Supplemental Material

for

Identification of Novel Small-Molecule Agonists for Human Formyl Peptide Receptors and Pharmacophore Models of their Recognition

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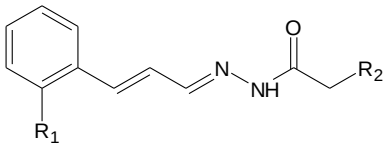
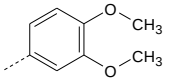
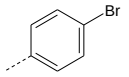
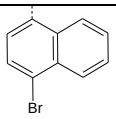
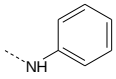
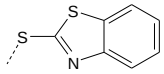
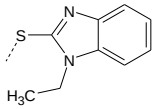
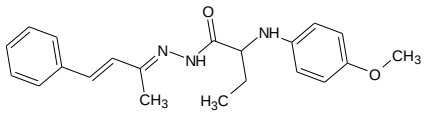
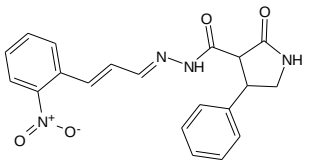
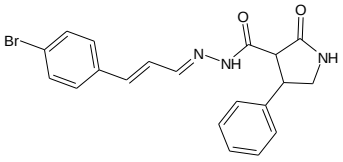
Supplemental Table S1. Structure and Activity of Acetohydrazide Derivatives

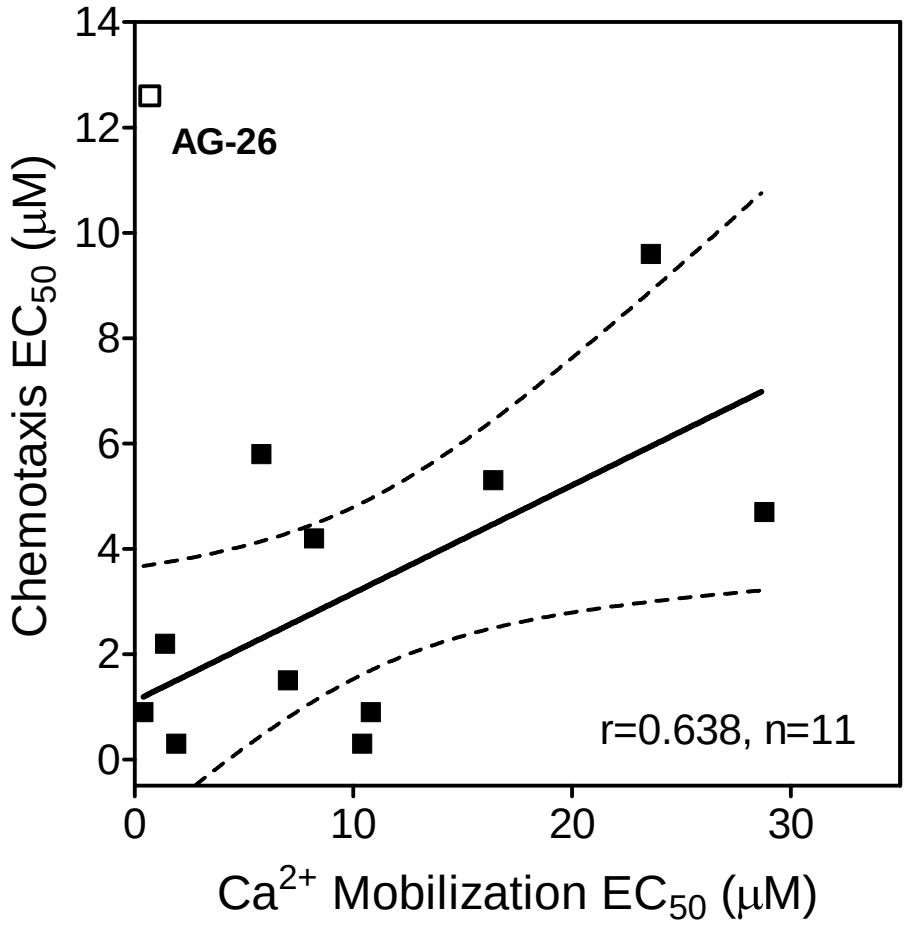


#	R_1	R_2	Ca^{2+} Mobilization EC_{50} (μM) and Efficacy (%)[a]				
			FPR1		FPR2		PMN
			RBL	HL60	RBL	HL-60	
AG-09/92	NO_2		N.A.	N.A.	6.0 (30)	1.6 (20)	3.7 (80)
AG-09/93	NO_2		N.A.	N.A.	2.9 (80)	2.9 (30)	2.2 (30)
AG-09/94	NO_2		N.A.	N.A.	N.A.	N.A.	N.A.
AG-09/95	NO_2		N.A.	N.A.	N.A.	N.A.	N.A.
AG-09/96	NO_2		N.A.	N.A.	17.2 (30)	14.3 (40)	35.4 (100)
AG-09/97	NO_2		N.A.	N.A.	N.A.	N.A.	N.A.
AG-09/98	NO_2		N.A.	N.A.	N.A.	N.A.	17.0 (110)
AG-09/99	-O-CH_3		N.A.	N.A.	N.A.	N.A.	N.A.
AG-09/100	NO_2		N.A.	N.A.	N.A.	N.A.	N.A.
AG-09/101			N.A.	N.A.	3.9 (95)	2.6 (105)	1.1 (110)
AG-09/7			N.A.	N.A.	5.4 (70)	11.2 (50)	10.8 (35)
AG-09/102			N.A.	N.A.	N.A.	N.A.	N.A.

[a]Median effective concentration values (EC_{50}) were determined by nonlinear regression analysis of the dose-response curves (5-6 points) generated using GraphPad Prism 5 with 95% confidential interval (p<0.05). Efficacy (in parentheses) is expressed as % of the response induced by 5 nM fMLF (FPR1) or 5 nM WKYMVm (FPR2). N.A., very low response (efficacy <20% of positive control) or no activity (no Ca^{2+} flux response was observed during the 3 min after addition of compounds under investigation).

Supplemental Figure S1

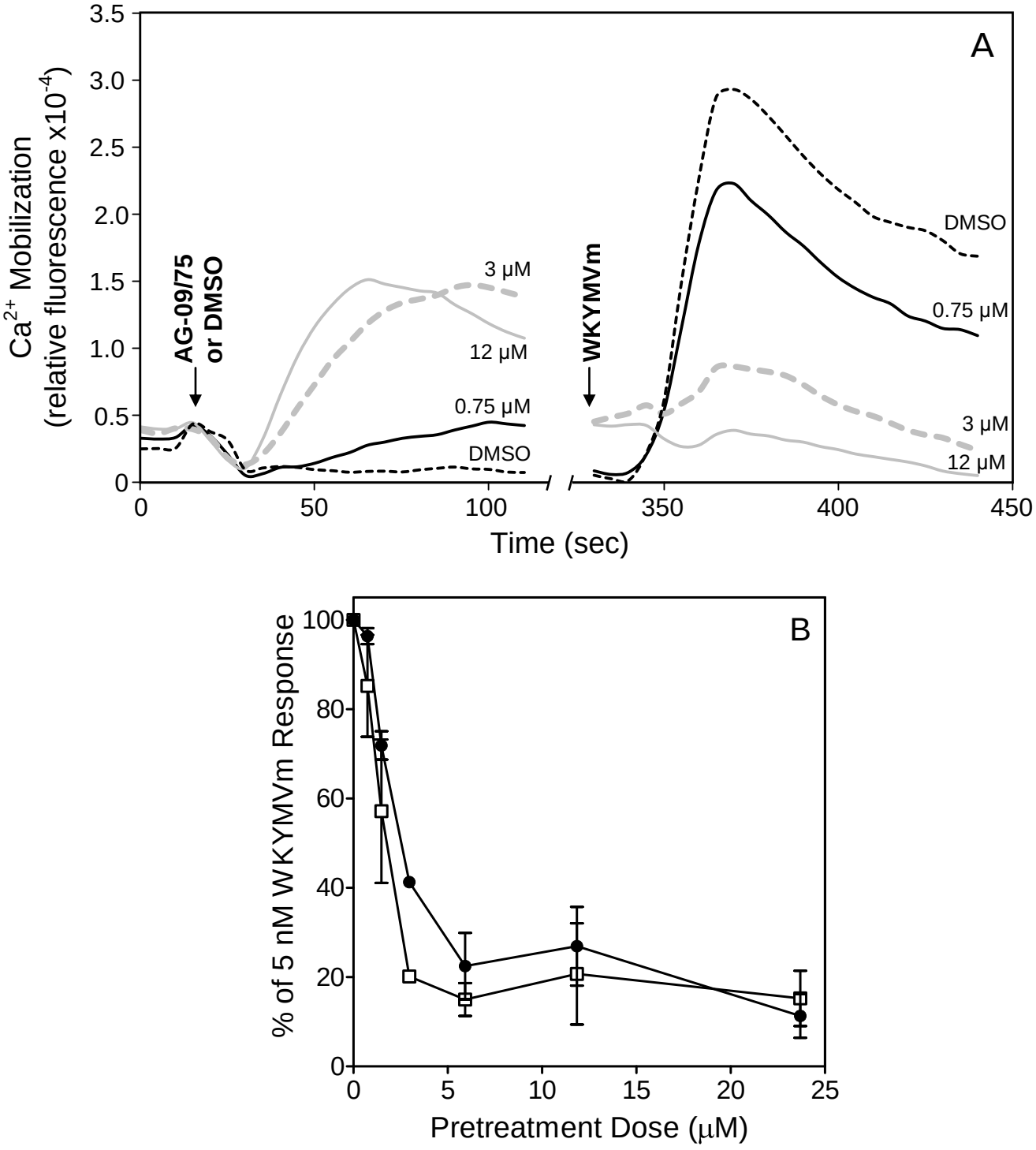
Correlation of Ca^{2+} mobilization and Chemotaxis in Human Neutrophils Treated with the Selected FPR1/FPR2 Agonists



Legend: EC_{50} values for Ca^{2+} mobilization in human neutrophils were plotted versus EC_{50} values for chemotactic activity in human neutrophils for the selected compounds (see Figure 1 and Table 1). Compound **AG-26** was omitted from the regression calculation and is shown as outlier. Dashed lines indicate area of the 95% confidence band.

Supplemental Figure S2

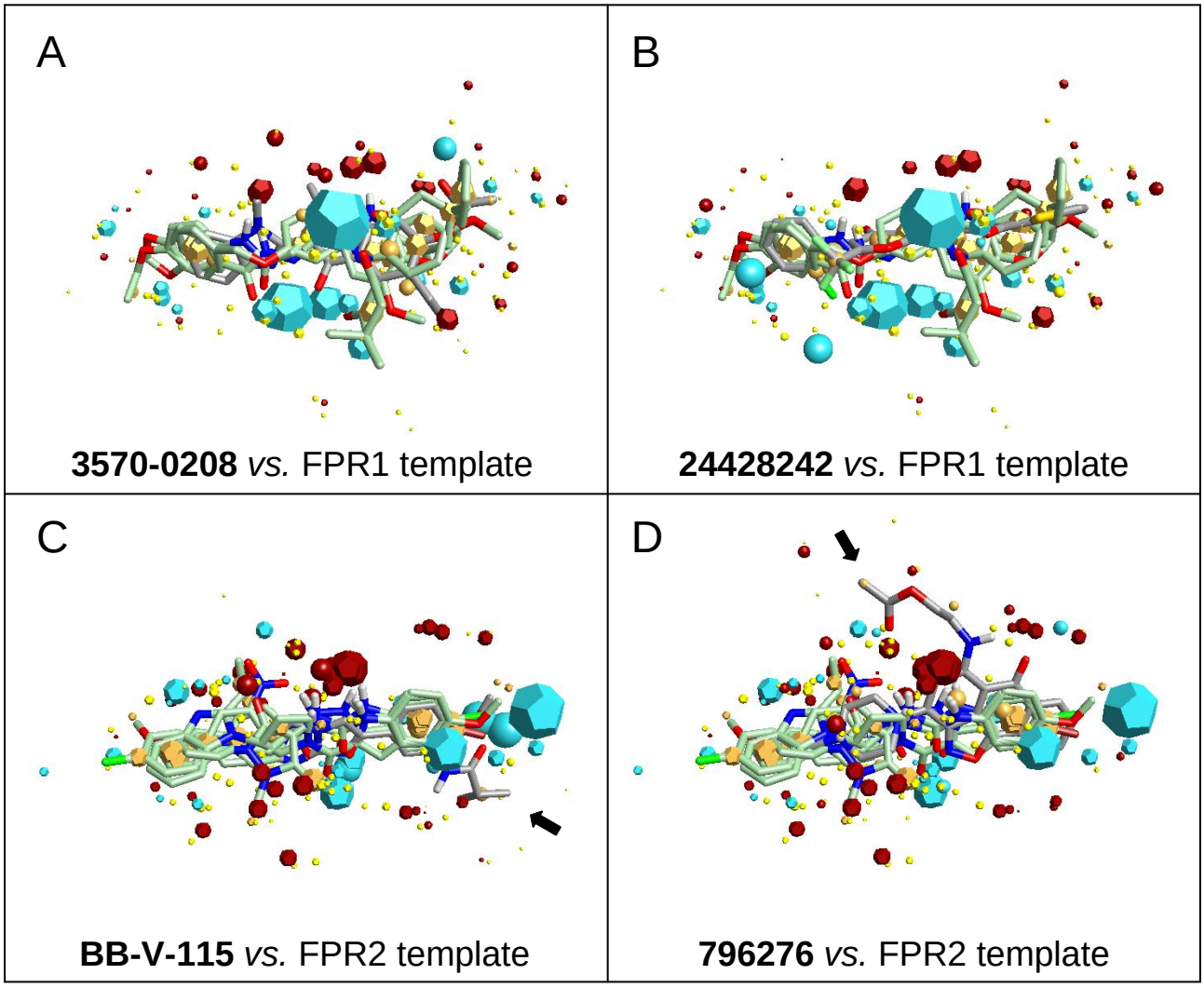
Desensitization of FPR2 Response by Low Efficacy Agonists



Legend: **Panel A.** HL-60 FPR2 cells were loaded with Fluo-4AM dye and pretreated with 0.75, 3, or 12 µM **AG-09/75** or vehicle (DMSO), and calcium flux was monitored. The same wells were then treated with 5 nM WKYMVm, and calcium flux was monitored following this second treatment. **Panel B.** HL-60 FPR2 cells were loaded with Fluo-4AM dye and pretreated for 5 minutes with the indicated concentrations of **AG-09/75** (□) and **AG-09/76** (●). Control cells were pretreated with DMSO. Following pretreatment, 5 nM WKYMVm was added, and calcium flux was monitored as described. The data are presented as mean ± S.D. of duplicate samples. In both panels, the data are representative of three experiments.

Supplemental Figure S3

The Best Overlays of Antagonists on FPR1 and FPR2 Templates



3570-0208 *vs.* FPR1 template

24428242 *vs.* FPR1 template

BB-V-115 *vs.* FPR2 template

796276 *vs.* FPR2 template

Legend: Overlay of **3570-0208** (panel A) and **24428242** (panel B) on the FPR1 template and **BB-V-115** (panel C) and **796276** (panel B) on the FPR2 template. Field points of the FPR1/FPR2 templates are shown by polyhedra, field points of antagonist molecules are shown by spheres, and inhibitor conformations are depicted with grey skeletons. Field points are colored as follows: blue = electron-rich (negative); red = electron-deficient (positive); yellow = van der Waals attractive (steric); and orange = hydrophobic. Arrows indicate fragments of antagonist molecules that don't overlap with the agonist template.