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3′-Functionalized Adamantyl Cannabinoid Receptor Probes

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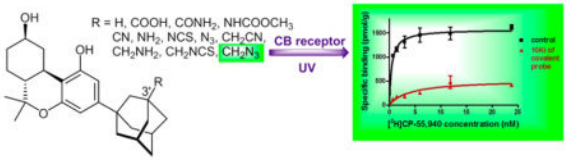
Abstract

The aliphatic side chain plays a pivotal role in determining the cannabinergic potency of tricyclic classical cannabinoids, and we have previously shown that this chain could be substituted successfully by adamantyl or other polycyclic groups. In an effort to explore the pharmacophoric features of these conformationally fixed groups, we have synthesized a series of analogues in which the C3 position is substituted directly with an adamantyl group bearing functionality at one of the tertiary carbon atoms. These substituents included the electrophilic isothiocyanate and photoactivatable azido groups, both of which are capable of covalent attachment with the target protein. Our results show that substitution at the 3′-adamantyl position can lead to ligands with improved affinities and CB1/CB2 selectivities. Our work has also led to the development of two successful covalent probes with high affinities for both cannabinoid receptors, namely, the electrophilic isothiocyanate AM994 and the photoactivatable aliphatic azido AM993 analogues.



INTRODUCTION

The discovery of the first cannabinoid receptor, CB1, by Howlett and co-workers in 1988 marked the beginning of a renaissance in cannabinoid research.[1] CB1, which is implicated in

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Notes

The authors declare no competing financial interest.

Supporting Information

Saturation binding curves using [^{3}H]CP-55,940 for CB1 and CB2 receptors preincubated with the 3′-azido (**20**) and 3′-isothiocyanato (**19**) analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

the CNS effects of cannabinoids in humans and other mammals, is found in greatest abundance in the hippocampus, cerebellum, and striatum. Humans, rats, and mice share a 97–99% amino acid sequence homology for CB1. In 1993 Munro and co-workers identified a second cannabinoid receptor.[2] This new receptor exhibited 44% amino acid sequence homology overall and 68% homology with the transmembrane region of CB1. This receptor would later come to be known as CB2, and it is associated with the peripheral organs of the immune system. CB2 is found primarily in the spleen, tonsils, and thymus and with low expression in the CNS.[3] Activation of CB2 is therefore not expected to lead to any of the CNS effects that are associated with cannabis abuse. Furthermore, its emerging role in immunomodulation makes it an attractive therapeutic target.

The discovery by one of us of the high affinity adamantyl cannabinoid **1a** (AM411)[4] suggested that rather than excluding the ligand from the receptor active site, the sterically demanding adamantyl group conferred high affinity for CB1 and modest (7-fold) selectivity in favor of CB1 (Figure 1).[4] *endo*-Norbornyl cannabinoid **1b** (AM735)[5] and *exo*-norbornyl **1c** (AM731)[5] underscore some of the subtleties inherent in this structural motif. Both **1c** and **1b** have greater CB2 affinity than **1a**, but **1c** also has inverted selectivity compared to **1a**, with a 10-fold preference for CB2 over CB1.[5] These data suggested that small structural modifications of the adamantyl group may lead to substantial variations in the overall pharmacological profiles within this class of analogues. This motivated us to further explore the pharmacophoric features of these unusual cannabinoids in which the flexible side chain of their classical structure has been substituted by conformationally fixed cyclic substituents.[6–8] In this effort, we have now synthesized a series of novel analogues in which the 3-position of the adamantyl ring is substituted with different moieties. For the tricyclic component of the new analogues we used the 9*β*-hydroxyhexahydrocannabinol structure that we had earlier shown to confer an optimal pharmacophoric profile within the classical cannabinoid structure.[6,8,9] In an effort to explore the pharmacophoric features of the cannabinoid binding domain within each of the two receptors' active site(s), we also included in our series analogues with suitable electrophilic and photoactivatable substituents, as we have done in previous work to obtain structural and functional information on the ligand–receptor complex using an approach involving receptor mutants and LC/MS/MS methods which we named ligand assisted protein structure (LAPS).[10–17] To that effect we used the isothiocyanato group as our favored electrophile, having shown that under the experimental conditions of our receptor binding experiments this group reacted exclusively with the cysteine thiol group(s) within the receptor's ligand binding domain(s).[13] Also, as with earlier work, our choice of a photoreactive covalent group was the aliphatic azide moiety that upon photoactivation is transformed into a nitrene group capable of covalent attachment with amino acid residues at or in the vicinity of the active site. This SAR exploration led to a series of analogues with a wide range of affinities and functional CB1/CB2 profiles. Additionally, we obtained four novel covalently reacting analogues, two of which, **17** and **16**, exhibit excellent covalent binding profiles. In future work both of these novel analogues will serve as biochemical tools to study the CB1/CB2 ligand–receptor complex using the LAPS approach developed in our laboratory.[10–17]

CHEMISTRY

We considered two distinct strategies to prepare the novel adamantyl analogues. We failed to implement our first approach, which was to functionalize one of the tertiary adamantyl carbon atoms after coupling to 2,6-dimethoxyphenol **1** (Scheme 1); therefore, we explored the coupling reactions of **1** with bifunctional adamantanes **2**, **3**, and **4**. A number of attempts to couple **1** with 1,3-dihydroxyadamantane **2** or with 1-bromo-3-hydroxyadamantane **3** failed to provide the desired products, leading in each case to intractable mixtures. Fortunately, the Friedel–Crafts alkylation of **1** with 3-hydroxyadamantane-1-carboxylic acid **4** took place cleanly and in nearly quantitative yield.[4] We found that it was essential to first dissolve **4** at 40–50 °C in the methanesulfonic acid that served as the reaction solvent and to add 2,6-dimethoxyphenol **1** subsequently.[18] At higher reaction temperatures we observed a redox process that led to adamantane carboxylic acid as a byproduct.

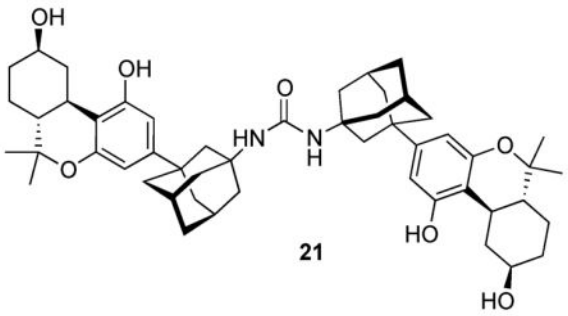
Exposure of **5** to (trimethylsilyl)diazomethane produced the methyl ester that was immediately converted to phenolic triflate **6** in 91% overall yield from **4**. Formation of the triflate in the presence of the free carboxylate had proved to be difficult, hence the need to protect the acid as the methyl ester. The catalytic reductive cleavage of the phenolic triflate in the presence of $PdCl_2(PPh_3)_2$ and dppp[19] had been difficult to reproduce, often leading to incomplete reactions and the appearance of precipitated metallic palladium. Careful purification of **6** to remove traces of *N*-phenyltriflamide byproduct from the preceding reaction was necessary, as was the inclusion of a small amount of polymethylhydrosiloxane (PHMS) in the reaction mixture.[20] When this attention was given to the reaction conditions, the reductive cleavage to **7** took place reproducibly in high yield (89%). Global demethylation of **7** with boron tribromide led to resorcinol **8** in 89% yield.[21]

We chose to apply the excellent procedure that had been developed at the Eli Lilly Company during their synthesis of nabilone for the assembly of the tricyclic cannabinoid nucleus from **8**.[22] We were aware from our earlier work that the Lilly reaction represents a finely optimized process and that any deviation from the preferred reaction solvent, chloroform, inevitably led to erosion of the yield. The difficulty we faced was the sparing solubility of **8** in chloroform. Indeed, when we performed the condensation of **8** with the mixture of diacetates **9** and **10** in chloroform solvent in the presence of tosic acid, we were able to isolate only ~5% of **11** after several days of reaction. Inspection of the reaction vessel revealed a substantial quantity of insoluble solid material that was determined to be the polar and poorly soluble resorcinol **8**. The solution to the problem was to dissolve **8** in the minimum volume of dry acetone and to then add the mixture of diacetates and tosic acid in chloroform. Under these conditions **8** remained in solution and adduct **11** was isolated in 78% yield after 2.5 days. Traces of tricyclic compound **12** were also detected by TLC in the product mixture. The cyclization of **11** to **12** was accomplished in 96% yield by exposure to trimethylsilyl triflate in nitromethane. This reagent has proven to be superior to tin tetrachloride that was used by the Lilly group to assemble the tricyclic cannabinoid nucleus. The isolation of the product from the thick matrix of insoluble tin salts that forms during workup had been problematic. Reduction of the C9 keto group in **12** with sodium borohydride led to alcohol **13** in 95% yield as a 95/5 mixture of β-equatorial and α-axial

diastereoisomers. Ketone **12** and acid **13** were the common intermediates for the synthesis of the entire series of functionalized adamantyl cannabinoids that is described in what follows.

Scheme 2 summarizes the conversion of **13** to isothiocyanatomethylene analogue **16** and azidomethylene analogue **17**. The carboxylic acid function in **13** was converted to the amide **14** by treatment with aqueous ammonia in THF in the presence of EDCI and HOBT (84% yield).[23] Reduction of carboxamide **14** with lithium aluminum hydride followed by workup with either Glauber's salt or careful addition of water followed by aqueous NaOH did not lead to amine **15** in satisfactory yield. Fortunately, reduction of **14** with borane–dimethyl sulfide complex proceeded cleanly and led to **15** in 90% yield.[24] The reaction of amine **15** with carbon disulfide, tosyl chloride, and triethylamine led to isothiocyanate **16** in 89% yield.[25] The synthesis of azidoadamantyl cannabinoid **17** from **15** was accomplished through the straightforward application of Wong's diazo transfer reaction using triflyl azide.[26,27] This reaction worked extraordinarily well leading to azide **17** in 93% yield. The absence of any protecting groups at any point in the synthesis is noteworthy.

The synthesis of isothiocyanate **19** and azide **20** is shown in Scheme 3. Exposure of ketocarboxylate **12** to diphenylphosphoryl azide (DPPA)[28] and triethylamine in refluxing toluene led to an intermediate isocyanate through the Curtius rearrangement of the intermediate acyl azide.[29] Typical conditions for hydrolysis of the isocyanate to the amine, refluxing in aqueous acid or in aqueous base, led only to decomposition of the isocyanate. After some experimentation we found that exposure of the isocyanate to mercuric acetate in aqueous THF followed by reduction of the mercury salt with sodium borohydride led to amine **18** in 76% overall yield from **12**.[30] Simultaneous reduction of the C9 keto group during this step led to the to C9-β alcohol group in **18**, saving one step. Dimeric urea **21** was formed in ~5% yield as a side product during this reaction. Conversion of the primary amino group in **18** to the isothiocyanate was carried out in the same way as shown in Scheme 2, leading to **19** in 86% yield. Azido transfer from triflyl azide as in the case of **15** converted amine **18** to azide **20** in 98% yield.



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The remaining analogues **22**–**26** were prepared from ketocarboxylate **12** as summarized in Scheme 4. The challenging task was the preparation of cyanomethyl analogue **23**. A model study employing hydroxymethyl adamantane unsurprisingly revealed that S_N2 displacement of the derived mesylate by cyanide was uniformly unsuccessful even under forcing conditions. This made it necessary to adapt our strategy, so Arndt–Eistert homologation of carboxylate **12** was carried out. Sequential treatment of **12** with oxalyl chloride and catalytic

DMAP in THF to produce the acid chloride was followed by exposure to diazomethane.[31] The resulting α-diazoketone was heated to reflux in THF in the presence of catalytic silver benzoate and ammonium hydroxide to give carboxamide **22** in low overall yield (27% from **12**).[32] The conversion of the crude amide **22** to nitrile **23** was accomplished in two steps. Treatment first with trifluoroacetic anhydride and pyridine in dioxane led to dehydration of the carboxamide to the nitrile and also converted the free phenolic hydroxyl group to the trifluoroacetate ester.[33] Hydrolysis of trifluoroacetate with methanolic potassium carbonate followed by addition of sodium borohydride led to **23** in 86% overall yield for the two steps from **22**.

Ketoacid **12** was converted to carboxamide **24** in 81% yield by exposure to a mixture of EDCI and HOBT in THF in the presence of ammonium hydroxide. The preparation of nitrile **25** was carried out in the same way as for **23**. The overall yield of **25** was 71% for the two steps from **24**.

Ketoacid **12** was also used to prepare methyl carbamate **26** (Scheme 4). Reduction with sodium borohydride led to C9 alcohol **13**. Exposure of **13** to diphenylphosphoryl azide and triethylamine in toluene at reflux led initially to an acyl azide that underwent Curtius rearrangement to an intermediate isocyanate that was immediately treated with sodium methoxide in methanol at reflux. It is noteworthy that no protecting groups were used in any part of the chemistry.

CB1/CB2 BINDING AFFINITIES

These were obtained by measuring the abilities of the new analogues to displace the radiolabeled CB1/CB2 agonist [^{3}H]CP-55,940 from suitably prepared membrane preparations (Table 1). As in previous work for CB1, we used rat brain membranes while for CB2 we used both mouse (mCB2) and human (hCB2) preparations obtained from HEK293 cells expressing these receptors.[9,34] The use of two CB2 receptor preparations was aimed at addressing species differences that we observed earlier.[35] In addition, because of the covalent nature of the binding of the isothiocyanates **16** and **19**, the affinities for CB receptors are presented as "apparent K_i" (K_i*) values, and these are expected to be dependent on the time during which the preparation is pretreated with the ligand. When such time dependent experiments were performed with **19** in CB1 receptor preparations, we observed (data not shown) that increasing the incubation time from 15 to 90 min increases the CB1 affinity of **19** by 3-fold ($K_{i(15\ min)}$ = 96 nM; $K_{i(30\ min)}$ = 58.6 nM; $K_{i(60\ min)}$ = 35.4 nM; $K_{i(90\ min)}$ = 35.7 nM). Thus, under the assay conditions the lower K_i value for **19** (35.4 nM, Table 1) was reached in 60 min.

Our data show that the analogues with the highest affinities for both receptors were those bearing the isothiocyanato, azido, and cyano groups (**19**, **20**, and **25**, respectively) as well as their methylene homologues **16**, **17**, and **23**, respectively. Also exhibiting relatively high affinity was the methyl carbamate analogue **26**. Conversely, compounds with free carboxylic acid (**13**) or amine (**18**, **15**) substituents as well as carboxamide **14** had very low affinities for both receptors, suggesting that strong hydrogen bond donating groups could not be accommodated within the binding site(s).

Compounds with the highest affinities were the homologues in which a methylene group is introduced between the adamantyl ring and the NCS, N_3, or CN functional groups. Also observed among the analogues was a certain level of CB1 vs CB2 selectivity as exemplified by the methylisothiocyanato **16** which showed 3- or 10-fold selectivity in favor of CB1 and methyl carbamate **26** with a 4- to 15-fold selectivity for CB2. The parent compound (**27**) bearing an unsubstituted adamantyl group had generally lower affinities for both receptors when compared to the higher affinity substituted analogues, suggesting the existence of favorable interactions of some of the 3′-adamantyl substituents with the CB1/CB2 receptor binding domain.

FUNCTIONAL CHARACTERIZATION

We focused on those analogues possessing the highest CB1/CB2 affinities, and the experiments were carried out by measuring changes in forskolin-stimulated cAMP, as detailed earlier.[9,34] Our testing results show that compounds **16**, **19**, **20**, **23**, and **25**–**27** potently decreased the levels of cAMP, indicating that within this signaling mechanism these compounds behaved as potent agonists at the CB1 receptor with the methyl isothiocyanato **16** and methylcyano **23** analogues being more potent than their methylazido counterpart **17** (Table 2). Surprisingly, when tested with hCB2 preparations, all compounds failed to decrease the levels of cAMP with **19**, **20**, and **17** showing no change in the cAMP measured, thus behaving as neutral antagonists. The remaining analogues showed increases in cAMP levels and behaved as inverse agonists. The dose–response curves of representative analogues for both the CB1 and CB2 receptors are depicted in Figures 2 and 3, respectively, where data for the standard cannabinoid agonist CP-55,940 are also shown for comparison. We observe that **17**, **23**, and **26** have high binding affinities for CB2 (Table 1), thus resembling the standard CB1/CB2 agonist CP-55,940. However, unlike CP-55,940, which behaves as a full agonist at CB2, the adamantyl analogues described here act as antagonists/inverse agonists at CB2 in the cAMP functional test. We have no immediate explanation for this striking observation.

COVALENT LABELING OF THE CB1/CB2 RECEPTORS

The ability of the compounds carrying covalently reacting groups to label each of the two receptors was obtained by measuring reductions in the binding for the radioligand [^{3}H]CP-55,940 when the preparation was pretreated with the covalent ligand, compared to the untreated sample (details in Experimental Section). Following earlier work from our laboratory, the experiment was conducted by pretreating the sample with a concentration equal to 10-fold the compound's K_i value for the receptor in question and subsequently measuring the decrease of B_{max} obtained from a saturation curve using [^{3}H]CP-55,940. Our results clearly show that all four of our covalent ligands (**16**, **17**, **19**, **20**) are capable of covalently labeling both receptors with the best results being obtained by the 3′-isothiocyanatomethyl (**16**) and the 3′-azidomethyl (**17**) analogues (data shown in Figure 4 and Table 3, while saturation binding curves for analogues **19** and **20** are provided in Supporting Information). Thus, **16** and **17** appear to be optimal probes for studying the structures of the ligand–receptor binding complexes to be carried out in future work.

As negative controls we used the cyano analogue **23** and its azido counterpart **17** prior to irradiation, both of which were not expected to interact covalently with the CB1/CB2 receptor preparations. We observed no reductions in the levels of specific binding (B_{max}) of [^{3}H]CP-55,940 under conditions identical to those used for the covalent labeling (data not shown).

To better understand the receptor labeling characteristics of the electrophilic isothiocyanato analogues, we carried out a time-dependent study using CB1 receptor membranes, a 10K_i nM concentration of **19** and varied the incubation time (Figure 5). According to our experimental conditions, the termination of the ligand–receptor covalent reaction is accomplished by centrifugation. Unreacted ligand was removed by repeatedly pelleting and washing the membrane preparation after the incubation period was completed. Our studies showed that with a 15 min incubation 26% of the receptor sites were occupied irreversibly. By increasing the incubation time to 30 min, covalent binding increased to 54% with no further increase seen if the incubation time was extended to 60 min. Thus, at the ligand concentration used, covalent binding had reached its plateau at 30 min of incubation time. The control experiment was a 60 min incubation with no added **19**. However, we note that the true kinetic behavior of the covalent reaction between **19** and the cannabinoid receptor is not necessarily reflected by this time-dependent receptor labeling, since the incubation time does not represent the precise reaction time of the ligand with the receptor.[12]

CONCLUSION

In our current effort to explore the pharmacophoric requirements of the “adamantyl subsite” within the CB1/CB2 binding domain we have synthesized a family of (1-adamanyl)-9β-hydroxyhexahydrocannabinols carrying C3′-substituents on the adamantyl ring. Our studies show that within this subsite, properly chosen C3′-substituents are capable of engaging in favorable interactions with both CB1 and CB2 receptors with some substantial level of CB1/CB2 selectivity. Arguably, such substitutions could be further extended to develop more selective CB1 or CB2 analogues. All successful compounds when tested in vitro in the cAMP assay were potent agonists for the CB1 receptor. Conversely, all of these ligands behaved as CB2 inverse agonists or neutral antagonists in the same functional test. This unexpected discovery opens the door for developing highly functionally selective CB1 agonists. Importantly, our study has led to the development of a new pair of potentially very successful covalent ligands **17** and **16**. In future work these will be tested by using our LAPS methodology to obtain structural information on the active CB1 and inactive CB2 receptor conformations. The two covalent probes are also currently being explored for X-ray crystallographic work involving the CB1/CB2 ligand–receptor complexes and for labeling work with other cannabinoid-related GPCRs.

EXPERIMENTAL SECTION

Chemistry

^{1}H NMR and ^{13}C spectra were recorded either at 300 MHz (^{1}H) and 75 MHz (^{13}C) or at 500 MHz (^{1}H) and 126 MHz (^{13}C). Chemical shifts are reported in parts per million (δ) and are referenced to the solvent, i.e., 7.26/77.0 for $CDCl_3$. Multiplicities are indicated as br

(broadened), s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sept (septet), or m (multiplet). Coupling constants (*J*) are reported in hertz (Hz). Thin layer chromatography (TLC) was performed on glass plates 250 μm, particle size 5–17 μm, pore size 60 Å. Flash column chromatography was performed on silica gel, 200–400 mesh, or premium silica gel, 60 Å, 40–75 μm. All moisture sensitive reactions were performed under a static atmosphere of nitrogen or argon in oven-dried or flame-dried glassware. Purity and homogeneity of all materials were determined to be at least 95% from TLC, ^{1}H NMR, and ^{13}C NMR spectra as well as by LC/MS analysis using a Waters MicroMass ZQ system [electrospray ionization (ESI) with Waters-2525 binary gradient module coupled to a photodiode array detector (Waters-2996) and ELS detector (Waters-2424) using a XTerra MS C18, 5 μm, 4.6 mm × 50 mm column and acetonitrile/water]. All optical rotations were measured on a JASCO digital polarimeter in a 0.1 dL cell.

Methyl (1*r*,3*r*)-3-(4-Hydroxy-3,5-dimethoxyphenyl)-adamantane-1-carboxylate (5)—To a solution of 3-hydroxyada-mantane-1-carboxylic acid **4** (5.0 g, 25.5 mmol) in methanesulfonic acid (23.0 mL) heated to 40 °C and stirred under argon atmosphere in a reaction flask equipped with stir bar and reflux condenser was added of 2,6-dimethoxyphenol **1** (3.6 g, 23.2 mmol), and the mixture was stirred at 50 °C for 3.0 h. The reaction was allowed to cool to room temperature, diluted with CH_2Cl_2 (100 mL), poured onto ice–water (300 mL), and was stirred overnight until both organic and aqueous layers were clear. The organic layer was removed, and the aqueous layer was back-extracted with CH_2Cl_2 (2 × 50 mL). The organic layers were combined, washed with water (2 × 50 mL) followed by brine (20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to give **5** as a lavender solid in quantitative yield (7.706 g, 23.20 mmol). The crude product was taken on to the next step without purification. An analytical sample was obtained via flash chromatography on silica gel (10–30% acetone in hexanes). ^{1}H NMR (300 MHz, CD_3OD) δ 6.49 (s, 2H), 3.71 (s, 6H), 2.06 (br, 2H), 1.85 (br, 2H), 1.79 (br, 4H), 1.75 (br, 2H), 1.62 (br, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 181.4, 148.9, 142.7, 134.7, 103.5, 56.8, 45.8, 43.6, 42.8, 39.4, 37.5, 36.7, 30.3; mp 174–176 °C; IR (neat; cm^{-1}) 3371, 2907, 2854, 1693, 1605, 1521, 1451, 1214, 1115; HRMS (ESI^+) calculated for $C_{19}H_{24}O_5$ [M + H^+] 333.1703, found 333.1717.

Methyl (1*r*,3*r*)-3-[3,5-Dimethoxy-4-(trifluoromethylsulfonyloxy)phenyl]adamantane-1-carboxylate (6)—To a solution of **5** (7.7 g, 23.2 mmol) in benzene (92.8 mL) and MeOH (23.2 mL) was added $TMSCHN_2$ (12.8 mL of 2.0 M in hexanes, 25.5 mmol), and the mixture was stirred at room temperature under air atmosphere for 30 min. The progress of the reaction was monitored by TLC (R_f = 0.45 in 30% acetone in hexanes). The crude product was concentrated in vacuo to yield a yellow oil, which was directly taken on to the subsequent reaction. To a solution of the methyl ester in CH_2Cl_2 (128 mL) in a reaction flask equipped with stir bar and reflux condenser were added $PhNTf_2$ (9.1 g, 25.5 mmol), Et_3N (3.6 mL, 25.5 mmol), and catalytic DMAP. The reaction was heated to reflux overnight, cooled to room temperature, then washed with a 3 N NaOH solution (2 × 30 mL). The aqueous layer was back-extracted with CH_2Cl_2 (2 × 20 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and adsorbed onto Celite. The product was purified via flash

chromatography on silica gel (0% to 5% to 10% acetone in hexanes, TLC R_f = 0.30 in 15% acetone in hexanes) to give triflate **6** as a white solid in 96% yield (10.6 g, 22.2 mmol) over two steps. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.59 (s, 2H), 3.89 (s, 6H), 3.68 (s, 3H), 2.25 (br, 2H), 2.00 (br, 2H), 1.93 (m, 4H), 1.87 (br, 4H), 1.74 (m, 2H); ^{13}C (125 MHz, $CDCl_3$) δ 177.6, 151.9, 151.1, 126.0, 118.7 (q, J_{C-F} = 320.1 Hz, 1C), 101.8, 56.2, 51.8, 44.1, 42.0, 41.8, 38.0, 37.1, 35.4, 28.5; mp 148.9–150.1 °C; IR (neat; cm^{-1}) 2936, 1729, 1607, 1454, 1417, 1248, 1205, 1136, 1080, 886, 872; HRMS (ESI$^+$) calculated for $C_{21}H_{26}F_3O_7S$ [M + H$^+$] 479.1352, found 479.1369.

Methyl (1*r*,3*r*)-3-(3,5-Dimethoxyphenyl)adamantane-1-carboxylate (7)—To solution of triflate 6 (5.2 g, 10.9 mmol) in DMF (44.0 mL) were added $PdCl_2(PPh_3)_2$ (418 mg, 0.6 mmol), dppp (450 mg, 1.1 mmol), *n*-Bu_3N (13.0 mL, 54.5 mmol), HCOOH (88% w/v in H_2O, 1.03 mL, 27.3 mmol), and catalytic polymethylhydrosiloxane (PMHS) in a reaction flask equipped with stir bar, reflux condenser, and a three-way tap, and the mixture was stirred for 5 min under argon atmosphere at room temperature for 5 min. The reaction vessel was subjected to three cycles of vacuum/argon, then heated to 95 °C under argon pressure for 16 h. The reaction mixture was cooled to room temperature, diluted with Et_2O (25.0 mL) and 1 N HCl (10 mL), stirred for 30 min, and then filtered through a plug of Celite to remove insoluble palladium. The organic layer was extracted, and the aqueous layer was back-extracted with Et_2O (3 × 20 mL). The organic layers were combined, washed with 1 N HCl (2 × 10 mL) followed by brine (20 mL), dried over $MgSO_4$, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (0% to 5% to 10% EtOAc in hexanes) to give **7** as an off-white solid in 89% yield (3.2 g, 9.7 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52 (d, J = 2.2 Hz, 2H), 3.32 (t, J = 2.2 Hz, 1H), 3.80 (s, 6H), 3.66 (s, 3H), 2.22 (br, 2H), 2.01 (br, 2H), 1.91 (m, 8H), 1.72 (br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 160.6, 152.7, 103.5, 97.2, 55.2, 51.7, 44.1, 42.0, 41.8, 38.1, 36.7, 35.5, 20.6; mp 60.7–63.2 °C; IR (neat; cm^{-1}) 2907, 2854, 1728, 1596, 1455, 1423, 1309, 1242, 1204, 1153, 1087, 1068, 1013, 830; HRMS (ESI$^+$) calculated for $C_{20}H_{27}O_4$ [M + H$^+$] 331.1909, found 331.1922.

(1*r*,3*r*)-3-(3,5-Dihydroxyphenyl)adamantane-1-carboxylic Acid (8)—A solution of dimethoxy aromatic **7** (3.5 g, 10.6 mmol) in CH_2Cl_2 (53.0 mL) under N_2 atmosphere was cooled to 0 °C and stirred for 5 min. To the cooled solution was added BBr_3 (5.12 mL, 53.2 mmol) dropwise, and the mixture was stirred and gradually warmed to room temperature over 3 h. The reaction was cooled to 0 °C before quenching with ice cold H_2O (20 mL) and was taken up in Et_2O (50 mL). The organic layer was washed with 1 N HCl (2 × 10 mL), and the aqueous layer was back-extracted with Et_2O (2 × 20 mL). The organic layers were combined, washed with brine (10 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (20% to 30% to 40% acetone in hexanes) to give resorcinol **8** as a white foam in 89% yield (2.7 g, 9.5 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.32 (d, J = 2.1 Hz, 2H), 6.10 (t, J = 2.1 Hz, 1H), 2.17 (br, 2H), 1.95 (br, 2H), 1.90 (br, 4H), 1.83 (br, 4H), 1.74 (br, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 181.5, 159.3, 154.1, 104.5, 101.0, 45.6, 43.4, 42.8, 39.5, 37.5, 36.8, 30.3; mp 194.8–195.9 °C; IR (neat; cm^{-1}) 3234 (br), 2907, 2853, 1690, 1602, 1513, 1450, 1278, 1156, 1005; HRMS (ESI$^+$) calculated for $C_{17}H_{21}O_4$, [M + H$^+$] 289.1440, found 289.1449.

(1*R*,3*r*)-3-{4-[(1*R*,2*R*,5*R*)-6,6-Dimethyl-4-oxobicyclo[3.1.1]-heptan-2-yl]-3,5-dihydroxyphenyl}adamantane-1-carboxylic Acid (11)—A solution of resorcinol **8** (609 mg, 2.11 mmol) dissolved in a minimal volume of acetone (~2 mL) by heating to 55 °C in a reaction flask equipped with a stir bar and reflux condenser was degassed with argon, and to it was added a mixture of *p*-TSA·H_2O (442 mg, 2.32 mmol) and diacetates **9** and **10** (609 mg, 2.54 mmol) in $CHCl_3$ (6.0 mL). The reaction was degassed with argon for an additional 10 min and then stirred at 55 °C for 2.5 days. The progress of the reaction was monitored by multiple elution TLC. Upon completion, the reaction was allowed to cool to room temperature and taken up in with Et_2O (20 mL) and washed with 1 N HCl (10 mL). The aqueous layer was back-extracted with Et_2O (2 × 10 mL), and organic phases were combined, washed with brine, dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (10% to 20% to 30% to 50% acetone in hexanes) to give condensation product **11** as an off-white foam in 78% yield (698 mg, 1.64 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.32 (s, 2H), 4.00 (t, J = 8.0 Hz, 1H), 3.73 (dd, J = 18.8, 7.6 Hz, 1H), 2.65–2.58 (m, 1H), 2.49–2.46 (m, 2H), 2.42 (dd, J = 18.8, 8.7 Hz, 1H), 2.18–2.16 (m, 3H), 1.94–1.73 (m, 12H), 1.36 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 219.9, 181.6, 157.6, 151.0, 114.5, 105.0, 59.3, 48.6, 45.4, 43.4, 43.2, 42.7, 39.5, 38.5, 37.2, 36.8, 30.4, 30.3, 26.6, 25.0, 22.5; $[\alpha]^{23}_D$ +56.2 (*c* 1.99, CH_3OH); IR (neat; cm^{-1}) 3387 (br), 2930, 2855, 1693, 1621, 1421, 1024; HRMS (ESI^+) calculated for $C_{26}H_{33}O_5$ [M + H^+] 425.2329, found 425.2338.

(1*R*,3*r*)-3-[(6a*R*,10a*R*)-1-Hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-adamantane-1-carboxylic Acid (12)—To a solution of condensation product **11** (50 mg, 0.12 mmol) in $MeNO_2$ (12.0 mL) cooled to 0 °C under N_2 atmosphere was added TMSOTf (0.108 mL, 0.59 mmol) dropwise. The reaction was allowed to gradually warm to room temperature and stirred for 2 h. Upon completion of the reaction, monitored by TLC, 1 N HCl (3 mL) was added and stirred for 5 min and the reaction mixture was concentrated in vacuo. The product was taken up in Et_2O (3 mL), and the organic phase was washed with 1 N HCl (2 × 2 mL). The aqueous layer was back-extracted with Et_2O (2 × 3 mL), and the organic layers were combined, washed with brine (1 × 2 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (10% to 20% to 30% acetone in hexanes) to give the ketone **12** as an off-white foam in 96% yield (48 mg, 0.11 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.34 (d, J = 1.8 Hz, 1H), 6.28 (d, J = 1.8 Hz, 1H), 3.85 (dd, J = 14.9, 3.4 Hz, 1H), 2.80 (td, J = 12.8, 3.5 Hz, 1H), 2.49 (dd, J = 9.5, 5.1 Hz, 2H), 2.22–1.69 (m, 16H), 1.60–1.24 (m, 2H), 1.44 (s, 3H), 1.08 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 214.1, 181.2, 157.1, 155.5 151.6 109.7, 106.1, 104.8, 77.4, 46.0, 45.2, 43.0, 42.4, 41.3, 39.2, 37.0, 36.9, 36.5, 35.8, 30.0, 27.9, 27.6, 18.8; $[\alpha]^{23}_D$ −85.2 (*c* 0.95, CH_3OH); IR (neat; cm^{-1}) 3360 (br), 2907, 2855, 1694, 1621, 1452, 1360; HRMS (ESI^+) calculated for $C_{26}H_{33}O_5$ [M + H^+] 425.2329, found 425.2322.

(1*R*,3*r*)-3-[(6a*R*,9*R*,10a*R*)-1,9-Dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-adamantane-1-carboxylic Acid (13)—To a solution of keto acid **12** (183 mg, 0.043 mmol) in MeOH (2.1 mL) cooled to 0 °C was added $NaBH_4$ (16 mg, 0.43 mmol). The reaction flask was warmed to room temperature,

stirred for 1.5 h, cooled to 0 °C, quenched with 1 N HCl (0.5 mL), and concentrated in vacuo. The crude product was taken up in Et_2O (5 mL) and washed with 1 N HCl (2 × 1 mL). The aqueous layer was back-extracted with Et_2O (2 × 3 mL), and the organic layers were combined, washed with brine (1 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash column chromatography on silica gel (10–40% acetone in hexanes) to give C9-β hydroxy acid **13** as an off-white amorphous solid in 95% yield (17 mg, 0.041 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.33 (d, J = 1.9 Hz, 1H), 6.23 (d, J = 1.9 Hz, 1H), 3.72 (dt, J = 15.3, 5.4 Hz, 1H), 3.52 (m, 1H), 2.44 (td, J = 11.2, 2.4 Hz, 1H), 2.21–2.07 (m, 3H), 1.96–1.71 (m, 14H), 1.48–1.11 (m, 4H), 1.35 (s, 3H), 1.03 (s, 3H); ^{13}C (125 MHz, CD_3OD) δ 181.5, 157.5, 155.9, 151.2, 110.8, 106.3, 105.0, 77.7, 71.4, 50.3, 45.5, 43.3, 42.7, 39.9, 39.5, 37.2, 36.8, 36.7, 34.9, 30.3, 28.3, 27.2, 19.2; $[\alpha]^{22}_D$ −87.9 (*c* 2.83, MeOH); IR (neat; cm^{-1}) 3215 (br), 2932, 2856, 2661, 1695, 1621, 1514, 1055, 830; HRMS (ESI^+) calculated for $C_{26}H_{35}O_5$ [M + H+] 427.2484, found 427.2489. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98% and retention time of of 4.5 min for the title compound.

(1*R*,3*r*)-3-[(6a*R*,9*R*,10a*R*)-1,9-Dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-adamantane-1-carboxamide (14)—To a solution of hydroxy acid **13** (86 mg, 0.20 mmol) and HOBT (32 mg, 0.24 mmol) in THF (2.0 mL), stirred at room temperature for 5 min under ambient atmosphere, was added a suspension of EDCI (41 mg, 0.24 mmol), and the reaction mixture was stirred overnight. After 16 h, NH_4OH (0.5 mL) was added dropwise over 5 min, and the reaction mixture was stirred for an additional 1 h. Additional EDCI (41 mg, 0.24 mmol) was added to the reaction and was stirred overnight. The reaction was quenched with 1 N HCl (1 mL) and extracted with EtOAc (3 × 1 mL). The organic layers were combined, washed with phosphate buffer (1 mL) followed by brine (1 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (5–10% EtOH in CH_2Cl_2 with NH_4OH_{sat}) to give amide **14** as an off-white semisolid in 81% yield (69 mg, 0.072 mmol), mp 174–176 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 6.32 (d, J = 1.9 Hz, 1H), 6.25 (d, J = 1.9 Hz, 1H), 3.74 (m, 1H), 3.57–3.48 (m, 1H), 2.48–2.42 (m, 1H), 2.21 (m, 2H), 2.12 (m, 1H), 1.90–1.74 (m, 12H), 1.48–1.28 (m, 3H), 1.34 (s, 3H), 1.24–1.15 (m, 1H), 1.18 (m, 1H), 1.04 (s, 3H), 0.95 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 183.8, 157.5, 155.9, 151.2, 110.8, 106.3, 105.1, 77.7, 71.4, 50.3, 45.7, 43.3, 42.9, 40.0, 39.6, 37.4, 36.8, 36.7, 34.9, 30.4, 28.3, 27.2, 19.2; $[\alpha]^{25}_D$ −90.6 (*c* 0.64, CH_3OH); IR (neat; cm^{-1}) 3354 (br), 2937, 2861, 1625, 1421, 1035; HRMS (ESI^+) calculated for $C_{26}H_{36}NO_4$ [M + H^+] 426.2645, found 426.2650. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 95% and retention time of 4.2 min for the title compound.

(6a*R*,9*R*,10a*R*)-3-[(1*r*,3*R*)-3-(Aminomethyl)adamantan-1-yl]-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]-chromene-1,9-diol (15)—To a solution of amide **14** (27 mg, 0.064 mmol) in THF (1.3 mL) cooled to 0 °C under N_2 was added $BH_3{\cdot}S(CH_3)_2$ in THF (0.064 mL, 0.64 mmol) dropwise, and the reaction mixture was stirred. The reaction mixture was allowed to warm to room temperature over 3 h. Then the reaction was quenched by addition of EtOH (1 mL) dropwise and refluxed overnight. The crude reaction mixture was concentrated in vacuo, taken up in EtOAc (2 mL), and washed

with pH 7 phosphate buffer (1 mL). The aqueous layer was back-extracted with EtOAc (2 × 1 mL). The organic layers were combined and washed with brine (2 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (5–10% EtOH in CH_2Cl_2 with sat. aq NH_4OH) to give homologated amine 15 as a clear glass in 90% yield (24 mg, 0.058 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.32 (d, J = 1.9 Hz, 1H), 6.23 (d, J = 1.9 Hz, 1H), 3.73 (m, 1H), 3.52 (m, 1H), 3.05 (s, 2H), 2.43 (td, J = 11.2, 2.4 Hz, 1H), 2.19–1.08 (m, 19H), 1.34 (s, 3H), 1.02 (s, 3H), 0.93 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 157.4, 155.8, 151.8, 110.6, 106.4, 105.2, 77.6, 71.4, 64.6, 50.3, 47.5, 43.8, 41.3, 40.0, 37.6, 37.4, 36.6, 36.5, 34.9, 30.7, 28.3, 27.2, 19.2; $[\alpha]^{25}_D$ −41.9 (c 0.51, CH_3OH); IR (neat; cm^{-1}) 3395 (br), 2944, 1581, 1574, 1477, 1402, 1279, 1131, 1001; HRMS (ESI^+) calculated for $C_{26}H_{38}NO_3$ [M + H^+] 412.2852, found 412.2846. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 95% and retention time of 4.1 min for the title compound.

(6a*R*,9*R*,10a*R*)-3-[(1*r*,3*R*)-3-(Isothiocyanatomethyl)-adamantan-1-yl]-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromene-1,9-diol (16)—To a solution of homologated amine 15 (5 mg, 0.012 mmol) and Et_3N (~8 μL, 0.061 mmol) in THF (1 mL) cooled to 0 °C under N_2 was added CS_2 (3 μL, 0.049 mmol), and the reaction mixture was stirred for 2 h at 0 °C. To the reaction flask was added TsCl (8 mg, 0.043 mmol), and the solution was stirred for an additional 2 h while gradually warming to room temperature. The reaction was quenched with pH 7 phosphate buffer (1 mL) and the mixture diluted with Et_2O (5 mL). The organic phase was washed with phosphate buffer (2 × 2 mL), and the aqueous phase was back-extracted with Et_2O (2 × 3 mL). The organic layers were combined, washed with brine (4 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (10–20% acetone in hexanes) to give isothiocyanate **16** as a white amorphous solid in 89% yield (5 mg, 0.011 mmol). ^{1}H NMR (500 MHz, CD_3OD) δ 6.33 (d, J = 1.9 Hz, 1H), 6.23 (d, J = 1.9 Hz, 1H), 3.73 (m, 1H), 3.52 (m, 1H), 3.28 (s, 2H), 2.44 (td, J = 11.3, 2.5 Hz, 1H), 2.20 (m, 2H), 2.11 (m, 1H), 1.91–1.54 (m, 13H), 1.45–1.29 (m, 2H), 1.35 (s, 3H), 1.18 (m, 1H), 1.04 (s, 3H), 0.94 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.6, 155.9, 151.1, 131.3, 110.9, 106.3, 105.1, 77.7, 71.4, 57.6, 50.3, 46.4, 43.5, 40.1, 39.9, 37.5, 37.0, 36.9, 36.7, 34.9, 30.4, 28.3, 27.2, 19.2; $[\alpha]^{23}_D$ −54.0 (c 0.98, CH_3CN); IR (neat; cm^{-1}) 3333 (br), 2925, 2850, 2181, 2104, 1701, 1620, 1573, 1516, 1448, 1416, 1384, 1273, 1141, 1040; HRMS (EI^+) calculated for $C_{27}H_{35}NO_3S$ [M^+] 453.2338, found 453.2323. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 97% and retention time of 5.3 min for the title compound.

(6a*R*,9*R*,10a*R*)-3-[(1*r*,3*R*)-3-(Azidomethyl)adamantan-1-yl]-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]-chromene-1,9-diol (17)—To a mixture of amine 15 (~6 mg, 0.014 mmol), Et_3N (~6 μL, 0.041 mmol), and $CuSO_4$ (cat.) in H_2O (0.50 mL) at room temperature under air was added dropwise a 0.1 M solution of TfN_3 (0.27 mL, 0.027 mmol) in CH_2Cl_2 and to it was added additional CH_2Cl_2 (0.25 mL) followed by MeOH (1.5 mL) until a homogeneous solution was obtained. The reaction mixture was stirred for 12 h, then concentrated in vacuo and taken up in Et_2O (5 mL). The organic layer was washed with pH 7 phosphate buffer (2 × 3 mL) and the aqueous layer back-extracted with Et_2O (2 × 5 mL). The organic layers were combined, washed with brine (3 mL), dried

over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (10–20% acetone in hexanes) to give azide **17** as an orange amorphous solid in 93% yield (~6 mg, 0.013 mmol). ^{1}H NMR (500 MHz, CD_3OD) δ 6.31 (d, J = 1.8 Hz, 1H), 6.21 (d, J = 1.8 Hz, 1H), 3.73 (m, 1H), 3.52 (m, 1H), 3.06 (s, 2H), 2.44 (td, J = 11.2, 2.2 Hz, 1H), 2.15 (m, 2H), 2.10 (m, 1H), 1.91–0.90 (m, 17H), 1.35 (s, 3H), 1.03 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.5, 155.8, 151.4, 110.7, 106.3, 105.1, 77.7, 71.4, 65.1, 50.3, 46.6, 43.6, 40.4, 39.9, 37.4, 37.1, 36.7, 36.7, 34.9, 30.4, 28.3, 27.2, 19.2; $[\alpha]^{23}_D$ −73.2 (*c* 0.56, CH_3CN); IR (neat; cm^{-1}) 3356 (br), 2925, 2850, 2098, 1703, 1621, 1573, 1449, 1416, 1363, 1275, 1143, 1054; HRMS (EI^+) calculated for $C_{26}H_{35}N_3O_3$ [M^+] 437.2678, found 423.2688. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98% and retention time of 5.1 min for the title compound.

(6a*R*,9*R*,10a*R*)-3-[(1*r*,3*R*)-3-Aminoadamantan-1-yl]-6,6-di-methyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromene-1,9-diol (18)—To a solution of keto acid **12** (0.11 g, 0.27 mmol) and Et_3N (45 μL, 0.032 mmol) in PhMe (2.7 mL) at room temperature under air in a reaction flask equipped with a stir bar and reflux condenser was added DPPA (70 μL, 0.032 mmol) dropwise, and the solution was refluxed for 3 h. Upon completion of the reaction, the reaction mixture was concentrated in vacuo and taken up in THF/H_2O (1:1, v/v) (1.3 mL) and stirred. A solution of $Hg(OAc)_2$ (0.10 g, 0.32 mmol) in THF/H_2O (1:1, v/v) (1.3 mL) was added to the reaction mixture, and stirring was continued overnight. To the reaction mixture was added a solution of $NaBH_4$ (51 mg, 1.4 mmol) in 5% NaOH (w/v), and stirring was continued for an additional 3 h. The reaction was quenched by the addition of 1 N HCl (~1 mL) until gas evolution ceased, and the solution was concentrated in vacuo. The crude product was taken up in EtOAc (2 mL) and washed with pH 7 phosphate buffer (2 mL). The aqueous phase was back-extracted with EtOAc (2 × 1 mL), and the organic layers were combined, washed with brine (2 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (5–10% EtOH in CH_2Cl_2 with sat. aq NH_4OH) to give amine **18** as a clear glass in 76% yield (82 mg, 0.21 mmol). ^{1}H NMR (500 MHz, CD_3OD) δ 6.31 (d, J = 1.8 Hz, 1H), 6.22 (d, J = 1.8 Hz, 1H), 3.72 (m, 1H), 3.52 (m, 1H), 2.42 (td, J = 11.2, 2.3 Hz, 1H), 2.20–2.05 (m, 3H), 1.90–1.83 (m, 1H), 1.78–1.47 (m, 11H), 1.45–1.11 (m, 4H), 1.33 (s, 3H), 1.01 (s, 3H), 0.94 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.5, 155.8, 150.9, 110.7, 106.3, 105.2, 77.6, 71.3, 51.5, 50.3, 49.5, 45.2, 43.1, 39.9, 39.2, 36.7, 36.5, 34.9, 31.5, 28.3, 27.2, 19.2; $[\alpha]^{23}_D$ −57.4 (*c* 0.67, CH_3OH); IR (neat; cm^{-1}) 3415 (br), 2973, 2848, 1651, 1593, 1574, 1437, 1384, 1234, 1203, 1042; HRMS (ESI^+) calculated for $C_{25}H_{36}NO_3$ [$M + H^+$] 398.2696, found 398.2690. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 95% and retention time of 4.0 min for the title compound.

(6a*R*,9*R*,10a*R*)-3-[(1*r*,3*R*)-3-Isothiocyanatoadamantan-1-yl]-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]-chromene-1,9-diol (19)—To a solution of amine **18** (~5 mg, 0.012 mmol) and Et_3N (8 μL, 0.058 mmol) in THF (1 mL) cooled to 0 °C under N_2 was added CS_2 (~3 μL, 0.046 mmol), and the solution was stirred for 2 h at 0 °C. To the reaction mixture was added TsCl (8 mg, 0.041 mmol), and stirring was continued for an additional 2 h while gradually warming to room temperature. The reaction was quenched with pH 7 phosphate buffer (1 mL) and the mixture diluted with Et_2O (5 mL). The organic

phase was washed with phosphate buffer (2 × 2 mL) and the aqueous phase back-extracted with Et_2O (2 × 3 mL). The organic layers were combined, washed with brine (4 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (10–20% acetone in hexanes) to give isothiocyanate **19** as a white amorphous solid in 86% yield (5 mg, 0.010 mmol). ^{1}H NMR (500 MHz, CD_3OD) δ 6.30 (d, J = 1.8 Hz, 1H), 6.22 (d, J = 1.8 Hz, 1H), 3.74 (m, 1H), 3.52 (m, 1H), 2.44 (td, J = 11.3, 2.3 Hz, 1H), 2.26 (m, 2H), 2.15–1.55 (m, 13H), 1.49–1.26 (m, 3 H), 1.35 (s, 3H), 1.18 (m, 1H), 1.03 (s, 3H), 0.94 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.6, 156.0, 149.7, 129.3, 111.1, 106.2, 104.9, 77.7, 71.3, 60.7, 50.3, 50.0, 44.0, 42.4, 39.9, 39.2, 36.6, 35.8, 34.9, 31.3, 28.2, 27.2, 19.2; $[\alpha]^{23}_D$ −67.0 (c 0.88, CH_3CN); IR (neat; cm^{-1}) 3338 (br), 2930, 2857, 2093(br), 1701, 1620, 1573, 1452, 1416, 1384, 1273, 1054; HRMS (EI^+) calculated for $C_{26}H_{33}NO_3S$ [M^+] 439.2181, found 439.2183. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 99% and retention time of 5.2 min for the title compound.

(6a*R*,9*R*,10a*R*)-3-[(1*r*,3*R*)-3-Azidoadamantan-1-yl]-6,6-di-methyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromene-1,9-diol (20)—To a mixture of amine **18** (5 mg, 0.012 mmol), Et_3N (~5 μL, 0.037 mmol), and $CuSO_4$ (cat.) in H_2O (0.5 mL) at room temperature under air was added dropwise a 0.1 M solution of TfN_3 (0.25 mL, 0.025 mmol) in CH_2Cl_2, and to it was added additional CH_2Cl_2 (0.25 mL) followed by MeOH (1.5 mL) until a homogeneous solution was obtained. The reaction was stirred for 12 h, then concentrated in vacuo and taken up in Et_2O (5 mL). The organic layer was washed with pH 7 phosphate buffer (2 × 3 mL) and the aqueous layer back-extracted with Et_2O (2 × 5 mL). The organic layers were combined, washed with brine (2 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (10–20% acetone in hexanes) to give azide **20** as an orange amorphous solid in 98% yield (5 mg, 0.012 mmol). ^{1}H NMR (500 MHz, CD_3OD) δ 6.31 (d, J = 1.8 Hz, 1H), 6.21 (d, J = 1.8 Hz, 1H), 3.74 (m, 1H), 3.52 (m, 1H), 2.44 (td, J = 11.2, 2.2 Hz, 1H), 2.29 (m, 2H), 2.11 (m, 1H), 1.95–1.67 (m, 12H), 1.48–1.13 (m, 4H), 1.35 (s, 3H), 1.03 (s, 3H), 0.94 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 158.0, 156.0, 150.1, 111.1, 106.0, 105.1, 77.7, 71.3, 60.8, 50.3, 48.0, 42.8, 41.8, 39.9, 39.6, 36.7, 36.2, 34.9, 31.7, 28.2, 27.2, 19.2; $[\alpha]^{23}_D$ −33.3 (c 1.02, CH_3CN); IR (neat; cm^{-1}) 3312 (br), 2924, 2754, 2088, 1704, 1620, 1574, 1455, 1416, 1364, 1249, 1138, 1055; HRMS (EI^+) calculated for $C_{25}H_{33}N_3O_3$ [M^+] 423.2522, found 423.2510. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98% and retention time of 5.0 min for the title compound.

2-{(1*R*,3*r*)-3-[(6a*R*,9*R*,10a*R*)-1,9-Dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-adamantan-1-yl}acetonitrile (23)—To a solution of ketoacid 12 (42 mg, 0.099 mmol) in THF (2.0 mL) cooled to 0 °C were added a catalytic amount of DMF and $(COCl)_2$ (42 μL, 0.49 mmol), and the reaction mixture was allowed to warm to room temperature over 45 min. The reaction mixture was again cooled to 0 °C, and to it was added a solution of CH_2N_2 in ether (1.0 mL, 0.5 M). The reaction mixture was stirred for 2 h while gradually warming to room temperature. Excess CH_2N_2 was quenched with acetic acid. The contents were dissolved in Et_2O (5 mL) and extracted with 1 N HCl (2 × 1 mL). The aqueous layer was back-extracted with Et_2O (2 × 1 mL). The

organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude α-diazoketone was carried onto the next reaction.

To a solution of the intermediate α-diazoketone (~0.099 mmol) in THF (5.0 mL) were added silver benzoate (0.090 g, 0.39 mmol), Et_3N (0.055 mL, 0.39 mmol), and NH_4OH (0.13 mL, 0.97 mmol), and the reaction mixture was stirred at room temperature for 2 h. The contents were dissolved in Et_2O (5 mL) and extracted with 1 N HCl (2 × 1 mL). The aqueous layer was back-extracted with Et_2O (2 × 1 mL), and the organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and absorbed onto Celite. The crude product was purified via flash chromatography on silica gel (20–40% acetone in hexanes) to give the homologated amide **22** as an amorphous solid in 27% yield (12 mg, 0.026 mmol) over the two steps.

To a solution of amide **22** (11 mg, 0.025 mmol) in dioxane (1 mL) at room temperature under N_2 atmosphere was added pyridine (40 μL, 0.50 mmol), and the mixture was stirred for 10 min and followed by the addition of TFAA (34 μL, 0.25 mmol). The reaction mixture was stirred for 16 h and quenched with the addition of pH 7 phosphate buffer (0.5 mL). The reaction mixture was taken up in Et_2O (1 mL) and the organic phase washed with phosphate buffer (2 × 1 mL) and the aqueous phase back-extracted with Et_2O. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was redissolved in MeOH (1 mL), and to the mixture was added K_2CO_3 (35 mg, 0.25 mmol). The mixture was stirred at room temperature under ambient atmosphere for 3 h. The reaction mixture was cooled to 0 °C, and to it was added $NaBH_4$ (92 mg, 0.25 mmol). The mixture was stirred for 3 h. The reaction mixture was quenched with 1 N HCl (1 mL) and was concentrated in vacuo and taken up in Et_2O (3 mL). The organic phase was extracted with 1 N HCl (2 mL), and the aqueous phase was back-extracted with Et_2O (2 × 2 mL). The organic layers were combined, washed with brine (2 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (10% to 20% to 30% acetone in hexanes) to yield nitrile **23** as a clear colorless film in 86% yield (9.1 mg, 0.021 mmol) from amide **22**. ^{1}H (300 MHz, CD_3OD) δ 6.33 (d, J = 1.8 Hz, 1H), 6.23 (d, J = 1.8 Hz, 1H), 3.71 (m, 1H), 3.51 (m, 1H), 2.43 (td, J = 11.2, 2.2 Hz, 1H), 2.30 (s, 2H), 2.23–2.02 (m, 3H), 1.95–1.53 (m, 12H), 1.48–1.10 (m, 4H), 1.35 (s, 3H), 1.03 (s, 3H), 1.05–0.85 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 155.9, 151.0, 119.1, 111.2, 110.9, 106.3, 105.0, 77.7, 71.4, 50.3, 48.3, 43.2, 43.2, 41.9, 39.9, 37.7, 36.7, 34.9, 34.0, 32.0, 30.6, 28.2, 27.2, 19.2; $[\alpha]^{24}_D$ −73.1 (c 0.41, CH_3OH); IR (neat; cm^{-1}) 3377 (br), 2975, 2909, 2852, 2247, 1620, 1574, 1448, 1416, 1055, 840; HRMS (ESI^+) calculated for $C_{27}H_{36}NO$ [M + H^+] 422.2695, found 422.2700. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 97% and retention time of 4.8 min for the title compound.

(1*R*,3*r*)-3-[(6a*R*,10a*R*)-1-Hydroxy-6,6-dimethyl-9-oxo-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-adamantane-1-carboxamide (24)—To a solution of keto acid **12** (14 mg, 0.20 mmol) and HOBT (6 mg, 0.048 mmol) in THF (1.6 mL), stirred at room temperature for 5 min under ambient atmosphere, was added a suspension of EDCI (9 mg, 0.048 mmol), and the mixture was stirred overnight. After 16 h, NH_4OH (0.5 mL) was added dropwise over 5 min and the reaction mixture was stirred for an additional 1 h. EDCI (9 mg, 0.048 mmol) was added, and the reaction mixture was stirred

overnight. The reaction was quenched with 1 N HCl (1 mL) and extracted with EtOAc (3 × 1 mL). The organic layers were combined, washed once with phosphate buffer (1 mL) followed by brine (1 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (5–10% EtOH in CH_2Cl_2 with sat. aq NH_4OH) to yield amide **24** as an off-white semisolid in 76% yield (10 mg, 0.024 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.34 (d, J = 1.8 Hz, 1H), 6.28 (d, J = 1.8 Hz, 1H), 3.85 (dd, J = 14.9, 3.4 Hz, 1H), 2.80 (m, 1H), 2.51 (m, 2H), 2.24–1.75 (m, 16H), 1.60–1.24 (m, 2H), 1.45 (s, 3H), 1.08 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 214.4, 183.7, 157.4, 155.8, 151.9, 110.0, 106.4, 105.1, 77.7, 46.3, 45.7, 43.2, 43.1, 42.9, 41.6, 39.6, 37.5, 36.8, 36.1, 30.4, 28.2, 27.9, 19.0; $[\alpha]^{25}_D$ −93.3 (*c* 0.31, CH_3OH); IR (neat; cm^{-1}) 3478, 3345, 2925, 2854, 1679, 1634, 1572, 1415, 1355; HRMS (ESI^+) calculated for $C_{25}H_{34}NO_4$ [M + H^+] 424.2489, found 424.2488.

(1*R*,3*r*)-3-[(6a*R*,9*R*,10a*R*)-1,9-Dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-adamantane-1-carbonitrile (25)—To a solution of ketoamide **24** (6 mg, 0.014 mmol) in dioxane (2.5 mL) at room temperature under N_2 atmosphere was added pyridine (~24 μL, 0.28 mmol), and the mixture was stirred for 10 min followed by the addition of TFAA (~19 μL, 0.14 mmol). The reaction mixture was stirred for 16 h and quenched with the addition of pH 7 phosphate buffer (0.5 mL) and diluted with Et_2O (1 mL). The organic phase was washed with phosphate buffer (2 × 1 mL) and the aqueous phase back-extracted with Et_2O (1 mL). The organic layers were combined, washed with brine (2 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was redissolved in MeOH (1 mL), and to it was added K_2CO_3 (20 mg, 0.14 mmol). The mixture was stirred at room temperature under ambient atmosphere for 16 h. The reaction mixture was cooled to 0 °C, and $NaBH_4$ (5 mg, 0.14 mmol) was added. The mixture was stirred for 3 h. The reaction was quenched with 1 N HCl (1 mL), and the reaction mixture was concentrated in vacuo. The reaction mixture was taken up in Et_2O (3 mL). The organic phase was extracted with 1 N HCl (2 mL), and the aqueous phase was back-extracted with Et_2O (2 × 2 mL). The organic layers were combined, washed with brine (2 mL), dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (10% to 20% to 30% acetone in hexanes) to yield nitrile **25** as a clear colorless film in 71% yield (5 mg, 0.010 mmol) from ketoamide **24**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.30 (d, J = 1.8 Hz, 1H), 6.23 (d, J = 1.8 Hz, 1H), 3.73 (m, 1H), 3.51 (m, 1H), 2.44 (td, J = 11.2, 2.2 Hz, 1H), 2.24–1.69 (m, 15H), 1.50–1.10 (m, 4H), 1.35 (s, 3H), 1.03 (s, 3H), 0.94 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.1, 155.0, 148.6, 124.9, 109.8, 106.3, 103.9, 71.0, 70.9, 48.2, 44.8, 41.3, 39.2, 38.5, 35.7, 35.5, 34.9, 33.4, 31.2, 27.9, 27.8, 26.0, 19.0; $[\alpha]^{24}_D$ −91.8 (*c* 0.74, CH_3OH); IR (neat; cm^{-1}) 3380 (br), 2931, 2859, 2235, 1700, 1620, 1575, 1452, 1416, 1273, 1192, 1141, 1056, 827; HRMS (ESI^+) calculated for $C_{26}H_{34}NO_3$ [M + H^+] 408.2539, found 408.2532. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98% and retention time of 4.7 min for the title compound.

Methyl {(1*R*,3*r*)-3-[(6a*R*,9*R*,10a*R*)-1,9-Dihydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl]-adamantan-1-yl}carbamate (26)—To a solution of hydroxy acid **13** (19 mg, 0.045 mmol) in PhMe (1.6 mL) were added Et_3N (~9

μL, 0.068 mmol) and DPPA (14 μL, 0.0068 mmol), and the reaction was heated to reflux for 2 h. The reaction was concentrated in vacuo, and the crude isocyanate was taken up in MeOH (1.6 mL). To it was added NaOMe (24 mg, 0.45 mmol). The reaction mixture was heated to reflux overnight. Upon completion, as determined by TLC, the reaction mixture was concentrated in vacuo. The contents were taken up in Et_2O (5 mL) and extracted with 1 N HCl (2 × 1 mL). The aqueous layer was back-extracted with Et_2O (2 × 1 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and adsorbed onto Celite. The crude product was purified via flash chromatography on silica gel (20–40% acetone in hexanes) to give the methyl carbamate **26** as a yellow amorphous solid in 65% yield (13 mg, 0.029 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.32 (d, J = 1.8 Hz, 1H), 6.23 (d, J = 1.8 Hz, 1H), 3.73 (m, 1H), 3.56 (s, 3H), 3.52 (m, 1H), 2.44 (td, J = 11.2, 2.2 Hz, 1H), 2.21 (m, 2 H), 2.12 (m, 1H), 2.03–1.67 (m, 12H), 1.48–1.13 (m, 4H), 1.35 (s, 3H), 1.03 (s, 3H), 0.94 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.6, 157.5, 155.9, 150.9, 110.8, 106.3, 105.1, 77.6, 71.3, 52.5, 51.7, 50.3, 47.9, 43.2, 41.9, 39.9, 39.0, 36.66, 36.70, 34.9, 31.4, 28.3, 27.2, 19.2; $[\alpha]^{23}_D$ −64.7 (*c* 0.58, CH_3OH); IR (neat; cm^{-1}) 3362, 2910, 2856, 1703, 1621, 1574, 1519, 1453, 1416, 1361, 1249, 1135, 1071; HRMS (ESI^+) calculated for $C_{27}H_{38}NO_5$ [M + H^+] 456.2750, found 456.2749. LC/MS analysis (Waters MicroMass ZQ system) showed purity of 98% and retention time of 4.6 min for the title compound.

Radioligand Binding Assays

The affinities (K_i) of the new compounds for rat CB1 receptor as well as for mouse and human CB2 receptors were obtained by using membrane preparations from rat brain or HEK293 cells expressing either mCB2 or hCB2 receptors, respectively, and [^{3}H]CP-55,940 as the radioligand, as previously described.[9,34] Results from the competition assays were analyzed using nonlinear regression to determine the IC_{50} [36] values for the ligand; K_i values were calculated from the IC_{50} (Prism by GraphPad Software, Inc.). Each experiment was performed in triplicate, and K_i values were determined from three independent experiments and are expressed as the mean of the three values. Results for the covalently reacting isothiocyanato electrophiles are expressed as pseudoaffinities (K_i*).

cAMP Assay.[9,34]

HEK293 cells stably expressing rCB1 receptor were used for the studies. The cAMP assay was carried out using PerkinElmer's Lance Ultra cAMP kit following the protocol of the manufacturer. Briefly, the assays were carried out in 384-well plates using 1000–1500 cells/well. The cells were harvested with non-enzymatic cell dissociation reagent Versene, washed once with HBSS, and resuspended in the stimulation buffer. The various concentrations of the test compound (5 μL) in forskolin (2 μM final concentration) containing stimulation buffer were added to the plate followed by the cell suspension (5 μL). Cells were stimulated for 30 min at room temperature. Eu-cAMP tracer working solution (5 μL) and Ulight-anti-cAMP working solution (5 μL) were then added to the plate and incubated at room temperature for 60 min. The data were collected on a PerkinElmer Envision instrument. The EC_{50} values were determined by nonlinear regression analysis using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

Photoaffinity Covalent Labeling

Rat brain membranes (rCB1, Pel-Freez Biologicals, Rogers, AR) were prepared following the previously described and appropriately modified procedures.[37] Membranes from human CB2 receptors (hCB2) expressed in HEK293[38] were incubated with the azido ligands (**20** and **17**) in concentrations of 10-fold their K_i values for 30 min at 37 °C in a water bath with gentle agitation and then exposed to UV (254 nm) for 1 min to activate the ligand.[39] Unbound excess ligand was washed out twice with 1% BSA in TME. This was followed by an additional washing to remove residual BSA, and the membranes were isolated by centrifugation (Beckman Coulter, JA 20, 17 000 rpm, 10 min, 25 °C). A blank membrane sample was treated in parallel using the same procedure and used as a control.

Electrophilic Covalent Labeling

CB1 and CB2 membranes were incubated with the isothiocyanato ligands (**19** and **16**) in a concentration of 10-fold their K_i values at 37 °C in a water bath with gentle agitation for 1 h and treated as above without the photoirradiation step. Unbound excess ligand was washed out twice with 1% BSA in TME and once with TME alone to remove BSA, and the membranes were isolated by centrifugation.

Saturation Binding Assay

Protein concentrations were determined by using a Bio-Red Bradford protein assay kit,[40] and saturation binding assays were performed in a 96-well format.[16] Membrane pellets were resuspended in TME containing 0.1% BSA. A total of 25 μg of protein was added to each well, and [^{3}H]CP-55,940 was diluted in 0.1% BSA/TME buffer to yield ligand concentrations ranging from 0.5 to 23.8 nM. Nonspecific binding was determined in the presence of 4 μM unlabeled CP-55,940. The assay plates were incubated at 30 °C with gentle agitation for 1 h. The resultant mixture was then transferred to Unifilter GF/B filter plates, and the bound ligand was separated from unbound using a Packard Filtermate-96 cell harvester (PerkinElmer Packard, Shelton, CT). Filter plates were washed five times with ice-cold wash buffer (50 mM Tris-base, 5 mM magnesium chloride with 0.5% BSA, pH 7.4). Bound radioactivity was quantitated in a Packard TopCount scintillation counter. Nonspecific binding was subtracted from the total bound radioactivity to calculate the specific binding of [^{3}H]CP-55,940 (measured as pmol/g in saturation curves). Saturation assays were performed in triplicate, and data points were presented as the mean ± SEM. B_{max} and K_d values were calculated by nonlinear regression using GraphPad Prism 4.0 (one-site binding analysis equation $Y = B_{max}X/(K_d + X)$, GraphPad Software, San Diego, CA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

CB1	cannabinoid receptor 1
CB2	cannabinoid receptor 2
(−)-Δ^9-THC	(−)-Δ^9-tetrahydrocannabinol
CNS	central nervous system
HEK293	human embryonic kidney cell line
SAR	structure–activity relationship
NMR	nuclear magnetic resonance
HOBT	hydroxybenzotriazole
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
HPLC	high-performance liquid chromatography
LAPS	ligand assisted protein structure
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DPPA	diphenylphosphorylazide
TFAA	trifluoromethylacetic anhydride
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
***p*-TSA**	*p*-toluenesulfonic acid
TsCl	*p*-toluenesulfonyl chloride

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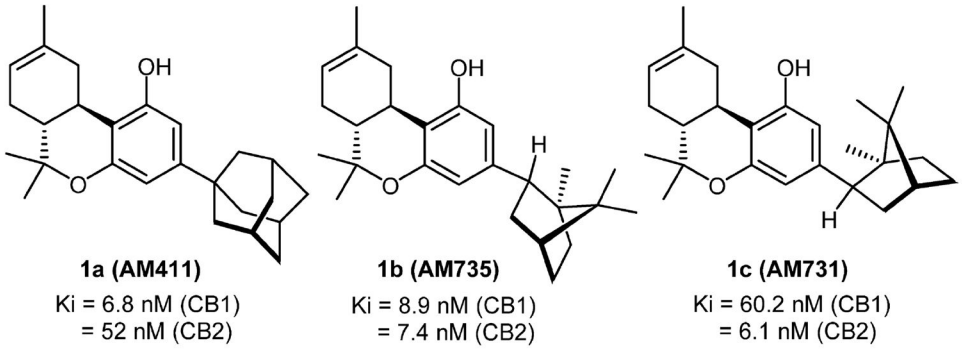


Figure 1.

Structures of the adamantyl (**1a**) and the *endo*- and *exo*-norbonyl-(−)-Δ^8-THC analogues **1b** and **1c**.

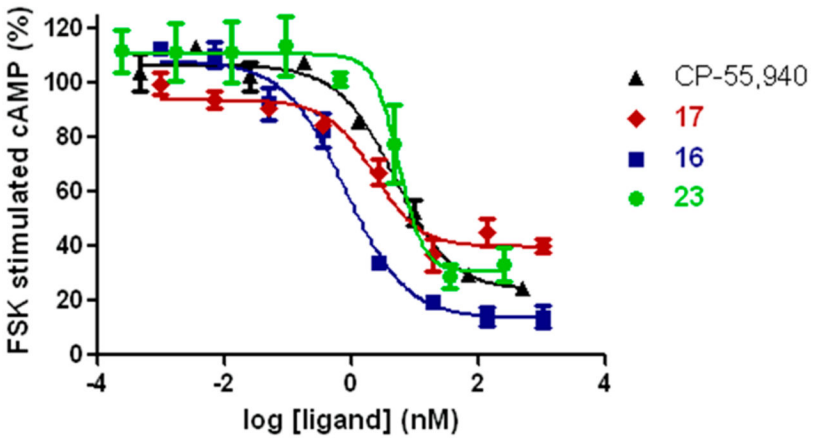


Figure 2.

Concentration-dependent inhibition of forskolin-stimulated cAMP accumulation in HEK293 cells expressing rCB1 receptors by representative agonists. All compounds behave as full agonists.

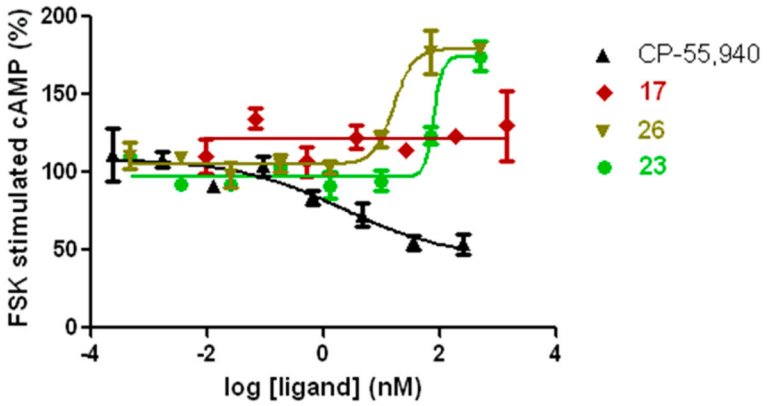


Figure 3.

Concentration-dependent response of forskolin-stimulated cAMP accumulation in HEK293 cells expressing hCB2 receptors by representative ligands. The cannabinoid standard CP-55,940 behaves as a potent agonist decreasing the levels of cAMP, while compounds **23** and **26** behave as inverse agonists. Compound **17** showed no response up to a 3 μM concentration.

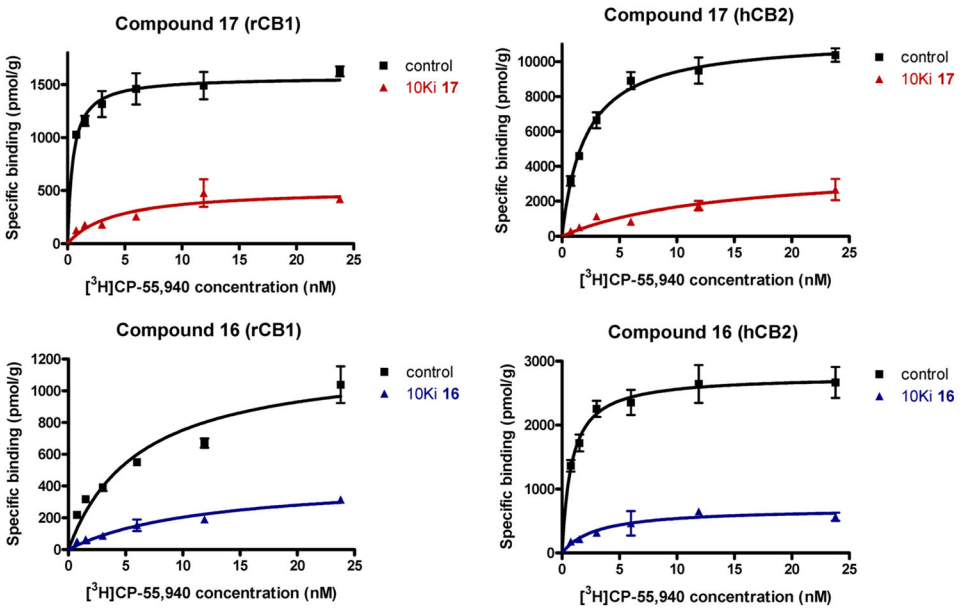


Figure 4.

Saturation binding curves using [^{3}H]CP-55,940 for CB1 (left panel) and CB2 (right panel) receptors preincubated with the 3′-azidomethyl (red, **17**) and 3′-isothiocyanatomethyl (blue, **16**) analogues. Control membranes processed in parallel but without prior exposure to covalent ligands are shown in black. Data represent the mean values ± SEM of at least two independent experiments performed in triplicate. Both compounds inhibit the specific binding of the radioligand to CB1 and CB2 receptors.

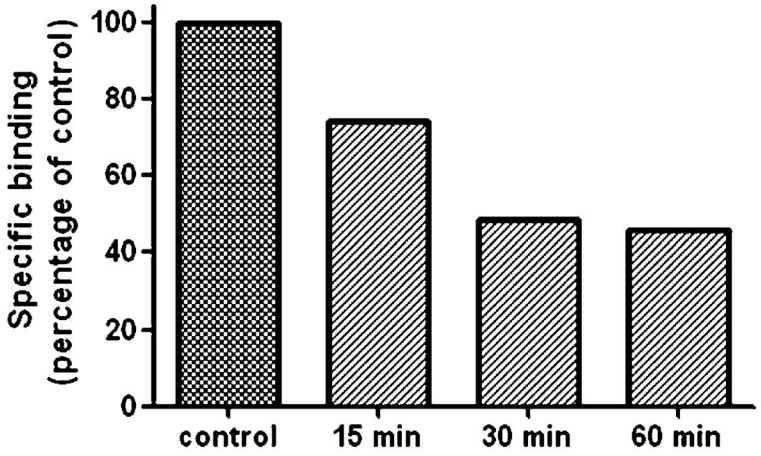
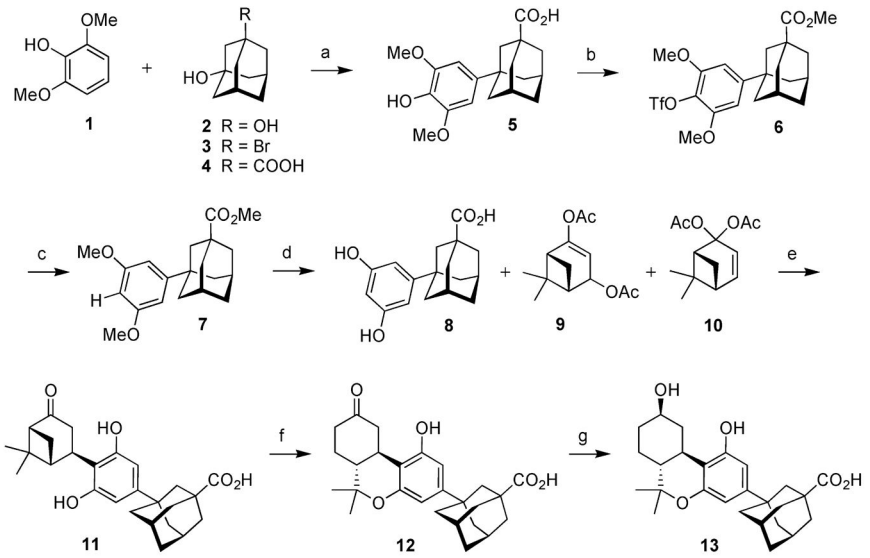


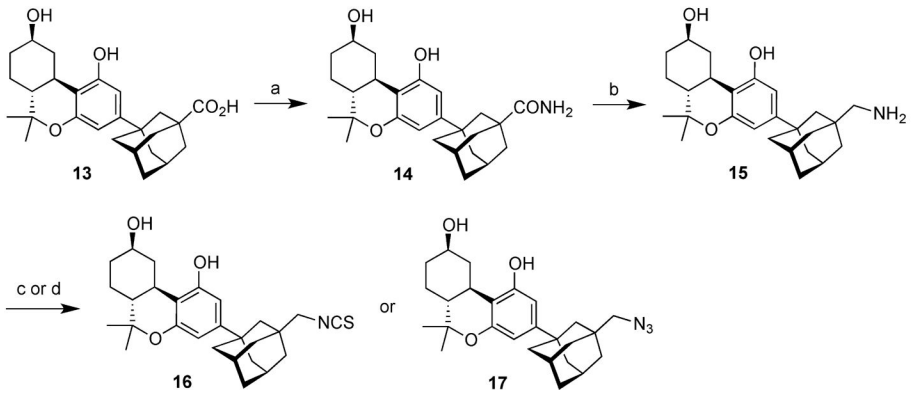
Figure 5.

Time course for CB1 receptor labeling with isothiocyanato probe **19**. The membranes were preincubated with 10-fold the K_i value of compound **19** for 15, 30, and 60 min. Membranes in the control experiment were preincubated for 60 min without compound **19**. The [^{3}H]CP-55,940 specific bindings were determined by the saturation binding method reported in Experimental Section.



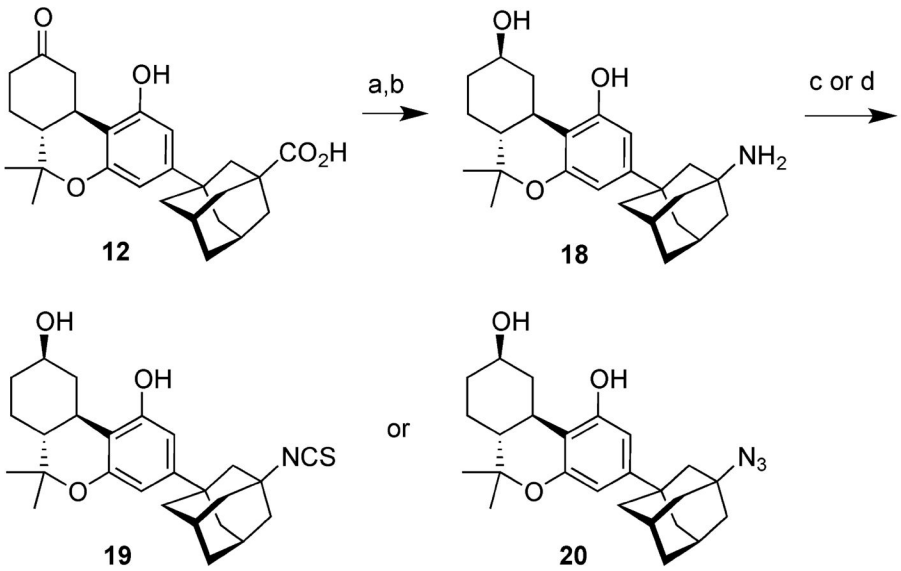
Scheme 1. Synthesis of C9 Ketone 12 and β-C9 Alcohol 13[a]

[a]Reagents and conditions: (a) MeSO3H, 50 °C; 100%; (b) $TMSCHN_2$, PhH, MeOH, rt; Tf_2NPh, Et_3N, DMAP (cat.), CH_2Cl_2, reflux; 91%; (c) $PdCl_2(Ph_3P)_2$, dppp, *n*-Bu_3N, HCOOH, PMHS (cat.), DMF, reflux; 89%; (d) BBr_3, CH_2Cl_2, 0 °C to rt; 89%; (e) *p*-TSA·H_2O, $CHCl_3$/acetone (4:1), 55 °C; 78%; (f) TMSOTf, $MeNO_2$, 0 °C to rt; 96%; (g) NaBH4, MeOH, 0 °C to rt; 95%.



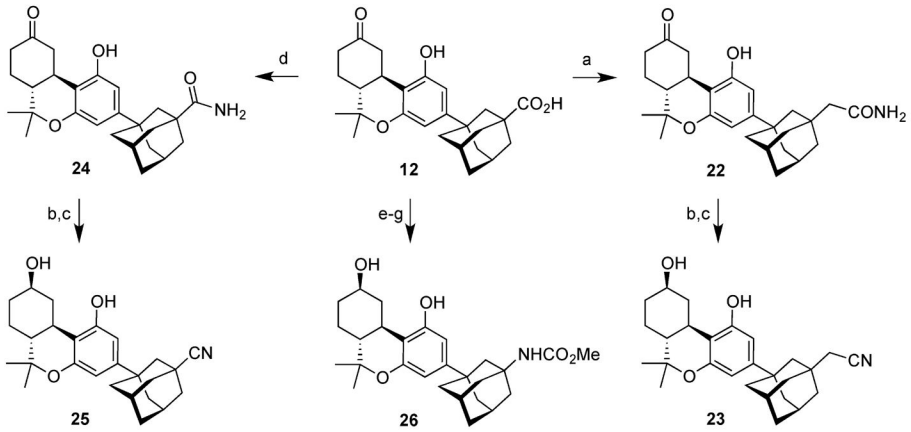
Scheme 2. Synthesis of Isothiocyanante 16 and Azide 17[a]

[a]Reagents and conditions: (a) HOBT, EDCI, NH_4OH, THF, rt; 84%; (b) BH_3·DMS, 0 °C to rt; 90%; (c) Et_3N, CS_2, TsCl, THF, 0 °C to rt; 89%; (d) TfN_3, Et_3N, $CuSO_4$, H_2O, CH_2Cl_2, MeOH, rt; 93%.



Scheme 3. Synthesis of Isothiocyanate 19 and Azide 20[a]

[a]Reagents and conditions: (a) DPPA, Et_3N, PhMe, reflux; (b) $Hg(OAc)_2$, THF, H_2O (1/1), rt; $NaBH_4$, 5% aq KOH, rt; 76% from **12**; (c) Et_3N, CS_2, TsCl, THF, 0 °C to rt; 86%; (d) TfN_3, Et_3N, $CuSO_4$, H_2O, CH_2Cl_2, MeOH, rt; 98%.

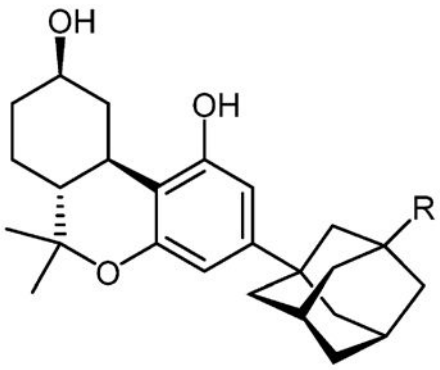


Scheme 4. Synthesis of Functionalized Adamantyl Cannabinoids 22 –26[a]

[a]Reagents and conditions: (a) $(COCl)_2$, DMAP (cat.), THF; CH_2N_2, rt; $PhCO_2Ag$, Et_3N, NH_4OH, THF, reflux; 27%; (b) TFAA, pyr, dioxane, 0 °C to rt; (c) K_2CO_3, MeOH, rt; $NaBH_4$, 0 °C to rt; 86% of **23** from **22**; 71% of **25** from **24**; (d) HOBT, EDCI, NH_4OH, THF, rt; 81%; (e) $NaBH_4$, MeOH, 0 °C to rt; 95%; (f) DPPA, Et_3N, PhMe, reflux; (g) NaOMe, MeOH, reflux; 65% for two steps.

Table 1

CB1/CB2 Affinities (K_i) of 3′-Functionalized Adamantyl Cannabinoid Analogues (95% Confidence Limits)



		K_i (nM)[a]		
compd	**R**	**rCB1**	**mCB2**	**hCB2**
27	-H	23.9[b]	39.4[b]	40.5[b]
13	-COOH	>1000	>1000	>1000
14	-$CONH_2$	297	947	337
26	-$NHCOOCH_3$	29.1	2.1	8.4
25	-CN	45.8	2.8	4.2
18	-NH_2	>1000	>1000	>1000
19	-NCS	35.4[c]	31.7[c]	13.1[c]
20	-N_3	18.6	38.4	24.8
23	-CH_2CN	2.6	4.4	4.6
15	-CH_2NH_2	>1000	>1000	>1000
16	-CH_2NCS	3.0[c]	34.6[c]	10.3[c]
17	-CH_2N_3	4.4	26.4	9.6

[a] Affinities for CB1 and CB2 receptors were determined using rat brain (CB1) preparations or membranes from HEK293 cells expressing mouse or human CB2 receptors and [^{3}H]CP-55,940 as the radioligand following previously described procedures.[6,9] Data were analyzed using nonlinear regression analysis. K_i values were obtained from three independent experiments run in triplicate and are expressed as the mean of the three values (SD < ±20%).

[b] Reported previously.[8]

[c] Apparent K_i (K_i*) value determined in this study.

Table 2

CB1/CB2 Functional Potencies (EC_{50}) of Selected 3′-Functionalized Adamantyl Cannabinoid Analogues

	rCB1	hCB2
compd	**EC_{50} (nM),[a] $E_{(max)}$ (%)[b] classification**	**EC_{50} (nM),[a] $E_{(max)}$ (%)[b] classification**
27	2.7 (2.1–3.3), 69 agonist	NR
26	1.6 (1.3–1.9), 71 agonist	15.8 (12.5–19.1), −74 inverse agonist
25	95.2 (75.4–114.9), 82 agonist	90.7 (71.8–109.6), −93 inverse agonist
19	12.0 (9.5–14.5), 68 agonist	NR
20	4.4 (3.4–5.5), 73 agonist	NR
23	5.4 (4.3–6.5), 78 agonist	83.1 (65.8–100.4), −78 inverse agonist
16	0.8 (0.6–1.0), 94 agonist	>400, −91 inverse agonist
17	2.4 (1.9–2.9), 45 agonist	NR

[a] Functional potencies at rCB1 and hCB2 receptor were determined by measuring the decrease in forskolin-stimulated cAMP levels.[9,34] EC_{50} values were calculated using nonlinear regression analysis. Data are the average of two independent experiments run in triplicate, and 95% confidence intervals for the EC_{50} values are given in parentheses.

[b] Forskolin stimulated cAMP levels were normalized to 100%. $E_{(max)}$ is the maximum inhibition of forskolin stimulated cAMP levels and is presented as the percentage of CP-55,940 response at 500 nM. NR: no response up to 3 μM.

Table 3

Reductions in the Specific Binding of [^{3}H]CP-55,940 after Covalent Ligand Pretreatment

	covalent labeling (%)[a]	
compd	**CB1**	**CB2**
20	61 (58–67)	26 (21–32)
19	54 (49–60)	25 (22–29)
17	67 (60–75)	60 (53–75)
16	63 (57–72)	74 (69–82)

[a]The receptor membranes were pretreated with a concentration equal to 10-fold the compound's K_i value. Percent covalent labeling was calculated as $\{[B_{max}(\text{control}) - B_{max}(\text{ligand})]/B_{max}(\text{control})\} \times 100$. B_{max}(ligand) was calculated as described in Experimental Section. The B_{max}(control) for [^{3}H]CP-55,940 was determined by conducting the assay as described but in the absence of the test ligand. Confidence intervals (95%) are shown in parentheses.