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Development of novel tail-modified anandamide analogs

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Abstract

To explore the hydrophobic groove subsite within the CB1 cannabinoid receptor we have designed and synthesized a group of tail-substituted anandamide analogs. Our design involves the introduction of aryl or heterocyclic ring as terminal substituents that are connected to the last *cis*-arachidonyl double bond through aliphatic chains of variable lengths. Our results indicate that there are strict stereochemical requirements for the interaction of such analogs with the CB1 receptor. The optimal pharmacophore includes the phenyl, *p*-substituted phenyl or 3-furyl substitutents attached to the *cis*-double bond through a four methylene chain.

Keywords

anandamide; tail modification; CB1 receptor; cannabinoid; endocannabinoid

Anandamide,[1] a key endogenous cannabinoid ligand, is a partial CB1 cannabinoid receptor agonist and is known to produce a concentration-dependent inhibition of the electrically evoked twitch response of the mouse vas deferens,[2,3] as well as antinociception, hypothermia, hypomotility, and catalepsy in mice.[4–8] In brain and liver, anandamide is hydrolyzed enzymatically to yield arachidonic acid and ethanolamine. The reaction is catalyzed by a membrane-bound amidohydrolase[9–12] (anandamide amidohydrolase or fatty acid amide hydrolase, FAAH) which has been cloned and fully characterized.[13] This enzyme was shown to be co-localized in the same brain regions as the CB1 cannabinoid receptor.[12] The hydrolytic breakdown of anandamide can be prevented *in vitro* by phenylmethanesulfonyl fluoride (PMSF), a general serine protease inhibitor.[9,10] PMSF can be included in the competitive binding assays of anandamide analogues where amidase-catalyzed anandamide hydrolysis might be a complicating factor.[14] There is also evidence pointing to the existence of carrier-mediated anandamide transport[15] which is essential for the termination of the biological effects of anandamide. Thus, anandamide interacts not only with the CB receptors but also possibly with endocannabinoid transporter system and FAAH.

Anandamide exhibits selectivity toward the CB1 cannabinoid receptor. Additionally, structure activity relationship (SAR) studies of anandamide analogs have provided insight into the stereoelectronic requirements for interaction with the CB1 receptor.[14,16–27] Although much of the reported work has addressed the head group, SAR of the arachidonoyl

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side chain has revealed that this hydrophobic chain is also very sensitive to structural modifications.[18,22,23] Complete saturation, or replacement of the olefins with alkynes, results in the total loss of receptor affinity. Substitution of the arachidonoyl chain with other fatty acid chains with ω-olefinic bonds, with a *trans* double bond or analogs with non ω-6 structure leads to a major reduction in affinity for CB1. Earlier studies on the hydrophobic tail of anandamide suggested that the pentyl chain may mimic the five carbon side chain of (−)-Δ^9-tetrahydrocannabinol, the principal active ingredient of cannabis.[16–18,28] Substitution of the terminal pentyl group of anandamide with a 1,1-dimethyl moiety leads to significant enhancement of its affinity for CB1, as has also been observed with (−)-Δ^9-THC.[17] The present communication further explores this terrain through the design and synthesis of novel chain modified anandamide analogs.

In earlier work, we have shown that introduction of ω-isothiocyanato and ω-azido groups within the anandamide structure lead to substantial enhancement of CB1 affinity and potency.[29] The present work describes the synthesis and evaluation of a series of anandamide analogs of variable chain lengths in which the terminal carbon is functionalized with a phenyl, substituted phenyl or heterocyclic rings. All the compounds carry the *R*-methylethanolamine moiety, a headgroup which was earlier demonstrated to provide analogs (e.g. methanandamide, AM356) with optimized affinity for the CB1 receptor as well as robust metabolic stability.[14] Our results confirm that, as with anandamide, all analogs exhibit CB1 selectivity over CB2. Additionally, our data reveal that the stereochemical features of the anandamide tail may have a major impact on ligand's affinity for CB1.

The synthesis of the anandamide analogs is illustrated in Scheme 1. Methyl 5-hexynoate **1** was coupled with 4-chloro-butyn-1-ol in the presence of copper (I) iodide[30] and the alcohol intermediate was converted to the corresponding bromide using CBr_4/PPh_3. Bromide **2** was coupled in an analogous procedure with 3-butyn-1-ol to afford alcohol **3** which underwent hydrogenation with Lindlar's catalyst in the presence of quinoline to afford triene **4** in high yield.[31,32] Dess-Martin periodinane[33] oxidation of alcohol **4** resulted in the formation of the unstable aldehyde **5** which was immediately subjected to Wittig olefination with various phosphonium salts to furnish *cis*-alkenes **6**. The methanandamide analogs were obtained by direct amidation of the corresponding esters with R-(−)-alaninol catalyzed by sodium cyanide.[34] The iodo-substituted compound **7j** was prepared from the corresponding bromo compound **9** via the tin intermediate.

Scheme 2 illustrates the preparation of the phosphonium salts utilized in Scheme 1. Starting with the coupling of properly functionalized iodobenzene **8** and hex-5-yn-1-ol **9** catalyzed by tetrakistriphenylphosphinepalladium(0),[35] the resulting alcohols were hydrogenated to give ω- phenyl alcohols **11**. Iodination of alcohol **11** by I_2/PPh_3 followed by heating with PPh_3 gave the desired phosphonium salts **13** in high yields (85–95%). All the other phosphonium salts were obtained from commercially available iodide and triphenylphosphine, and were used for the Wittig reaction without further purification. The furyl derivatives **7o–7s** were synthesized by utilizing an approach published earlier by our research group.[29] This involves the coupling of furyl substituted terminal alkynes (**14** or **15**) with 13-bromo-trideca-5,8,11-triynoic acid methyl ester **16** followed by partial hydrogenation with Lindlar's catalyst to give esters **17** (Scheme 3) which were converted to the corresponding amides using the same method described in the scheme 1.

Binding affinities for the CB1 and CB2 receptors were determined according to previously reported procedures.[36,21,23] For the CB1 receptor, binding data were obtained using a rat brain membrane in the presence of phenylmethanesulfonyl fluoride (PMSF) a general serine protease inhibitor to protect the analogues from the hydrolytic activity of fatty acid amide hydrolase. CB2 data were obtained using a membrane preparation from mouse spleen

known to be rich in CB2 receptor. [^{3}H]CP-55,940, a most widely used radioligand, was chosen as a competing ligand for the assays as it has high affinity for both CB1 and CB2 receptors and is nonselective.[37]

The binding affinities of novel anandamide analogues are summarized in Table 1, where (R)-methanandamide is included for comparison. It is apparent from the CB2 affinities reported here that these novel anandamide analogues show selectivity for the CB1 receptor. Analogs **7a**–**7e** were synthesized to assess the optimal length for the phenyl group at the hydrophobic tail for receptor affinity. The binding results show that **7d** with four methylene carbons separating C_{15} from the phenyl group exhibited comparable activity (K_i = 13.4 nM) for the CB1 receptor as (R)-methanandamide (K_i = 17.9 nM in the presence of PMSF). Decreasing the number of methylene carbons between C_{15} *cis*-double bond and the phenyl group gave analogs **7a–7c** and showed progressively weaker affinities for the CB1 receptor with decreasing length of the alkyl chain. Conversely, compound **7e** which differs from **7d** by one extra methylene group displayed approximately over 5-fold lower affinity compared to **7d**. These results clearly indicate that a four methylene linker is optimal between the distal arachidonyl double bond and the pendant phenyl moiety. In an effort to enhance the polar features of ananadamide analogs, we have substituted the phenyl ring with the more polar furan moiety. A similar trend was observed with the 3-furyl derivative (**7o**). However, surprisingly the corresponding 2-furyl derivative (**7q**) showed a substantially weaker affinity. Introduction of terminal α-naphthyl group (**7f**) resulted in the loss of CB1 receptor affinity.

Earlier data has suggested the presence of a hydrophobic groove subsite capable of accommodating the side chain of classical cannabinoids and possibly the terminal five carbon alkyl group of anandamide.[38] Our present results are congruent with the existence of the hydrophobic subsite for the anandamide tail within the CB1 receptor capable of accommodating a terminal phenyl group. It can be argued that a short chain linking the phenyl ring with the last double bond as seen in **7a** (n=1) and **7b** (n=2), may not occupy a sufficient portion of this groove, resulting in low binding affinity. Conversely, a linker that is too long, as seen in **7e** (n=5), may be too large to be fully encompassed by the subsite. The ideal length, given by **7d**, completely fills the groove and yet does not incur any steric penalties. The groove also accommodates the 3-furyl moiety, but not the 2-furyl isomer suggesting that the relative position of the furyl oxygen within the receptor subsite may affect the overall ability of the ligand for an optimal interaction with the receptor. Substitution of the phenyl ring with an α-naphthyl moiety results in severe loss in affinity in CB1, an effect which is probably due to its large size.

To further probe this CB1 receptor subsite, different substituents were introduced on the pendent phenyl ring. Our results indicate that, while *p*-phenyl substituents are tolerated in the receptor subsite, *meta* or *ortho* substitution leads to a substantial reduction in affinity. The most potent analog is that with the *p*-bromophenyl group (**7n**). Substitution at the *meta* and *ortho* postion is not tolerated. However, the introduction of bromine at the *para* position results in an increase in CB1 receptor affinity. The above stereochemical postulate for the anandamide distal tail pharmacophore is pictorially summarized in figure 1.

In conclusion, new anandamide analogues with structural modifications at the hydrophobic tail were synthesized and evaluated for their affinities for the cannabinoid receptors. Introduction of a pendant phenyl, aryl or furyl group separated from the last *cis* double bond of arachidonic acid by methylene chains of variable lengths were used to probe the cannabinoid side-chain subsite with CB1 and CB2 receptors. Our results suggest that, as with the anandamide head group, the stereochemical requirements for the anandamide hydrophobic tail are stringent.

The series of analogs described here provides further insight into the SAR of the hydrophobic tail in anandamide. The results also reveal the presence of a hydrophobic subsite at the distal end of the groove capable of accommodating a phenyl or furyl substituent as well as a *p*-substituted phenyl group. Such information will further the understanding of the binding motif of anandamide and aid in the design of novel molecular probes for the CB1 receptor.

Acknowledgments

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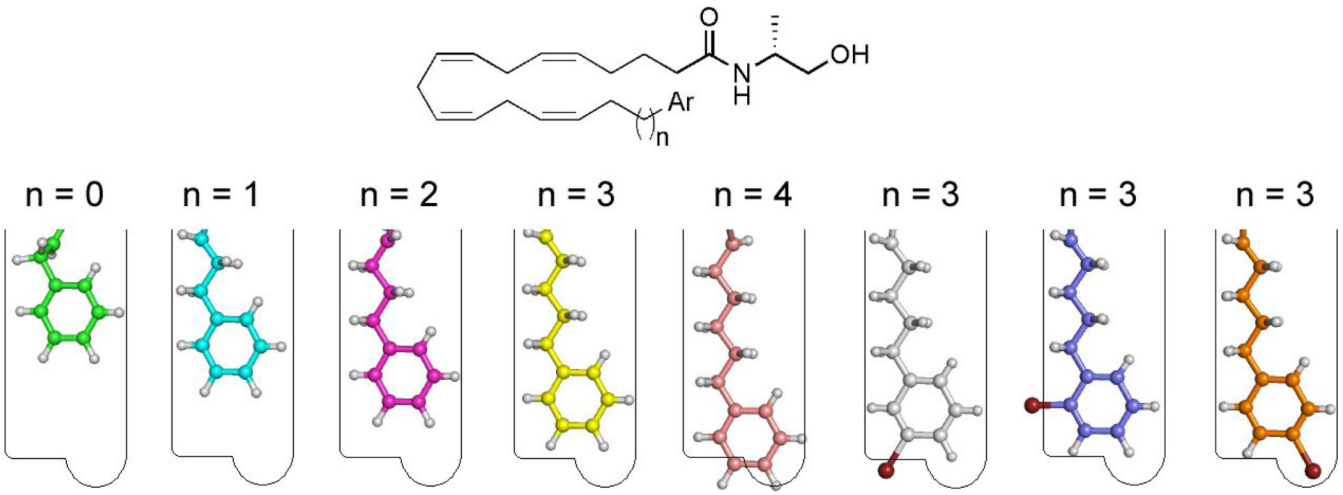
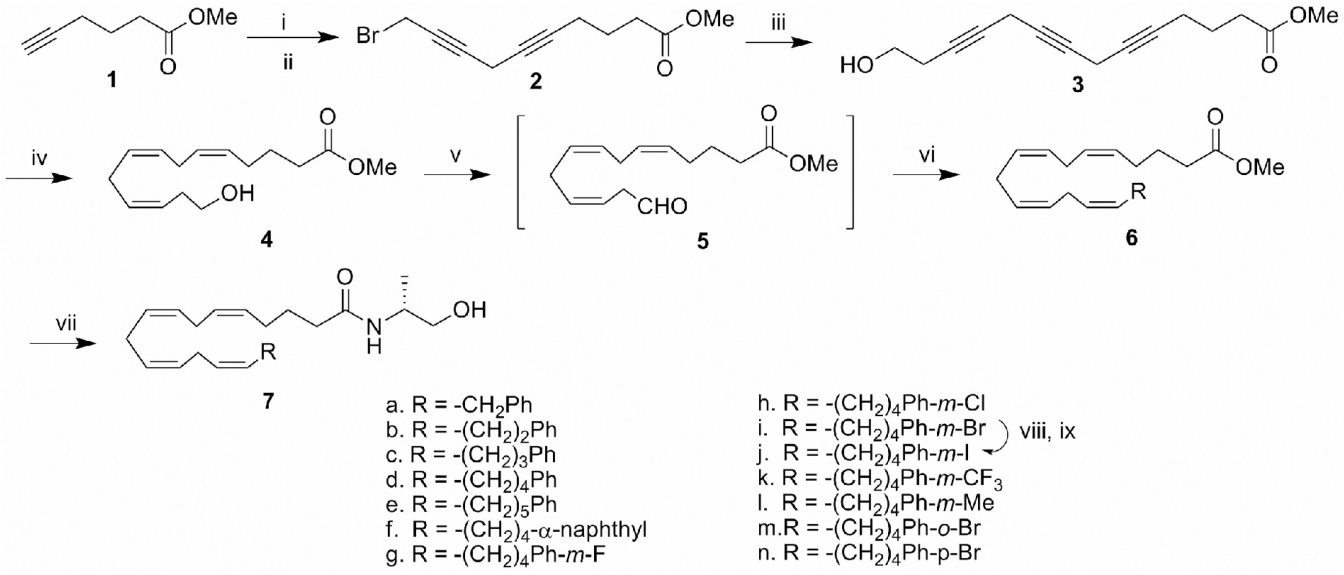


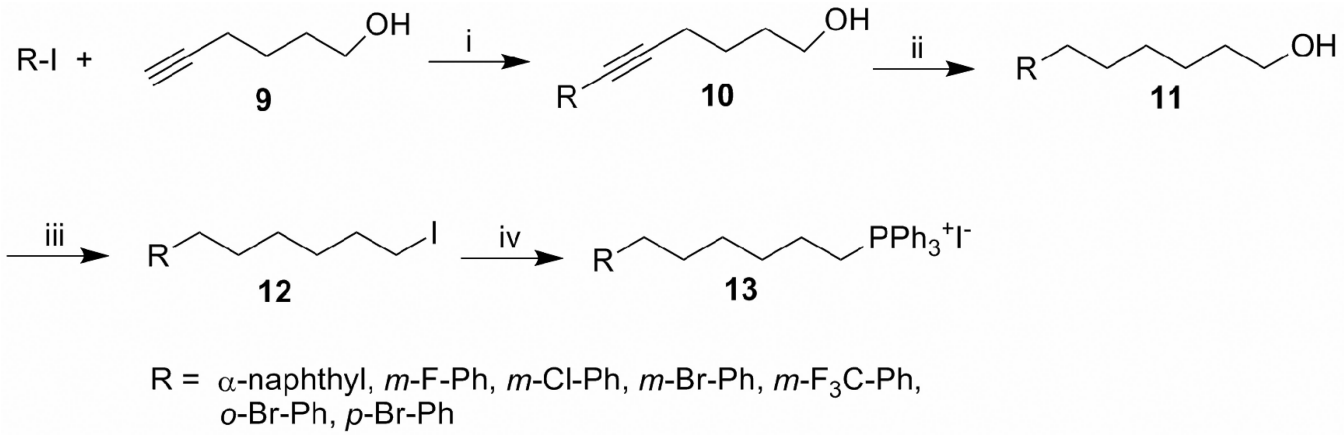
Figure 1.

Pictorial representation of the CB1 binding groove for the tail of (R)-methanandamide analogs. **7a** (green) **7b** (cyan), **7c** (magenta), **7d** (yellow), **7e** (pink), **7i** (grey), **7m** (blue), **7n** (orange). Structures were minimized with the OPLS_2005 force field in Macromodel.[39]



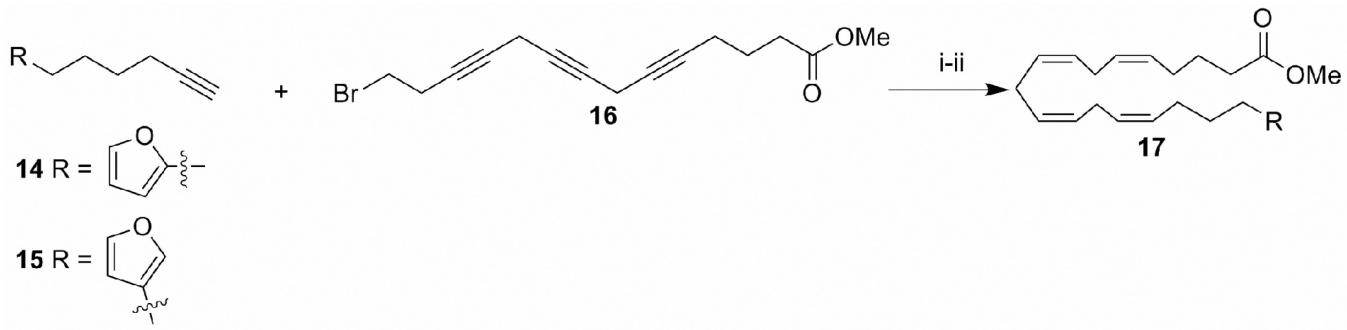
Scheme 1.

Reagents and conditions: (i). 4-chloro-butyn-1-ol, CuI, NaI, K_2CO_3, DMF, rt, overnight, 84%; (ii). PPh_3, CBr_4, rt, 95%; (iii). 3-butyn-1-ol, CuI, NaI, K_2CO_3, DMF, rt, overnight, 80%; (iv). H_2, Lindlar catalyst, quinoline, ether, 0–10 °C, 86%; (v). Dess-Martin reagent, CH_2Cl_2, rt, 30 min, 86%; (vi). Wittig reagent from the corresponding phosphonium salt (see scheme 2) with n-BuLi in THF at −78° C, 60–80%;(vii). *R*-(−)-2-amino-propanol, NaCN (cat), Methanol, 55°C, sealed vial, 74%; (viii). *bis*-tributyltin, $Pd(PPh_3)_4$, toluene, reflux, 40%; (ix). I_2, CH_2Cl_2, rt, 95%.



Scheme 2.

Reagents and conditions: (i). $Pd(PPh_3)_4$, CuI, piperidine, DMF, 0 °C-rt, 50–82%; (ii). H_2, 5% Pd-C, methanol, rt, 85–90%; (iii). I_2, PPh_3, imidazole, 0 °C, CH_2Cl_2, rt, 80–92%; (iv). PPh_3, neat, 110 °C, 80–92%.

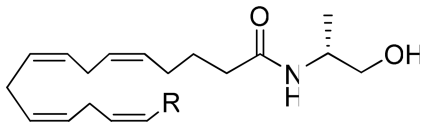


Scheme 3.

Reagents and conditions: (i) CuI, NaI, K_2CO_3, DMF, rt, overnight, 60%; (ii). H_2, Lindlar catalyst, quinoline, ether, 0–10 °C, 94%.

Table 1

Affinity (K_i)[a] of new anandamide analogues for CB1 and CB2 receptors



Analogues	R	CB1 K_i (nM) (PMSF)	CB2 K_i (nM)
(*R*)-methanandamide	$(CH_2)_4CH_3$	17.9	868
7a	CH_2Ph	348.5	826.3
7b	$(CH_2)_2Ph$	241.2	335
7c	$(CH_2)_3Ph$	69.2	533.7
7d	$(CH_2)_4Ph$	13.3	1117
7e	$(CH_2)_5Ph$	76.6	240.6
7f	$(CH_2)_4$-α-naphthyl	678.3	3996
7g	$(CH_2)_4$Ph-*m*-F	30.4	987.3
7h	$(CH_2)_4$Ph-*m*-Cl	89.4	605.1
7i	$(CH_2)_4$Ph-*m*-Br	95.3	1054
7j	$(CH_2)_4$Ph-*m*-I	533.3	1564
7l	$(CH_2)_4$Ph-*m*-Me	344.3	839.7
7m	$(CH_2)_4$Ph-*o*-Br	170.0	N.R.
7n	$(CH_2)_4$Ph-*p*-Br	8.9	250.4
7o	$(CH_2)_4$(3-furyl)	12.0	1027
7p	$(CH_2)_5$(3-furyl)	170	869
7q	$(CH_2)_4$(2-furyl)	325	749.8
7s	$(CH_2)_5$(2-furyl)	561.2	3255

[a] CB1 affinities were determined using rat brain membranes and 0.8 nM [^{3}H] CP-55,940 as the radioligand. Mouse spleen was used as source of CB2 receptor. Data were analyzed using nonlinear regression analysis. K_i values were obtained from a minimum of two independent experiments run in duplicate.