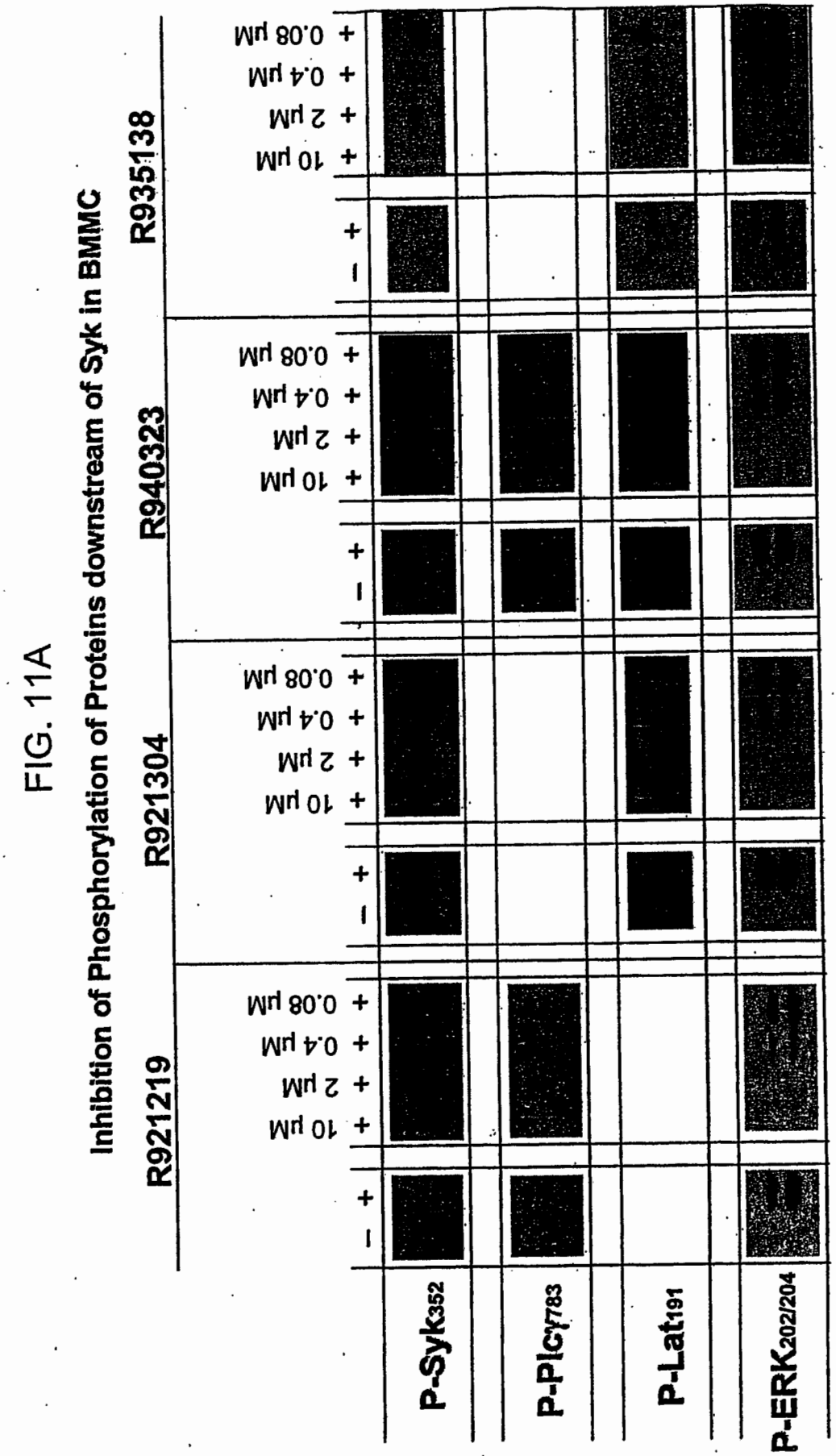

Inhibition of Phosphorylation of Proteins downstream of Syk in BMMC
R921219
R921304
R940323
R935138
- + 10 μM + 2 μM + 0.4 μM + 0.08 μM
P-Syk352
P-PLCγ783
P-Lat191
P-ERK202/204

FIG. 11B

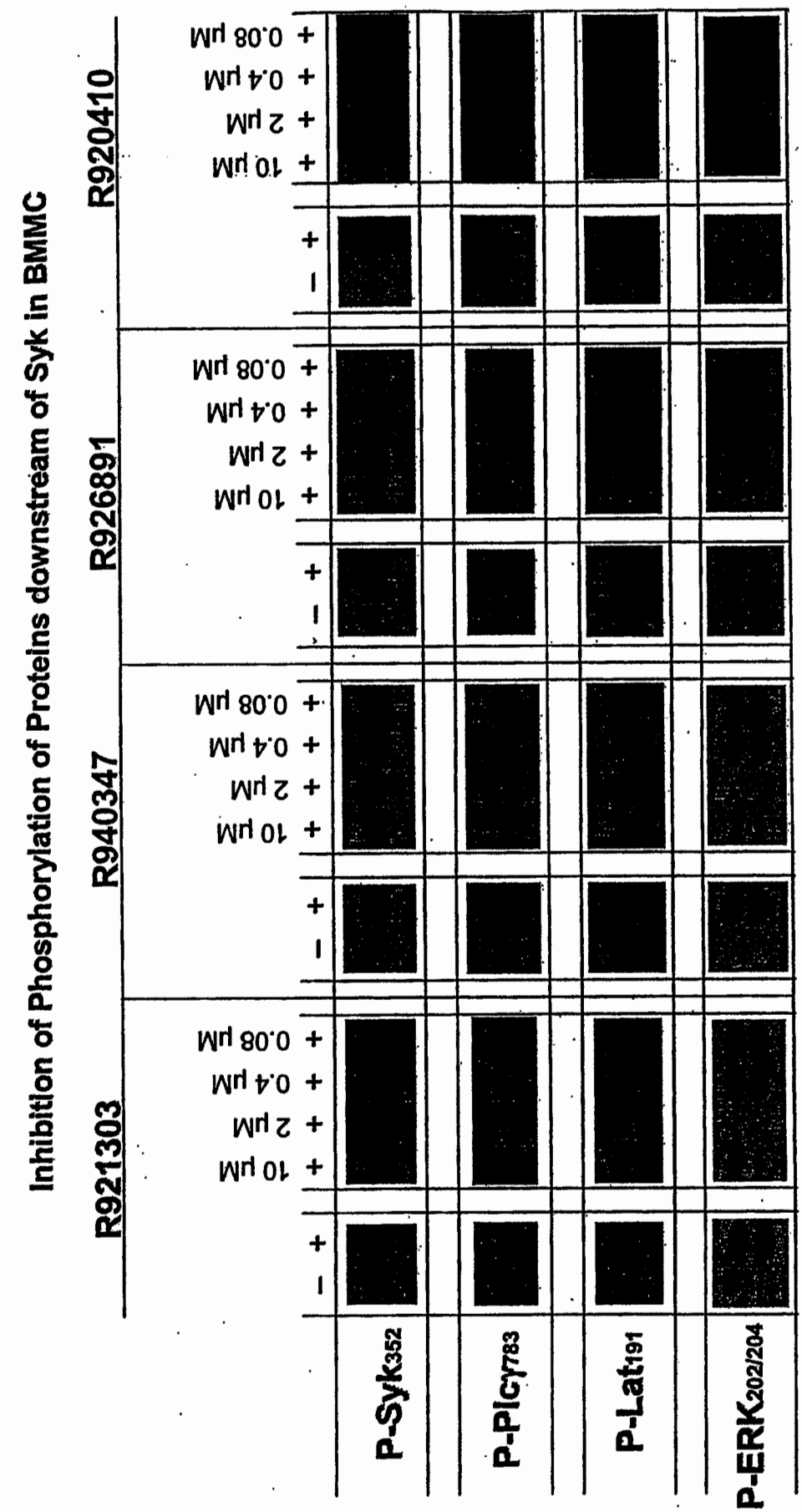

Inhibition of Phosphorylation of Proteins downstream of Syk in BMMC
R921303
R940347
R926891
R920410
- + 10 µM + 2 µM + 0.4 µM + 0.08 µM
P-Syk352
P-PlCγ783
P-Lat191
P-ERK202/204

FIG. 11C

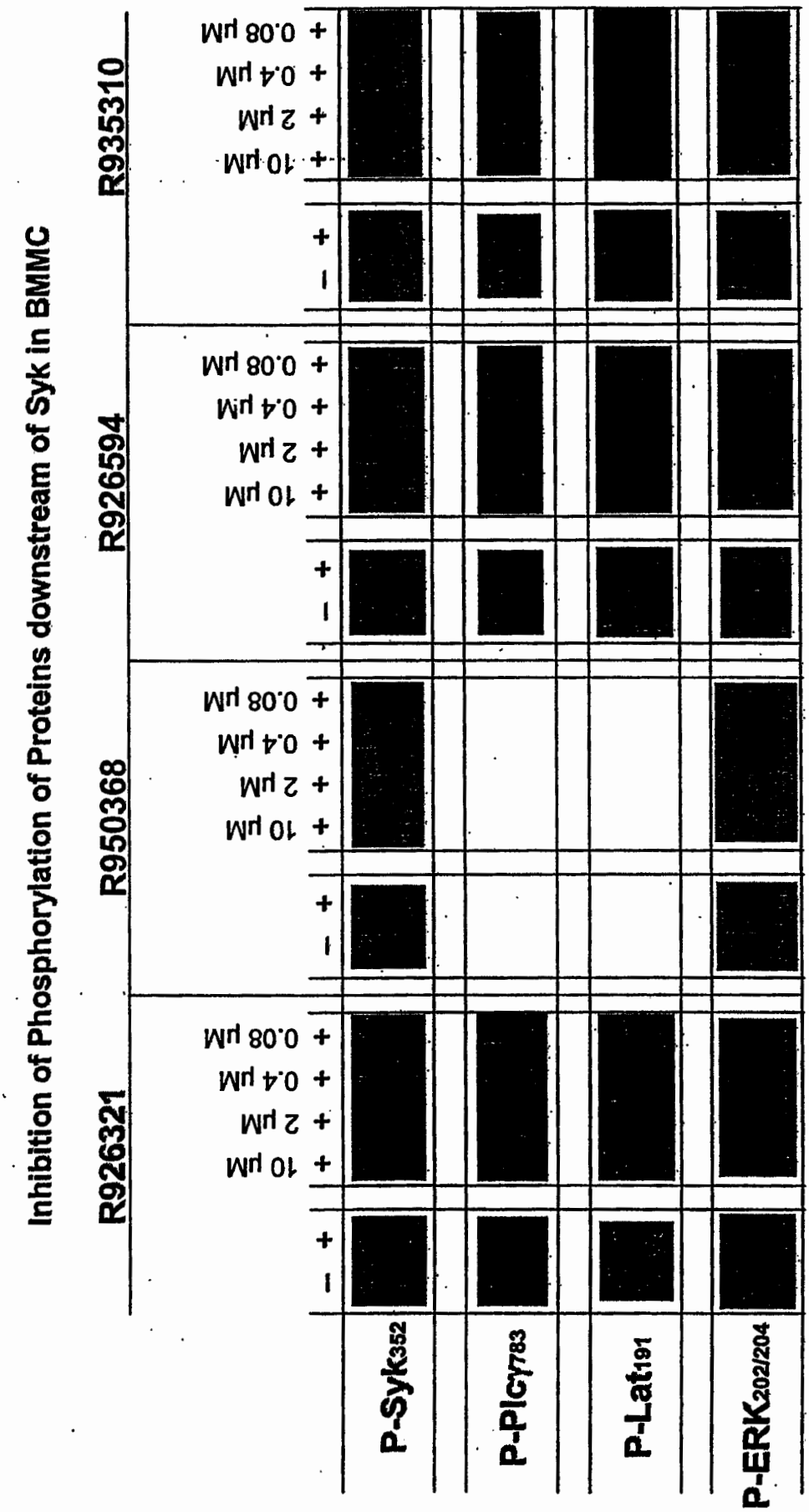

Inhibition of Phosphorylation of Proteins downstream of Syk in BMMC
R926321
R950368
R926594
R935310
- + 10 μM + 2 μM + 0.4 μM + 0.08 μM
P-Syk352
P-PLCγ783
P-Lat191
P-ERK202/204

FIG. 11D

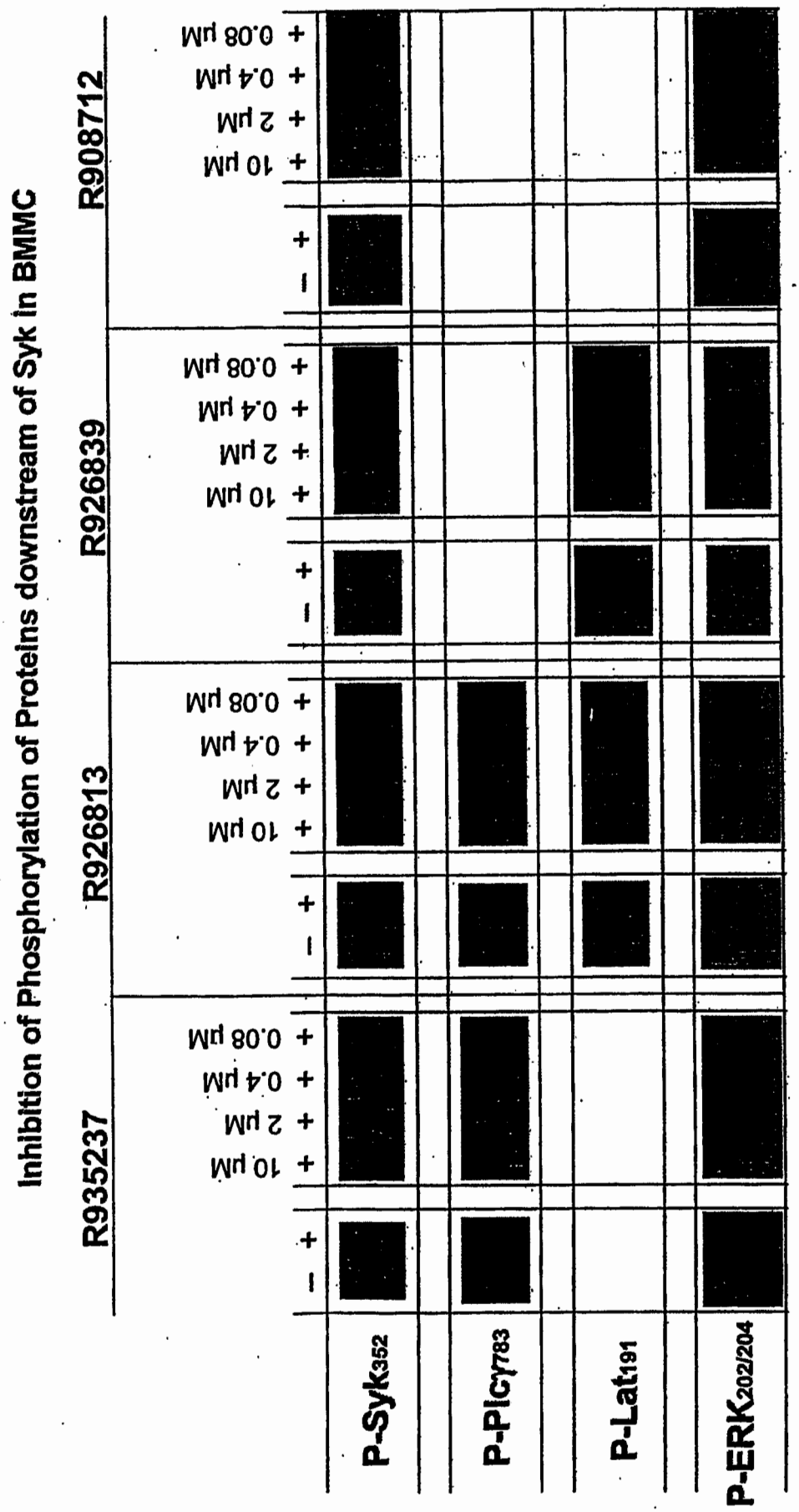

Inhibition of Phosphorylation of Proteins downstream of Syk in BMMC
R935237
R926813
R926839
R908712
- + 10 μM + 2 μM + 0.4 μM + 0.08 μM
P-Syk352
P-Plcγ783
P-Lat191
P-ERK202/204

FIG. 12

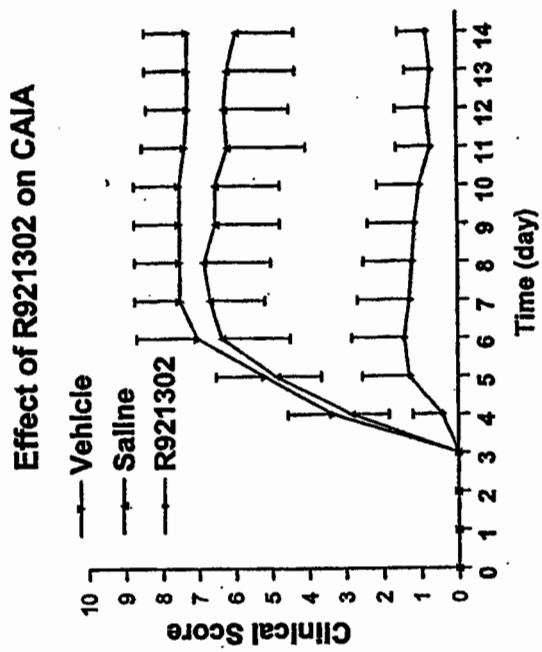

Effect of R921302 on CAIA
Vehicle
Saline
R921302
Clinical Score
Time (day)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

FIG. 13

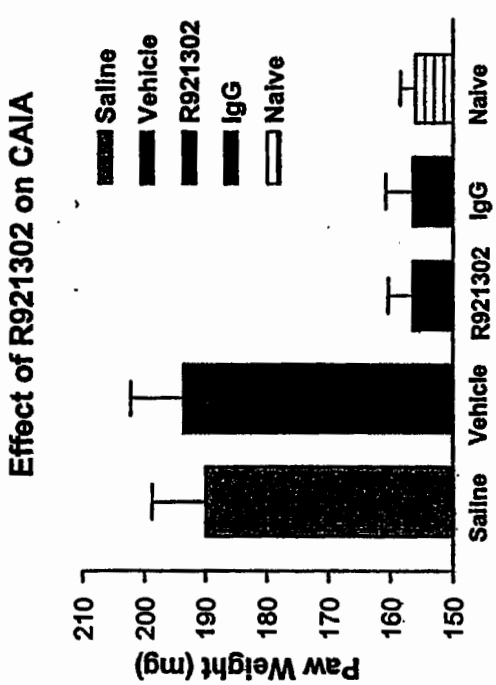

Effect of R921302 on CAIA
Saline
Vehicle
R921302
IgG
Naive
Paw Weight (mg)
210
200
190
180
170
160
150
Saline
Vehicle
R921302
IgG
Naive

FIG. 14

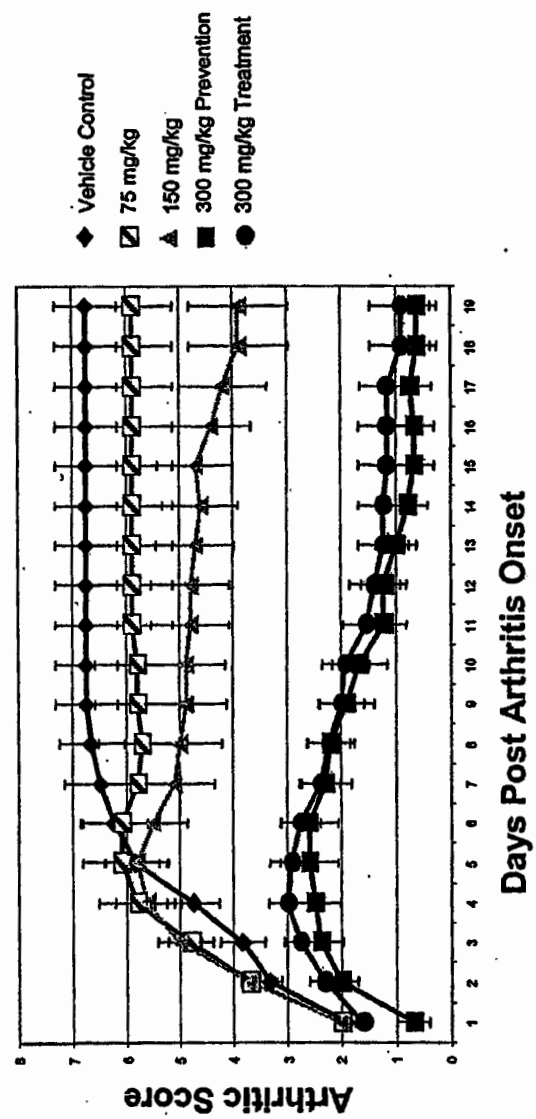

Vehicle Control
75 mg/kg
150 mg/kg
300 mg/kg Prevention
300 mg/kg Treatment
Arthritic Score
Days Post Arthritis Onset

FIG. 15

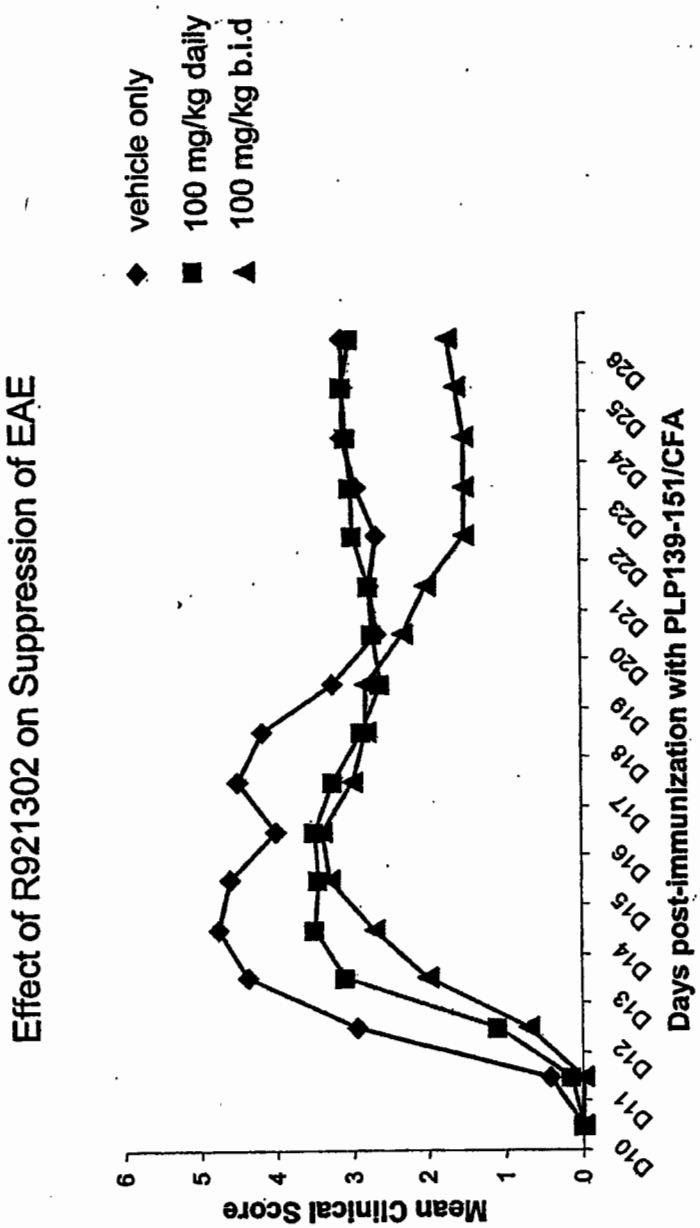
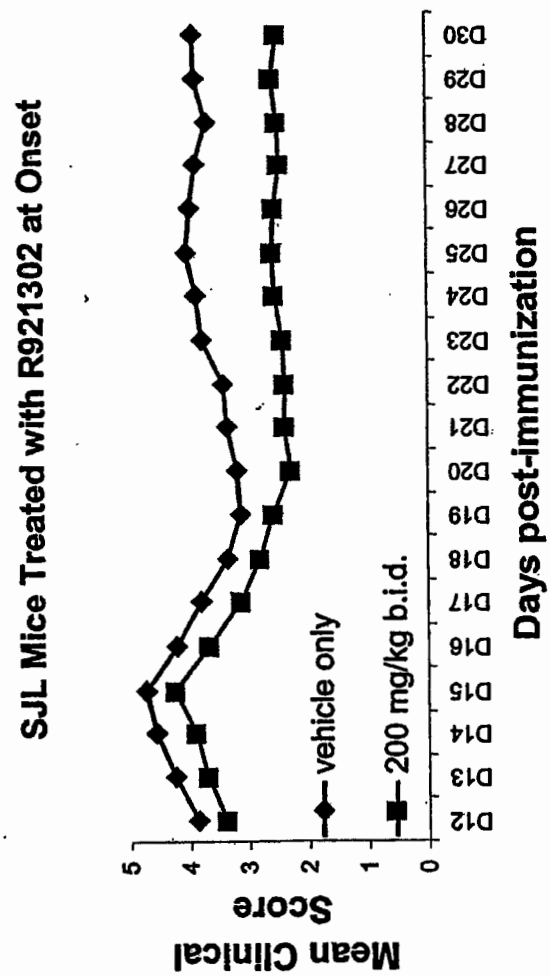

Effect of R921302 on Suppression of EAE
vehicle only
100 mg/kg daily
100 mg/kg b.i.d
Mean Clinical Score
0
1
2
3
4
5
6
D10
D11
D12
D13
D14
D15
D16
D17
D18
D19
D20
D21
D22
D23
D24
D25
D28
Days post-immunization with PLP139-151/CFA

FIG. 16


SJL Mice Treated with R921302 at Onset
Mean Clinical Score
0
1
2
3
4
5
vehicle only
200 mg/kg b.i.d.
D12
D13
D14
D15
D16
D17
D18
D19
D20
D21
D22
D23
D24
D25
D26
D27
D28
D29
D30
Days post-immunization