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Novel Tail and Head Group Prostanamide Probes

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Abstract

We report the design and synthesis of novel prostaglandin-ethanolamide (PGE₂-EA) analogs containing head and tail group modifications to aid in the characterization of a putative prostanamide receptor(s). Our synthetic approach utilizes Horner-Wadsworth-Emmons and Wittig reactions to construct the head and the tail moieties of the key PGE₂ precursor, which leads to the final products through a peptide coupling, Swern oxidation and HF/pyridine assisted desilylation. The synthesized analogs were shown not to interact significantly with endocannabinoid proteins and recombinant EP1, EP3 and EP4 receptors and suggest a yet to be identified prostanamide receptor as their site(s) of action.

Keywords

Prostanamides; Cyclooxygenase-2; Endocannabinoids

The endocannabinoid system has broad physiological function and is involved in pain, weight management, neuroprotection and the immune system.¹ This is accomplished by modulating the levels of endocannabinoids, one of which, anandamide (AEA, Figure 1), is produced on demand and metabolized by a number of enzymes including COX-2,² fatty acid amide hydrolase (FAAH),^{3,4} *N*-acyl ethanolamide acid amidase (NAAA)⁵ and monoacylglycerol lipase (MGL).⁶ Enzymatic transformation of AEA by COX-2 leads to the

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Supplementary data

Experimental procedures, characterization data for final compounds and methods associated with this article can be found, in the online version.

production of a new family of endogenous substances namely prostamides (prostaglandin-ethanolamides, PG-EA) with distinct biological activities from either AEA or prostaglandins.⁷ Biological testing of the PGE₂, PGF_{2α} and PGD₂ related prostamides exhibited physiological properties that ranged from contraction of feline iris to the relaxation of rabbit jugular vein.⁸ Although a number of studies related to their biological activity have been reported, the full picture regarding their mechanism of action and their molecular targets remains to be elucidated.⁸⁻¹⁰

Yu and co-workers identified the first prostamide (PGE₂-EA, Figure 1), during studies of anandamide deactivation.¹¹ In their studies, arachidonic acid ethanolamide was submitted to both COX-1 and COX-2 to test whether this reaction is involved in the biotransformation of a significant portion of AEA. They showed that AEA was not a substrate for COX-1 but underwent oxidative cyclization with COX-2 to produce a PGE₂ related prostamide.² Earlier, amide derivatives of prostaglandins were studied and shown to be inactive in a number of receptor specific preparations.^{12,13} The drug Latanoprost (Figure 1), a PGF_{2α} ester derivative approved to treat glaucoma was reported to act as a pro-drug for PGF_{2α}^{14,15} while the receptors for the related amide (Bimatoprost) and the PGF_{2α}-EA, have been identified as heterodimers of the wild type FP receptor and one of its splice variants.¹⁰ It has also been reported that possibly the actions of PGE₂ and PGD₂ related prostamides may be associated with distinct yet unidentified receptors.^{8,16} Further work after the endogenous nature of prostamides was established revealed a marked difference in their activity compared to the prostaglandins.¹⁶⁻¹⁸

Access to prostamide analogs with improved pharmacological properties, such as enhanced stability toward hydrolytic and oxidative pathways, would greatly aid in understanding their molecular mechanism of action. In this communication we describe the synthesis of a set of novel aromatic tailed prostamide probes basing our design on the chemical structures of the endogenous PGE₂-EA and the drugs Bimatoprost and Latanoprost. Because PGE₂-EA is the major product of prostamide biosynthesis,¹¹ the E₂ structure was selected as the template in our design. As with earlier work on anandamide probes (e.g. AM356, Figure 1) we included in our synthesis analogs with *R*- or *S*-methyl groups at the ethanolamide moiety of the prototype to increase the stability of the compound toward enzymatic hydrolysis (Scheme 4).¹⁹ The aromatic tail of the drugs Bimatoprost and Latanoprost was included because it maintains biological activity while it is expected to have enhanced stability for P450 induced ω-oxidation when compared to the *n*-pentyl tail of the PGE₂ prototype (Scheme 3). Biological testing results indicate that the analogs reported here do not exhibit significant interactions with the proteins of the endocannabinoid system. Also, *in vitro* testing of a representative analog employing electrical cell-substrate impedance sensing system revealed that the compound is inactive at the recombinant EP1, EP3 and EP4 receptors.

The synthetic approach we used as outlined below is a modification of the Corey prostaglandin synthesis^{20,21} and begins with the Fisher esterification of commercially available acid **1a** to form methyl ester **2a** in 56% yield (Scheme 1).²² Compound **2a** was converted to the β-keto-phosphonate **3a** by exposure to dimethyl(lithiomethyl)phosphonate which is generated *in situ* from dimethylphosphonate and *n*-BuLi (82% yield).²³ Submitting phosphonate **3a** and commercially available (–)-Corey-aldehyde/lactone **4** to the Masamune-

Roush modification of the Horner-Wadsworth-Emmons reaction yielded the key enone **5a** in 92% yield.²⁴ Under the experimental conditions used this reaction gave the E geometrical isomer exclusively, as confirmed by ¹H NMR data (³J_{CH=CH} = 15.8 Hz). Reduction of compound **5a** followed two different paths (Scheme 2). The first uses (*R*)-2-methyl-CBS-oxazaborolidine and catechol borane to provide alcohols **6a** and **6b** in 65% yield with an 1:5 enrichment in favor of the naturally occurring diastereomer **6a**.²⁵ Alternatively, standard Luche reduction procedures could be used to furnish alcohols **6a** and **6b** in higher yield (87%) with an enrichment ratio of 1:1.6.²⁶ Subsequently, the two diastereomers were separated by flash column chromatography on silica gel. It should be noted that assignment of the absolute stereochemistry at C15 of the alcohols **6a** and **6b** was based on our work with close related analogs where the “Mosher's method” was used.²⁷ With the individual compounds **6a** and **6b** in hand, the synthesis could be completed (Scheme 3). Thus, alcohol **6a** was protected with TBSOTf to provide lactone **7a** in 79% yield.²⁸ Standard DIBAL-H reduction gave lactol **8a** in 82% yield. This compound underwent a Wittig olefination reaction to yield acid **9a** as a single geometrical isomer under the experimental conditions used (68% yield).^{14,29} Intermediate **9a** was coupled with the 2-(*tert*-butyldimethylsilyloxy)ethanamine³⁰ to give compound **10a** (58% yield) which was subsequently oxidized to ketone **11a** in excellent yield (89%) using Swern conditions.³¹ The synthesis was completed by global desilylation of ketone **11a** with hydrofluoric acid/pyridine to give the final product **12a** (AM6905) in 49% yield.²⁷ Similarly, the 15*R*-isomer (**12b**, AM6909, Scheme 3) was synthesized in six steps starting from alcohol **6b**. Following the completion of AM6905 and AM6909 various analogs were synthesized to allow for a study of these intriguing compounds. Thus, readily available 3-(4-iodophenyl)propionic acid³² was converted to the key 15*S* alcohol **6c** in four steps through the Luche reduction approach (Schemes 1 and 2). Subsequently, the iodo-substituted tail analog **12c** was synthesized from **6c** in six steps (Scheme 3). The intermediate prostaglandin acid derivatives **9a**, **9b** and **9c** served as the starting points for the synthesis of the head group modified analogs **15a**, **15b** and **15c** through our well established three step process which involves amide coupling, Swern oxidation and HF/pyridine assisted desilylation (Scheme 4).

Because of their structural and functional proximity with the endocannabinoid system all synthesized compounds (**12a-12c** and **15a-15c**) were tested for their inhibitory activities on three key endocannabinoid enzymes namely, FAAH, MGL, and NAAA as well as for their binding affinities at CB1 and CB2 cannabinoid receptors using procedures we described earlier.^{30,33} Our testing results (Table 1) revealed that all analogs reported here have no significant inhibitory activity exhibiting IC₅₀s greater than 100 μM for all three endocannabinoid deactivating enzymes. The testing results for FAAH are consistent with those reported earlier for the endogenous prototype PGE₂-EA.⁸ In addition, all analogs exhibited weak binding affinities for both the CB1 and CB2 cannabinoid receptors with K_i values greater than 620 nM, which is in agreement with earlier studies on PGE₂-EA derivatives.³⁴

To assess whether AM6905, a representative of this new class of PGE₂-EA analogs, has direct interactions with specific prostaglandin receptors or may interact with distinct functional proteins, we next carried out *in vitro* screening assays using recombinant EP1,

EP3 and EP4 receptors in an electrical cell-substrate impedance sensing system (ECIS). The prototypes PGE₂ and PGE₂-EA were also tested for comparison (see supporting information and Table 2). ECIS can be used to investigate the signal transduction of G protein-coupled receptors such as the EP receptors.³⁵ G proteins (G_s, G_i, or G_q) coupling to the receptor give diverse characteristic profiles of impedance change upon ligand binding. Measurement of cell impedance gave a rapid change in impedance in these cells that was PGE₂ dependent, conversely, AM6905 and PGE₂-EA by direct comparison did not activate either the EP1, EP3, or EP4 receptors each expressed separately in CHO cells.

In summary, we have described the synthesis of six prostamide analogs with structural modifications at the tail, and at the head group of the endogenous prototype PGE₂-EA. These novel compounds may be considered as hybrid ligands with contributions from both the endocannabinoid and prostaglandin biochemical systems. However, our biological testing results indicate that these analogs do not exhibit significant interactions with the proteins of the endocannabinoid system. Also, further *in vitro* testing of a representative analog employing electrical cell-substrate impedance sensing system revealed that the compound is inactive at the prostaglandin EP1, EP3 and EP4 receptors. These results which parallel those observed with PGE₂-EA suggest a yet to be identified target protein as their site(s) of action.⁹ We are now planning to utilize these new compounds as probes for the characterization of such a target.

Supplementary Material

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Acknowledgments

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References and notes

1. Wang J, Ueda N. Prostag. Oth. Lipid M. 2009; 89:112.
2. Rouzer CA, Marnett LJ. J. Biol. Chem. 2008; 283:8065. [PubMed: 18250160]
3. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Nature. 1996; 384:83. [PubMed: 8900284]
4. Wei BQQ, Mikkelsen TS, McKinney MK, Lander ES, Cravatt BF. J. Biol. Chem. 2006; 281:36569. [PubMed: 17015445]
5. Tsuboi K, Takezaki N, Ueda N. Chem. Biodivers. 2007; 4:1914. [PubMed: 17712833]
6. Dinh TP, Kathuria S, Piomelli D. Mol. Pharmacol. 2004; 66:1260. [PubMed: 15272052]
7. Woodward DF, Liang Y, Krauss AHP. Br. J. Pharmacol. 2008; 153:410. [PubMed: 17721551]
8. Matias I, Chen J, De Petrocellis L, Bisogno T, Ligresti A, Fezza F, Krauss AHP, Shi L, Protzman CE, Li C, Liang Y, Nieves AL, Kedzie KM, Burk RM, Di Marzo V, Woodward DF. J. Pharmacol. Exp. Ther. 2004; 309:745. [PubMed: 14757851]
9. Alhouayek M, Muccioli GG. Trends Pharmacol. Sci. 2014; 35:284. [PubMed: 24684963]
10. Woodward DF, Wang JW, Poloso NJ. Pharmacol. Rev. 2013; 65:1135. [PubMed: 23893067]
11. Yu M, Ives D, Ramesha CS. J. Biol. Chem. 1997; 272:21181. [PubMed: 9261124]
12. Maddox YT, Ramwell PW, Shiner CS, Corey EJ. Nature. 1978; 273:549. [PubMed: 661964]
13. Schaaf TK, Hess HJ. J. Med. Chem. 1979; 22:1340. [PubMed: 533881]

14. Feng ZX, Hellberg MR, Sharif NA, McLaughlin MA, Williams GW, Scott D, Wallace T. *Bioorg. Med. Chem.* 2009; 17:576. [PubMed: 19101156]
15. Rautio J, Kumpulainen H, Heimbach T, Oliyai R, Oh D, Jarvinen T, Savolainen J. *Nat. Rev. Drug Discov.* 2008; 7:255. [PubMed: 18219308]
16. Ross RA, Craib SJ, Stevenson LA, Pertwee RG, Henderson A, Toole J, Ellington HC. *J. Pharmacol. Exp. Ther.* 2002; 301:900. [PubMed: 12023517]
17. Woodward DF, Krauss AHP, Chen J, Lai RK, Spada CS, Burk RM, Andrews SW, Shi L, Liang Y, Kedzie KM, Chen R, Gil DW, Kharlamb A, Archeampong A, Ling J, Madhu C, Ni J, Rix P, Usansky J, Usansky H, Weber A, Welty D, Yang W, Tang-Liu DDS, Garst ME, Brar B, Wheeler LA, Kaplan LJ. *Surv. Ophthalmol.* 2001; 45:S337. [PubMed: 11434936]
18. Woodward DF, Krauss AHP, Chen J, Liang Y, Li C, Protzman CE, Bogardus A, Chen R, Kedzie KM, Krauss HA, Gil DW, Kharlamb A, Wheeler LA, Babusis D, Welty D, Tang-Liu DDS, Cherukury M, Andrews SW, Burk RM, Garst ME. *J. Pharmacol. Exp. Ther.* 2003; 305:772. [PubMed: 12606640]
19. Khanolkar AD, Abadji V, Lin SY, Hill WAG, Taha G, Abouzid K, Meng ZX, Fan PS, Makriyannis A. *J. Med. Chem.* 1996; 39:4515. [PubMed: 8893848]
20. Corey EJ, Ohuchida S, Hahl R. *J. Am. Chem. Soc.* 1984; 106:3875.
21. Corey EJ, Shiraham H, Yamamoto H, Terashim S, Venkates A, Schaaf TK. *J. Am. Chem. Soc.* 1971; 93:1490. [PubMed: 5548346]
22. Kolotuchin SV, Meyers AI. *J. Org. Chem.* 1999; 64:7921.
23. Delamarche I, Mosset P. *J. Org. Chem.* 1994; 59:5453.
24. Ghosh S, Kumar SU, Shashidhar J. *J. Org. Chem.* 2008; 73:1582. [PubMed: 18201100]
25. Schrader TO, Snapper ML. *J. Am. Chem. Soc.* 2002; 124:10998. [PubMed: 12224945]
26. Brinkmann Y, Oger C, Guy A, Durand T, Galano JM. *J. Org. Chem.* 2010; 75:2411. [PubMed: 20218561]
27. Shelnut, E. Ph. D. Thesis. Northeastern University; Jun. 2012
28. Pandya BA, Snapper ML. *J. Org. Chem.* 2008; 73:3754. [PubMed: 18416578]
29. Zaroni G, D'Alfonso A, Porta A, Feliciani L, Nolan SP, Vidari G. *Tetrahedron.* 2010; 66:7472.
30. Papahatjis DP, Nahmias VR, Nikas SP, Schimpfen M, Makriyannis A. *Chem. Eur. J.* 2010; 16:4091. [PubMed: 20187040]
31. Schule A, Liang H, Vors JP, Ciufolini MA. *J. Org. Chem.* 2009; 74:1587. [PubMed: 19199657]
32. Rosen BM, Wilson DA, Wilson CJ, Peterca M, Won BC, Huang C, Lipski LR, Zeng X, Ungar G, Heiney PA, Percec V. *J. Am. Chem. Soc.* 2009; 131:17500. [PubMed: 19904947]
33. Alapafuja SO, Nikas SP, Bharathan IT, Shukla VG, Nasr ML, Bowman AL, Zvonok N, Li J, Shi X, Engen JR, Makriyannis A. *J. Med. Chem.* 2012; 55:10074. [PubMed: 23083016]
34. Berglund BA, Boring DL, Howlett AC. *Adv. Exp. Med. Biol.* 1999; 469:527. [PubMed: 10667378]
35. Peters MF, Scott CW. *J. Biomol. Screen.* 2009; 14:246. [PubMed: 19211780]

Figure 1.
Chemical structures of the endogenous AEA and PGE₂-EA and their synthetic derivatives AM356, Bimatoprost and Latanoprost.

Scheme 1.
Synthesis of the key enones **5a** and **5b**.

Scheme 2.

Reduction of enones **5a** and **5b** to introduce the 15*S*- and 15*R*- stereochemistry.

Scheme 3.

Synthesis of the tail modified prostamide analogs AM6905, AM6909 and AM6910.

Scheme 4.

Synthesis of the head group modified analogs AM6906, AM6907 and AM6908.

Table 1

Enzyme inhibition data and cannabinoid receptor binding affinities for synthesized compounds.

Compd	Enzyme inhibition data (IC ₅₀ , μM)			Cannabinoid receptor binding affinities (K _i , nM)	
	FAAH human rat	MGL human	NAAA human	CB1 (rat) with PMSF without PMSF	CB2 mouse human
12a	>100μM	~100μM	>100μM	>1,000	>1,000
	>100μM			>1,000	>1,000
12b	>100μM	>100μM	>100μM	~740	>1,000
	>100μM			~740	>1,000
12c	>100μM	>100μM	>100μM	~620	>1,000
	>100μM			~830	>1,000
15a	>100μM	>100μM	>100μM	~730	>1,000
	>100μM			~770	>1,000
15b	>100μM	>100μM	>100μM	~950	>1,000
	>100μM			>1,000	>1,000
15c	>100μM	>100μM	>100μM	>1,000	>1,000
	>100μM			>1,000	>1,000

PMSF: Phenylmethanesulfonyl fluoride.

Table 2

Changes of impedance for PGE₂, PGE₂-EA, and compound 12a using recombinant EP1, EP3 and EP4 receptors.

Compd	<u>Changes of impedance (Ω)</u>		
	EP1	EP2	EP3
12a	no	no	no
PGE₂-EA	no	no	no
PGE₂	yes	yes	yes