Journal of Medicinal Chemistry

BRIEF ARTICLE

pubs.acs.org/jmc

Development of Endocannabinoid-Based Chemical Probes for the Study of Cannabinoid Receptors†

Lidia Martín-Couce, Mar Martín-Fontecha, Samanta Capolicchio, María L. López-Rodríguez,* and Silvia Ortega-Gutiérrez*

Departamento de Química Orgánica I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, E-28040 Madrid, Spain

Supporting Information

ABSTRACT: We report the synthesis of new chemical probes (**1a**,**b**, **2a**−**c**, **3a**−**c**) based on the structure of the main endocannabinoids for their use in biological systems directly or via click chemistry. As proof of concept, 2-arachidonyl glyceryl ether based biotinylated **3b** enables direct visualization of CB_1 receptor in cells. These results represent the starting point for the development of advanced small molecule chemical probes able to generate valuable information about the cannabinoid receptors.

INTRODUCTION

Endocannabinoids are lipid signaling molecules that regulate a wide range of physiological functions in mammals including pain, inflammation, neurodegeneration, feeding, and cognitive and emotional states.[1−3] Although the pharmacology and bioactivity of these molecules have been extensively studied during the past decade, many basic questions remain unanswered. For instance, while most of their effects are thought to be mediated through CB_1 and CB_2 cannabinoid receptors (CBRs), thorough pharmacological studies indicate that cannabinoids regulate cell functions independently of these two receptors.[4] However, the evidence is rather indirect and the molecular characterization of these proposed receptors is still lacking. Therefore, tools that enable acquisition of direct information about the location, levels, and any other relevant aspect of the CBRs would be of utmost importance. Currently such tools are basically limited to the use of antibodies that recognize CB_1 and CB_2 receptors. Nonetheless, they exhibit some shortcomings such as limited sensitivity, specificity, or selectivity in certain biological settings that make difficult the unequivocal interpretation of results;[5] they are not suitable for in vivo or ex vivo applications, and the availability of antibodies requires the molecular characterization of the receptor under study. In this context, small molecule ligands with appropriate affinity and selectivity can greatly contribute to the study of a given receptor or target protein even if it has not been molecularly characterized by their conversion into chemical probes.[6] This strategy has been successfully applied to the study of enzymes with the development of activity-based probes[7] (ABPs) that take advantage of the inherent chemical activity of the enzymes. Hence, extension of this approach to other superfamilies of proteins such as G-protein-coupled receptors (GPCRs), main drug targets in medicinal chemistry programs,[8] currently constitutes an important challenge. In general, efforts have been made toward the development of noninvasive imaging probes,[9] although in the case of GPCRs this goal normally requires the expression of tagged receptors,[10] a fact that hinders direct observation of GPCRs in native systems. In this context, we have recently started a project aimed at the development of small molecule probes suitable for the labeling of native GPCRs. As an initial proof of concept, in our research group we have already reported the first efficacious fluorescent probes for the specific labeling of human serotonin receptors 5-HT_{1A} and 5-HT_6 in transfected cells.[11,12] This fact has led us to extend these efforts to CBRs, where development of tagged small-molecule probes would greatly improve our understanding of the endogenous cannabinoid system, its physiology and its therapeutic potential. Some previous attempts have addressed the development of fluorescent ligands directed toward CB_2R. Among them, the attempts made to modify the CB_2 agonist (2-methyl-1-propyl-1*H*-indol-3-yl)(1-naphthyl)methanone (JWH-015) with a fluorophore (although this modification leads to a complete loss of affinity[13]) or the CB_2 antagonist-based probe 1-(4-{[(6-aminohexyl)amino]methyl}benzyl)-5-(4-chloro-3-methylphenyl)-*N*-(1,3,3-trimethylbicyclo[2.2.1]hept-2-yl)-1*H*-pyrazole-3-carboxamide (MBC94) coupled to a near-infrared dye, which exhibits a K_i for this receptor of 260 nM and enables cell visualization, deserve special attention.[14,15]

In this work we report the synthesis of a set of new chemical probes **1a**,**b**, **2a**−**c**, and **3a**−**c** that bear different tags (biotin, benzophenone, terminal alkyne) and are based on the structures of the main endocannabinoids anandamide (AEA), 2-arachidonoylglycerol (2-AG), and 2-arachidonyl glyceryl ether (**13**, 2-AGE, noladin ether) (Figure 1). Among the synthesized compounds, **3b** stands out as a CB_1 ligand (K_i = 221 nM) suitable for direct visualization of this receptor in cell systems in a comparable manner to available commercial antibodies. These results provide the basis for further development of these or other derivatives as probes for endocannabinoid-target proteins that enable not only visualization in native systems but also to identify other target protein(s) of endocannabinoids, studies that are underway in our laboratory.

RESULTS AND DISCUSSION

Visualization and profiling of endocannabinoid binding sites, including the known cannabinoid receptors CB_1 and CB_2, have

Received: April 13, 2011
Published: June 15, 2011

ACS Publications © 2011 American Chemical Society

raised much attention. To aid this objective, we envisioned the possibility of developing biotinylated derivatives that could be tracked using the adequate avidin or streptavidin conjugate (such as a fluorophore for visualization or solid support for enrichment and identification). Therefore, our first objective was to synthesize a set of AEA-, 2-AG-, and 2-AGE-based biotinylated probes. On the basis of the AEA structure, we envisaged the possibility of introducing the different tags in the tail region or in the polar headgroup (Figure 1). Reported docking studies proposed different possibilities for the AEA bioactive conformation in the CB_1 binding site.[16] Moreover, previous structure–affinity relationship studies on AEA analogues provided some clues on the requirements involved in ligand–receptor recognition.[17] In this regard, replacement of the ethanolamine moiety of AEA for cyclopropylamine or (*R*)-2-hydroxy-1-methylethylamine leads to an increase of CB_1 affinity and metabolic stability (as in the case of (*R*)-methanandamide).[18,19] Therefore, we chose these two moieties as headgroups when biotin was introduced as tag in the tail region (probes **1a**,**b**, Figure 1). Conversely, to assess whether the head position would tolerate the incorporation of chemical tags, we synthesized probes **2a**–**c** (Figure 1). In this case, besides biotin, a photoactivable cross-linker (benzophenone) and a bioorthogonal chemical reporter (terminal alkyne) were also used as chemical tags. The terminal alkyne would be useful for performing click chemistry, while the presence of a benzophenone moiety introduces the possibility of covalent binding between the probe and the receptor after UV irradiation.

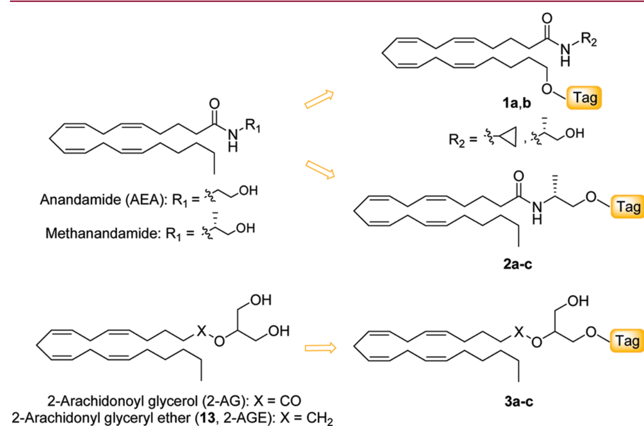
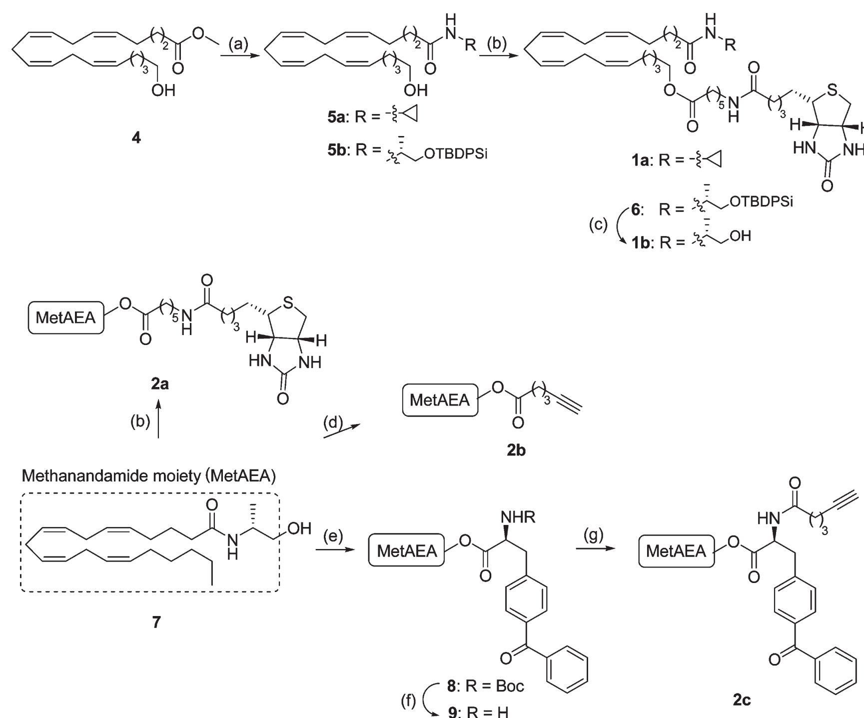


Figure 1. Structures of the main endocannabinoids anandamide, 2-arachidonoylglycerol, and 2-arachidonyl glyceryl ether and designed probes **1**–**3**.

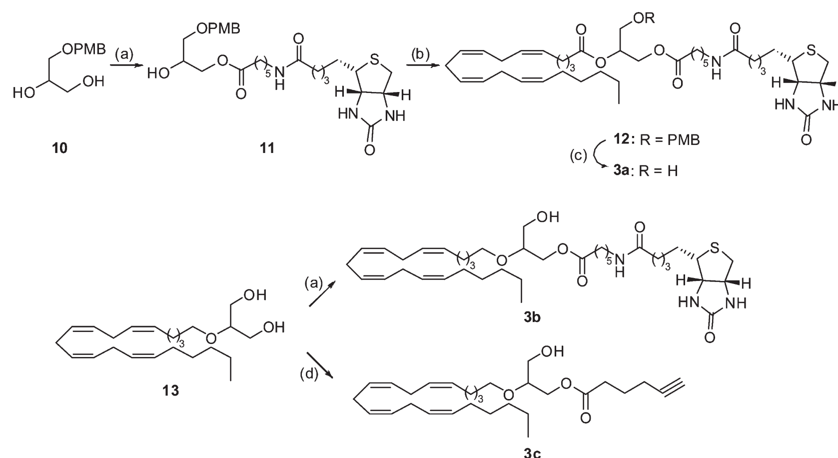
Synthesis of probes **1a**,**b** and **2a**–**c** is depicted in Scheme 1. Direct amidation of methyl ester **4** with the appropriate amine in the presence of trimethylaluminium yielded the corresponding hydroxyamides **5a**,**b**, whose esterification with *N*-(+)-biotinyl-6-aminohexanoic acid, followed by deprotection in the case of **6**, yielded target compounds **1a**,**b**. Because of the lack of significant affinity of these compounds for the CBRs, we next focused our efforts on the AEA-based probes substituted in the polar head (**2a**–**c**) to determine whether the tag attachment at this position could keep the affinity of the probe for the CBRs. For this purpose (*R*)-methanandamide (**7**)[18] was derivatized with biotin (**2a**) and a terminal alkyne (**2b**) by coupling reaction with the corresponding commercial carboxylic acids, whereas **2c** was prepared by esterification of **7** with Boc-4-benzoyl-L-phenylalanine, followed by Boc cleavage and further condensation with

Scheme 1. Synthesis of Compounds 1a,b and 2a–c[a]



[a] Reagents and conditions: (a) R-NH_2, $Al(CH_3)_3$, CH_2Cl_2, 0 °C to rt, 39–79%; (b) *N*-(+)-biotinyl-6-aminohexanoic acid, EDC, DMAP, DMSO, CH_2Cl_2, −10 °C to rt, 17–48%; (c) $(Bu)_4NF$, THF, rt, 46%; (d) 5-hexynoic acid, DCC, DMAP, CH_2Cl_2, 0 °C to rt, 98%; (e) Boc-4-benzoyl-L-phenylalanine, DCC, DMAP, CH_2Cl_2, 0 °C to rt, 47%; (f) TFA, CH_2Cl_2, rt, 85%; (g) 5-hexynoic acid, EDC, HOBt, DMF, CH_2Cl_2, rt, 26%.

Scheme 2. Synthesis of Compounds 3a−c[a]



[a] Reagents and conditions: (a) *N*-(+)-biotinyl-6-aminohexanoic acid, DCC, DMAP, HOBt, DMF, CH_2Cl_2, 60 °C to rt, 20−90%; (b) arachidonic acid, DCC, DMAP, DMF, CH_2Cl_2, −20 °C to rt, 15%; (c) TFA, CH_2Cl_2, rt, 60%; (d) 5-hexynoic acid, DCC, DMAP, CH_2Cl_2, 0 °C to rt, 44%.

Table 1. CB_1 and CB_2 Binding Affinities for Compounds 3a−c

	receptor affinity[a] (K_i ± SEM) (nM)	
compd	CB_1	CB_2
3a	>5000	379 ± 90
3b	221 ± 8	450 ± 11
3c	84.7 ± 0.8	84.9 ± 0.6
2-AG	480 ± 12	1300 ± 215
2-AGE	25 ± 7	>1000

[a] Affinity of compounds was evaluated using HEK293EBNA cells transfected with human CB_1 or CB_2 receptors, respectively, and [^{3}H]CP55940. K_i values are the mean ± SEM from two to four independent experiments performed in triplicate.

5-hexynoic acid. For the different coupling reactions, conditions and reagents were optimized for each case.

Affinity of **1a**,**b** and **2a**−**c** for CBRs was evaluated by radioligand competitive binding assays using membranes of HEK-293-EBNA cells transfected with human CB_1 or CB_2 receptors and [^{3}H]CP55940. The affinity constant K_i was calculated from the inhibitory concentration 50 (IC_{50}), using the Cheng−Prusoff equation.[20] All these derivatives exhibited very poor affinity toward both receptors (K_i > 2000 nM), results that indicate that AEA does not admit tags without a concomitant decrease of affinity for CBRs.

Since the low affinity could be an important shortcoming in terms of specificity and background noise for the intended application of these probes, we decided to explore whether the scaffolds of the other endocannabinoids 2-AG and 2-AGE (Figure 1) could incorporate a tag without dramatically affecting their affinity. These ligands contain two equivalent hydroxyl groups that could potentially be used for introducing the biotin tag. 2-AG-biotinylated probe **3a** was synthesized by consecutive esterifications of monoprotected glycerol **10**[21] with *N*-(+)-biotinyl-6-aminohexanoic acid and arachidonic acid, followed by further cleavage of the *p*-methoxybenzyl (PMB) protecting group of intermediate **12** with trifluoroacetic acid (TFA) (Scheme 2). On the other hand, condensation of 2-AGE (**13**)[22] with *N*-(+)-biotinyl-6-aminohexanoic acid or 5-hexynoic acid afforded the desired **3b** or **3c**, respectively.

Notably, these probes exhibited moderate to good affinities, with K_i in the nanomolar range (Table 1), and derivative **3c** showed the best affinity values for both CBRs [$K_i(CB_1)$ = 84.7 nM; $K_i(CB_2)$ = 84.9 nM], a feature that makes it a promising candidate for click chemistry attachment of more complex tags within biological samples. Also of note, the 2-AG derived probed **3a** is selective for CB_2 [$K_i(CB_1)$ > 5000 nM; $K_i(CB_2)$ = 379 nM] while the 2-AGE-based probe **3b** maintains a moderate affinity for both CBRs [$K_i(CB_1)$ = 221 nM; $K_i(CB_2)$ = 450 nM]. Given that the highest affinity was obtained for **3b** at the CB_1R, we chose this probe to assess its suitability for in vitro cell visualization of the CB_1 receptor. The mouse hippocampal cell line HT-22 was transiently transfected with CB_1 receptor. Cells were incubated with **3b** and streptavidin-Alexa 488 was used to detect the probe and visualized by fluorescence microscopy. Figure 2 shows CB_1 labeling (green). To evaluate the specificity of the labeling, parallel experiments were carried out under the same conditions in nontransfected cells (Figure 2C) and in the presence of an excess of the high affinity (K_i = 0.06 nM) CBR ligand (6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-9-(hydroxymethyl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (HU210)[23] (Figure 2D), showing only low background levels of staining. Although in this model it does not seem to be an issue, we cannot rule out that this staining could represent nonspecific binding or other binding sites different from CB_1 or CB_2. To confirm that the labeling was indeed due to CB_1 binding and to assess the sensitivity of this method, we compared it to antibody-based detection. In this case, transfected HT-22 cells were sequentially treated with **3b**, streptavidin-Alexa 488, anti-CB_1 antibody, and anti-rabbit Alexa 633 conjugate. The fluorescence image shows that labeling with a small molecule chemical probe can be as sensitive as antibody labeling for in vitro detection of CB_1 (Figure 2E,F).

Taken together, these results confirm that the synthesized probe **3b** is a useful visualization tool able to label CB_1 receptor in vitro in a comparable manner to existing antibodies. Considering that antibodies sometimes have important problems of specificity and background, this new probe complements the available tools

dx.doi.org/10.1021/jm2004392 | *J. Med. Chem.* 2011, 54, 5265–5269

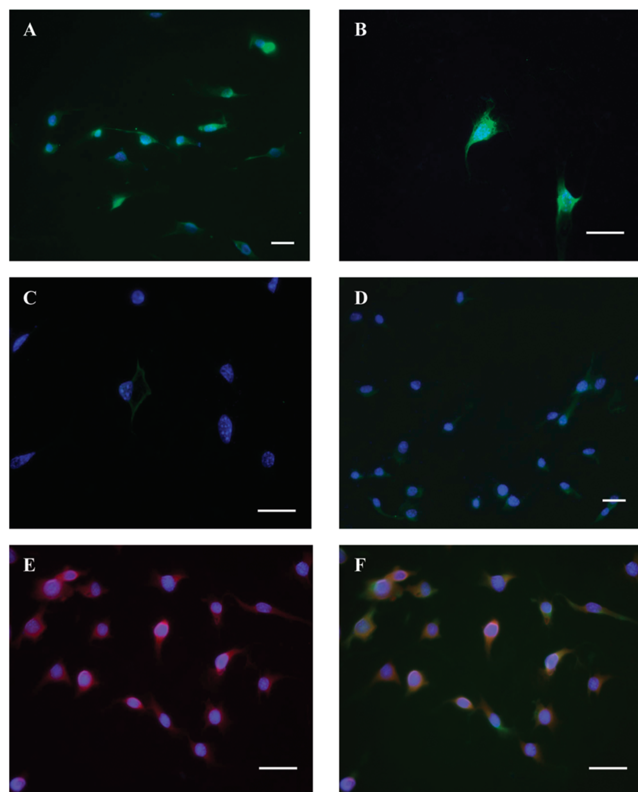


Figure 2. Labeling of HT-22 cells transiently transfected with CB_1 receptor with probe **3b**. Cells were incubated in the presence of **3b** (1 μM) for 1 h. Excess of the probe was removed, and the bound probe was detected with streptavidin-Alexa Fluor 488 (green). Nuclei were stained with Höechst 33258 (blue), and cell samples were mounted and imaged using a Zeiss inverted fluorescence microscope (A, B). To assess specificity, the same experiment was carried out using nontransfected cells (C) or in the presence of an excess of HU210 (D). Colabeling studies of **3b** (green) and anti-CB_1 antibody detected with the corresponding secondary antibody conjugated to Alexa 633 (red): red emission channel (E) and merge (F). Bars: 25 μm.

for the study of the CBRs. In addition, this approach could be extended to other GPCRs, superfamily that lacks available antibodies for many of its members, and therefore, information about expression of protein must be indirectly inferred from mRNA expression and autoradiography images.

We are currently extending the number of probes for CBRs using different synthetic cannabinoid ligands aimed at obtaining selective probes with higher affinities. All these results will be reported in due course.

■ EXPERIMENTAL SECTION

Chemistry. Full synthetic details and characterization data, including HPLC−MS purity analysis of final compounds **1a**,**b**, **2a**−**c**, and **3a-c**, and synthesis of all intermediates are described in the Supporting Information. All compounds tested were >95% pure by HPLC.

General Procedure for the Synthesis of 1a and 2a. To a solution of the corresponding alcohol (1 equiv) in dry dichloromethane (DCM, 90 mL/mmol) with activated 4 Å molecular sieves (30 mg), a mixture of *N*-(+)-biotinyl-6-aminohexanoic acid (2 equiv) in DMSO (10 mL/mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 3.5 equiv) in dry DCM (10 mL/mmol) was added at −10 °C and under an argon atmosphere. The mixture was stirred for 5 min before 4-(dimethylamino)pyridine (DMAP, 1.5 equiv) was added, and the mixture was stirred at room temperature (rt) for 48 h. Then the solution was washed sequentially with saturated $NaHCO_3$ and NH_4Cl solutions. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (DCM/MeOH, 9:1) to afford **1a** (48% yield from 0.10 mmol of alcohol **5a**) and **2a** (17% yield from 0.10 mmol of alcohol **7**).

Synthesis of 1b. To a solution of protected alcohol **6** (29 mg, 30 μmol) in anhydrous tetrahydrofuran (THF, 1.1 mL), tetrabutylammonium fluoride (10 mg, 3 μmol) was added. The mixture was stirred at rt for 2 h. Then the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (DCM/MeOH, 9:1) to afford **1b** (10 mg, 46%).

General Procedure for the Synthesis of 2b and 3c. To a solution of 5-hexynoic acid (1.1 equiv) in dry DCM (2 mL/mmol), a solution of *N*,*N*′-dicyclohexylcarbodiimide (DCC, 1.1 equiv) and DMAP (0.2 equiv) in dry DCM (1.9 mL/mmol) was added at 0 °C and under an argon atmosphere. The mixture was stirred at this temperature for 30 min before a solution of alcohol **7** or **13** (1 equiv) in dry DCM (0.3 mL/mmol) was added. The mixture was stirred at rt for 10 h, and the solvent was evaporated under reduced pressure. The residue was dissolved in DCM and washed with 5% $NaHCO_3$ solution. The aqueous layer was extracted with DCM, and the organic layers were dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc, 6:4) to afford **2b** (98% yield from 0.28 mmol of alcohol **7**) and **3c** (44% yield from 0.10 mmol of alcohol **13**).

Synthesis of 2c. A mixture of 5-hexynoic acid (7 mg, 0.06 mmol), 1-hydroxybenzotriazole (HOBt, 8 mg, 0.06 mmol), and EDC (11 mg, 0.06 mmol) in anhydrous DMF (0.25 mL) was stirred at rt and under an argon atmosphere for 1 h. Then a solution of amine **9** (30 mg, 0.05 mmol) in anhydrous DMF (0.5 mL) was added. The mixture was stirred for 10 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc and washed with 10% $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc, 1:1) to yield **2c** (9 mg, 26%).

Synthesis of 3a. A solution of PMB protected alcohol **12** (40 mg, 0.05 mmol) in 2.7 mL of 10% TFA in DCM was stirred at rt for 5 min. The reaction was quenched by pouring the mixture into 50 mL of saturated $NaHCO_3$ (pH $\approx$ 8). The aqueous layer was extracted with DCM, the combined organic layers were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by column chromatography (DCM/MeOH, 95:5 to 8:2) to afford **3a** (22 mg, 60%).

Synthesis of 3b. A suspension of *N*-(+)-biotinyl-6-aminohexanoic acid (39 mg, 0.11 mmol) and HOBt (3 mg, 0.02 mmol) in anhydrous DMF (1 mL) with activated 4 Å molecular sieves was heated until a clear solution was obtained (60 °C, 20 min). Once the mixture was cooled to rt, a solution of DCC (25 mg, 0.12 mmol) in DCM (0.2 mL) was added dropwise. The mixture was stirred at rt for 3 h. Then a solution of **13** (80 mg, 0.22 mmol) and DMAP (0.12 mg, 0.001 mmol) in DMF was added, and the mixture was stirred at 60 °C for 4 h and at rt for 24 h. The mixture was filtered and washed with DCM/MeOH (1:1), and the solvents were removed under reduced pressure. The residue was purified by column chromatography (DCM/MeOH, 95:5 to 8:2) to afford **3b** (15 mg, 20%).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Additional synthetic procedures, analytical and spectral data for all intermediates, spectral data and HPLC−MS results for all final compounds (**1a**,**b**, **2a**−**c**, **3a**−**c**), binding assays, and cell visualization experiments.

This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*For M.L.L.-R.: phone, +34 913944239; fax, +34 913944103; e-mail, mluzlr@quim.ucm.es. For S.O.-G.: phone, +34 913944894; fax, +34 913944103; e-mail, siortega@quim.ucm.es.

DEDICATION

†Dedicated to the memory of Professor Rafael Suau.

ACKNOWLEDGMENT

This work was supported by grants from the Spanish Ministerio de Ciencia e Innovación (MICINN, Grant SAF2010-22198-C02-01, and predoctoral fellowship to L.M.-C.) and Comunidad Autónoma de Madrid (Grant S-SAL-249-2006). The authors thank the Spanish Society of Medicinal Chemistry (SEQT) and GlaxoSmithKline for the Young Researcher Award to L.M.-C. S.O.-G. is a Ramon y Cajal Scholar funded by MICINN and the European Social Fund.

ABBREVIATIONS USED

ABP, activity-based probe; AEA, anandamide; 2-AG, 2-arachidonoyl-glycerol; 2-AGE, 2-arachidonyl glyceryl ether; CBR, cannabinoid receptor; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; GPCR, G-protein-coupled receptor; HOBt, 1-hydroxybenzotriazole; SEM, standard error of the mean; rt, room temperature

REFERENCES

(1) Fowler, C. J. "The tools of the trade"—an overview of the pharmacology of the endocannabinoid system. *Curr. Pharm. Des.* **2008**, *14*, 2254–2265.

(2) Pachar, P.; Bátkai, S.; Kunos, G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* **2006**, *58*, 389–462.

(3) Bellocchio, L.; Lafenêtre, P.; Cannich, A.; Cota, D.; Puente, N.; Grandes, P.; Chaouloff, F.; Piazza, P. V.; Marsicano, G. Bimodal control of stimulated food intake by the endocannabinoid system. *Nat. Neurosci.* **2010**, *13*, 281–283.

(4) Mackie, K.; Stella, N. Cannabinoid receptors and endocannabinoids: evidence for new players. *AAPS J.* **2006**, *8*, E-298−306.

(5) Grimsey, N. L.; Goodfellow, C. E.; Scotter, E. L.; Dowie, M. J.; Glass, M.; Graham, E. S. Specific detection of CB_1 receptors; cannabinoid CB_1 receptors antibodies are not all created equal!. *J. Neurosci. Methods* **2008**, *171*, 78–86.

(6) Simon, G. M.; Cravatt, B. F. Challenges for the "chemical-systems" biologist. *Nat. Chem. Biol.* **2008**, *11*, 639–642.

(7) Simon, G. M.; Cravatt, B. F. Activity-based proteomics of enzyme superfamilies: serine hydrolases as a case study. *J. Biol. Chem.* **2010**, *285*, 11051–11055.

(8) Lagerström, M. C.; Schiöth, H. B. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat. Rev. Drug Discovery* **2008**, *7*, 339–357.

(9) Park, D.; Don, A. S.; Massamiri, T.; Karwa, A.; Warner, B.; Macdonald, J.; Hemenway, C.; Naik, A.; Kuan, K. T.; Dilda, P. J.; Wong, J. W.; Camphausen, K.; Chinen, L.; Dyszlewski, M.; Hogg, P. J. Non-invasive imaging of cell death using an Hsp90 ligand. *J. Am. Chem. Soc.* **2011**, *133*, 2832–2835.

(10) Nonaka, H.; Fujishima, S. H.; Uchinomiya, S. H.; Ojida, A.; Hamachi, I. Selective covalent labeling of tag-fused GPCR proteins on live cell surface with a synthetic probe for their functional analysis. *J. Am. Chem. Soc.* **2010**, *132*, 9301–9309.

(11) Alonso, D.; Vázquez-Villa, H.; Gamo, A. M.; Martínez-Esperón, M. F.; Tortosa, M.; Viso, A.; Fernández de la Pradilla, R.; Junquera, E.; Aicart, E.; Martín-Fontecha, M.; Benhamú, B.; López-Rodríguez, M. L.; Ortega-Gutiérrez, S. Development of fluorescent ligands for the human $5\text{-}HT_{1A}$ receptor. *ACS Med. Chem. Lett.* **2010**, *1*, 249–253.

(12) Vázquez-Villa, H.; González-Vera, J. A.; Benhamú, B.; Viso, A.; Fernández de la Pradilla, R.; Junquera, E.; Aicart, E.; López-Rodríguez, M. L.; Ortega-Gutiérrez, S. Development of molecular probes for the human $5\text{-}HT_6$ receptor. *J. Med. Chem.* **2010**, *53*, 7095–7106.

(13) Yates, A. S.; Doughty, S. W.; Kendall, D. A.; Kellam, B. Chemical modification of the naphtoyl 3-position of JWH-015: in search of a fluorescent probe to the cannabinoid CB_2 receptor. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3758–3762.

(14) Bai, M.; Sexton, M.; Stella, N.; Bornhop, D. J. MBC94, a conjugable ligand for cannabinoid CB_2 receptor imaging. *Bioconjugate Chem.* **2008**, *19*, 988–992.

(15) Sexton, M.; Woodruff, G.; Horne, E. A.; Hsing Lin, Y.; Muccioli, G. G.; Bai, M.; Stern, E.; Bornhop, D. J.; Stella, N. NIR-mbc94, a fluorescent ligand that binds to endogenous CB_2 receptors and is amenable to high-throughput screening. *Chem. Biol.* **2011**, *18*, 563–568.

(16) Padgett, L. W.; Howlett, A. C.; Shim, J.-Y. Binding mode prediction of conformationally restricted anandamide analogs within the CB_1 receptor. *J. Mol. Signaling* **2008**, *3*, 5.

(17) Pavlopoulos, S.; Thakur, G. A.; Nikas, S. P.; Makriyannis, A. Cannabinoid receptors as therapeutic targets. *Curr. Pharm. Des.* **2006**, *12*, 1751–1769.

(18) Abadji, V.; Lin, S.; Taha, G.; Griffin, G.; Stevenson, L. A.; Pertwee, R. G.; Makriyannis, A. (*R*)-Methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. *J. Med. Chem.* **1994**, *37*, 1889–1893.

(19) Hillard, C. J.; Manna, S.; Greenberg, M. J.; Dicamelli, R.; Ross, R. A.; Stevenson, L. A.; Murphy, V.; Pertwee, R. G.; Campbell, W. B. Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB_1). *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1427–1433.

(20) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(21) Lim, Z.-Y.; Thuring, J. W.; Holmes, A. B.; Manifava, M.; Ktistakis, N. T. Synthesis and biological evaluation of a $PtdIns(4,5)P_2$ and a phosphatidic acid affinity matrix. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1067–1075.

(22) Hanus, L.; Abu-Lafi, S.; Fride, E.; Breuer, A.; Vogel, Z.; Shalev, D. E.; Kustanovich, I.; Mechoulam, R. 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB_1 receptor. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3662–3665.

(23) Howlett, A. C.; Barth, F.; Bonner, T. I.; Cabral, G.; Casellas, P.; Devane, W. A.; Felder, C. C.; Herkenham, M.; Mackie, K.; Martin, B. R.; Mechoulam, R.; Pertwee, R. G. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.* **2002**, *54*, 161–202.