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Asymmetric synthesis of novel *N*-(1-phenyl-2,3-dihydroxypropyl)arachidonylamides and evaluation of their anti-inflammatory activity

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ABSTRACT

Aims: To design and synthesize novel *N*-(1-phenyl-2,3-dihydroxypropyl)arachidonylamides and evaluate their analgesic and anti-inflammatory potential.
Main methods: The murine macrophage cell line RAW 264.7 has been widely used as a model for inflammatory responses in vitro. Our model consists of cultured monolayers of RAW 264.7 cells in which media concentrations of 15-deoxy-$\Delta^{13,14}$-PGJ_2 (PGJ) are measured by ELISA following LPS (10 ng/ml) stimulation and treatment with 0.1, 0.3, 1.0, 3.0 and 10 μM concentrations of the compounds.
Key findings: Our data indicate that several of our compounds have the capacity to increase production of PGJ and may also increase the occurrence of programmed cell death (apoptosis).
Significance: Thus these agents are potential candidates for the therapy of conditions characterized by ongoing (chronic) inflammation and its associated pain.

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Introduction

Arachidonylethanolamide (anandamide, A) (Fig. 1) was isolated from porcine brain and was identified as a putative endogenous ligand for the cannabinoid receptor. This identification was mainly based on the ability of anandamide to inhibit both the specific binding of a tritiated cannabinoid ligand to synaptosomal membranes and the electrically evoked twitch response of the mouse vas deferens (Dewane et al., 1992). Further research in this area has shown that the pharmacological activity of anandamide, when administered in vivo, parallels that of other cannabinoid receptor agonists (Fride and Mechoulam, 1993). Furthermore, like other cannabimimetic agents, anandamide was found to be capable of inhibiting forskolin stimulated adenylate cyclase both in neuroblastoma cell lines that naturally express cannabinoid receptors and in cells transfected with plasmids carrying cannabinoid receptor DNA (Vogel et al., 1993).

Anandamide has also been found to exhibit cross-tolerance with (−)−Δ^9-tetrahydrocannabinol (Δ^9-THC) in the mouse vas deferens and to bind, albeit with a lower affinity to a second type of cannabinoid receptor expressed in the periphery (Pertwee et al., 1993; Munro et al., 1993). In addition to this, structure–activity relationship (SAR) studies of anandamide analogs have provided insights into the stereoelectronic requirements for interaction with the CB_1 receptor (Yao et al., 2008; Abadji et al., 1994; Ryan et al., 1997; Adams et al., 1995; Khanolkar et al., 1996; Lin et al., 1998; Sheskin et al., 1997; Goutopoulos et al., 2001). Likewise, 2-arachidonylglycerol (2-AG, B) (Fig. 1) which is an endogenous cannabinergic ligand that interacts with both CB_1 and CB_2 receptors, was found to possess various biological activities, such as binding to CB_1 and CB_2 receptors, inhibition of adenylyl cyclase in mouse spleen cells, and induction of hypothermia, reduction of spontaneous activity, analgesia and immobility in mice. 2-Arachidonylglycerol (2-AG) acts as a cannabinergic agonist, and the structure of 2-AG is recognized by the cannabinoid receptors (CB_1 and CB_2) (Vadivel et al., 2011). Anandamide possesses only a moderate affinity for the receptor ($Ki = 78$ nM) and it has a short metabolic half-life (Abadji et al., 1994).

The synthesis of pure 2-AG is problematic because of the migration of the arachidonyl group from the secondary to the primary hydroxyl group, resulting in the formation of the more stable 1-arachidonyl glycerol. Nearly all known methods for the synthesis of cannabinoid receptor ligands reported so far suffer from extended reaction times, harsh conditions for the removal of protecting groups as well as extensive work up procedures and purification methods (Vadivel et al., 2011). According to the literature, some of the research groups reported the design and synthesis of anandamide analogs with high metabolic stability, with the aim of improving the affinity of the anandamide ligand forthe receptor (Abadji et al., 1994; Mahadevan and Razdan, 2005; Razdan and Mahadevan, 2002).

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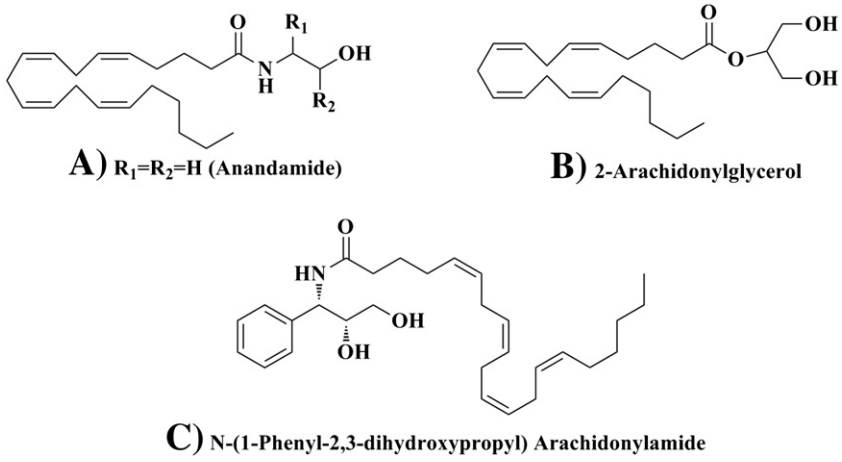


Fig. 1. The structures of anandamide, arachidonylglycerol and *N*-(1-phenyl-2,3-dihydroxypropyl)arachidonylamide.

There are several reports in the literature showing that, structural modifications on the arachidonyl side chain resulted in changes in the receptor affinity. According to these reports, complete saturation, or replacement of the alkenes with alkynes, resulted in the complete loss of receptor affinity. Also, when the arachidonyl chain was substituted with other fatty acid chains, with ω-olefinic bonds and with a *trans* double bond, obvious reduction in affinity for CB_1 was observed (Yao et al., 2008). The affinity of anandamide for CB_1 can be significantly enhanced by substituting the terminal pentyl group with a 1,1-dimethyl moiety, as has also been observed with (−)–Δ^9-THC (Seltzman et al., 1997). Anandamide analogs of variable chain lengths in which the terminal carbon is functionalized with a phenyl, substituted phenyl, or heterocyclic rings showed that the stereochemical features of the anandamide tail may have a big impact on the ligand's affinity for CB_1 (Yao et al., 2008). Taking all the above factors into consideration, we envisioned that the introduction of an aromatic ring and two hydroxyl groups in the main carbon chain of arachidonyl amide may have a significant effect on CB_1 or CB_2 affinity and potency. The present work describes the synthesis and evaluation of *anti*-inflammatory activity of *N*-(1-phenyl-2,3-dihydroxypropyl)arachidonylamide (C) (Fig. 1) and its analogs.

Materials and methods

Chemicals

All the cinnamic acids were purchased from Sigma Aldrich chemical company and Acros Organics. All the solvents were obtained from Acros Organics, Mallinckrodt Chemicals and Fisher Scientific. Lithium aluminum hydride (LAH) and palladium on activated carbon was obtained from Acros Organics. Thionyl chloride ($SOCl_2$) was obtained from Sigma Aldrich chemical company. Triethylamine was obtained from Acros Organics. Arachidonic acid and palmitic acid were purchased from Nu-Chek Prep. Inc. (Elysian, MN). Chemicals for the amino hydroxylation reaction were obtained from Sigma Aldrich and Macron chemical companies.

Reagents

Chemical preparation of chiral arachidonyl amides and related analogs

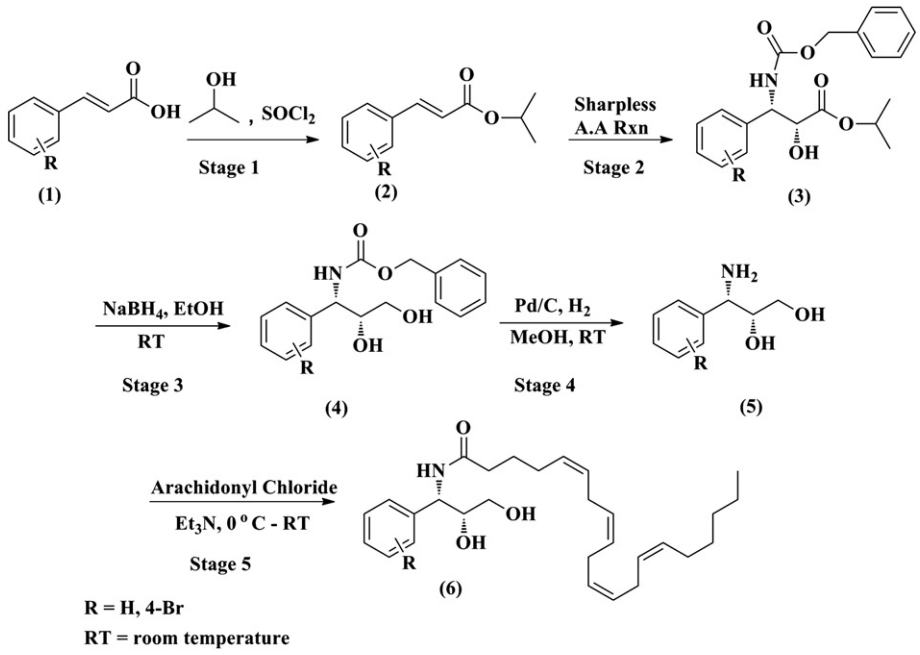
A series of *N*-(1-phenyl-2,3-dihydroxypropyl)arachidonylamides were synthesized and characterized by using cinnamic acid or substituted cinnamic acids as starting materials. Sharpless asymmetric aminohydroxylation (AA) is the key step involved in the synthesis scheme. The synthesis of *N*-(1-phenyl-2,3-dihydroxypropyl) arachidonylamides is illustrated in Scheme 1.

(**2**) *Isopropyl cinnamic ester.* Cinnamic acid (10.00 g, 67.50 mmol) was dissolved in isopropanol (100 mL) and thionyl chloride (8.37 mL, 114.74 mmol) was added dropwise with constant stirring at room temperature. After the completion of addition, the reaction mixture was heated to reflux over night. Then, the reaction mixture was brought to room temperature and an excess amount of isopropanol was removed under reduced pressure. The crude reaction mixture was dissolved in dichloromethane and washed with saturated sodium bicarbonate solution followed by water (3 × 100 mL). The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The product is a yellow, viscous, oily liquid (12.4 g, 65.18 mmol, 97% yield).

(**3**) *(2R,3S)-isopropyl 3-(((benzyloxy)carbonyl)amino)-2-hydroxy-3-arylpropanoate.* (2R,3S)-isopropyl 3-(((benzyloxy)carbonyl)amino)-2-hydroxy-3-phenylpropanoate was prepared in accordance with the procedure described in the literature (Li et al., 1996). The product was obtained as a white solid.

(**4**) *Benzyl ((1S,2R)-2,3-dihydroxy-1-arylpropyl)carbamate.* (2R,3S)-isopropyl 3-(((benzyloxy)carbonyl)amino)-2-hydroxy-3-phenylpropanoate (0.8 g, 2.23 mmol) was dissolved in absolute ethanol (50 mL) and stirred at room temperature. To the stirring reaction mixture, sodium borohydride (0.254 g, 6.71 mmol) was added slowly and stirring continued overnight at room temperature. The progress of reaction was monitored by TLC. After confirming the completion, EtOH was removed under reduced pressure, and water was added to the crude product, which was extracted with ethyl acetate. The product was purified by column chromatography and isolated as a white solid (0.51 g, 1.69 mmol, 76% yield).

(**5**) *(2R,3S)-3-amino-3-arylpropane-1,2-diol.* The CBZ group present in **(4)** was removed by the treatment with Pd/C in MeOH under hydrogen atmosphere. Compound **(4)** (0.33 g, 1.09 mmol) was placed in a round bottomed flask and under inert atmosphere Pd/C (0.033 g, 10% by weight of the starting material) catalyst was added. To the resulting mixture, anhydrous MeOH (10 mL/g of the starting material) was slowly added under argon. Then, after degassing the round bottomed flask containing the reaction mixture, a hydrogen balloon was attached to the flask and was stirred overnight. After confirming the completion of reaction, Pd/C was filtered off on a celite pad and the filtrate was concentrated under reduced pressure. The crude product was purified



Scheme 1. General procedure for the synthesis of *N*-(1-aryl-2,3-dihydroxypropyl)arachidonylamide and its analogs.

by column chromatography using methanol/dichloromethane solvent mixture. The product was obtained as an off-white solid (0.095 g, 0.568 mmol, 52% yield).

(**6**) *N-(1-Aryl-2,3-dihydroxypropyl)arachidonylamide*. To the solution of arachidonic acid (0.063 g, 0.21 mmol) in anhydrous dichloromethane (10 mL), oxalyl chloride (36 μL, 0.41 mmol) dissolved in dichloromethane (1 mL) was added slowly under stirring. *N,N*-dimethylformamide (1 drop) was added next. The reaction mixture was stirred at room temperature for 1 h and concentrated to give crude arachidonyl chloride (Casida et al., 2010). This crude arachidonyl chloride was dissolved in 5 mL of THF and added slowly dropwise to an ice-cold solution of **(5)** (0.044 g, 0.26 mmol) and triethylamine (45 μL, 0.31 mmol) in 15 mL of THF. The temperature of the reaction mixture was slowly brought to room temperature and stirred overnight. Progress of the reaction was monitored by TLC. After confirming the completion of the reaction, the reaction solution was diluted with hexanes and the triethylamine hydrochloride salt was filtered. The filtrate was concentrated, dissolved in chloroform, washed with water, dried over anhydrous magnesium sulfate and concentrated to give the crude product as viscous pale yellow oil (Casida et al., 2010). The product was purified using column chromatography (0.090 g, 0.19 mmol, 75% yield).

N-((1S,2R)-2,3-dihydroxy-1-phenylpropyl)palmitamide

The procedure for the synthesis of palmitoyl chloride and *N*-((1S,2R)-2,3-dihydroxy-1-phenylpropyl)palmitamide (**E**) is the same as described above for the synthesis of compound **(6)**. Compound **(5)** (0.040 g, 0.23 mmol), palmitoyl chloride (0.0986 g, 0.35 mmol) and triethyl amine (40 μL, 0.28 mmol) were used for this reaction. The final product *N*-((1S,2R)-2,3-dihydroxy-1-phenylpropyl)palmitamide (**E**) was obtained as a white solid after purification using column chromatography (0.075 g, 0.18 mmol 77% yield).

The compound characterization data and NMR spectra of all the compounds discussed above are provided in the supporting information.

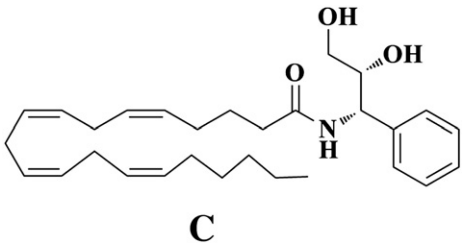
Procedure for stimulation of prostaglandin J adapted from (Burstein et al., 2012)

Cells used were RAW 264.7 and were obtained from ATCC. The base medium is Gibco DMEM with 10% fetal bovine serum added. Cells are grown in a T-75 flask in 15 mL of medium; medium is replaced on day four and sub cultured on day seven. Cells are removed by scraping without the aid of trypsin. A sub cultivation ratio of 1:3–1:6 was used. ELISA assay kits for PGJ were obtained from Assay Designs, Inc. (Ann Arbor, MI). The identity of the PGJ analyte in the culture medium was confirmed by mass spectrometry (Wood and Makriyannis, data not shown). Treatments were carried out in 48 well plates with 50,000 cells/500 μl DMEM + FCS media/well and TNFa (10 nM) added. Cells were incubated for 20 h at 37 °C and 5% CO_2. Media were changed to 500 μl of serum free DMEM, treated for 2 h and 100 μM removed for assays. NAgly [10 lM] control; 16,300 pg/mL. DMSO control: <16.0 pg/mL. N = 4.

Results

Anti-inflammatory activity (PGJ stimulation)

The murine macrophage cell line RAW 264.7 has been widely used as a model for inflammatory responses in vitro. LPS is a potent inducer of inflammatory responses including the arachidonic acid cascade; in a wide variety of models, some of which have been used to



C

Fig. 2. Structure of the compound tested for PGJ stimulation.

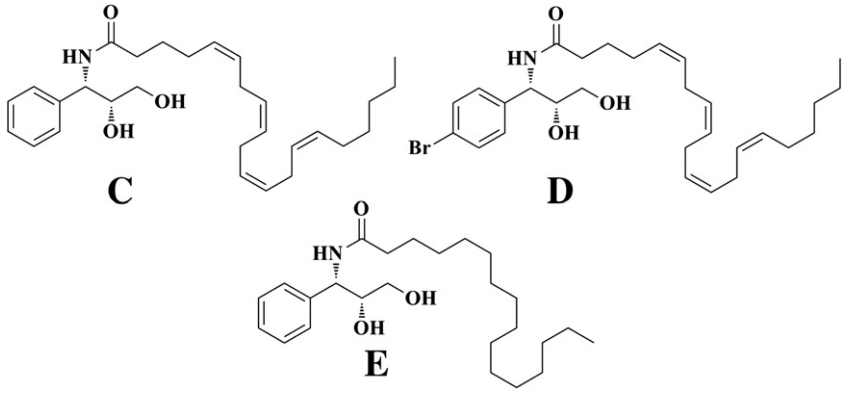


Fig. 3. Structures of the compounds tested for PGJ stimulation in RAW cells.

evaluate drug candidates. Thus, our model consisted of cultured monolayers of RAW 264.7 cells in which media concentrations of prostaglandins were measured by ELISA following LPS (10 ng/mL) stimulation and treatment with 0.1, 0.3, 1.0, 3.0 and 10 μM concentrations of the test compounds for 2 h (Figs. 2–3). To confirm the identity of the analyte by an independent method, mass spectral analysis of selected samples was performed using procedures previously done (Burstein et al., 2002).

The use of immunoassay procedures always raises questions of specificity. Therefore, we have indicated that we measured immunoreactive prostaglandin as defined by the cross reactivities of the antiserum used by the manufacturer.[1] The major cross reactants (>1%) are all precursors of the analyte and would not substantially change the conclusions on *anti*-inflammatory action of our compounds since the precursors all show *anti*-inflammatory actions. Regardless of the particular species that the assay detects, the preliminary results suggest that an empirical relationship exists between the ELISA data and the in vivo anti-inflammatory effects of the analogs tested thus far (Burstein, 2008) (Table 1).

Induction of apoptosis

Based on the results of the PGJ stimulation response from previous studies, studies for the induction of apoptosis were done using these analogs.

We speculate that these effects are due to programmed cell death or apoptosis rather than necrosis. An important consequence of apoptosis in certain cell types including macrophages is the resolution of chronic inflammation.

Experimental protocol

- Seed 2 (12-well) plates (24 wells total) with 150,000 RAW cells/mL 10% FBS medium (DMEM).
- Add 10 μL of LPS (100 μL/10 mLs) to all wells.
- Incubate the flasks for 18 h at 37 °C and 5% CO_2.
- Wash cells with serum free DMEM, then add 1 mL serum free DMEM to wells.
- Add treatments (10 μL) to each well as shown in Table 2 and incubate for 3 h.
- Scrape wells with cell scraper and spin in centrifuge 1350 rpm for 5 min. Remove 800 μL supernate and triturate remaining 200 μL. Take 20 μL and add 20 μL of 1/5 trypan blue stain. Count in hemocytometer, 4 corner squares and center square (N = 3).

[1] Cross reactivities (Assay Designs, Ann Arbor, MI): 15-deoxy-$\Delta^{12,14}$-PGJ_2 100%; PGJ_2 49.2%; delta12-PGJ_2 5.99%; PGD_2 4.92%; arachidonic acid 0.03%; <0.01%: PGF_2alpha/9alpha, 11beta-PGF_2alpha/PGE_2/thromboxane B_2/2-arachidonylglycerol/anandamide.

Discussion

Based on recent reports, it is suggested that members of the elmiric acid (EMA) family are candidates for drugs to treat various inflammatory conditions (Burstein et al., 2007). In the process of our ongoing effort to design and prepare novel analgesic and *anti*-inflammatory agents, we developed a mechanism based on in vitro assay for screening libraries of EMAs for potential *anti*-inflammatory activity based on their stimulatory action on PGJ levels. It is noteworthy that, in contrast to the nonsteroidal *anti*-inflammatory drugs (NSAIDS), the elmiric acids are not COX-2 inhibitors. Thus, it is expected that their side effect profile, if any, would be different.

Several literature reports indicate that an elevation of tissue concentrations of PGJ is associated with the resolution of an inflammatory condition (Gilroy et al., 2003, 2004). This is believed to come about through the binding and activation of the transcription factor PPAR-γ followed by increased expression of *anti*-inflammatory factors.

Table 1
Stimulation of PGJ production by compound C in RAW cells.

Conc. C (μM)	OD	Cell #	PGJ (pg/mL)	SEM	PGJ/10^3 cells
0	0.284	39,800	0	0	0
0.1	0.287	40,200	0	0	0
0.3	0.274	38,300	0	0	0
1.0	0.271	38,000	1376	172	36.2
3.0	0.266	37,300	8831	413	238
10	0.253	35,400	15,534	1220	439

Experimental protocol (see Materials and methods section).
Based on these results, analogs of the compound **C** were synthesized and PGJ stimulation studies were done.

Table 2
Stimulation of prostaglandin J_3 production by compounds **C**, **D** and **E** in RAW cells. Values shown represent the ratio of analog treated/NAGly control (NAGly = 1.00). NT = not tested.

Conc. (μM)	**C**	**D**	**E**
0.10	0.00	0.00	0.00
0.30	0.02	NT	NT
0.74	NT	0.00	NT
1.00	0.62	NT	0.00
2.20	NT	0.016	NT
3.00	3.56	NT	0.00
6.70	NT	0.029	NT
10.00	6.33	NT	0.00
20.00	NT	2.18	NT
30.00	NT	NT	0.00
60.00	NT	6.83	NT

Experimental protocol (see Materials and methods section).

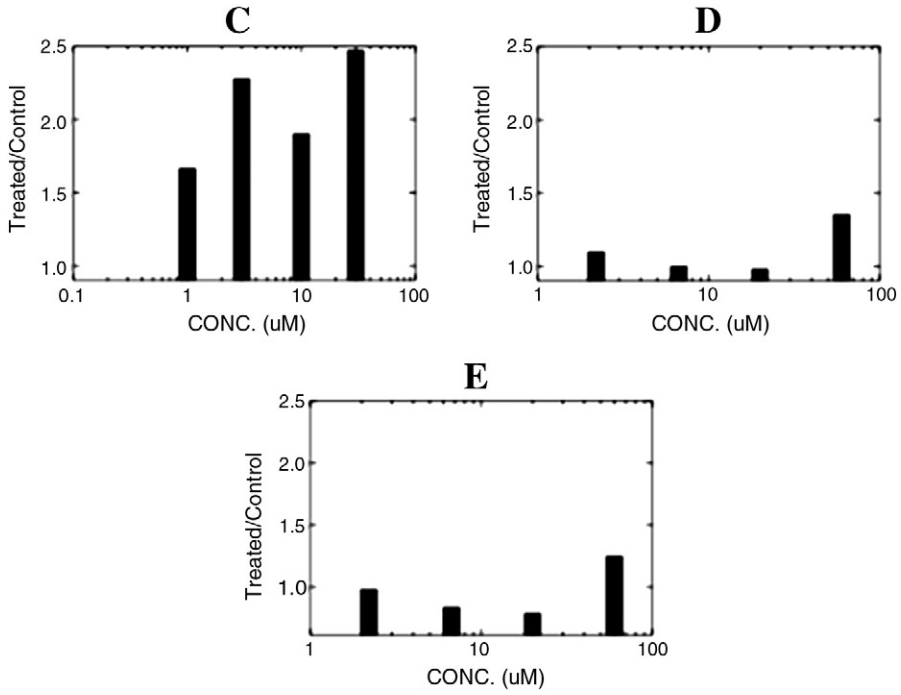


Fig. 4. Increase in trypan blue exclusion by **C**: possible occurrence of apoptosis.

Previous studies showed that the synthetic cannabinoid analog ajulemic acid elevates PGJ in similar models, i.e. fibroblast-like synovial cells (Stebulis et al., 2008). More recent data demonstrated that a similar response can be observed with *N*-arachidonyl glycine in vivo (Burstein et al., 2011).

The concentration of PGJ, formed from the dehydration of PGD_2, increases during the resolution phase of chronic inflammation, and acts as a brake on inflammation by, among other actions, inducing apoptosis of inflammatory cells. Programmed cell death (apoptosis) of inflammatory cells is an important part of the resolution process. Inflammatory and synovial cells from joints of patients with rheumatoid arthritis are resistant to apoptosis, which interferes with resolution of acute inflammation and leads to chronic inflammation and joint tissue injury. Our data indicate that several of our compounds have the capacity to increase production of PGJ and possibly also increase the occurrence of apoptosis (Fig. 4). The parent compound *N*-arachidonylglycine has recently been reported to induce apoptosis in macrophages (Takenouchi et al., 2012). Thus, these agents (analogs) are potential candidates for the therapy of conditions characterized by ongoing (chronic) inflammation and its associated pain.

Conclusion

From the above studies and results we conclude that there is no correlation between PGJ stimulation and induction of apoptosis. However, it is interesting to note that compound **C** was the most active one in both studies. These data suggest that there may be a mechanistic connection, and compound **C** may show in vivo activity in the resolution of chronic inflammation.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2012.06.040.

References

Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, et al. (R)-Methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. J Med Chem 1994;37:1889–93.
Adams IB, Ryan W, Singer M, Razdan RK, Compton DR, Martin BR. Pharmacological and behavioral evaluation of alkylated anandamide analogs. Life Sci 1995;56:2041–8.
Burstein S. The elmiric acids: biologically active anandamide analogs. Neuropharmacology 2008;55(8):1259–64.
Burstein S, McQuain C, Salmonsen R, Seicol B. N-Amino acid linoleoyl conjugates: anti-inflammatory activities. Bioorg Med Chem Lett 2012;22:872–5.
Burstein SH, Adams JK, Bradshaw HB, Fraioli C, Rossetti RG, Salmonsen RA. Potential anti-inflammatory actions of the elmiric (lipoamino) acids. Bioorg Med Chem 2007;15:3345–55.
Burstein SH, Huang SM, Petros TJ, Rosetti RG, Walker JM, Zurier RE. Regulation of anandamide tissue levels by N-arachidonylglycine. Biochem Pharmacol 2002;64:1147–50.
Burstein SH, McQuain CA, Ross AH, Salmonsen RA, Zurier RE. Resolution of inflammation by N-arachidonylglycine. J Cell Biochem 2011;112:3227–33.
Casida JE, Gulevich AG, Sarpong R, Bunnelle EM. S-Arachidonyl-2-thioglycerol synthesis and use for fluorimetric and colorometric assays of monoacylglycerol lipase. Bioorg Med Chem 2010;18:1942–7.
Dewane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992;258:1946–9.
Fride E, Mechoulam R. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. Eur J Pharmacol 1993;231:313–4.
Gilroy DW, Colville-Nash PR, McMaster S, Sawatzky DA, Willoughby DA, Lawrence T. Inducible cyclogenase-derived 15-deoxy(delta)$^{12,14}PGJ_2$ brings about acute inflammatory resolution in rat pleurisy by inducing neutrophil and macrophage apoptosis. FASEB J 2003;17:2269–71.
Gilroy DW, Lawrence T, Perretti M, Rossi AG. Inflammatory resolution: new opportunities for drug discovery. Nat Rev 2004;3:401–16.

Goutopoulos A, Fan P, Khanolkar AD, Xie XQ, Lin S, Makriyannis A. Stereochemical selectivity of methanamides for the CB_1 and CB_2 cannabinoid receptors and their metabolic stability. Bioorg Med Chem 2001;9:1673–84.

Khanolkar AD, Abadji V, Lin S, Hill WA, Taha G, Abouzid K, et al. Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. J Med Chem 1996;39:4515–9.

Li G, Angert HH, Sharpless KB. N-Halocarbamate salts lead to more efficient catalytic asymmetric aminohydroxylation. Angew Chem Int Ed Engl 1996;35: 2813–7.

Lin S, Khanolkar AD, Fan P, Goutopoulos A, Qin C, Papahadjis D, et al. Novel analogues of arachidonyl ethanolamide (anandamide): affinities for the CB_1 and CB_2 cannabinoid receptors and metabolic stability. J Med Chem 1998;41: 5353–61.

Mahadevan A, Razdan RK. Further advances in the synthesis of endocannabinoid-related ligands. AAPS J 2005;7:E496–502.

Munro S, Thomas KL, Abu-shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–5.

Pertwee RG, Stevenson LA, Griffin G. Cross-tolerance between delta-9-tetrahydrocannabinol and the cannabimimetic agents CP-55,940, WIN 55,212-2 and anandamide. Br J Pharmacol 1993;110:1483–90.

Razdan RK, Mahadevan A. Recent advances in the synthesis of endocannabinoid related ligands. Chem Phys Lipids 2002;121:21–33.

Ryan WJ, Banner WK, Wiley JL, Martin BR, Razdan RK. Potent anandamide analogs: the effect of changing the length and branching of the end pentyl chain. J Med Chem 1997;277:3617–25.

Seltzman HH, Fleming DN, Thomas BF, Gilliam AF, McCallion DS, Pertwee RG, et al. Synthesis and pharmacological comparison of dimethylheptyl and pentyl analogs of anandamide. J Med Chem 1997;40:3626–34.

Sheskin T, Hanus L, Slager J, Vogel Z, Mechoulam R. Structural requirements for binding of anandamide-type compounds to the brain cannabinoid receptor. J Med Chem 1997;40:659–67.

Stebulis JA, Johnson DR, Rossettic RG, Burstein ZH, Zurierc RB. Ajulemic acid, a synthetic cannabinoid acid, induces an anti-inflammatory profile of eicosanoids in human synovial cells. Life Sci 2008;83:666–70.

Takenouchi R, Inoue K, Kambe Y, Miyata A. N-arachidonyl glycine induces macrophage apoptosis via GPR18. Biochem Biophys Res Commun 2012;418:366–71.

Vadivel SK, Whitten KM, Makriyannis A. Chemoenzymatic synthesis of 2-arachidonylglycerol, an endogenous ligand for cannabinoid receptors. Tetrahedron Lett 2011;52:1149–50.

Vogel Z, Berg J, Levy R, Saya D, Heldman E, Mechoulam R. Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. J Neurochem 1993;61:352–5.

Yao F, Li C, Vadivel SK, Bowman AL, Makriyannis A. Development of novel tail-modified anandamide analogs. Bioorg Med Chem Lett 2008;18:5912–5.