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Synthesis of new analogs of tetraiodothyroacetic acid (tetrac) as novel angiogenesis inhibitors for treatment of cancer

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ABSTRACT

In the angiogenesis process, integrins, which are members of a family of cell surface transmembrane receptors, play a critical role particularly in blood vessel formation and the local release of vascular growth factors. Thyroid hormones such as L-thyroxine (T_4) and 3,5,3′-triiodo-L-thyronine (T_3) promote angiogenesis and tumor cell proliferation via integrin αvβ3 receptor. At or near an arginine-glycine-aspartate (RGD) recognition site on the binding pocket of integrin $\alpha_v\beta_3$, tetraiodothyroacetic acid (tetrac, a deaminated derivative of T_4) is a thyrointegrin receptor antagonist and blocks the actions of T_3 and T_4 as well as different growth factors-mediated angiogenesis. In this study, we synthesized novel tetrac analogs by modifying the phenolic moiety of tetrac and tested them for their anti-angiogenesis activity using a Matrigel plug model for angiogenesis in mice. Pharmacological activity results showed that tetrac can accommodate numerous modifications and maintain its anti-angiogenesis activity.

Tumor angiogenesis is the formation of new blood vessel growth from the existing vasculature by cell adhesion to the extra cellular matrix (ECM), which results in tumor progression.[1,2] Rapid tumor cell proliferation produces environmental stresses such as a hypoxic, glucose-deprived environment that begins the angiogenic switch whereby tumor cells produce angiogenic activators including angiogenin, transforming growth factor (TGF)-α, TGF-β, tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor, basic fibroblast growth factor (bFGF, FGF2), platelet-derived endothelial growth factor, and hepatocyte growth factor.[3]

Integrins are important transmembrane receptors that play a critical role in the angiogenesis process, particularly in blood formation and local release of vascular growth factors. They are members of a family of cell surface receptors that are immunoglobulin superfamily molecules or ECM proteins,[4] and consist of α and β chain units. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are the main receptor types involved in the angiogenesis process, especially in binding with angiogenesis modulators containing an arginine-glycine-aspartate (RGD) recognition site that binds to the integrin receptor.[5] There are several natural RGD-containing proteins such as fibronectin, fibrinogen, λ-receptor on E. coli, sindbis coat protein, and α-lytic protease protein[6] as well as cyclic RGD tripeptide (c-RGD) and c-RGD peptidomimetics[7] that show high binding affinity to the integrin recognition site.

Many studies have reported the effects of thyroid hormone analogs like L-thyroxine (T_4), which is a prohormone antecedent to 3,5,3′-triiodo-L-thyronine (T_3), on thyroid hormone receptor (TR) via binding to integrin $\alpha_v\beta_3$ and the effects of the angiogenesis activity of T_4 and T_3 with an interaction site that is located at or near the RGD recognition site of integrins (Fig. 1a). Tetraiodothyroacetic acid (tetrac), a deaminated derivative of T_4, inhibited the pro-angiogenesis response of thyroid hormone by inhibiting the cell surface-initiated actions of T_4 and T_3 in the chick chorioallantoic membrane (CAM) and many other angiogenesis models.[8,9] Anti-proliferative activity of tetrac against other cancer cell lines such as human non-small cell lung cancer[10] have been investigated both *in vitro* and in xenografts and results showed anti-angiogenic activity at the integrin $\alpha_v\beta_3$ receptor-binding site. For example, Yoshida et al. found tetrac to be an effective inhibitor of retinal angiogenesis and of the pro-angiogenic effect of both erythropoietin (EPO) and VEGF on retinal endothelial cells; this suggested that tetrac (and antagonism of integrin $\alpha_v\beta_3$) is a viable therapeutic strategy for proliferative diabetic retinopathy.[11]

In order to limit tetrac to the cell surface thyroid hormone receptor and to provide optimized exposure of the biphenyl structure and acetic acid side chain of its inner ring to the receptor site on αvβ3, our group has conjugated tetrac to the polymeric

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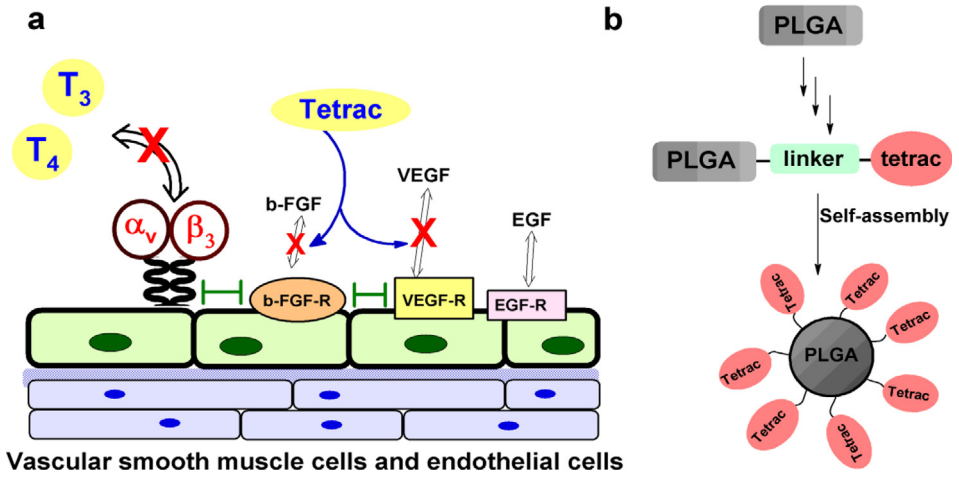
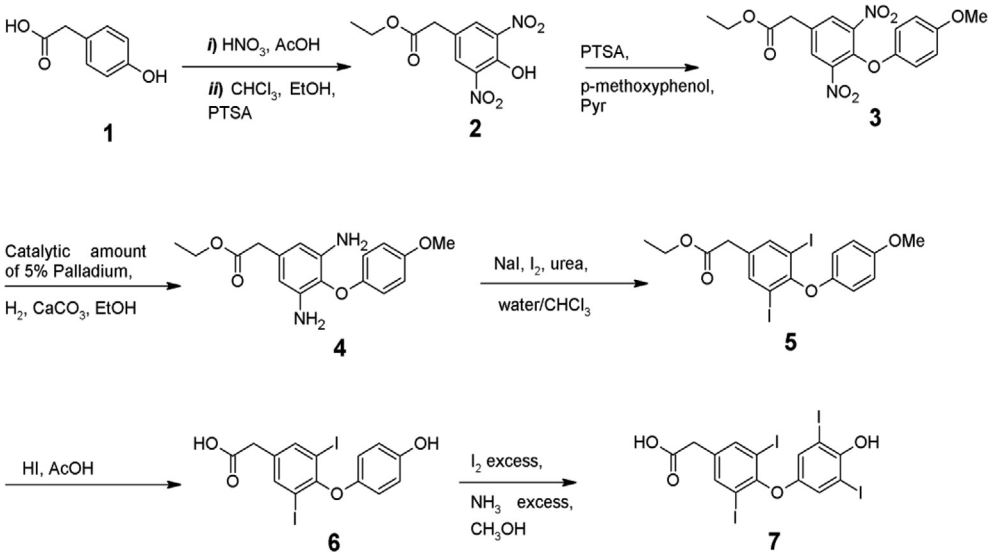


Fig. 1. (a) Pro-angiogenic activity of thyroid hormones T_4 and T_3 on vascular smooth and endothelial cells is initiated at the cell surface receptor (integrin) for the hormone on the extracellular domain of integrin $\alpha_v\beta_3$. Tetrac, a thyroid hormone analog, is inhibitory at the $\alpha_v\beta_3$ integrin receptor and is anti-angiogenic. (b) Conjugation of tetrac to PLGA nanoparticle results in tetrac-nanoparticle that has shown anti-angiogenic activity.



Scheme 1. Synthetic route for the preparation of tetrac, **7**.

nanoparticle (NP) poly (lactic-*co*-glycolic acid) (PLGA) via covalent binding (an amide bond formation) between tetrac's outer ring hydroxyl to NHS-modified PLGA, resulting in tetrac NP (Nano-diamino-tetrac; NDAT; Nanotetrac). This NP showed anti-angiogenic activity, confirming the role of the integrin receptor in the results explained above (Fig. 1b).[8] Results from both *in vitro* and *in vivo* experiments for the treatment of drug-resistant breast cancer showed that NDAT is not able to enter into the cell nucleus but will enhance inhibition of tumor proliferation at a low-dose equivalent of free tetrac.[12] It has been concluded that NDAT has high potential as an anticancer agent,[13–15] with possible applications in the treatment of drug-resistant cancer. NDAT also inhibits the PI3-K and MAPK pathways[16,17] and blocks the expression of a panel of genes critical to cancer cell survival pathways and the epidermal growth factor receptor (*EGF-R*) gene.[18,19] We also designed and synthesized a novel derivative of tetrac that showed pro-angiogenic activity rather than anti-angiogenic activity by mimicking the action of the iodothyronine deiodinases (the enzymes that convert T_4 to T_3). In this regard, the phenolic OH group of tetrac was initially deprotonated, and subsequent anion extraction of a proton formed a tautomeric dienone. Finally, nucleophilic attack on the iodine atom resulted in a deiodinated product of tetrac, designated MR-49.[20]

Knowing that the angiogenesis activity of tetrac is stimulated by FGF or VEGF without influencing the pre-existing blood vessels, we synthesized new tetrac analogs by modifying the phenolic moiety of tetrac and studied the structure-activity relationship. The phenolic hydroxyl group (–OH) of thyroid analogs is an important site for their modification and is a target site for converting tetrac to an integrin antagonist without any changes to the carboxylic acid moiety of tetrac. We previously synthesized diamino-tetrac, which showed anticancer/anti-angiogenic activity and was able to target the thyroid hormone-tetrac receptor on the extracellular domain of integrin $\alpha_v\beta_3$. Wu et al.[21] showed that conjugation of the phenolic hydroxy group (4′-OH) of T_4 with sulfate or glucuronic acid yielded the corresponding sulfated (T_4S) and glucuronidated (T_4G) hormone[22]; sulfate and glucuronic acid conjugations are useful to enhance the water solubility of many hydrophobic drugs and to enhance their excretion through urine and/or bile.[23] Similar to T_4S and T_4G, tetrac also undergoes sulfonation and glucuronidation, thus suggesting that the body can metabolize tetrac.[22]