Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1913–1916

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# 5-HETE Congeners as Modulators of Cell Proliferation

Thomas A. Miller,[a] Jagadananda Ghosh,[b] Charles E. Myers[b] and Timothy L. Macdonald[a,*]

[a]*Department of Chemistry, University of Virginia, McCormick Road, Charlottesville, VA 22901, USA*
[b]*Cancer Center, University of Virginia, PO Box 334, Charlottesville, VA 22908, USA*

Received 26 May 2000; accepted 16 June 2000

**Abstract**—The synthesis and assessment of the mitogenic properties of 5-HETE congeners are reported. These studies represent an effort to develop a structure–activity profile for ligands of the 5-HETE/5-oxoETE G-protein coupled receptor(s). Many of these agents possess mitogenic activity that equals or exceeds that of racemic 5-HETE family constituents in prostate cancer cell lines.


Malignant prostate cancer occurs in 80% of men over age 70. However, metastatic forms of prostate cancer are much less prevalent, with ~50,000 presentations in the United States per year. Terminal prostate cancer typically exhibits tumor doubling times of 3–4 months. The most extreme cases are associated with tumor doubling times of 2 weeks or less. The nature of the factors associated with the transition from malignant to metastatic prostate cancer has led to speculation that dietary fat has an enormous impact on the development of lethal prostate cancer. For example, it has been shown that supplemental arachidonic acid induces rapid proliferation in prostate cellular assays and in implanted prostate carcinoma.[1,2] More recently, we demonstrated that 5-lipoxygenase (5-LO) products of arachidonic acid possess potent mitogenic activity in prostate cancer cell lines and that cell survival was dependent on the presence of constituents of the 5-HETE family of eicosanoids (Fig. 1). These studies revealed that inhibition of the metabolic production of the 5-HETE family of eicosanoids from arachidonic acid in prostate cancer cell lines results in apoptosis for essentially all the cells within 1 h and death of the entire population within 2 h.[3,4] The 5-HETE family constituents were able to rescue serum starved prostate cell cultures when introduced exogenously. The leukotrienes, which are also 5-LO products of arachidonic acid metabolism, did not exhibit similar mitogenic activities, nor was cell survival leukotriene dependent.[5]

In light of these findings, we have developed a program dedicated to the study of agents that may provide insight into the relationship between function and structure for the 5-HETE family of eicosanoids, with the ultimate goal of understanding the consequence of dietary fat composition on the induction of prostate cancer. This work should prove useful in the pharmacophoric deconvolution of agonistic and antagonistic activities at the 5-HETE family receptor(s).[6] Our current efforts have been focused on the development of agonist structure–activity profiles through the synthesis and evaluation of 5-HETE congeners that possess modified steric and electronic properties.

## Design

Unlike receptor discovery, the pharmacologic characterization of receptors relies on defining the relationships between ligand structure and receptor response. The current understanding of the structure–response profile for the 5-HETE family is limited and thereby warrants further study. Several postulates about the observed activities of the members of the 5-HETE family of eicosanoids were considered in the development of our synthetic program. The mitogenic activity of the 5-HETE family may result from, among other things, the recognition of 5-HETE by the proposed receptor(s) or from metabolism of 5-HETE into other active agents. Notably, 5-HETE and 5-oxoETE (Fig. 1) have been shown to rapidly interconvert in human polymorphonuclear leukocytes.[7] Also, 5-HETE lactone (Fig. 1) possesses mitogenic activity in prostatic cancer cell lines and induces a 2-fold maximal growth enhancement over

*Corresponding author. Tel.: +1-804-924-7718; fax: +1-804-982-2302; e-mail: tlm@virginia.edu

0960-894X/00/$ - see front matter 
PII: S0960-894X(00)00370-X

that of the parent, 5-HETE. It is currently unclear if this activity can be attributed to slow hydrolysis of 5-HETE lactone to 5-HETE or if 5-HETE lactone itself is active at the receptor(s).[4,7] We have attempted to address the issues of interconversion of the members of the 5-HETE family by assessing agents that should possess attenuated lability in metabolic conversions into alternative structures.

In these initial studies, we have modified two of the substructures of the 5-HETE/5-oxoETE molecules: the 5-oxo-region and the acyl-region. This study has produced a variety of agents and many of these have been shown to possess potent agonistic activity.

## Chemistry[8]

The 5-HETE derivatives possessing altered acyl region functionalities were produced directly from 5-HETE lactone by nucleophilic ring opening (Fig. 2). Racemic 5-HETE lactone was conveniently synthesized from arachidonic acid by the method of Corey et al.[9] Ring opening of 5-HETE lactone with excess nucleophile afforded targets **1**–**5** and **10**–**18** in good yields.[9,10] Thus, 5-HETE methyl ester **2** and a wide variety of amide derivatives could be accessed with a single, easily obtainable intermediate. Those derivatives modified at the 5-position were produced from 5-oxoETE methyl ester. 5-oxoETE was produced from 5-HETE methyl ester **2** by treatment with $MnO_2$ (15 equiv) in $CH_2Cl_2$ for 24 h.[11] Subsequent reductive amination followed by saponification afforded the corresponding amines **6** and **7**.[11] These were readily converted to the corresponding lactams **8** and **9**, respectively, in good overall yield.[11]

## Discussion

All of the compounds synthesized were screened for growth enhancement activity using LNCap prostate cell cultures. Growth enhancement data for the racemic

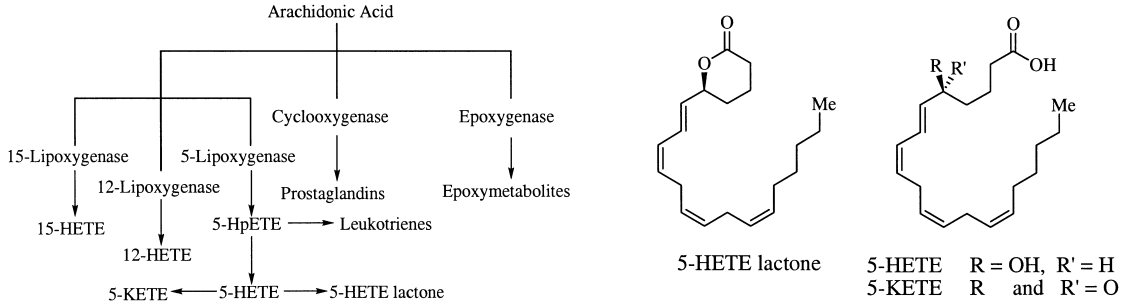


**Figure 1.** Major pathways of arachidonic acid metabolism (left) 5-HETE, 5-KETE and 5-HETE lactone (right).

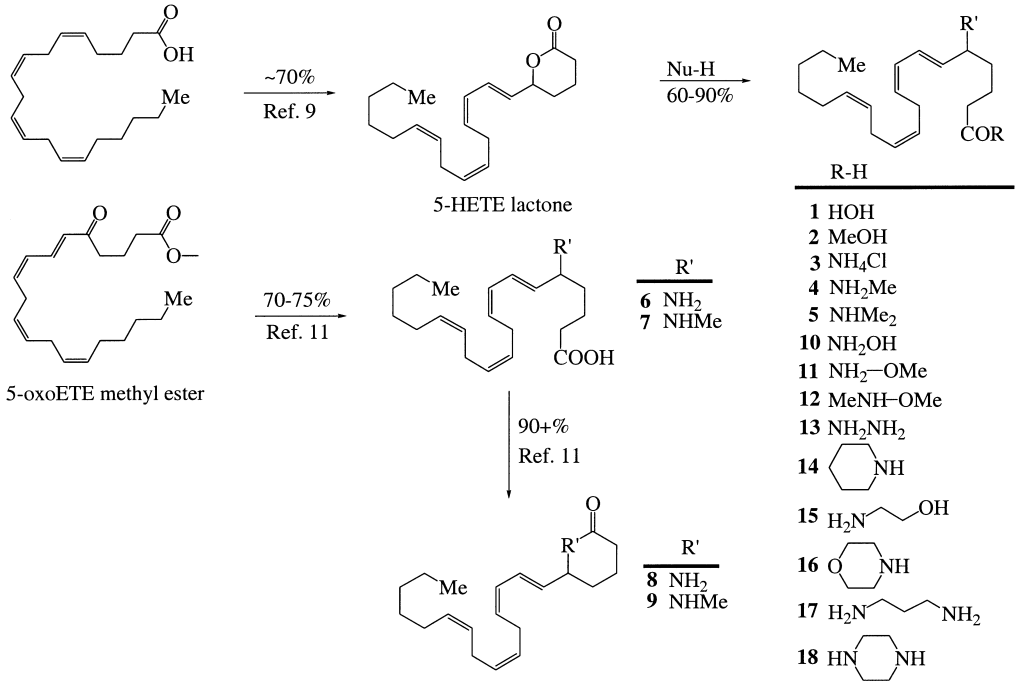


**Figure 2.** First generation eicosanoids.

**Table 1.** Growth enhancement data[a]

| Compound | Growth enhancement[b] | R[c] | R′[d] | Compound | Growth enhancement | R[c] | R′[d] |
|---|---|---|---|---|---|---|---|
| **1** | 116 | OH | OH | **11** | 133 | N(OMe)H | OH |
| **2** | 111 | OMe | OH | **12** | 115 | N(OMe)Me | OH |
| **3** | 108 | $NH_2$ | OH | **13** | 60 | NH-$NH_2$ | OH |
| **4** | 108 | NHMe | OH | **14** | 123 | Piperdine | OH |
| **5** | 138 | $NMe_2$ | OH | **15** | 125 | Ethanolamine | OH |
| **6** | 116 | OH | $NH_2$ | **16** | 106 | Morpholine | OH |
| **7** | 125 | OH | NHMe | **17** | 116 | 1,3-Diaminopropane | OH |
| **8** | 121 | NH lactam | | **18** | 113 | Pyrazine | OH |
| **9** | 131 | NMe lactam | | Anandamide[e] | 112 | Ethanolamine | — |
| **10** | 3 | NH-OH | OH | 18:1 Ethanolamide[f] | No effect | Ethanolamine | — |

[a]Assay conditions were as decribed in ref 4.
[b]Growth enhancement in LNCAP cell lines as a percent of control.
[c]R = Substituent at acyl position on the eicosanoid chain.
[d]R′ = substituent at 5-position on the eicosanoid chain.
[e]Archidonate fatty acid chain.
[f]18:1 fatty acid chain.

target compounds are summarized in Table 1. Like the endogenous 5-HETE family constituents, many of the agents in this study were capable of rescuing 5-HETE family depleted, serum starved cell cultures and thereby serve as 5-HETE family surrogates. Of the congeners tested, some displayed significant mitogenic activity relative to that of racemic constituents of the 5-HETE family of eicosanoids. Additionally, given the chirality requirements for ligands that bind at the 5-HETE receptor, only one enantiomer is expected to bind effectively.[7] Therefore, as is the case for 5-HETE family constituents, it is likely that these agents will elicit greater activity in their optically pure forms. Our continuing studies will address this issue experimentally. In general, the activities of the majority of the synthetic derivatives were comparable to the activities of the racemic 5-HETEs.

Growth enhancement data for the modified 5-HETE derivatives in this study ranged from 3–138% relative to control experiments. Racemic 5-HETE **1** possesses growth activity of 116% over control under the assay conditions. The most active derivatives included in this study were disubstituted amides **5**, **9** and **14**, which possess 138, 131, and 123% growth enhancement activity relative to the control experiments. The most potent of the monosubstituted amides were 5-aminomethyl-ETE **7**, 5-HETE methoxylamide **11**, and 5-HETE ethanolamide **15**, which possess growth enhancement activities at 125, 133, and 125% of control, respectively. In general, the 5-amino-substituted derivatives were more active than the corresponding 5-hydroxy congeners. Also, the lactams were more active than the corresponding 5-hydroxy substituted amides. Activity is increased with increased substitution for both the lactams and the acyclic amides.

With regard to deconvoluting the issues surrounding the interconversion of the constituents of the endogenous 5-HETEs, the activity of amides and the lactams suggest that 5-HETE and 5-HETE lactone are both active at the receptor. The issue of the ability of 5-oxoETE to activate the receptor, however, has not been unambiguously resolved by this study.

Other agents that contain the arachidonate sidechain also induce mitogenesis and may act at the 5-HETE receptor(s). For example, anandamide also induced mitogenic responses in neoplastic prostate cell lines (Table 1). This suggests that other dietary fats with polyunsaturation can also play a role in the induction of mitogenesis and other arachidonate derivatives will be examined in this regard. This may also prove to be mediated by 5-LO metabolism as suggested by the activity of derivative **15**. The use of 5-LO inhibitors can suppress the activity of derivatives possessing the arachidonate chain, presumably by inhibiting metabolism of the unsaturated chain.[4] Other unsaturated fatty acids, such as 18:1 (oleic acid), 18:1 ethanolamide, the cyclooxygenase families and the leukotrienes did not induce mitogenesis.[4] Activity, therefore, appears to be highly sensitive to changes in the fatty acid chain, while it is modulated more generally by changes to the 5-oxo position and acyl functionalities.

## Conclusion

We have disclosed the synthesis and evaluation of a panel of 5-HETE congeners for growth enhancement activity. Our work has provided insight into the mitogenic activity of 5-HETE derivatives, which may be a consequence of agonism at the 5-HETE receptor. We have demonstrated that *N*-substituted amides possess greater mitogenic activity than racemic 5-HETE and are capable of acting as 5-HETE family constituent surrogates. This work has also revealed that the polyunsaturated fatty acid anandamide possesses similar activity, while monounsaturated oleic acid (18:1) does not enhance growth in cultured prostate cells. Thus, these studies have provided insight into the relationship between structure and mitogenic activity for potential ligands of the 5-HETE receptor(s) and provided a basis on which to explore other polyunsaturated fatty acids with regard to the induction of mitogenesis in prostate cancer. These studies lend support to exploring the use of 5-LO inhibitors and 5-HETE family receptor antagonists to inhibit fatty acid associated mitogenesis in prostate cancer.

## References and Notes

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