Discovery of a Slow Tight Binding LPA1 Antagonist (ONO-0300302) for the Treatment of Benign Prostatic Hyperplasia

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S Supporting Information

ABSTRACT: Scaffold hopping from the amide group of lead compound ONO-7300243 (**1**) to a secondary alcohol successfully gave a novel chemotype lysophosphatidic acid receptor 1 (LPA₁) antagonist **4**. Wash-out experiments using rat isolated urethra showed that compound **4** possesses a tight binding feature to the LPA₁ receptor. Further modification of two phenyl groups of **1** to pyrrole and an indane moiety afforded an optimized compound ONO-0300302 (**19**). Despite its high *i.v.* clearance, **19** inhibited significantly an LPA-induced increase of intraurethral pressure (IUP) in rat (3 mg/kg, *p.o.*) and dog (1 mg/kg, *p.o.*) over 12 h. Binding experiments with [³H]-ONO-0300302 suggest that the observed long duration action is because of the slow tight binding character of **19**.

KEYWORDS: LPA₁ antagonist, GPCR, slow tight binding antagonist, benign prostatic hyperplasia, SAR, lead optimization

Improved *in vivo* efficacy is a major goal of drug discovery based lead optimization campaigns. To increase the *in vivo* potency and pharmacokinetic (PK) profiles, some general strategies are known, such as blocking of the metabolic labile part¹ and conformational restriction.² Recently, residence time, which implies that a compound binds tightly to the target protein, has attracted attention because of the long biological effect of the compound *in vivo*.³ In this report, we describe *in vitro* and *in vivo* structure–activity relationship (SAR) studies during the lead optimization campaign of an LPA₁ antagonist for the treatment of benign prostatic hyperplasia (BPH). We also discuss the relationship between the slow and tight binding character and the *in vivo* efficacy of the LPA₁ antagonist.

As described in our previous paper,⁴ we have successfully obtained the lead compound ONO-7300243 (**1**), which is a new chemotype LPA₁ antagonist.^{5–8} Compound **1** is orally active against the lysophosphatidic acid (LPA) induced rat intraurethral pressure (IUP) model^{9,10} in a dose-dependent manner. Moreover, this compound (30 mg/kg, *p.o.*) significantly reduced the IUP in a conscious rat without the LPA stimulation, and it did not affect the mean blood pressure (MBP). Tamsulosin (an α₁ adrenoceptor antagonist) is clinically used for BPH treatment but induces postural hypotension at clinical doses. We found that tamsulosin also reduces IUP in the same *in vivo* model at 1 mg/kg oral administration and also showed significant MBP reduction at the same doses. These results suggest that an LPA₁ antagonist is required as an alternative option for BPH treatment, which does not affect blood pressure. Generally, most of medicines for

BPH, such as tamsulosin or doxazosin, are clinically used as a single daily dose. Therefore, our main goal is to develop a novel LPA₁ antagonist that shows highly potent IUP reduction efficacy and good duration of action to meet the single daily dose requirement.

For this purpose, the PK profile of the lead compound **1** required improvement to ensure better *in vivo* efficacy. As we already reported,⁴ **1** has a rapid clearance (CL_{tot} = 15.9 mL/min/kg at 3 mg/kg *i.v.*) and a short half-life (0.3 h). The *in vitro* metabolic study in rat and human liver microsomes indicated that the 3-phenylpropyl moiety of **1** is metabolically labile. We initially tried to modify the 3-phenylpropyl part of **1** to improve its metabolic stability. Modification of the 3-phenylpropyl group to a 3-phenyl-2-propenyl group (**2**) did increase the microsomal stability. However, introduction of the sp² carbon caused an unfavorable loss of antagonistic activity (Figure 1). Furthermore, we attempted to introduce various substituents into the phenyl ring but failed to improve the metabolic stability. Thus, modification of this moiety proved difficult with respect to maintaining a balance between the antagonist activity and the metabolic stability. Consequently, an alternative approach to improve the *in vivo* activity was required.

We next tried to modify the amide part to a different template. Scaffold hopping is generally considered an effective approach to change the properties of compounds.¹¹ Various

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Figure 1. Modification of lead compound 1. ^a IC_{50} values were determined by nonlinear regression analysis of the dose–response curves (4 points) generated using GraphPad Prism ver. 5.04 with 95% confidence intervals in parentheses. The LPA₁ receptor stably expressed in CHO cells was used. ^brMS, rat microsomal stability; hMS, human microsomal stability; 0.5 mg/mL NADPH.

attempts of introducing bioisosteres including five-membered aromatics failed. However, during this process we found that transformation of the amide group to a ketone group (3) retained antagonist activity (Figure 1, see Supporting Information Scheme S1 for synthesis of compound 3). Reduction of the ketone 3 gave two diastereomers, each a 50:50 mixture of enantiomers, which also retained antagonist activity. The four stereoisomers were separated using HPLC (Scheme S2) and the antagonist activity was tested *in vitro*. One isomer (4) was found to have increased antagonist activity *in vitro* when compared with that of compound 1. Determination of the absolute and relative stereochemistry of the active isomer was accomplished by an alternative synthesis, as depicted in Schemes S3 and S4.

Compound 4 was tested via oral administration for evaluating the effects on the LPA induced rat IUP model. Compound 4 inhibited the LPA-induced IUP increase in a dose-dependent manner ($ID_{50} = 0.97 \text{ mg/kg p.o.}$) after 1 h of an oral dose. In addition, we sought to identify the difference in the mode of action between amide 1 and the secondary alcohol 4. The biological activities of 1 and 4 were examined in rat-isolated urethras using a Magnus apparatus. Both compounds inhibited LPA induced rat urethra contraction. However, inhibition by amide 1 was found to reduce gradually as the wash-out was repeated (Figure 2). However, inhibition by alcohol 4 was not affected by the wash-out process. These results suggest that alcohol 4 has a tight binding feature to the LPA₁ receptor. We speculate that this character improved the *in vivo* efficacy of compound 4. This observation may be due to the different hydrogen bond networks formed by amide 1 and alcohol 4. The secondary amide group of compound 1 only acts as a hydrogen bond acceptor. The alcohol group, which is present in compound 4, acts as a hydrogen bond donor and/or acceptor. We hypothesize that this difference in binding mode leads to tight binding of compound 4 to the LPA₁ receptor.

Thus, we successfully changed the core structure from the amide to the secondary alcohol scaffold.

Figure 2. Wash-out experiments of compounds 1 and 4 using rat isolated urethras (y axis shows % inhibition of LPA induced rat isolated urethra contraction, means $\pm$ SD, $n = 3$). Ten micromolar LPA was added into a medium to measure the extent of a rat isolated urethra contraction (100% control). After exchanging the medium, a test compound (1 μM) was added and incubated for 30 min. Ten micromolar LPA was added to record the extent of contraction (Compound). The urethra was washed out with media (no compound) three times every 10 min. Ten micromolar LPA was added to record the extent of contraction (Wash-out 1). The wash-out procedure was repeated (Wash-out 2). (A) Inhibition by compound 1 was reduced after the wash-out procedure. (B) Inhibition by compound 4 was unaffected after two wash-outs.

Further modification was conducted using this secondary alcohol structure as a template. Various *N*-alkylated hetero-aromatic analogs of compound 4 were synthesized using the intermediate S16 (Scheme S3, see Supporting Information) and tested for *in vitro* LPA₁ antagonist activity. The results are summarized in Table 1. Imidazole analog 5 resulted in a three-fold decrease in the potency when compared with that of 4. Changing to 1,2-pyrazole analogues (6 and 8) increased the potency of 4 by ~ 2 -fold. The addition of a dimethyl group to compound 6 resulted in the loss of potency (7). This is postulated to be due to steric hindrance. Changing to a pyrrole derivative (9) caused a further increase in potency. The relative order of the antagonist activity is pyrrole (9) > 1,2-pyrazole (6, 8) > benzene (4) > imidazole (5).

We next changed the tether length between the aromatic ring and the carboxylic acid. One carbon elongation of the 1,2-pyrazole analogues (6, 8) gave the propionic acid derivatives (11, 12), respectively, which showed similar antagonist activity as the parent species. In the case of the pyrrole derivative, the propionic acid analog 13 had significantly reduced antagonist activity when compared with the acetic analog 9. Shortening compound 9 by one carbon gave compound 10, which resulted in a significant loss of potency. At any tether length, the pyrrole derivatives gave better antagonist activity among the hetero-aromatic rings synthesized.

To define the functional role of the pyrrole ring, a docking study using the X-ray crystal structure of LPA₁ (PDB ID: 4z34)¹² with compound 9 was conducted. The result is shown in Figure 3. The binding mode of compound 9 is almost the same as the one of the original ligand bound in the X-ray crystal structure.¹² In addition, the amine group of Lys39 was located above the pyrrole group of compound 9. The interaction distance between the pyrrole ring and the amine group of Lys39 is 3.7 Å. As described above, the antagonist activity becomes stronger in the order pyrrole > 1,2-pyrazole > benzene > imidazole. This order correlates with the order of cation– π interactions, which has been reported in the literature.¹³ Under the physiological conditions studied, we speculate that the amine residue of Lys39 is protonated and the pyrrole of compound 9 interacts with the cationic amine. We hypothesize

Table 1. Effects of Various Five- Membered Heteroaromatics and Tether Lengths

Cmpd	R	IC ₅₀ (μM) ^a	Cmpd	R	IC ₅₀ (μM) ^a
5		0.18 (0.15 - 0.22)	10		0.12 (0.10 - 0.13)
6		0.030 (0.022 - 0.041)	11		0.034 (0.031 - 0.038)
7		0.52 (0.35 - 0.78)	12		0.040 (0.031 - 0.052)
8		0.032 (0.025 - 0.041)	13		0.022 (0.020 - 0.024)
9		0.013 (0.012 - 0.014)			

^aIC₅₀ values were determined by nonlinear regression analysis of the dose–response curves (4 points) generated using GraphPad Prism ver. 5.04 with 95% confidence intervals in parentheses. The LPA₁ receptor stably expressed in CHO cells was used.

Figure 3. Docking results for compound **9** using the LPA₁ structure (PDB ID: 4z34).¹² Hydrogen atoms are omitted for clarity.

that this interaction makes the pyrrole analog **9** the most potent.

The *in vivo* potency of compound **9** was evaluated against the LPA induced rat IUP model, and an ID₅₀ of 0.6 mg/kg was determined after 1 h oral dosing. This ID₅₀ is very similar to the value determined for compound **4**. The duration of action of **9** was not sufficient (33% inhibition for 6 h using rats) due to its poor PK profile (CL_{tot} = 50 mL/min/kg). Thus, we attempted to modify the 3-phenylpropyl moiety of compound **9**, which is suspected to be labile to hepatic metabolism, as discussed previously.⁴

The *in vitro* activities of these modified compounds are summarized in Table 2. Most substitutions on the phenyl ring and side chain were found to be detrimental to the antagonist activity. In the alcohol series, however, it was discovered that 2- or 3-substituted thiophenes (**14**–**17**) retained antagonist activity. Moreover, surprisingly, the incorporation of the indane structure into this moiety was tolerated for activity. Acetic acid type compounds (**14**, **16**, and **18**) tended to have stronger activity than the corresponding propionic acids (**15**, **17**, and **19**).

The metabolic stability of the compounds using rat and human microsomes were also examined (Table 2). The metabolic stabilities of the compounds against human microsomes were found to be generally good. However, some

compounds (**18** and **19**) showed moderate stability against rat microsomes, which suggests a poor *in vivo* efficacy. However, as mentioned above for compound **4**, the alcohol template has a tight binding feature that should give good *in vivo* efficacy. Thus, all compounds in Table 2 were evaluated in the rat IUP model (Figure 4A).

When LPA was injected intravenously into rat, the IUP increased by ~6 mmHg (Figure 4A, vehicles). Compound **14** decreased significantly the LPA-induced IUP increase to 1.31 mmHg (75% inhibition *p* < 0.001 vs vehicle, see Supporting Information Table S1) after 1 h at 3 mg/kg oral dosing. The effect of compound **14** was weaker than the effect of compound **9** at 3 h and was not observed at 6 h (23% inhibition, no significance vs vehicle). Other thiophene analogues (**15**–**17**) were evaluated at 3 and 6 h to determine if these compounds have a longer duration of action when compared with compound **14** (Figure 4A). All compounds were significantly effective at 3 h with a reduction in the IUP increase to ~2 mmHg (~70% inhibition). However, the inhibition was not retained to 6 h, probably because of poor metabolic stability (Table 2).

The best result was obtained by incorporation of the indane structure into the 3-phenylpropyl moiety. Compound **18** significantly reduced the IUP increase by up to 3.33 mmHg (52% inhibition, *p* < 0.05 vs vehicle) until 12 h. Compound **19** (a propionic acid analog of **18**) showed even better reduction of the IUP increase (47% inhibition at 18 h, *p* < 0.01 vs vehicle). Moreover, compounds **18** and **19** were evaluated in a dog IUP model (Figure 4B) because the BPH in human and dog has common features.¹⁴ In dog, compounds **18** and **19** gave good efficacy and duration of action (Figure 4B). In particular, compound **19** showed 30% inhibition after 24 h at 1 mg/kg oral dosing. Thus, our approach has successfully obtained the optimized compound **19** (ONO-0300302).

We were interested in the possible excellent *in vivo* activity of **19**, despite its moderate *in vitro* antagonist activity and nonsustainable PK profile in rat. We conducted further experiments to investigate the differences among the derivatives to explain the PK/PD correlation.

Table 2. Exploration of the 3-Phenylpropyl Moiety

Cmpd	X	R3	LPA1	metabolic stability	rat iv
			IC ₅₀ (μM) ^a	T _{1/2} (min) human/rat MS ^b	clearance CL _{tot} (mL/min/kg) ^c
9	-CH ₂ -		0.013 (0.012 - 0.014)	49 / <15	50.1 ± 1.4
14	-CH ₂ -		0.023 (0.020 - 0.026)	60 / <15	16.4 ± 3.5
15	-CH ₂ CH ₂ -		0.038 (0.033 - 0.045)	66 / <15	N.T. ^d
16	-CH ₂ -		0.029 (0.024 - 0.034)	82 / <15	N.T. ^d
17	-CH ₂ CH ₂ -		0.045 (0.041 - 0.049)	75 / <15	N.T. ^d
18	-CH ₂ -		0.061 (0.046 - 0.082)	65 / 34	19.2 (n = 2)
19	-CH ₂ CH ₂ -		0.086 (0.044 - 0.16)	79 / 15	20.5 ± 2.1

^aIC₅₀ values were determined by nonlinear regression analysis of the dose–response curves (4 points) generated using GraphPad Prism ver. 5.04 with 95% confidence intervals in parentheses. The LPA₁ receptor stably expressed in CHO cells was used. ^b0.5 mg/mL, NADPH. MS: microsomes. ^ci.v. dose of 1 mg/kg. ^dN.T. = not tested.

Figure 4. *In vivo* efficacy and duration of action against LPA induced rat and dog IUP. (A) Compound or vehicle (20% STPG) was orally administered at 3 mg/kg in conscious rats. After the prescribed time (1, 3, 6, 9, 12, 18, and 24 h), LPA (300 μg/kg) was injected intravenously and the IUP measured in short-term anesthetized rats. (B) Compound was orally administered at 1 or 3 mg/kg in conscious dogs. LPA (300 μg/kg) was injected intravenously at each point (0.5, 1, 2, 4, 6, 9, 12, and 24 h), and the IUP was measured in conscious dogs. Each datum was shown as a % response, which is compared with the IUP of predosing (0 h) in each dog (%pre).

Figure 5. Binding experiments. Saturation binding for tritium labeled compounds to membranes of the LPA₁ receptor expressed in CHO cells. K_d values were determined by fitting curves with one-site binding (hyperbola) using GraphPad Prism ver. 5.04. (A) Specific binding curves for [³H]-compound **19** at different incubation times. (B) Specific binding curves for [³H]-compound **1** at different incubation times. (C) Effect of temperature. (D) Correlation between binding (K_i) and *in vivo* $i.v.$ efficacy (ID_{50}). K_i values were measured at 37 °C for 2 h using 1 nM [³H]-ONO-0300302 and calculated with the Cheng–Prusoff equation ($K_i = IC_{50}/(1 + [radioligand]/K_d)$). ID_{50} was estimated in the LPA induced rat IUP model via *i.v.* dosing (3 doses, $n = 1$).

Currently, GPCR downstream Ca²⁺ mobilization at 10 min incubation time is used as the indicator of compound affinity with the LPA₁ receptor. However, no correlation was found between Ca²⁺ mobilization and *in vivo* activities, as described above. Thus, a binding experiment was conducted to measure the affinity directly between a compound and the LPA₁ receptor. Tritium labeled lead compound **1** ([³H]-ONO-7300243) and the optimized compound **19** ([³H]-ONO-0300302) were synthesized via tritiation of the corresponding olefin analogs of compounds **1** and **19**.¹⁵ The binding experiments were conducted with different incubation times at room temperature. Saturation binding curves to the membrane fraction of LPA₁ expressed CHO cells are presented in Figure 5A,B. Dissociation constants (K_d) are summarized in the tables in Figure 5A–C. The K_d values for [³H]-compound **19** were found to be lower as the incubation time increased (K_d

= 0.49 nM at 4 h). This indicates that the binding affinity of compound **19** increases with time, which means compound **19** is a slow binding inhibitor. Conversely, the K_d values of the amide compound **1** did not change with the incubation time.

The minimum K_d value of compound **19** ($K_d = 0.34$ nM) was observed at 37 °C and after 2 h (Figure 5C). Using these binding assay conditions (37 °C, 2 h, 1 nM [³H]-ONO-0300302), the K_i values of our in-house LPA₁ antagonists were measured. At the same time, their *in vivo* potency (ID_{50}) was evaluated in the rat LPA induced IUP model via *i.v.* dosing. Figure 5D shows a good correlation between the *in vivo* ID_{50} and the *in vitro* K_i values ($R^2 = 0.68$). The human LPA₁ receptor shares high sequence homology (97.3% identity) with the rat LPA₁ receptor (Figure S1, see Supporting Information). In addition, 61/62 residues within 8 Å of ligand binding site are conserved in human and rat LPA₁ receptors (Figure S2). These

analyses ensure the usefulness of the rat and dog IUP models. We believe this slow tight binding feature to the LPA₁ receptor leads to the strong *in vivo* efficacy and the long duration of action of the optimized compound **19**.

Finally, the IC₅₀ value of **19** was re-evaluated in the LPA₁ receptor expressed CHO cells by taking into account the slow binding. The compound was tested using a long time incubation method and wash-out experiment in CHO cells (Figure S3, see [Supporting Information](#)). Compound **19** was added into the assay wells at the beginning of cell seeding and incubated with the CHO cells for 24 h. The concentration of the compound was held at the same concentration during a loading Ca²⁺ indicator and after stimulation by LPA. This long time incubation without the wash-out step gave an IC₅₀ value for **19** of 0.16 nM (0.094–0.27 nM, 95% confidence intervals). When the compound was washed out before LPA stimulation, the IC₅₀ value of **19** was 0.19 nM (0.10–0.36 nM, 95% confidence intervals). Thus, no significant difference in IC₅₀ values was observed between the two conditions. These results indicate that **19** is a tight binding inhibitor.

In conclusion, scaffold hopping from the amide group of lead compound ONO-7300243 (**1**) gave a secondary alcohol moiety, which was essential for displaying the tight binding feature to the LPA₁ receptor. Incorporation of an indane moiety and pyrrole ring afforded the most potent LPA₁ antagonist ONO-0300302 (**19**) among the analogues examined. Compound **19** shows excellent *in vivo* efficacy because of the slow tight binding feature, despite its moderate PK profile, and it represents the best research tool available to shed light on BPH patients and LPA-related diseases.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmedchemlett.7b00383](https://doi.org/10.1021/acsmedchemlett.7b00383).

Experimental procedures and characterization for the synthesis of **3–19**, biological assay protocols, Table S1, and Figures S1–S3 ([PDF](#))

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Nassar, A. E.; Kamel, A. M.; Clarimont, C. Improving the decision-making process in structural modification of drug candidates: reducing toxicity. *Drug Discovery Today* **2004**, *15*, 1055–64.
- (2) Fang, Z.; Song, Y.; Zhan, P.; Zhang, Q.; Liu, X. Conformational restriction: an effective tactic in 'follow-on'-based drug discovery. *Future Med. Chem.* **2014**, *6*, 885–901.

- (3) Tummino, P. J.; Copeland, R. A. Residence time of receptor-ligand complexes and its effect on biological function. *Biochemistry* **2008**, *47*, 5481–5492.

- (4) Terakado, M.; Suzuki, H.; Hashimura, K.; Tanaka, M.; Ueda, H.; Kohno, H.; Fujimoto, T.; Saga, H.; Nakade, S.; Habashita, H.; Takaoka, Y.; Seko, T. Discovery of ONO-7300243 from a novel class of lysophosphatidic acid receptor 1 antagonists: from hit to lead. *ACS Med. Chem. Lett.* **2016**, *7*, 913–918.

- (5) Ohta, H.; Sato, K.; Murata, N.; Damirin, A.; Malchinkhuu, E.; Kon, J.; Kimura, T.; Tobo, M.; Yamazaki, Y.; Watanabe, T.; Yagi, M.; Sato, M.; Suzuki, R.; Murooka, H.; Sakai, T.; Nishitoba, T.; Im, D. S.; Nochi, H.; Tamoto, K.; Tomura, H.; Okajima, F. Ki16425, a subtype-selective antagonist for EDG-family lysophosphatidic acid receptors. *Mol. Pharmacol.* **2003**, *64*, 994–1005.

- (6) Swaney, J. S.; Chapman, C.; Correa, L. D.; Stebbins, K. J.; Bunday, R. A.; Prodanovich, P. C.; Fagan, P.; Baccei, C. S.; Santini, A. M.; Hutchinson, J. H.; Seiders, T. J.; Parr, T. A.; Prasit, P.; Evans, J. F.; Lorrain, D. S. A novel, orally active LPA₁ receptor antagonist inhibits lung fibrosis in the mouse bleomycin model. *Br. J. Pharmacol.* **2010**, *160*, 1699–1713.

- (7) Qian, Y.; Hamilton, M.; Sidduri, A.; Gabriel, S.; Ren, Y.; Peng, R.; Kondru, R.; Narayanan, A.; Truitt, T.; Hamid, R.; Chen, Y.; Zhang, L.; Fretland, A. J.; Sanchez, R. A.; Chang, K.-C.; Lucas, M.; Schoenfeld, R. C.; Laine, D.; Fuentes, M. E.; Stevenson, C. S.; Budd, D. C. Discovery of highly selective and orally active lysophosphatidic acid receptor-1 antagonists with potent activity on human lung fibroblasts. *J. Med. Chem.* **2012**, *55*, 7920–7939.

- (8) Schaffer, M.; Pernerstorfer, J.; Kadereit, D.; Strobel, H.; Czechtizky, W.; Chen, L. C.; Safarova, A.; Weichsel, A.; Patek, M. Acylamino-substituted fused cyclopentanecarboxylic acid derivatives and their use as pharmaceuticals. Patent WO2009135590.

- (9) Akiyama, K.; Hora, M.; Tatemichi, S.; Masuda, N.; Nakamura, S.; Yamagishi, R.; Kitazawa, M. KMD-3213, a uroselective and long-acting α 1a-adrenoceptor antagonist, tested in a novel rat model. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 81–91. Rat IUP was measured according to this report using LPA instead of norepinephrine. Our modified procedure is described in ref 10.

- (10) Saga, H.; Ohhata, A.; Hayashi, A.; Katoh, M.; Maeda, T.; Mizuno, H.; Takada, Y.; Komichi, Y.; Ota, H.; Matsumura, N.; Shibaya, M.; Sugiyama, T.; Nakade, S.; Kishikawa, K. A novel highly potent Autotaxin/ENPP2 inhibitor produces prolonged decreases in plasma lysophosphatidic acid formation *in vivo* and regulates urethral tension. *PLoS One* **2014**, *9*, e93230.

- (11) Bohm, H. – J.; Flohr, A.; Stahl, M. Scaffold hopping. *Drug Discovery Today: Technol.* **2004**, *1*, 217.

- (12) Chrencik, J. E.; Roth, C. B.; Terakado, M.; Kurata, H.; Omi, R.; Kihara, Y.; Warshaviak, D.; Nakade, S.; Asmar-Rovira, G.; Mileni, M.; Mizuno, H.; Griffith, M. T.; Rodgers, C.; Han, G. W.; Velasquez, J.; Chun, J.; Stevens, R. C.; Hanson, M. A. Crystal structure of antagonist bound human lysophosphatidic acid receptor 1. *Cell* **2015**, *161*, 1633–1643.

- (13) Mecozzi, S.; West, A. P., Jr.; Dougherty, D. A. Cation-pi interactions in aromatics of biological and medicinal interest: Electrostatic potential surfaces as a useful qualitative guide. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 10566–10571.

- (14) Mahapokai, W.; Van Sluijs, F. J.; Schalken, J. A. Models for studying benign prostatic hyperplasia. *Prostate Cancer Prostatic Dis.* **2000**, *3*, 28–33.

- (15) The preparation of labeled compounds was conducted at a contract research laboratory, Sekisui Medical Co., Ltd. (former Daiichi Pure Chemicals Co., Ltd.).