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Original article

Effect of conjugates of *all-trans*-retinoic acid and shorter polyene chain analogues with amino acids on prostate cancer cell growth

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ABSTRACT

In the present work, a series of conjugates of amino acids with *all-trans*-retinoic acid (ATRA) and shorter polyene chain analogues were rationally designed, synthesized by coupling the succinimidyl active esters of the acidic retinoids with appropriately protected amino acids or peptides followed by deprotection, and examined for their possible effect on viability of human prostate cancer LNCaP cells. In contrast to ATRA, all conjugates bearing amino acids with polar side chains showed no inhibitory effect on LNCaP cell proliferation, while conjugates with α-amino acids with lipophilic side chain, such as **7**, or linear amino acids, such as **9**, significantly decreased prostate cancer LNCaP cell number. Interestingly, while the effect of ATRA was RARα-dependent, the effect of its active analogues was not inhibited by a selective RARα antagonist. Cell cycle analysis showed no effect on cell cycle, while quantitative analysis by annexin V-propidium iodide staining revealed that neither ATRA nor its analogues affected LNCaP cell apoptosis or necrosis. These results demonstrate that compounds **7** and **9** are potentially useful agents that warrant further preclinical development for treatment of prostate cancer.

1. Introduction

Retinoids are lipophilic molecules composed of three distinct structural domains: a β-ionone ring, an isoprenoid tail and a polar end group. These small molecules exhibit multiple and diverse biological activities through their nuclear receptors, Retinoic Acid Receptors (RARs) and Retinoid X Receptors (RXRs) [1]. RARs and RXRs are encoded from different genes, have many isoforms and control the expression of a large number of genes [2]. Moreover, retinoids inhibit the expression of several genes through inhibition of transcription factor activating protein-1, which seems to be responsible for the anti-tumor effects of retinoids [3]. The induction of differentiation [1,4,5], apoptosis [5–7] and cell cycle arrest [5,7] by retinoids have made them important pharmaceutical candidates against dermal dysfunctions and certain cancer types, such as chronic leukaemia, where the use of *all-trans*-retinoic acid (ATRA) leads to 90% complete remission rate [8]. Due to problems, however, such as development of resistance [9] or toxicity [10], analogues of ATRA are widely being tested as alternatives.

Shealy et al. have reported the synthesis of a series of *N*-(*all-trans*-retinoyl)amino acids, as analogues of ATRA (**1**) with extended polyene chain and increased water solubility, and tested their capacity to reverse keratinisation in vitamin A deficient hamster trachea in culture. The amino acids used were Gly, Ala, Leu, Phe, Tyr, and Glu. All of the thus examined conjugates were less active than ATRA [11]. In that bioassay, most active amino acid was Leu, incorporating a lipophilic side chain, and least active Glu, with a polar side chain. Even between Phe and Tyr most active was Phe,

Abbreviations: Asp, aspartic acid; ATRA, *all-trans*-retinoic acid; DCC, *N,N′*-dicyclohexylcarbodiimide; DCU, *N,N′*-dicyclohexylurea; HOBt, *N*-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; βAla, β-alanine; εAca, ε-aminocaproic acid; FCC, flash column chromatography; DEAD, diethyl azodicarboxylate; Lys, lysine; RT, room temperature; Boc, *tert*-butoxycarbonyl; DIEA, ethyldiisopropylamine; Tfa, trifluoroacetyl; DMAP, 4-dimethylaminopyridine; DCM, dichloromethane; TFA, trifluoroacetic acid; Trt, triphenylmethyl (trityl); RAR, Retinoic Acid Receptor; RXR, Retinoid X Receptor.

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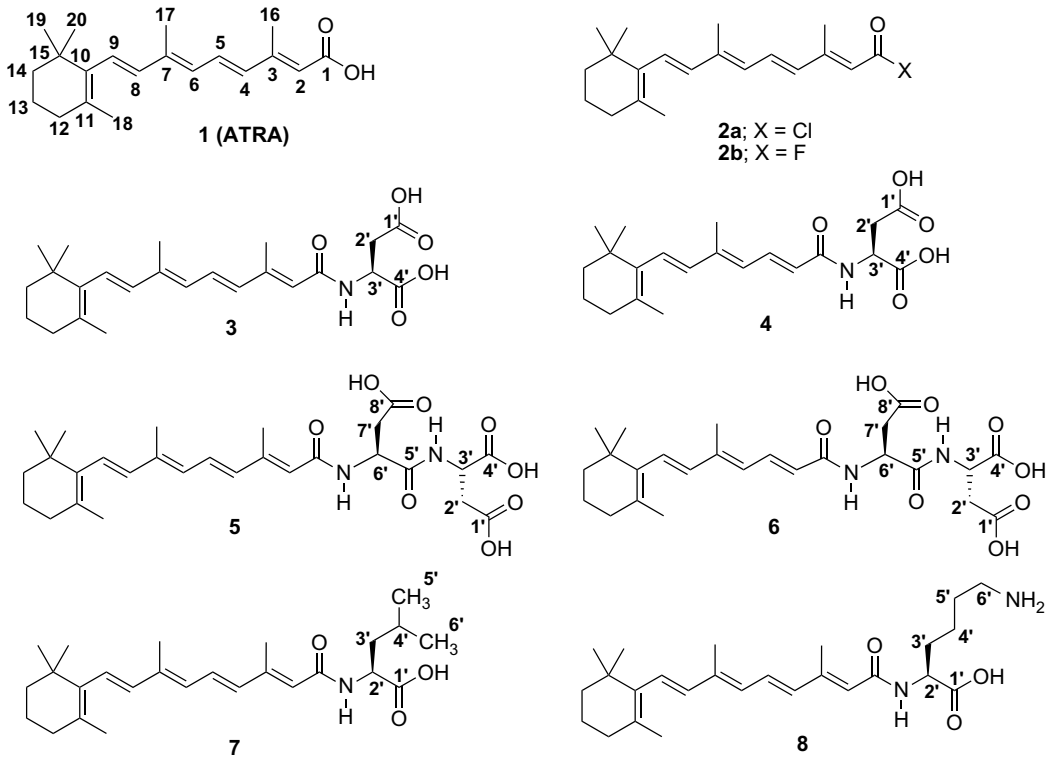

Fig. 1. Conjugates of ATRA and of its lower homologue **20** with acidic, neutral and basic α-amino acids and the dipeptide Asp–Asp.

lacking the polar hydroxyl group from position 4 of the aromatic ring of Tyr. *N*-(*all-trans*-retinoyl)-leucine and *N*-(*all-trans*-retinoyl)-glycine also inhibited the proliferation of two kinds of neoplastic cells in culture. The afore mentioned conjugates were all obtained through coupling of the *in situ* generated *all-trans*-retinoyl chloride (**2a**, Fig. 1) with methyl or ethyl esters of α-amino acids, followed by saponification [11]. In an alternative methodology, conjugates of ATRA with amino acids, such as Gly, Phe and Lys were obtained, however in low yields, by direct coupling of the isolable *all-trans*-retinoyl fluoride (**2b**) with free amino acids in aqueous solutions [12].

Taking into consideration that ATRA affects in various ways the differentiation, apoptosis and inhibition of cell proliferation in physiological and cancerous epithelial cells [5,13–15] and that retinoids have been widely tested for their effects in prostate cancer cells [16–18], we thought of interest to synthesize a series of conjugates of amino acids with ATRA, suitable for structure–activity relationship studies on the proliferation or/and apoptosis of human prostate cancer LNCaP cells. We were interested to identify alternative, more effective, ways of synthesis, as well as the possible role of (a) the nature of α-amino acid side chain, (b) the length of the chain in linear amino acids (lack of side chain), (c) the

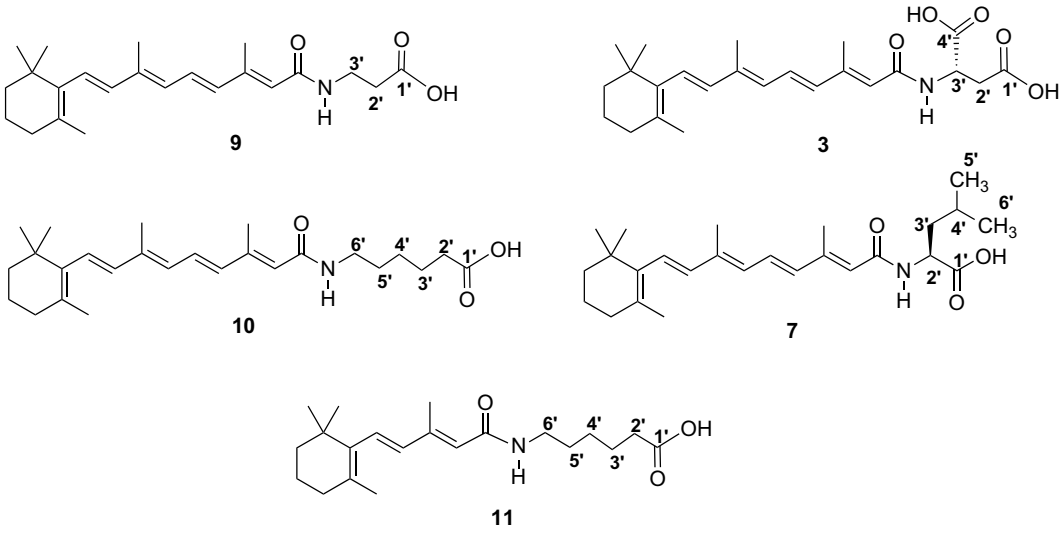

Fig. 2. Conjugates of ATRA and its lower homologue **14** with linear amino acids and their structural relationship to conjugates **3** and **7**.