

Supplemental Material

for

High-throughput Screening for Small-molecule Activators of Neutrophils: Identification of Novel *N*-Formyl Peptide Receptor Agonists

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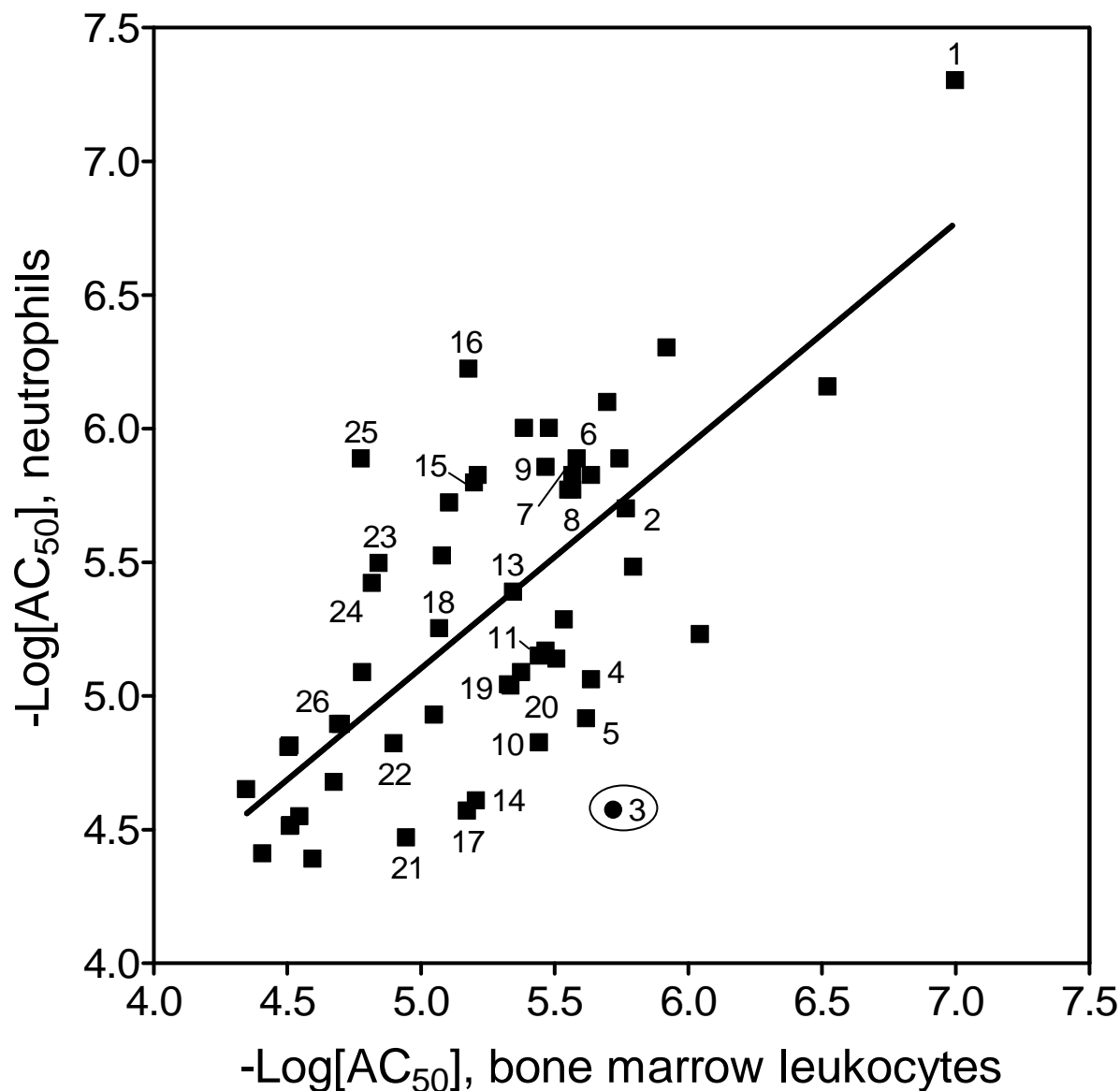
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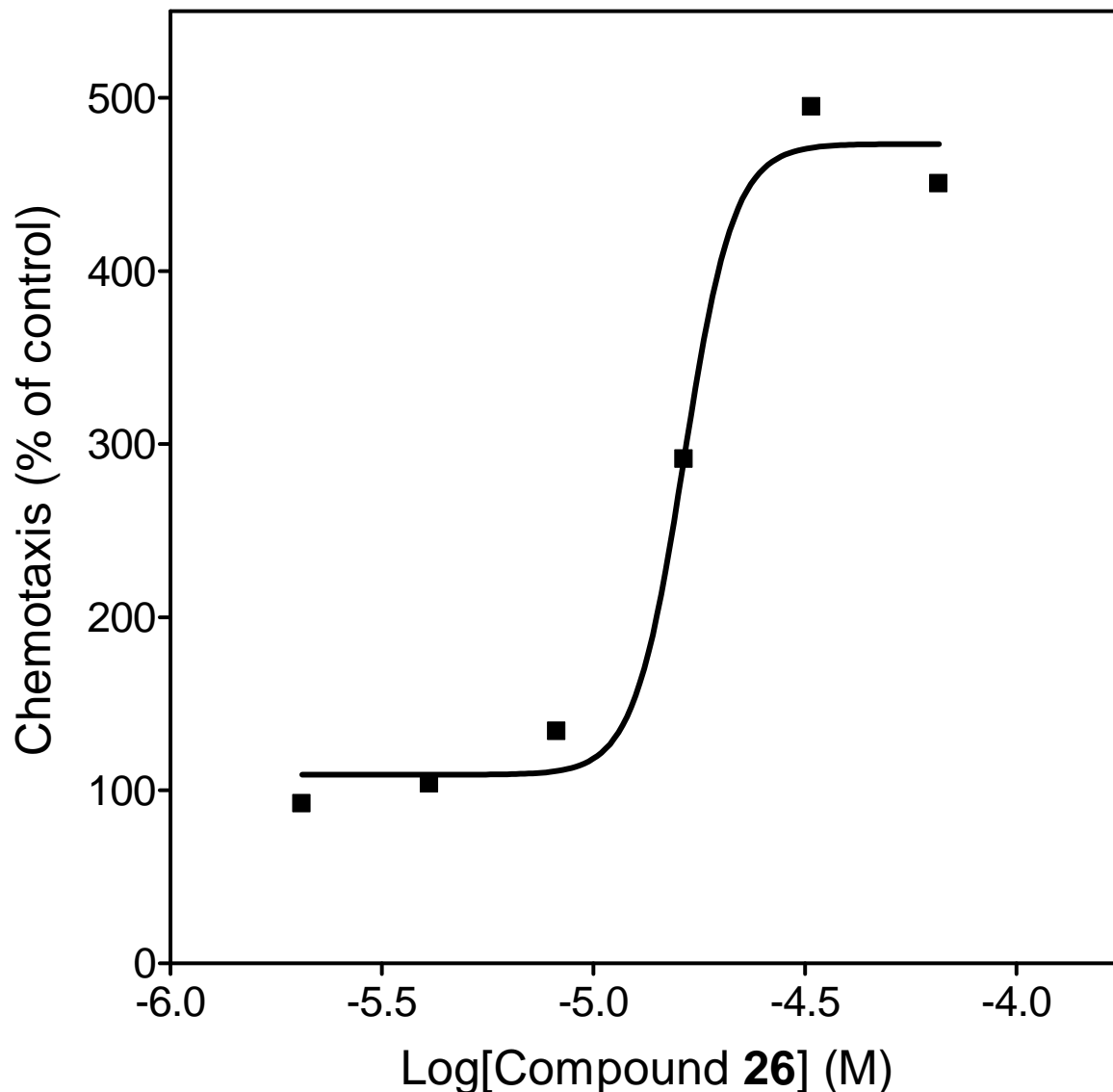
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Figure S1. Comparison of Test Compound Activity in Murine Bone Marrow Leukocytes Versus Isolated Murine Neutrophils



Cells were treated with a range of test compounds, and ROS production was measured to determine AC₅₀ values for each of the 56 hit compounds in murine bone marrow leukocytes and purified murine neutrophils. A line indicating the best-fit linear regression is shown (excluding the outlier point for compound 3). Compound numbers are shown for the 26 lead compounds selected for further analysis.

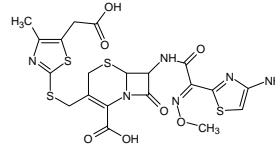
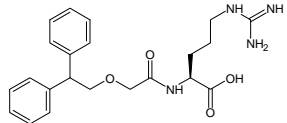
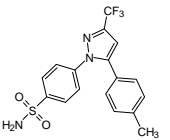
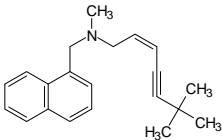
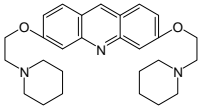
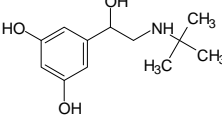
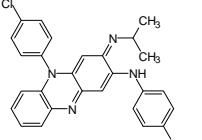
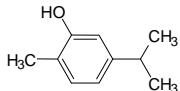
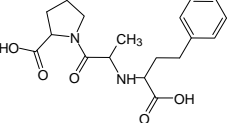
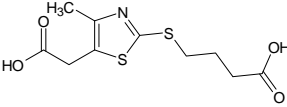
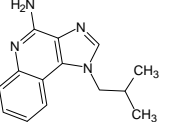
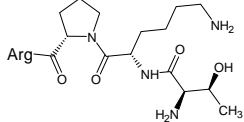
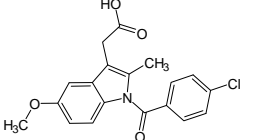
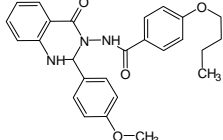
Figure S2. Representative Example of Human Neutrophil Chemotactic Response to a Test Compound



Human neutrophil chemotaxis towards the indicated concentrations of compound **26** was determined, as described under Materials and Methods. Note that higher concentrations of test compounds resulted in inhibition of chemotaxis. However, we did not include these data points due to potential for non-specific effects and issues of compound solubility. A representative experiment from three independent experiments is shown.

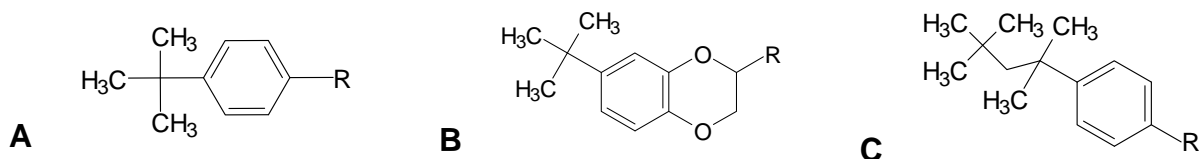
Table S1. Synthetic and Natural Small-molecule Compounds Previously Reported to Activate Phagocytes.

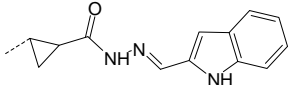
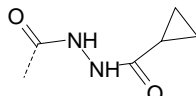
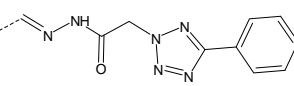
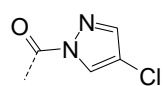
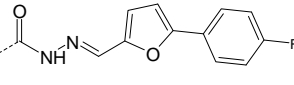
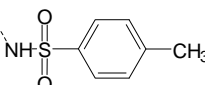
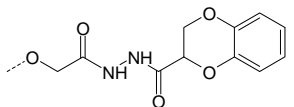
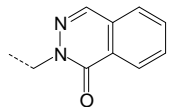
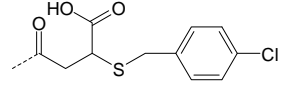
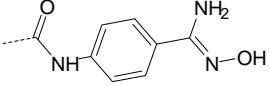
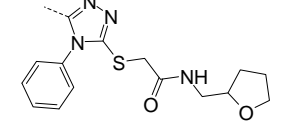
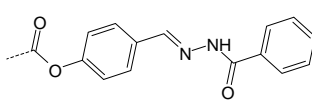
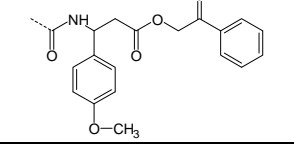
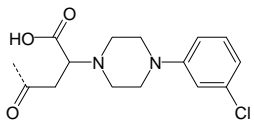
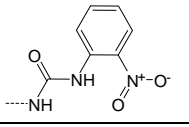
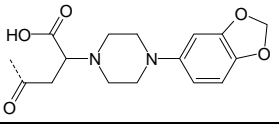
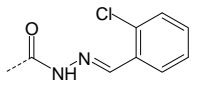
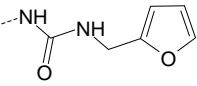
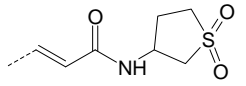
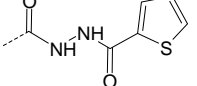
Compound name (drug class)	Structure	Mechanism and/or stimulation effect (cell type)	Ref.	Compound name (drug class)	Structure	Mechanism and/or stimulation effect (cell type)	Ref.
Amorolfin (antimycotic drug)		Phagocytosis (human PMN)	(26)	Kazinol B (plant derived COX-2 inhibitor)		PTX-insensitive, $\uparrow[\text{Ca}^{2+}]_i$, PKC & ROS production (rat PMN)	(34,35)
Anisomycin (antibiotic)		\uparrow p38 MAP kinase, degranulation (human PMN)	(13)	m-3M3FBS (nonspecific activator of PLC)		\uparrow PLC, ROS production (human PMN)	(1,15)
Artocarpol A (natural phenolic compound)		\uparrow PI3K, PLC & PKC, ROS production (rat PMN)	(16,17)	MK-886 (inhibitor of 5-LPO activating protein)		ROS production (human PMN)	(11)
Azimexon (synthetic immunoadjuvant)		ROS production (murine PMN)	(27)	No name (agonist of C5a receptor)		IP ₃ production (COS 7 ^a)	(4)
Benoxaprofen (mixed COX/5-LPO inhibitor)		\uparrow PKC, ROS production (human PMN)	(32)	Norfloxacin (antibiotic)		\uparrow p47-phox translocation, ROS production (murine M ϕ)	(6)
Bestatin (inhibitor of aminopeptidase N)		Phagocytosis (human PMN)	(20)	Pyriothioxine (neurotropic agent)		\uparrow [cGMP], chemotaxis (rabbit PMN)	(8)
Cannabidiol (plant-derived cannabinoid)		ROS production (human PMN)	(22)	Pidotimod (synthetic immunoadjuvant)		Phagocytosis. (human PMN)	(24)
Captopril (inhibitor of ACE)		G protein-dependent, \uparrow [cGMP] & degranulation (rabbit & human PMN)	(7,28)	Pro-Pro dipeptide (natural dipeptide)		\uparrow tyrosine kinase, priming of ROS production (human PMN)	(29,36)

Cefodizime (antibiotic)		Phagocytosis & ROS production (human PMN)	(3,25)	SB 290157 (agonist of C3a receptor)		[Ca ²⁺] _i (RBL cells ^b)	(19)
Celecoxib (selective COX-2 inhibitor)		G-protein- dependent, ↑[Ca ²⁺] _i & PKC, ROS production (human PMN)	(5)	Terbinafine (inhibitor of squalene epoxidase)		↑[Ca ²⁺] _i , priming of ROS production (human PMN)	(2,31)
CL 246,738 (synthetic immunoajuvant)		Cytostatic activity (murine Mφ)	(33)	Terbutaline (β ₂ - receptor agonist)		ROS production & chemotaxis (human PMN)	(18)
Clofazimine (antibiotic)		↑PLA ₂ , priming of ROS production (human PMN)	(14,37)	Thymol (plant- derived antimicrobial agent)		ROS production (guinea pig PMN)	(30)
Enalaprilat (inhibitor of ACE)		Degranulation (human PMN)	(21)	Tiprotimod [HBW 538] (synthetic immunoajuvant)		ROS production (human PMN)	(25)
Imiquimod (synthetic immunoajuvant)		Production of IFN-α, IL-12 & TNF-α (human PMN)	(10,12)	Tuftsins (natural IgG- derived peptide)		Phagocytosis (human PMN)	(28)
Indomethacin (non-selective COX inhibitor)		↑[Ca ²⁺] _i & PKC, ROS & MPO release (human PMN)	(9)	Quin-C1 (FPRL1 agonist)		↑chemotaxis and degranulation (human PMN)	(23)

PMN, polymorphonuclear leukocytes; Mφ, macrophages; ACE, angiotensin converting enzyme; COX, cyclooxygenase; LPO, lipoxygenase; PLC, phospholipase C. ^aAssays were performed on COS-7 cells transfected with the C5a receptor. ^bAssays were performed on RBL cells transfected with the C3a receptor. References (Ref.) are listed below in this Supplement.

Table S2. Effect of t-Butyl-benzene Derivatives (n=42) on ROS Production in Bone Marrow Leukocytes and Isolated Murine and Human Neutrophils.



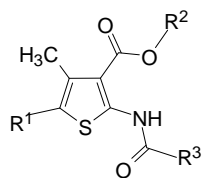
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			BM	PMN					BM	PMN	
				M	H					M	H
2	A		1.7	2.0	4.9	42	A		NA	NA	NA
19	A		8.6	12.0	17.3	43	A		NA	NA	NA
20	B		8.9	11.8	NA	44	A		NA	NA	NA
21	C		11.3	34.0	NA	45	A		NA	NA	NA
23	A		14.3	3.2	3.2	46	A		NA	NA	NA
25	A		16.7	1.3	NA	47	A		NA	NA	NA
27	A		21.1	21.1	10.6	48	A		NA	NA	NA
28	A		24.0	12.1	NA	49	A		NA	NA	NA
29	B		27.0	6.7	NA	50	A		NA	NA	NA
30	A		29.3	15.6	NA	51	A		NA	NA	NA

31	A		31.0	30.8	NA	52	A		NA	NA	NA
32	A		39.0	39.0	NA	53	A		NA	NA	NA
33	A		NA	13.3	13.3	54	B		NA	NA	NA
34	A		NA	13.7	NA	55	B		NA	NA	NA
35	A		NA	13.8	NA	56	A		NA	NA	NA
36	A		NA	14.9	NA	57	A		NA	NA	NA
37	A		NA	NA	6.4	58	A		NA	NA	NA
38	A		NA	NA	NA	59	A		NA	NA	NA
39	A		NA	NA	NA	60	A		NA	NA	NA
40	A		NA	NA	NA	61	A		NA	NA	NA
41	A		NA	NA	NA	62	A		NA	NA	NA

Compound activation of ROS production is indicated for bone marrow leukocytes (**BM**), murine neutrophils (**M PMN**), and human neutrophils (**H PMN**). **NA** indicates cell activation was <50% of control level over a concentration range of 0-40 μ M.

Table S3. Effect of Thiophene-2-amide-3-carboxylic Ester Derivatives (n=47) on ROS Production in Bone Marrow Leukocytes and Isolated Murine Neutrophils.

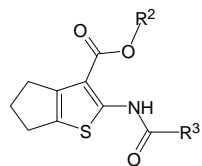
A



N	R ¹	R ²	R ³	AC ₅₀ (μM)		N	R ¹	R ²	R ³	AC ₅₀ (μM)	
				BM	PMN					BM	PMN
6	CH ₃	CH ₂ CH ₃		2.6	1.3	70		CH ₂ CH ₃		3.7	NA
7	CH ₃	CH ₂ CH ₃		2.7	1.7	71	CH ₃	CH ₂ CH ₃		NA	NA
8	CH ₃	CH ₂ CH ₃		2.8	1.7	72	CH ₃	CH ₂ CH ₃		NA	NA
63	CH ₃	CH ₃		1.3	2.1	73	CH ₃	CH ₂ CH ₃		NA	NA
64	CH ₃	CH ₂ CH ₃		1.4	1.2	74		CH ₃		NA	NA
65		CH ₃	CH ₃	1.7	NA	75		CH ₂ CH ₃		NA	NA

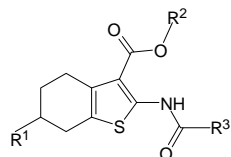
66		CH ₂ CH ₃		1.8	1.5	76		CH ₂ CH ₃		NA	NA
67		CH ₃	CH ₂ -CH ₃	7.6	2.6	77		CH ₂ CH ₃		NA	NA
68	CH ₃	CH ₂ CH ₃		2.7	NA	78		CH ₂ CH ₃		NA	NA
69		CH ₂ CH ₃		5.1	NA	79		CH ₂ CH ₃		NA	NA

B



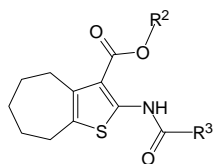
N	R ²	R ³	AC ₅₀ (μM)		N	R ²	R ³	AC ₅₀ (μM)	
			BM	PMN				BM	PMN
80	CH ₂ CH ₃		2.9	NA	84	CH ₂ CH ₃		NA	NA
81	CH ₃		3.6	NA	85	CH ₂ CH ₃		NA	NA
82	CH ₃		4.3	NA	86	CH ₂ CH ₃		NA	NA
83	CH ₃		NA	NA					

C



N	R ¹	R ²	R ³	AC ₅₀ (μM)		N	R ¹	R ²	R ³	AC ₅₀ (μM)	
				BM	PMN					BM	PMN
15	H			6.3	1.6	91	H	CH ₃		7.2	1.6
						92	CH ₃	CH ₂ CH ₃		3.7	NA
16	t-butyl	CH ₂ CH ₃		6.6	0.6	93	CH ₂ CH ₃	CH ₂ CH ₃		5.1	NA
87	t-butyl	CH ₂ CH ₃		2.6	1.3	94	H	CH ₃		NA	NA
88	CH ₃	CH ₃		0.1	2.3	95	CH ₃			NA	NA
89	H	CH ₂ CH ₃		17.6	NA	96	H	CH ₂ CH ₃		NA	NA
90	H	CH ₂ CH ₃		3.8	2.7	97	t-butyl	CH ₂ CH ₃		NA	NA

D



N	R ²	R ³	AC ₅₀ (μM)		N	R ²	R ³	AC ₅₀ (μM)	
			BM	PMN				BM	PMN
1	CH ₂ CH ₃		0.1	0.05	98	CH ₂ CH ₃		0.6	3.4

N	Structure	AC ₅₀ (μM)		N	Structure	AC ₅₀ (μM)	
		BM	PMN			BM	PMN
99		2.7	2.5	102		NA	NA
100		1.3	NA	103		NA	NA
101		NA	NA			NA	NA

Compound activation of ROS production is indicated for bone marrow leukocytes (**BM**) and murine neutrophils (**PMN**). **NA** indicates cell activation was <50% of control level over a concentration range of 0-20 μM.

References for the Table S1

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