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Inhibition of IGF-1R and lipoxygenase by nordihydroguaiaretic acid (NDGA) analogs

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Abstract—Herein, we pursue the hypothesis that the structure of nordihydroguaiaretic acid (NDGA) can be refined for selective potency against the insulin-like growth factor 1 receptor (IGF-1R) as a potential therapeutic target for breast cancer while diminishing its action against other cellular targets. Thus, a set of NDGA analogs (7a–7h) was prepared and examined for inhibitory potency against IGF-1R kinase and an alternative target, 15-lipoxygenase (15 LOX). The anti-cancer effects of these compounds were determined by their ability to inhibit IGF-1 mediated cell growth of MCF-7 breast cancer cells. The design of the analogs was based upon a cursory Topliss approach in which one of NDGA's aromatic rings was modified with various substituents. Structural modification of one of the two catechol rings of NDGA was found to have little effect upon the inhibitory potency against both kinase activity of the IGF-1R and IGF-1 mediated cell growth of MCF-7 cells. 15-LOX was found to be most sensitive to structural modifications of NDGA. From the limited series of NDGA analogs examined, the compound that exhibited the greatest selectivity for IGF-1 mediated growth compared to 15-LOX inhibition was a cyclic analog 7h with a framework similar to a natural product isolated from *Larrea divaricata*. The results for 7h are significant because while NDGA displays biological promiscuity, 7h exhibits greater specificity toward the breast cancer target IGF-1R with that added benefit of possessing a 10-fold weaker potency against 15-LOX, an enzyme which has a purported tumor suppressing role in breast cancer. With increased specificity and potency, 7h may serve as a new lead in developing novel therapeutic agents for breast cancer.

Nordihydroguaiaretic acid (NDGA, Fig. 1) is isolated from the resinous extract of the creosote bush *Larrea divaricata*.¹ It has also been reported that NDGA inhibits the growth of tumors, both in vitro and in vivo² but relatively high concentrations are required to achieve efficacy in most cases. Preliminary in vivo studies have revealed that NDGA inhibits tumor growth by inhibiting receptor tyrosine kinase (RTK) phosphorylation.³ NDGA has been shown to inhibit several RTKs overexpressed in certain cancer cells. In a previous study, we noted that NDGA inhibits both the IGF-1R and HER2/neu with moderate potency and impairs subse-



Figure 1. Structure of nordihydroguaiaretic acid (NDGA).

quent signaling through the anti-apoptotic pathway in breast cancer cell.^{3,4} These and other RTKs such as the epidermal growth factor receptor (EGFR) are over-expressed in breast and other cancers and contribute to a poor prognosis.^{5–7}

However, NDGA is known to directly interact with multiple non-RTK targets, several of which could contribute to its anti-cancer effects. NDGA directly inhibits the interaction of fatty acid synthase (FAS) with the substrate malonyl CoA.⁸ NDGA can also directly inhi-

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bit 5- α reductase,⁹ thereby blocking the conversion of testosterone into the biologically active dihydrotestosterone. Inhibitors of both FAS and 5-a reductase can inhibit growth or induce apoptosis in certain cancer cell lines.^{9,10} NDGA's reported antioxidant properties are attributed to its activity as a non-selective inhibitor of lipoxygenases (LOX).¹¹ The role of LOX isozymes in cancer has been questionable, as the anti-cancer effects of NDGA were originally attributed to reduction of cellular levels of leukotrienes generated from arachidonic acid by 5- and 12-LOX,¹²⁻¹⁴ but recent studies suggest that in breast cancer, 15-LOX has a tumor suppressing role.¹⁵. The multiplicity of NDGA's effects may, in some cases, enhance its anti-tumor actions, but may also antagonize its therapeutic effects or increase the undesirable side effects in non-tumor sites. Therefore, in designing potential therapeutics for breast cancer, compounds capable of inhibiting RTKs such as IGF-1R and HER2/ neu but lacking potency against 15-LOX or other targets could prove promising. The development of NDGA analogs with more specific potencies for various cellular targets is expected to result in an enhancement of its anti-cancer properties.

The focus of the present study was to demonstrate that the structure of NDGA could be refined for selective potency against the IGF-1R as a therapeutic target for breast cancer¹⁶ while diminishing its action against 15-LOX. Primarily based upon a cursory Topliss approach,^{17,18} our objective was to prepare a small set of NDGA analogs in which the substituents on one of NDGA's aromatic rings were adjusted to explore a range of hydrophobicity, electronics, and steric properties. We also pursued two simplified analogs of NDGA in which the methyl substituents on the bridging butyl chain and one of the hydroxyls of a catechol ring were absent.

The synthesis of NDGA analogs 7a-7g is outlined in Scheme 1. Carboxylic acids 1a and 1b were initially reduced to the corresponding alcohols with LiAlH₄¹⁹ and subsequently oxidized to the intermediate aldehydes 2a and 2b with pyridinium chlorochromate (PCC).²⁰ Next, treatment with substituted-aryl Grignard reagents (3a-3f) yielded alcohols²¹ 4a-4g, which were oxidized with PCC²⁰ to yield ketones 5a-5g prior to their complete reduction.

As halogenated aryl rings can undergo dehalogenation under the simple conditions of catalytic hydrogenation to reduce benzylic ketones,²² the halogen-containing aryl ketones 5d and 5e were subjected to adapted Wolff-Kishner conditions employing hydrazine hydrate²³ to generate an initial hydrazone intermediate. Hydrazones were subsequently reduced with KOH^{23,24} to vield alkanes 6d and 6e. The remaining non-halogen substituted aryl ketones (5a-5c, 5f, and 5g) were reduced by simple catalytic hydrogenation. Next, precursors 6a-6e were demethylated with BBr₃²⁵ to yield the NDGA analogs 7a-7e. 3,4-Methylenedioxy-protected catechol **6f** was selectively deprotected using PCl_5^{26} to yield **7f**. Similar conditions were applied to 6g, followed by subsequent demethylation with BBr₃ to generate 7g, the desmethyl analog of NDGA. Both 7a and 7g have been reported previously.27,28

A cyclic NDGA analog **7h** was prepared as outlined in Scheme 2. Ketone **5a** was reduced with Et_3SiH^{29} and TFA, and was expected to proceed through two steps of carbocation formation followed by reduction through



Scheme 1. Reagents and conditions: (a) LiAlH₄, CH₂Cl₂, rt, 3.5 h; (b) PCC, CH₂Cl₂, rt, 1 h; (c) Et₂O or THF, -78 °C, then rt, 30 min; (d) PCC, CH₂Cl₂, rt, 1 h; (e) H₂, 10% Pd/C, THF, rt, 1.5 h; (f) H₂NNH₂.H₂O and KOH, 165 °C, 4 h; (g) BBr₃, CH₂Cl₂, -78 °C, then rt, 30 min; (h) PCl₅, PhMe, 160 °C, then H₂O.



Scheme 2. Reagents and conditions: (a) TFA, Et₃SiH, 0 °C then rt, 2.5 h; (b) BBr₃, CH₂Cl₂, -78 °C, then rt, 30 min.

hydrogen transfer by the silane. Applied to this system, these conditions generated product **9** which may have occurred through cyclization of the benzylic carbocation intermediate **8**. Subsequent demethylation provided cyclic NDGA analog **7h**. Incidentally, this cyclic framework has also been isolated as a plant-derived natural product in *Larrea* spp.³⁰

Compounds **7a**–**7h** were screened along with NDGA in three separate assays for activity. The first assay tested direct inhibition of IGF-1R phosphorylation of a synthetic, non-specific, protein tyrosine kinase substrate. The second examined the inhibitory effect of the NDGA analogs on IGF-1 mediated cell growth of MCF-7 breast cancer cells. The third assay screened the analogs for the inhibition of 15-LOX, a non-target enzyme and one for which NDGA is a known inhibitor.³¹ The results from the three assays for NDGA and its synthetic analogs are summarized in Table 1.

In general, the analogs did not possess inhibitory potency against IGF-1R kinase activity distinct from

NDGA. These results suggest that with respect to the structure of NDGA, a single catechol ring is sufficient to affect inhibition of IGF-1R. The inhibition of IGF-1 mediated growth of MCF-7 cells by NDGA and the analogs presented an approximate 3-fold range in potency. The slight variation of potency compared to the results of IGF-1R inhibition may be due to variances in passive membrane permeability, metabolism, or interactions with other cellular targets. In contrast, NDGA and its analogs displayed larger differences in their inhibitory potency against 15-LOX. The desmethyl analog (7g) exhibited similar anti-lipoxygenase activity as NDGA and both these compounds were the most potent in the series that were examined. Given the symmetry of NDGA and 7g, the greater accessibility and probability of catechol rings binding in the enzyme may be responsible for their enhanced potency. Analogs possessing a single catechol ring and bearing sterically demanding substituents on the remote non-catechol ring exhibited weaker anti-lipoxygenase activity. The most dramatic loss of activity was observed for the cyclic analog 7h, especially when directly compared to its linear





Inhibitor	IGF-1R inhibition IC_{50} (μM)	Inhibition of IGF-1 mediated growth IC ₅₀ (μM)	15-Lipoxygenase inhibition IC_{50} (μM)
NDGA	0.9 (0.3)	24.6 (10.7)	3.8 (0.8)
7a	0.8 (0.1)	11. 2 (1.8)	7.3 (0.9)
7b	0.8 (0.1)	15.1 (4.1)	21.6 (0.5)
7c	1.0 (0.1)	36.2 (6.4)	9.4 (0.3)
7d	0.8 (0.4)	13.4 (3.3)	15.0 (5.5)
7e	1.0 (0.2)	21.5 (3.2)	33.6 (1.6)
7f	1.1 (0.1)	15.7 (4.0)	15.0 (1.4)
7g	0.6 (0.1)	25.4 (6.6)	3.9 (1.1)
7h	1.0 (0.4)	12.3 (2.4)	38.6 (1.7)

 a IC₅₀ values represent the means of a minimum of three determinations with standard deviation in parentheses.

congener 7a. These results suggest that steric congestion near the catechol ring diminishes15-lipoxygenase activity but has no effect upon the inhibition of IGF-1R and IGF-1R mediated cell growth of MCF-7 cells.

Significant structural modifications to one of the two catechol rings of NDGA had little effect upon the inhibitory potency against the kinase activity of the IGF-1R and only moderate differences in potency were observed when assayed for inhibition of IGF-1 mediated cell growth of MCF-7 cells. Of the three assays conducted, the inhibitory activity against 15-LOX was the most sensitive to structural modifications of NDGA. From the limited series of NDGA analogs examined, the compound that exhibited the greatest selectivity for IGF-1 mediated growth compared to 15-LOX inhibition was the cyclic analog **7h**. While the linear congener **7a** displayed similar potency as 7h against IGF-1 mediated growth of MCF-7 cells it retained similar potency as NDGA against 15-LOX. These findings are significant because while NDGA displays biological promiscuity, 7h exhibits greater specificity toward the breast cancer target IGF-1R with that added benefit of possessing a 10-fold weaker potency against 15-LOX, an enzyme which has a purported tumor suppressing role in breast cancer. With increased specificity and potency, 7h may serve as a new lead in developing novel therapeutic agents for breast cancer.

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Supplementary data

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