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Tetrahydro-4-quinolinamines identified as novel $P2Y_1$ receptor antagonists

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ABSTRACT

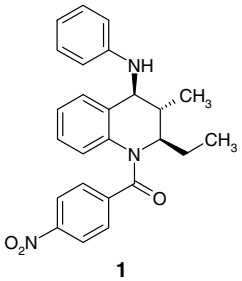
High-throughput screening of the GSK compound collection against the $P2Y_1$ receptor identified a novel series of tetrahydro-4-quinolinamine antagonists. Optimal substitution around the piperidine group was pivotal for ensuring activity. An exemplar analog from this series was shown to inhibit platelet aggregation.

Adenosine diphosphate (ADP) is a key activator of platelets and plays a central role in hemostasis and thrombosis. ADP activates platelets through interactions with two G-protein coupled P2 receptors, $P2Y_1$ and $P2Y_{12}$, producing two separate intracellular signals which synergize together to produce complete platelet activation.[1] Activation of the Gq-coupled $P2Y_1$ receptor leads to rapid Ca^{2+} entry and mobilization of intracellular Ca^{2+} stores resulting in platelet shape change and weak, transient aggregation. Activation of the Gi-coupled $P2Y_{12}$ receptor leads to the inhibition of cAMP production, resulting in the amplification of the platelet response and complete, irreversible aggregation.

The $P2Y_1$ receptor plays an integral role in thrombosis as evidenced by studies utilizing transgenic mice ($P2Y_1^{-/-}$), as well as rodents treated with nucleotide $P2Y_1$ antagonists (such as MRS2179 and MRS2500).[2] In both types of studies, ADP-induced platelet shape change, Ca^{2+} mobilization, and aggregation was abolished and the platelet response to other agonists was impaired.[3] Further, these animals were protected in models of systemic vascular thromboembolism as well as models of localized arterial and venous thrombosis.[3,4]

Taken together, these data suggest that a $P2Y_1$ antagonist could have significant utility in the treatment of a variety of thrombotic disorders. To date, there are few publications which describe selective, non-nucleotide, small molecule antagonists of $P2Y_1$,[5] therefore, an effort to discover such molecules was initiated.

High-throughput screening of the GSK compound collection in a FLIPR-based, HEK-293 cellular assay[8] identified tetrahydro-4-quinolinamine **1** as an antagonist of $P2Y_1$ with low micromolar activity (Fig. 1). Compound **1** also demonstrated low micromolar affinity in



1

FLIPR IC_{50} = 1.6 μM
Binding K_i = 0.5 μM

Figure 1. $P2Y_1$ antagonist, **1**, high-throughput screening hit.

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a radioligand ([^{33}P]-2-SMe-ADP) binding assay[8] using U2OS cell membranes expressing recombinant human $P2Y_1$ receptor.

Given the moderate potency of this new tetrahydroquinoline hit, early lead optimization chemistry efforts focused on SAR studies and confirmation of functional activity in a human platelet aggregation assay.

The synthesis of the *p*-nitro-aniline screening hit **1** was achieved following the sequence outlined in Scheme 1. A tandem condensation/cyclization of aniline and propionaldehyde resulted in the formation of *N*-phenyl-1,2,3,4-tetrahydro-4-quinolinamine **4** as a single diastereomer.[6] Quinolamine **4** was treated with excess *p*-nitrobenzoyl chloride and polymer-bounded *N*-methylmorpholine to afford the tetrahydroquinoline **1**.

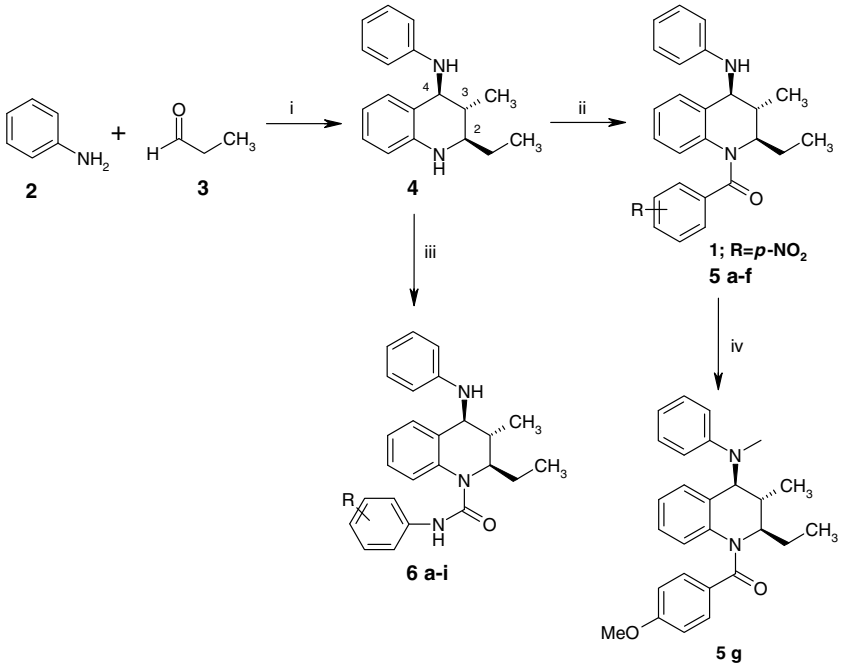
The efficient preparation of intermediate **4** facilitated rapid analoging and SAR studies around the benzamide moeity. Tetrahydroquinoline **4** was treated with commercially available benzoyl chlorides in the presence of polymer-bounded *N*-methylmorpholine to provide amides **5a–f** (Scheme 1). In the case of the 4-methoxy-benzamide analog **5b**, the secondary aniline was methylated by treatment with sodium hydride, followed by methyl iodide to provide **5g**. In addition, replacement of the amide group with urea was also investigated. Intermediate **4** was treated with commercially available aryl isocyanates in the presence of triethylamine to produce phenylureas **6a–i**.

Attempts were made to reduce the structural complexity around the piperidine ring by eliminating chiral centers. Scheme 2 illustrates the synthetic routes to the *des*-3-methyl analogs **9**/**10**, the *des*-2-ethyl-analog **13**, and tetrahydroquinoline **15** in which the 2 and 3 positions are unsubstituted. Ethyl lithium addition to quinoline **7**, followed by acylation of the resulting amine produced the corresponding 1,2-dihydroquinoline. Hydrobromination of the olefin, followed by dehalogenation of the bromohydrin, led to the formation of alcohol **8** as a single diastereomer. Treatment of the alcohol with iodotrimethylsilane provided an iodide intermediate which was rapidly treated with aniline under basic conditions to afford diastereomers **9** and **10**. The diastereomeric mixture was easily separated by reverse phase HPLC to cleanly provide the *cis* (**9**) and *trans* (**10**) C_2–C_4 isomers. Two dimensional NMR (nOe) studies highlighting the methine hydrogens confirmed the relative stereochemistry for analogs **9** and **10**. The synthesis of analog **13** was initiated via the acylation of dihydroquinolinone **11**. α-Alkylation and stereoselective reduction of ketone **11** produced quinoline **12**. Conversion of the cyclic benzylic alcohol to an azide using diphenylphosphoryl azide (DPPA), followed by reduction to the amine, and cross-coupling with phenyl bromide, produced tetrahydroquinoline **13**. Unsubstituted tetrahydroquinoline **15** was obtained in two steps from dihydroquinolinone **11**. N-Acylation followed by $TiCl_4$ mediated reductive amination with aniline provided target **15**.

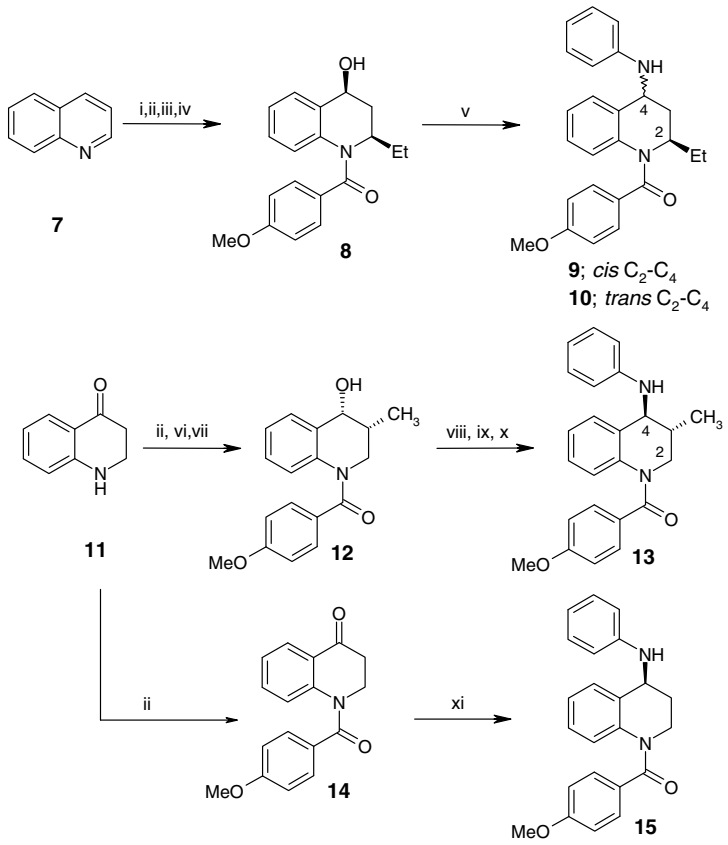
Given the potential for nitroarenes to generate reactive metabolites, lead optimization efforts focused on replacing this functionality in lead compound **1**. The SAR around the 4-nitro-phenyl substituent is summarized in Table 1. The 4-methoxy-phenyl, 4-chloro-phenyl, 4-trifluoromethoxy-phenyl, and 4-bromo-phenyl analogs (**5b**, **5c**, **5d**, and **5e**) were found to be suitable replacements for nitrophenyl, with all demonstrating slightly improved potency and binding affinity over compound **1**. Removal of the *para*-substituent resulted in a complete loss in activity as evidenced by analogs **5a** and **5f**.

Phenyl ureas **6a–i** demonstrated comparable activity to the benzamides (Table 2). In contrast to the benzamide series, substitution at the *meta*-position consistently offered the greatest potency. 3-Bromo-phenyl (**6f**), 3-chloro-phenyl (**6b**), and 3-methoxy-phenyl (**6d**) all demonstrated comparable potency to lead compound **1**. Interestingly, the unsubstituted phenyl analog, **6g**, offered similar potency to the *meta*-substituted analogs. The 2-chloro-phenyl analog, **6a**, was found to be inactive and the activity of the 4-methoxy-phenyl analog (**6e**) was attenuated relative to the 3-methoxy-analog (**6d**). 3,5-Disubstitution of the phenyl group resulted in good potency, as evidenced by **6h** and **6i**.

SAR exploration around the piperidine group focused on the various alkyl substituents as well as their relative stereochemistry (Table 3). Removal of the methyl group at C_3 led to a slight decrease in potency for the *cis* isomer **9**. There exists a strong preference for the C_2–C_4 relative stereochemistry to be *cis* (**9**) based on the loss of activity observed for the *trans* isomer (**10**). The ethyl group at C_2 also proved to be important for activity as evidenced by the 10-fold decrease in potency observed for analog **13**. Not



Scheme 1. Reagents and conditions: (i) EtOH, rt; (ii) a—R-Ph-COCl, PS-NMM, CH_2Cl_2, rt; b—PS-trisamine; (iii) R-Ph-NCO, NEt_3, CH_2Cl_2, rt; (iv) NaH, MeI, DMSO, 150 °C μwave, 5 min.



Scheme 2. Reagents and conditions: (i) EtLi, THF, −78 °C, 15 min; (ii) 4-MeO-Ph-COCl, NMM, CH_2Cl_2, rt; (iii) NBS, DMSO/H_2O, rt; (iv) AIBN (5 mol%), Bu_3SnH, toluene, 80 °C; (v) a—TMSI, CH_2Cl_2, 0 °C; b—aniline, Ba_2CO_3, THF, rt; (vi) LHMDS, THF, −78 °C, then MeI; (vii) L-selectride, THF, −78 °C; (viii) DPPA, DBU (5 mol%), CH_2Cl_2, 0 °C to rt; (ix) PPh_3, THF/H_2O, 70 °C; (x) Ph-Br, $Pd_2(dba)_3$ (3 mol%), NaO*t*-Bu, X-PHOS (6 mol%), *t*-BuOH, 120 °C μwave, 10 min; (xi) a—aniline, $TiCl_4$, CH_2Cl_2; b—$NaBH_4$, MeOH.

Table 1
SAR of endocyclic amides **1** and **5a–f**

Compound	R	FLIPR IC_{50}[a] (μM)	$P2Y_1$ K_i[a] (μM)
1	4-NO_2	1.6	0.5
5a	3-OCF_3	>25	ND
5b	4-OMe	0.8	0.4
5c	4-Cl	1.3	0.4
5d	4-OCF_3	1.0	0.1
5e	4-Br	0.8	0.1
5f	H	25	ND

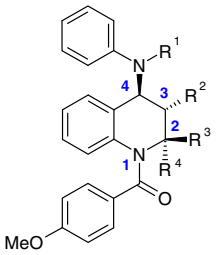
ND, not determined.
[a] Values are means of at least three determinations with a standard deviation ⩽ 0.3 log units.

Table 2
SAR of endocyclic phenyl ureas **6a–i**

Compound	R	FLIPR IC_{50}[a] (μM)	$P2Y_1$ K_i[a] (μM)
6a	2-Cl	>25	ND
6b	3-Cl	1.0	0.3
6c	4-Cl	2.0	0.3
6d	3-OMe	1.6	0.5
6e	4-OMe	6.0	ND
6f	3-Br	1.0	0.1
6g	H	1.6	0.4
6h	3,5-OMe	0.8	0.2
6i	3,5-Cl	0.6	0.07

ND, not determined.
[a] Values are means of at least three determinations with a standard deviation ⩽ 0.3 log units.

Table 3
Relative stereochemistry and substitution of the piperidine ring



Compound	R^1	R^2	R^3	R^4	FLIPR IC_{50}[a] (μM)	$P2Y_1$ K_i[a] (μM)
5b	H	CH_3	Et	H	0.8	0.4
5g	CH_3	CH_3	Et	H	>25	ND
9	H	H	Et	H	3.2	0.8
10	H	H	H	Et	>25	ND
13	H	CH_3	H	H	10	ND
15	H	H	H	H	>25	ND

ND, not determined.
[a] Values are means of at least three determinations with a standard deviation ⩽ 0.3 log units.

too surprisingly removal of both the 2-ethyl and 3-methyl groups led to a loss of activity as evidenced by analog **15**. Methylation of the aniline nitrogen of **5b** also led to a complete loss in activity (analog **5g**), suggesting that the aniline hydrogen may play a role in hydrogen bonding or in achieving a favorable binding conformation.

The importance of absolute stereochemistry on $P2Y_1$ activity is highlighted in Table 4. Separation of enantiomers for racemate **6i**

Table 4
Data for individual enantiomers of analog **6i**

Compound	Absolute stereochemistry (% purity)	FLIPR IC_{50}[a] (nM)	$P2Y_1$ K_i[a] (nM)	Inhibition of platelet aggregation IC_{50}[b] (nM)
16	2*R*,3*S*,4*S* (99.5)	600	70	504 ± 103
17	2*S*,3*R*,4*R* (97.8)	>25,000	ND	ND

ND, not determined.
[a] Values are means of at least three determinations with a standard deviation ⩽ 0.3 log units.
[b] Values are means ± SEM for $n = 3$ independent donors.

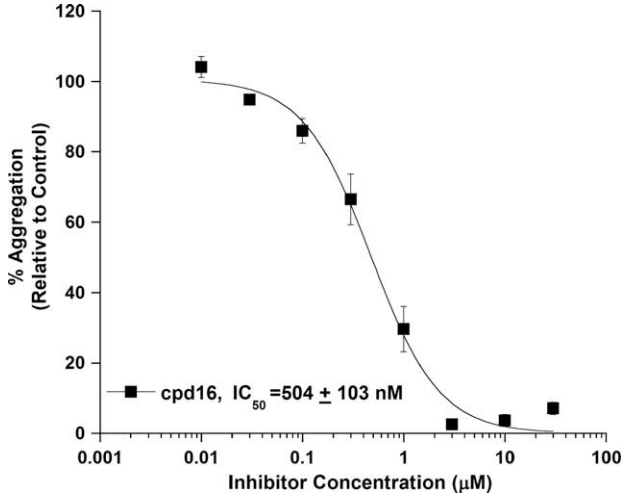


Figure 2. Inhibition of ADP-induced platelet aggregation by example **16**.

was carried out by chiral HPLC. Assignment of absolute configuration was determined by vibrational circular dichroism (VCD). All $P2Y_1$ activity is derived from a single enantiomer, as the 2*R*, 3*S*, 4*S* (**16**) isomer was active in all assays tested, and the 2*S*, 3*R*, 4*R* (**17**) isomer was inactive in the primary FLIPR screen.

The functional activity of compound **16** was subsequently evaluated in a platelet aggregation assay. Human washed platelets were incubated with compound for 5 min prior to the addition of 10 μM ADP and aggregation was monitored by standard light transmittance aggregometry.[7] Compound **16** inhibited ADP-induced aggregation with an IC_{50} of 504 ± 103 nM (Fig. 2).

In summary, a new series of tetrahydroquinoline $P2Y_1$ antagonists have been identified. *para*-Substituted benzamides and *meta*-substituted phenylureas provided enhanced activity. Within the piperidine ring of the tetrahydroquinoline, the *cis* stereochemistry for the C_2–C_4 substituents, and the *trans* stereochemistry for the C_2–C_3 and C_3–C_4 substituents also enhanced potency. In addition, $P2Y_1$ activity appears to reside in a single enantiomer (2*R*,3*S*,4*S*). One of the most potent analogs identified, **16**, demonstrated functional activity in a human platelet aggregation assay. Thus far poor aqueous solubility has hindered pharmacokinetic studies with urea **16** and similar analogs. Therefore future lead optimization efforts to improve in vivo exposure will be necessary prior to the evaluation of tetrahydro-4-quinolamines in thrombosis models.

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8. Experimental procedure for the preparation of compound **16**.Preparation of (*rac*)-2-ethyl-3-methyl-*N*-phenyl-1,2,3,4-tetrahydro-4-quinolinamine (**4**): In a 250 mL round bottom flask, aniline (18.6 g, 200 mmol) was dissolved in absolute ethanol (50 mL). The solution was cooled to 0 °C. To this solution was added propionaldehyde (11.6 g, 200 mmol) dropwise. Once the addition was completed, the reaction was allowed to warm to room temperature and stirred overnight (~14 h). The resulting yellow precipitate was filtered, washed with cold ethanol, and then allowed to dry at RT (under vacuum) for 24 h. The title compound was obtained as a white solid (9.3 g, 35 mmol, 35% yield). ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δppm 7.16–7.23 (3H, m) 7.02 (1H, t, *J* = 7.6 Hz) 6.62–6.68 (3H, m) 6.61 (1H, d, *J* = 1.0 Hz) 6.51 (1H, dd, *J* = 8.0, 1.1 Hz) 4.31 (1H, t, *J* = 9.0 Hz) 3.78–3.86 (2H, m) 3.13 (1H, td, *J* = 7.8, 3.4 Hz) 1.84–1.91 (1H, m) 1.68–1.75 (1H, m) 1.57–1.63 (1H, m) 1.08 (3H, d, *J* = 6.8 Hz) 0.98 (3H, t, *J* = 7.4 Hz).
Preparation of (2*R*,3*S*,4*S*)-2-ethyl-3-methyl-*N*-[3,5-(chloro)phenyl]-4-(phenylamino)-3,4-dihydro-1(2*H*)-quinolinecarboxamide (**16**): In a 100 mL round bottom flask (*rac*)-2-ethyl-3-methyl-*N*-phenyl-1,2,3,4-tetrahydro-4-quinolinamine **4** (1 g, 3.76 mmol) was dissolved in dichloromethane (13 mL). To this solution was added 3,5-dichlorophenylisocyanate (0.85 g, 4.51 mmol) followed by triethylamine (785 μL, 5.64 mmol) at room temperature. The reaction mixture was stirred overnight (~14 h). The resulting mixture was diluted with dichloromethane (50 mL) and quenched with water (25 mL). The phases were separated, and the aqueous phase was back-extracted with dichloromethane twice (15 mL each). The combined organic phases were dried over $MgSO_4$ and purified by flash chromatography (ISCO, 40 g SiO_2 cartridge, 0–15% ethyl acetate/hexanes as the eluents). The fractions corresponding to (*rac*)-2-ethyl-3-methyl-*N*-[3,5-(chloro)phenyl]-4-(phenylamino)-3,4-dihydro-1(2*H*)-quinolinecarboxamide (**6i**) were combined and concentrated under reduced pressure to provide the title compound as the racemate (1.2 g, 2.65 mmol, 70% yield).
The racemate (0.67 g) was separated by chiral HPLC (SFC with 25% MeOH as the modifier solvent; 10 μm Chiralpak OD, 10 mm × 250 mm, 10 ml/min, UV at 280 nm).
R_t = 7.1 min for 2*R*,3*S*,4*S*-enantiomer (99.4% purity), and R_t = 9.0 min for 2*S*,3*R*,4*R*-enantiomer (97.8% purity).
The absolute configuration for the (2*R*,3*S*,4*S*)-enantiomer was assigned by vibrational circular dichorism (VCD) studies (Bomem Chiral IR VCD spectrometer at 4 cm^{-1}; frequency range = 2000–800 cm^{-1}; dual PEM calibrated at 1400 cm^{-1}; PEM1 = 0.250λ; PEM2 = 0.275λ; Scan Method: single block scan 180 min; CCl_4 as solvent; Concentration 10 mg/125 μL).
(2*R*,3*S*,4*S*)-2-Ethyl-3-methyl-*N*-[3,5-(chloro)phenyl]-4-(phenylamino)-3,4-dihydro-1(2*H*)-quinolinecarboxamide (**16**): LC/MS: (M+H) = 454.0; ^{1}H NMR (400 MHz, $CHCl_3$-*d*) δ ppm 7.39–7.44 (3H, m) 7.36 (1H, d, *J* = 7.6 Hz) 7.24–7.31 (3H, m) 7.17–7.23 (2H, m) 7.02 (1H, m) 6.97 (1H, br s) 6.74 (1H, t, *J* = 7.3 Hz) 6.62 (2H, d, *J* = 7.8 Hz) 4.41 (1H, dt, *J* = 7.8, 5.6 Hz) 3.99 (1H, d, *J* = 9.6 Hz) 1.65–1.72 (1H, m) 1.59 (1H, ddd, *J* = 13.6, 7.6, 5.6 Hz) 1.35–1.39 (1H, m) 1.30 (3H, d, *J* = 6.7 Hz) 0.92 (3H, t, *J* = 7.4 Hz).
Screening Assay Protocols.
$P2Y_1$ FLIPR: HEK-293 MSRII cells endogenously expressing $P2Y_1$ were maintained in DMEM/F12 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, 15 mM Hepes, and 1% penicillin–streptomycin at 37 °C in a humidified 5% CO_2 incubator. Cells were seeded at a density of 20,000 cells/well (384-well format) and cultured for 48 h prior to experiment. On the day of the experiment, growth media was removed and the cells were loaded with Calcium 3 dye from Molecular Devices in HBSS, pH 7.4 containing 2.5 mM probenecid for 1 h at 37 °C. The dye loaded cells were then incubated with compound for 10 min prior to challenge with an EC80

concentration of ADP (determined daily; typically 2–6 nM). Intracellular calcium fluxes were measured on a Fluorescence Imaging Plate Reader (FLIPR). Compound IC_{50} values were subsequently determined by non-linear regression analysis using Activity Base.

$P2Y_1$ Binding: Membranes were prepared from BacMam transduced U2OS cells expressing human $P2Y_1$. [^{33}P]-2-MeS-ADP was utilized as the radioligand. Binding reactions were performed in 96-well plates in a volume of 130 μL containing 150 pM [^{33}P]-2-SMe-ADP, 0.5 μg $hP2Y_1$ expressing U2OS cell membranes pre-bound to 0.5 mg of WGA-SPA (wheat germ agglutinin-coupled scintillation proximity assay) beads in 15 mM Hepes, 145 mM NaCl, 0.1 mM $MgCl_2$, 5 mM EDTA, 5 mM KCl binding buffer, and various concentrations of test compound or dimethylsulfoxide vehicle control. Reactions were allowed to proceed to completion at room temperature for 1 h. Following centrifugation (2000*g*), supernatants were counted on a Perkin-Elmer Topcounter.