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Tetrahydroquinoline derivatives as CRTH2 antagonists

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ABSTRACT

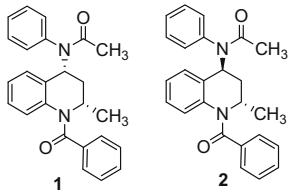
A series of tetrahydroquinoline-derived inhibitors of the CRTH2 receptor was discovered by a high throughput screen. Optimization of these compounds for potency and pharmacokinetic properties led to the discovery of potent and orally bioavailable CRTH2 antagonists.

CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells), also known as DP_2, is a G-protein coupled receptor related to the *N*-formyl peptide receptor (FPR) subfamily of chemoattractant receptors. Its endogenous ligand is prostaglandin D_2 (PGD_2). PGD_2 is the major cyclooxygenase product formed and secreted by activated mast cells during allergic reactions.[1–3] PGD_2 also signals through prostanoid D (DP or DP_1) receptor. The DP receptor is primarily expressed on airway epithelium, smooth muscle and platelets, while CRTH2 is selectively expressed on Th2 cells, T cytotoxic type 2 (Tc2) cells, eosinophils, and basophils.[4–6] Stimulation of CRTH2 by PGD_2 mediates multiple inflammatory responses, such as chemotaxis of eosinophils, basophils and Th2 cells, eosinophil activation and degranulation, cytokine production from Th2 T cells, and leukotriene production by mast cells.[7–13] Therefore, blockade of CRTH2 is likely to be beneficial in the treatment of allergic diseases triggered by PGD_2.

Several research groups, including ours, discovered that tetrahydroquinoline derivatives are potent CRTH2 antagonists.[14–19] These compounds were of special interest to us, because to our knowledge, it was the only series of CRTH2 antagonists devoid of a carboxylic acid moiety. Here we report the discovery, optimization and structure activity relationship (SAR) of the tetrahydroquinoline derivatives.

Tetrahydroquinoline **1** (Table 1), discovered in a high throughput screen, inhibited the binding of ^{3}H-PGD_2 to hCRTH2 receptors on 293 cells with an IC_{50} of 0.043 μM (Table 1).[20] Compound **1** also inhibited CRTH2 mediated cell migration in response to PGD_2 with an EC_{50} of 11 nM using hCRTH2 stably transfected CEM cells.[21] The

Table 1



1 2

Compd	Chiral Center	CRTH2 IC_{50}[a] in buffer (μM)	CRTH2 IC_{50}[a] in plasma (μM)
1	Racemic	0.043	1.05
2	Racemic	>10	
1a[b]	(2*S*,4*R*)	0.017	0.44
1b[b]	(2*R*,4*S*)	0.42	>10

[a] Displacement of ^{3}H-PGD_2 from the CRTH2 receptor expressed on 293 cells. Assay run in buffer containing 0.5% BSA or in 50% plasma. See Ref. 20 for assay protocol. Values are means of three experiments, standard deviation is ±30%.
[b] ee >99%.

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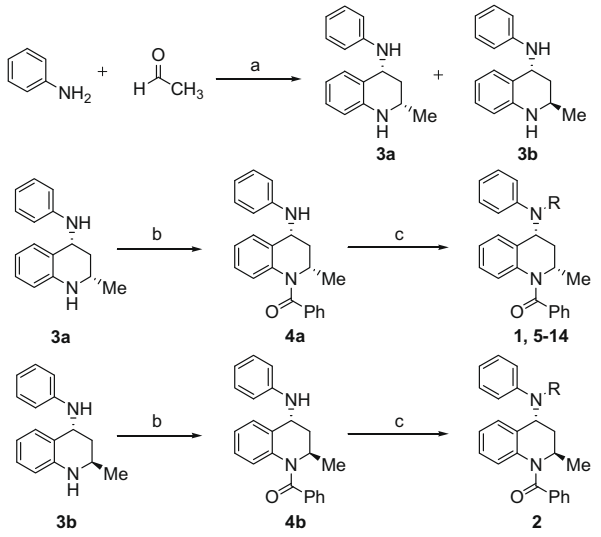
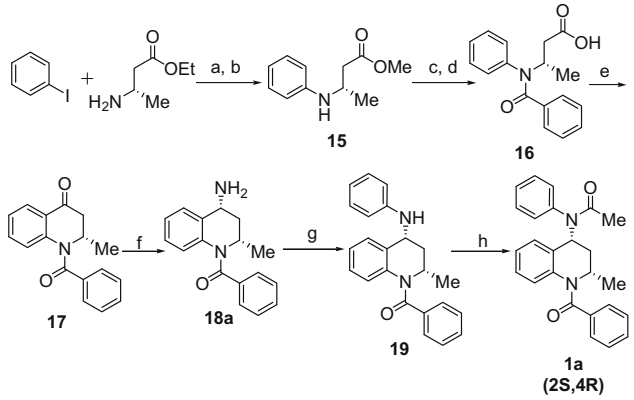
structure of compound **1** was determined to be *cis* by synthesis[22] (Scheme 1) and NMR studies.[23] Conversely, the *trans* isomer (**2**) had weak CRTH2 activity. Furthermore, the stereo-selective synthesis (Scheme 2) indicated the (2*S*,4*R*) enantiomer **1a** was responsible for the majority of the CRTH2 activity of racemic compound **1** (Table 1).

Compound **1** was synthesized according to Scheme 1. Reaction of aniline with acetaldehyde in ethanol at room temperature afforded a mixture of *cis*–*trans* isomers **3a** and **3b** in >95% yield. Separation of the *cis* isomers **3a** was achieved in 35% yield by recrystallization from 10% EtOAc/Hex. The *trans* isomer **3b** was obtained in 30% yield from the purification of the mother liquor using silica column chromatography. Selective acylation of **3a** and **3b** with benzoyl chloride at 1-*N* position afforded amides **4a** and **4b**, respectively, in 90% yield. Reaction of **4a** and **4b** with acetyl chloride afforded **1** and **2**, respectively, in 85% yield. Compounds **5–14** (Table 2) were synthesized from *cis* intermediate **4a** using reductive amination, sulfonylation or acylation.

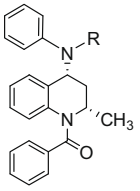


Scheme 1. Racemic synthesis of **1**, **2** and **5–14**. Reagents and conditions: (a) EtOH, rt, 24 h, 35% for compound **6** after recrystallization in 10% EtOAc/hexanes; (b) PhCOCl, triethylamine, DCM, rt, 24 h, 90%; (c) for amines: aldehydes, $Na(OAc)_3BH$, $ClCH_2CH_2Cl$, rt, 20 h, ~80%; for sulfonamides: sulfonyl chloride, DMAP, pyridine, rt, 3 h, 60%; for amides, acid chlorides, NaH, THF, rt, 20 h, 85%.



Scheme 2. Stereo-selective synthesis of **1a**. Reagents and conditions: (a) CuI, potassium carbonate, DMF, water, 90 °C, 48 h, 70%; (b) $SOCl_2$, MeOH, rt, 12 h, 90%; (c) PhCOCl, triethylamine, DCM, rt, 24 h, 90%; (d) LiOH, THF/MeOH/water, rt 4 h, 95%; (e) oxalyl chloride, DMF, DCM, 0 °C–rt, 3 h, then $AlCl_3$, DCM, 0 °C–rt, 12 h, 60%; (f) ammonium acetate, sodium cyanoborohydride, MeOH, 70 °C, 2 days, 85%; (g) phenyl boronic acid, pyridine, DMF, copper(II) acetate, air, 60 °C, overnight, 20%; (h) acetyl bromide, NaH, THF, 0 °C–rt, 3 h, 85%.

Table 2



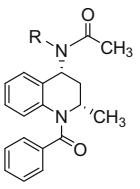
Compd[a]	R	CRTH2 IC_{50}[b] in buffer (μM)
1	–COMe	0.043
4a	–H	>50
5	$-CH_2CH_3$	3.17
6	$-SO_2Ph$	5.50
7	–COPh	0.105
8	$-CO(CH_2)_3CH_3$	0.064
9	$-CO(CH_2)_2CO_2H$	0.005
10	$-CO(CH_2)_2CO_2NH_2$	0.029
11	$-COCH_2CO_2H$	0.54
12	$-CO(CH_2)_3CO_2H$	0.022
13	$-COCH{=}CHCO_2H$	2.28
14	$-CO(1,3\text{-}Ph)CO_2H$	40.3

[a] Mixture of racemic mixture of (2*S*,4*R*) and (2*R*,4*S*) enantiomers.
[b] Displacement of ^{3}H-labeled PGD_2 from the CRTH2 receptor expressed on 293 cells. See Ref. 20 for assay protocol. Values are means of three experiments, standard deviation is ±30%.

The stereo-selective synthesis (Scheme 2) of **1a** began with a CuI catalyzed coupling of iodobenzene with the (*S*)-β-amino acid ester.[24] Amide formation of the coupling product (**15**) with benzoyl chloride followed by ester hydrolysis yielded acid **16**. Conversion of the carboxylic acid to the acid chloride followed by an intramolecular Friedel–Crafts acylation provided ketone **17**.[25] Reductive amination with ammonium hydroxide produced primary amine **18a**, which was coupled with phenyl boronic acid in the presence of copper acetate to give compound **19**.[14] Reaction of **19** with acetyl bromide afforded compound **1a** ((2*S*,4*R*)-enantiomer) with >99% ee. Compound **1b** ((2*R*,4*S*)-enantiomer) was obtained from chiral HPLC separation of racemate **1**.[26]

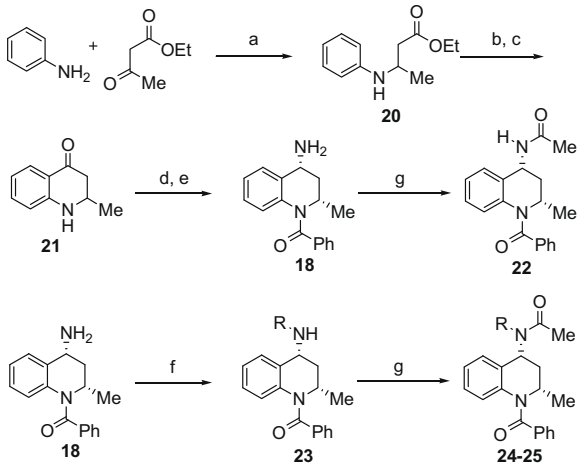
Compounds **22, 24** and **25** (Table 3) were synthesized according to Scheme 3. Reductive amination of ethyl acetoacetate with aniline afforded ester **20**. Saponification of **20** followed by intramolecular Friedel–Crafts acylation afforded ketone **21**.[27] Amide formation with benzoyl chloride followed by a reductive amination yielded compound **18**. Finally, compound **22** was obtained from acetamide formation of **18** with acetyl bromide. Compounds **24**

Table 3



Compd[a]	R	CRTH2 IC_{50}[b] in buffer (μM)
1	Ph	0.043
22	H	3.62
24	Et	0.25
25	Bn	0.43

[a] Mixture of racemic mixture of (2*S*,4*R*) and (2*R*,4*S*) enantiomers.
[b] Displacement of ^{3}H-labeled PGD_2 from the CRTH2 receptor expressed on 293 cells. See Ref. 20 for assay protocol. Values are means of three experiments, standard deviation is ±30%.



Scheme 3. Racemic synthesis of **22** and **24–25**. Reagents and conditions: (a) $Na(OAc)_3BH$, HOAc $ClCH_2CH_2Cl$, rt, 2 h, ~80%; (b) LiOH, THF/MeOH/water, rt 4 h, 95%; (c) PPA, 110 °C, 6 h, 50%; (d) PhCOCl, triethylamine, DCM, rt, overnight, 90%; (e) ammonium acetate, sodium cyanoborohydride, MeOH, 70 °C, 2 days, 85%; (f) aldehydes, $Na(OAc)_3BH$, $ClCH_2CH_2Cl$, rt, 2 h, 85%; (g) acetyl bromide, triethylamine, DCM, rt, overnight, 88%.

and **25** were obtained from reductive amination of **18** with the appropriate aldehydes followed by acetamide formation.

Compound **28** was a key intermediate needed to enable facile exploration of the 1-*N* position of tetrahydroquinoline **1** (Table 4 and 5). Synthesis of **28** started with the protection of **3a** at 1-*N* position by Cbz to give carbamate **26** (Scheme 4). Acetamide formation (**27**) and removal of Cbz delivered **28**. Final derivatization (reductive amination, sulfonylation or acylation) at 1-*N* position afforded compounds **29–42**.

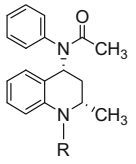
The lead optimization was guided by ^{3}H-PGD_2 displacement assays using hCRTH2 stably transfected 293 cells in buffer solution and plasma.[20] We evaluated most compounds as racemic mixtures and only resolved the enantiomers for compounds of interest.

Modification of the acetyl group of the 4-phenylamino of **1** showed that amides (e.g., **1**, **7** and **8**) were preferred over secondary amine **4a**, tertiary amine **5** and sulfonamide **6** (Table 2). A 3-carboxylpropionyl group significantly increased the binding affinity (**9**). The corresponding amide (**10**) of carboxylic acid **9** was less potent. The optimal distance between the acid and the amide carbonyl is two carbons, as in compound **9**. Either shortening (**11**) or extending the distance (**12**) decreased the CRTH2 activity. The activity is also sensitive to the composition of the linker between the acid and the amide. Other linkers, such as *trans* acetylene (**13**) and 1,3-phenylene (**14**), significantly reduced the binding affinity compared to the ethylene linker (**9**).

The aniline moiety at the 4-position of the tetrahydroquinoline core was studied briefly (Table 3). It was found that replacement of the *N*-phenyl of **1** by a hydrogen (**22**) or a small alkyl group (**24**), or extension of the phenyl by one methylene (**25**) all significantly decreased the CRTH2 activity.

Variation at 1-*N* position of the tetrahydroquinoline core (Table 4) demonstrated that a bulk at 1-*N* position was required for activity, as the compound (**28**) with no attachment at the position had little affinity for the CRTH2 receptor. In addition, the carbonyl of the benzoyl group (**1**) was preferred over a methylene (**29**) and a sulfonyl group (**30**). Extending the benzene of the benzoyl group from the carbonyl (**1**) by one or two atoms (**27**, **31** and **32**) resulted in decrease of the CRTH2 activity. The medium size *n*-alkyl amides, such as *n*-butyl amide (**33**), were also not as potent as the parental compound (**1**).

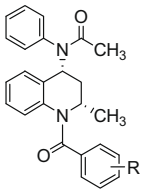
Table 4



Compd[a]	R	CRTH2 IC_{50}[b] in buffer (μM)
1	–COPh	0.043
28	–H	>50
29	$-CH_2Ph$	0.20
30	$-SO_2Ph$	62
31	$-COCH_2Ph$	1.75
27	$-COOCH_2Ph$	0.91
32	–COCH=CHPh	0.21
33	$-CO(CH_2)_3CH_3$	1.46

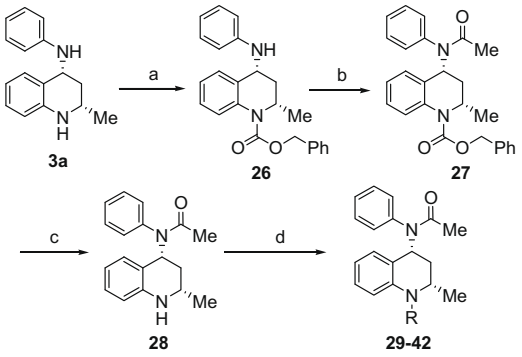
[a] Mixture of racemic mixture of (2*S*,4*R*) and (2*R*,4*S*) enantiomers.
[b] Displacement of ^{3}H-PGD_2 from the CRTH2 receptor expressed on 293 cells. See Ref. 20 for assay protocol. Values are means of three experiments, standard deviation is ±30%.

Table 5



Compd[a]	R	CRTH2 IC_{50}[b] in buffer (μM)
1	H	0.043
34	2-Me	1.42
35	3-Me	0.074
36	4-Me	0.041
37	3,4-Me	0.092
38	4-OMe	0.028
39	4-Cl	0.093
40	4-tBu	0.034
41	$4-OCF_3$	0.026
42	4-OPh	0.013

[a] Mixture of racemic mixture of (2*S*,4*R*) and (2*R*,4*S*) enantiomers.
[b] Displacement of ^{3}H-lPGD$_2$ from the CRTH2 receptor expressed on 293 cells. See Ref. 20 for assay protocol. Values are means of three experiments, standard deviation is ±30%.



Scheme 4. Racemic synthesis of **27–42**. Reagents and conditions: (a) Benzyl chloroformate, potassium carbonate, acetone, 55 °C, 5 h, 87%; (b) acetyl bromide, NaH, THF, 0 °C–rt, 3 h, 85%; (c) H_2, Pd/C, EtOH, rt, 1 h, 100%; (d) for amines: aldehydes, $Na(OAc)_3BH$, $ClCH_2CH_2Cl$, rt, 20 h, ~80%;%; for sulfonamides: sulfonyl chloride, DMAP, pyridine, rt, 3 h, 60%; for amides, acid chlorides, NaH, THF, rt, 20 h, 85%.

The substitution effect on the phenyl ring of the 1-*N* benzoyl moiety is shown in Table 5. Compounds with methyl substitution at the *para* and *meta* positions (**36** and **35**) displayed better potency than the *ortho*-substituted analog (**34**). 3,4-Dimethyl compound (**37**) was similar in potency to that of mono *para* or *meta*-methyl compounds (**36** and **37**). In general, substitutions at the *para*-position afforded compounds with good CRTH2 binding affinity regardless of electronic and steric effects (**36**, **38**–**42**). Some substitutions, such as phenoxy (**42**), methoxy (**38**) and trifluoromethoxy (**41**), at the *para*-position yielded more potent compounds compared to the unsubstituted compound (**1**).

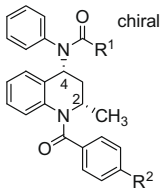
Based partially on their binding affinity, compounds **9**, **38** and **41** were selected and resolved by chiral HPLC and each enantiomer was evaluated (Table 6). Like compound **1**, the (2*S*,4*R*) enantiomers had greater affinity for the CRTH2 receptor. Even in the presence of plasma, these (2*S*,4*R*) enantiomers displayed strong inhibitory activity for the CRTH2 receptor.

In addition to having high affinity for the CRTH2 receptor, these compounds are also potent functional antagonists. Compounds **1a**, **9a**, **38a** and **41a** were potent inhibitors of PGD_2-mediated human eosinophil shape change[28] (Table 7). In particular, compound **9a** had an IC_{50} of 0.77 nM. The affinity of these compounds for the DP receptor was evaluated with a ^{3}H-PGD_2 displacement assay using 293 cells stably transfected with hDP receptor. These compounds were found to be selective for the CRTH2 receptor over DP.

The pharmacokinetics properties of compound **41a** were evaluated in male Sprague Dawley rats following IV (0.7 mg/kg) and oral (2.0 mg/kg) dosing. The total body clearance and the terminal half-life were 0.73 L/h/kg and 5.1 h, respectively. After oral administration the compound showed good bioavailability (38%) following administration of a solution formulation (1 mL/kg; 10% ethanol:90% PEG400).

In summary, we have discovered and optimized a series of tetrahydroquinoline derivatives as potent CRTH2 antagonists with selectivity over DP. Furthermore, we have identified compound **41a** as a potent CRTH2 antagonist with good pharmacokinetic properties rendering it a useful tool compound for in vivo studies of CRTH2 functions.

Table 6

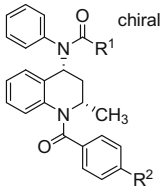


Compd	R^1	R^2	CRTH2 IC_{50}[a] in buffer (μM)	CRTH2 IC_{50}[a] in plasma (μM)
9 racemic	$-CO(CH_2)_2CO_2H$	H	0.005	0.028
9a (2*S*,4*R*)[b]	$-CO(CH_2)_2CO_2H$	H	0.003	0.009
9b (2*R*,4*S*)[b]	$-CO(CH_2)_2CO_2H$	H	0.40	>10
38 racemic	CH_3	OMe	0.028	0.13
38a (2*S*,4*R*)[b]	CH_3	OMe	0.015	0.039
38b (2*R*,4*S*)[b]	CH_3	OMe	0.40	>10
41 racemic	CH_3	OCF_3	0.026	0.18
41a (2*S*,4*R*)[b]	CH_3	OCF_3	0.025	0.106
41b (2*R*,4*S*)[b]	CH_3	OCF_3	0.217	>10

[a] Displacement of ^{3}H-PGD_2 from the CRTH2 or DP receptors expressed on 293 cells. Assay run in buffer containing 0.5% BSA or in 50% plasma. See Ref. 20 for assay protocol. Values are means of three experiments, standard deviation is ±30%.

[b] Stereochemistry assigned based on the retention times of chiral HPLC compared to **1a** and **1b** and CRTH2 activities, ee >99%.

Table 7



Compd	R^1	R^2	Eosinophil Shape Change[a] IC_{50} (nM)	DP IC_{50}[b] (μM)
1a[c]	CH_3	H	141	>10
9a[d]	$-CO(CH_2)_2CO_2H$	H	0.77	>10
38a[d]	CH_3	OMe	49.3	>10
41a[d]	CH_3	OCF_3	48.8	>10

[a] PGD_2-mediated human eosinophil shape change assay. See Ref. 28 for assay protocol. Values are means of two experiments.

[b] Displacement of ^{3}H-labeled PGD_2 from the DP receptors expressed on 293 cells. Assay run in buffer containing 0.5% BSA. See Ref. 29 for assay protocol. Values are means of three experiments, standard deviation is ±30%.

[c] Stereochemistry determined by synthesis (Scheme 2), ee >99%.

[d] Stereochemistry assigned based on the retention times of chiral HPLC compared to **1a** and **1b** and CRTH2 activities., ee >99%.

References and notes

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20. The CRTH2 radioligand binding assay was performed on 293 cells stably expressing human CRTH2. To measure binding, [^{3}H]-PGD_2 was incubated together with 293(hCRTH2) cells in the presence of increasing concentrations of compounds. After washing, the amount of [^{3}H]-PGD_2 that remained bound to the cells was measured by scintillation counting and the concentration of compounds required to achieve a 50% inhibition of [^{3}H]-PGD2 binding (the IC_{50}) was determined. The binding buffer contains either 0.5% BSA (buffer binding) or 50% human plasma (plasma binding).
21. CRTH2 mediated cell migration was analyzed in a transwell migration assay using hCRTH2 stably transfected CEM cells (a T lymphoblast cell line). The cells were incubated with increasing concentrations of compounds for 3 h in a 96-well migration chamber on top of a transwell filter and the number of cells

migrating through the filter in response to PGD_2 was counted and the IC_{50} of the compounds determined.
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26. Chiral HPLC conditions: column: ChiralPak AD-H, 20 × 250 mm; solvents: 40% isopropanol/hexanes; flow rate: 20 mL/min.
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28. Enriched human eosinophils were resuspended at a concentration of 10^6 cells/mL in assay buffer (PBS with Ca^{2+}/Mg^{2+}, 0.1% BSA, 10 mM Hepes, and 10 mM glucose). Eosinophils were incubated with antagonists or vehicle (0.05% DMSO) for 10 min at room temperature and subsequently stimulated with 10nM PGD_2 or vehicle for 10 min at 37 °C. Cells were immediately fixed with 1% paraformaldehyde. Samples were immediately analyzed on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). Live cells were gated using forward/side scatter parameters. Five thousand gated live events were acquired. Shape change responses were quantified as a percentage of the maximal PGD_2 response. CAY-10471 was used as a positive control. Its average IC_{50} in the two experiments was 0.20 nM.
29. The DP radioligand binding assay was performed on 293 cells stably expressing human DP. To measure binding, $[^3H]$-PGD_2 was incubated together with 293(hDP) cells in the presence of increasing concentrations of compounds. After washing, the amount of $[^3H]$-PGD_2 that remained bound to the cells was measured by scintillation counting and the concentration of compounds required to achieve a 50% inhibition of $[^3H]$-PGD2 binding (the IC_{50}) was determined. The binding buffer contains either 0.5% BSA (buffer binding).