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SYNTHESIS OF (+)- AND (-)-2-METHYLARACHIDONYL-2'-FLUOROETHYLAMIDE (O-689)

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Abstract: The enantiomers of 2-methylarachidonyl-2'-fluoroethylamide, (+)-**4a** (>98% ee) and (-)-**4b** (98% ee), were synthesized from methyl arachidonate and were shown to be nonenantioselective in binding to the cannabinoid receptor (CB_1) and in the mouse tetrad tests. © 1997 Elsevier Science Ltd.

Introduction

Since the discovery[1] of anandamide (AN) as a putative endogenous ligand of the cannabinoid receptor CB_1, considerable progress has been made in understanding and establishing the relationship that may exist between AN (an eicosanoid) and the cannabinoid Δ^9-tetrahydrocannabinol (THC).[1-13] Studies in vivo have shown that AN mimics the biological activities of Δ^9-THC, but with some differences. It has a faster onset and shorter duration of action, and is less potent than Δ^9-THC.[6] Although it can produce Δ^9-THC-like discriminative stimulus effects in rats, unlike Δ^9-THC it produces concomitant decreases in response rate.[7] Binding studies of AN have to be carried out in the presence of the enzyme inhibitor phenylmethylsulfonyl fluoride (PMSF) since it is susceptible to enzyme hydrolysis at the amide bond by amidase(s).[5,14-16]

In previous studies[10,11,13] we have demonstrated that introduction of substituents on the carbon in the 2-position of the arachidonic acid part of AN enhances metabolic stability, presumably by sterically hindering the amidase(s) responsible for AN's degradation. Of this series the methyl substituent was found to be optimal for both in vivo activity and for binding affinity. Thus we developed (±)-2-methylarachidonyl-2'-fluoroethylamide (O-689, (±)-**4**), which is a potent and metabolically stable AN analog. Its enhanced metabolic stability compared to AN is demonstrated by the facts that (1) the binding affinity is very similar with (K_i 5.7 ± 2.1 nM) and without (K_i 15 ± 6 nM) PMSF,[11] and (2) in trained rhesus monkeys, it produces a dose-dependent substitution for Δ^9-THC without accompanying changes in response rates, which AN does not.[17] Furthermore, while the agonist effects of AN are not antagonized by the CB_1 antagonist SR141716A, presumably also due to AN's rapid degradation, the agonist effects of O-689 are antagonized by SR141716A.[18] As the cannabinoid receptor (CB_1) is known to be stereoselective for ligands (e.g., THC, classical and non-classical THC analogs, aminoalkylindoles, 1'-methanandamide), we decided to resolve our racemate in order to examine if its biological activity resides in only one of the two enantiomers. Our synthetic method of resolution and the biological activities of the enantiomers are described in this report.

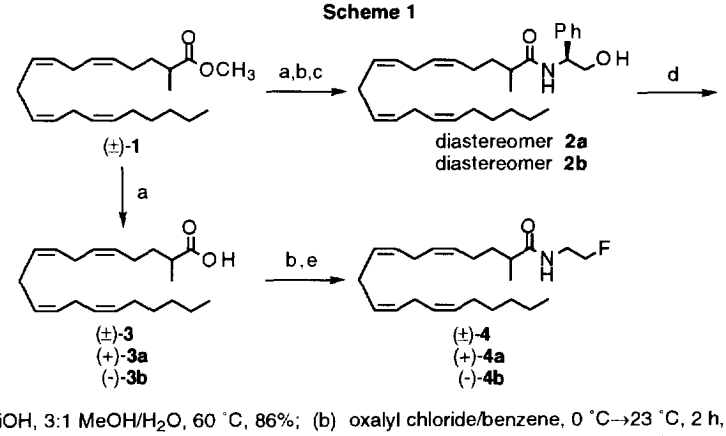
Chemistry

We synthesized compound O-689, (±)-2-methylarachidonyl-2'-fluoroethylamide (**4**),[11] (Scheme 1) from methyl arachidonate by α-alkylation with methyl iodide to give ester (±)-**1**, which was hydrolyzed to afford acid (±)-**3**. It was converted to its acid chloride and treated with excess fluoroethylamine to give (±)-**4**.[10,11,19]

Our current objective was accomplished by performing a resolution of the acid precursor and then converting it to the enantiomeric products. To resolve acid (±)-**3**, it was converted to its acid chloride and then treated with (S)-(+)-2-phenylglycinol[20] to give amide **2** as a diastereomeric mixture which was easily separated by flash chromatography to give diastereomers **2a** (87%; Rf 0.37 (50% ethyl acetate/hexanes); $[\alpha]^{21}_D$ +54° (*c* 0.14, MeOH)) and **2b** (75%; Rf 0.19 (50% ethyl acetate/hexanes); $[\alpha]^{21}_D$ +28° (*c* 0.15, MeOH)).[21] Each amide was hydrolyzed to give the corresponding enantiomeric acids **3a** (62%) and **3b** (77%). To check the enantiomeric purity of acids **3a** and **3b** (i.e., to verify that no racemization occurred during the amide hydrolysis), they were again converted to their respective diastereomeric amides **2** and analyzed by HPLC eluting with 55% ethyl acetate/hexanes. Regenerated diastereomer **2a** showed a single peak eluting at t_R 7.69 min, which indicated >98% de. The optical purity of **3b** was determined similarly—HPLC analysis of regenerated **2b** (t_R 15.82 min) indicated 98% de (i.e., 1% of diastereomer **2a** was detected).

The enantiomeric acids **3a** and **3b** were then converted to the target fluoroethylamides (+)-**4a** and (-)-**4b** as described above for the racemic compound. Enantiomer (+)-**4a** was obtained in 82% yield; $[\alpha]^{21}_D$ +19.9° (*c* 0.99, MeOH); MS *m/z* 364 (M+1). Similarly enantiomer (-)-**4b** was obtained in 89% yield; $[\alpha]^{21}_D$ -17.1° (*c* 0.98, MeOH); MS *m/z* 364 (M+1). Finally, assuming there was no further racemization during the conversion of acids **3a/3b** to fluoroethylamides **4a/4b**, to us a reasonable assumption considering that the same reaction procedures were used when regenerating amides **2a/2b**, we assume that amide **4a** was >98% ee and that amide **4b** was 98% ee, corresponding to the de's of the regenerated amides **2a/2b**, which is consistent with the optical rotations of **4a** and **4b** within experimental error. (An attempt to determine directly the ee of the final products by chiral HPLC was unsuccessful, as the enantiomers were not resolved by our chiral HPLC system).

Scheme 1



(a) LiOH, 3:1 MeOH/H_2O, 60 °C, 86%; (b) oxalyl chloride/benzene, 0 °C→23 °C, 2 h, 100%; (c) (S)-(+)-2-phenylglycinol/CH_2Cl_2, 0 °C→23 °C, 0.75 h, 75–87%; (d) 3 M HCl (aq), 1,4-dioxane, reflux, 2.5 h, 62%; (e) fluoroethylamine, 0 °C→23 °C, 0.75 h, 85%.

Pharmacology

For the cannabinoid receptor (CB_1) binding studies, displacement of radioligand [^{3}H]CP-55,940 binding to rat brain P_2 membrane preparations, in the presence of the enzyme inhibitor PMSF (50 μM), were used as described previously.[6,11] The in vivo studies were carried out in the mouse tetrad tests.[6,11] Inhibition of locomotor activity (SA) and antinociception by the tail-flick procedure (TF) were measured in the same group of animals, whereas hypothermia (RT) and ring immobility (RI) were determined in a separate group of animals following intravenous administration. The protocol used for these tests and the details of data analysis have been described previously.[6,11]

Table 1. Cannabinoid receptor (CB_1) binding affinity and mouse tetrad tests of anandamide analogs

Compound Number	Ki (nM)	ED_{50} (mg/kg)			
		SA	TF	RT	RI
(±)-**4**	21.2 ± 1.60	1.85 (0.91–3.77)	1.93 (1.12–3.33)	2.73 (0.42–17.5)	1.6 (1.06–2.43)
(+)-**4a**	22.4 ± 5.22	10.2 (5.93–17.6)	2.56 (1.40–4.67)	2.49 (1.42–4.36)	5.27 (3.93–7.07)
(-)-**4b**	15.3 ± 0.87	2.30 (1.37–3.87)	1.34 (0.69–2.59)	10.1 (4.17–24.3)	2.93 (2.01–4.27)

Discussion of Results

From an examination of both the in vitro and in vivo data (Table 1), it appears that there is little enantioselectivity between the two enantiomers. The confidence limits (shown in parentheses in Table 1) overlap for all three compounds with regard to tail-flick (TF) and ring immobility (RI), which suggests that they are not significantly different. However with regard to spontaneous activity (SA), the (+)-enantiomer is less potent than both the racemic (fivefold) and the (-)-enantiomer (fourfold). In the case of hypothermia (RT), only modest effects were seen for all three compounds. The maximal effect for the racemic compound (±)-**4** and the (+)- and (-)- enantiomers were only -1.8, -2.6, and -1.9 °C, respectively, and this is reflected in their wide confidence limits. Thus it is difficult to draw any conclusions from the RT data. We have previously reported[11] biological data for the racemic compound (±)-**4**; K_i = 5.7 ± 2.1 nM (PMSF), and an ED_{50} of 4.78 mg/kg in SA and 6.99 mg/kg in TF. It is difficult to comment on this discrepancy of results except that it is due to biological variation. However the results given in Table 1 were obtained for all the three analytes simultaneously.

We conclude that (1) we have developed an efficient method for resolving 2-methylarachidonic acid, which is a procedure that could potentially be used to resolve other 2-methyl long-chain acids, and (2) the introduction of a methyl substituent on the carbon in the 2-position of the arachidonic acid part of AN produces no observable enantioselectivity in its interaction with the cannabinoid receptor (CB_1), although it does enhance the metabolic stability of the adjacent amide bond.[10,11,13] The second conclusion is particularly interesting when compared with the enantioselectivity observed when a methyl substituent is introduced on the carbon adjacent to the nitrogen in the ethanolamine part of AN, to give the analog commonly known as 'methanandamide'.[9,13]

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18. Unpublished observation from our laboratory.

19. Compound (±)-**4**: ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 6.6 Hz, 3H), 1.16 (d, J = 6.8 Hz, 3H), 1.25–1.40 (m, 6H), 1.42–1.89 (m, 2H), 1.93–2.34 (m, 5H), 2.75–2.92 (m, 6H), 3.56 (ddt, J = 28.0, 5.1, 4.5 Hz, 2H), 4.47 (dt, J = 52.4, 4.9 Hz, 2H), 5.20–5.65 (m, 8H), 5.84 (br s, 1H); Anal. C, H, N.

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21. Diastereomer **2a**: ^{1}H NMR ($CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.04–1.54 (m, 6H), 1.16 (d, J = 6.8 Hz, 3H), 1.56–1.90 (m, 2H), 1.92–2.39 (m, 5H,), 2.70–2.92 (m, 6H), 3.88 (dd, J = <1.0, 5.2 Hz, 2H), 4.98–5.10 (m, 1H), 5.15–5.47 (m, 8H), 6.12 (d, J = 7.2 Hz, 1H), 7.23–7.35 (m, 5H). Diastereomer **2b** showed a similar ^{1}H NMR to **2a** except the coupling constant of the doublet of doublets at δ 3.87 was J = <1.0, and 4.6 Hz.

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