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Synthesis of Conjugates of Amino-Combretastatin with Tuftsin Derivatives as Potential Anticancer Agents

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A novel of 3'-*N*-(tuftsin or retro-tuftsin)-amino-combretastatin conjugates, has been synthesized as potential anticancer compounds. We hope that the conjugation of immunomodulators like tuftsin derivatives with amino-combretastatin A-4 would improve the therapeutical properties of combretastatin A-4.

Key words: combretastatin A-4, CA-4, amino-combretastatin, retro-tuftsin, tuftsin, synthesis

Combretastatin A-4 (CA-4) {(*Z*)-1-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxy-phenyl)ethane} **1** (Figure 1) is a potent antineoplastic and antiangiogenesis natural substance isolated from the South African tree *Combretum caffrum* [1]. It exhibits potent cytotoxicity against a broad spectrum of human cancer lines, including those that are multidrug resistance (MDR) pump, a cellular pump which rapidly transports out xenobiotics [2-5]. However, the limited water solubility of this compound complicates the drug application. Transformation of phosphate ester into ammonium, potassium, and sodium salts increased solubility in water. Currently one of them, sodium phosphate salts (CA-4P) **2** (Figure 1) is under clinical evaluation [6-8]. The studies of structure-activity relationship (SAR) of CA-4 **1** showed that 3,4,5-trimethoxy substitution on the A-ring and the 4'-methoxy group on the B-ring and the *Z*-olefin configuration are crucial for potent cytotoxicity, while the 3'-hydroxy group is optional [4]. A variety of analogues of CA-4 has been synthesized where to replace the double

bond into CA-4 have been introduced nonheterocyclic groups (*e.g*. ethers, olefins, ketones, sulfonates, sulfonamides, amide derivatives, amine, cyclopentanes) or heterocyclic groups containing five-membered rings (*e.g.* pyrazoles, thiazoles, triazoles, tetrazoles, oxazoles, furans, dioxolanes, thiophenes) and indoles [9-18]. Ohsumi *et al.* [19] synthesized a very interesting group of combretastatins by replacing the phenolic OH of CA-4 with NH_2. The authors obtained AC-7739 **3**, which suppressed solid murine tumor growth and its serine analogue AC-7700 **4** (Figure 1) [19,20]. AC-7700 is a unique tubulin binding agent, which exerts antivascular activity at tolerable doses with potent antitumor activity on solid tumors (murine colon26 adenocarcinoma (c26)) [21]. In 2003 Morinaga *et al* [22] described combination effect of AC-7700 and cisplatin (CDDP) against murine and human tumors *in vivo*. The obtained results suggest that AC-7700 may specifically augment the accumulation of CDDP in tumors, and thus has the potential to be useful in combination chemotherapy with CDDP.

Continuing our search for potential anticancer drug candidates, we synthesized a novel conjugates of amino-combretastatin containing chemically bonded immunomodulators such as tuftsin and retro-tuftsin derivatives as potential anticancer agents. Tuftsin, a natural tetrapeptide of sequence H-Thr-Lys-Pro-Arg-OH, occuring in the blood of humans and other mammals, capable of stimulating certain white blood cells (monocytes, macrophages, and neutrophils), was isolated at Tufts University in 1970 by Najjar and Nishioka [23-28]. Results of biological tests of these compounds will be reported in the future.

RESULTS AND DISCUSSION

The results of chemical experiments are shown in Scheme 1. The amino-combretastatin **9** was synthesized according to the method proposed by Ohsumi *et al.* [19]. The Wittig reaction of 3,4,5-trimethoxybenzylphosphonium bromide **6** with 3-nitro-4-methoxy-benzaldehyde **7** in

the presence of sodium hydride in toluene gave a mixture of (*Z*)- and (*E*)-stilbene derivatives **8a** and **8b**, respectively. The crude product, **8a** was reacted with Zn in acetic acid to give amino-compound **9** [19]. The tuftsin derivatives: Boc-Thr-Lys(Boc)-Pro-Arg(NO_2)-OCH_3, Boc-Arg(NO_2)-Pro-Lys(Boc)-Thr-OCH_3, Boc-Thr-Lys(BocAla)-Pro-Arg(NO_2)-OCH_3, Boc-Thr-Lys(BocVal)-Pro-Arg(NO_2)-OCH_3 and Boc-Arg(NO_2)-Pro-Lys(BocAla)-Thr-OCH_3 were obtained according to standard procedures used in peptide chemistry. For the synthesis of these peptides the mixed anhydride method with isobutyl chloroformate and *N*-methyl-morpholine (NMM) in anhydrous DMF was chosen [27,28]. Treatment of these peptides in alkaline media with an equimolar amount of 1N KOH/MeOH gave compounds **10a-e** with a free carboxyl group. For the synthesis of conjugates **11a-e** the DPPA method was chosen. The protected conjugates **11a-e** were purified with preparative TLC and their identities were confirmed by mass spectra (MS) (Table 1). Removal of protecting groups with HCl/dioxane afforded hydrochlorides **12a-e**.

EXPERIMENTAL

Melting points (uncorrected) were determined on the Kofler-block apparatus. ^{1}H-NMR spectra were measured in DMSO or $CDCl_3$ solutions with a Varian 500 and 200 NMR spectrometers. Preparative column chromatography was performed on silica gel (Kieselgel 60, 100-200 mesh) in solvent systems specified in the text. All chemicals and solvents were of reagent grade and were used without further purification. The reactions were monitored by TLC on Merck F_{254} silica gel precoated plates. The following solvent systems (by vol.) were used for TLC: (A) EtOAc-hexane, (B) n-BuOH-AcOH-H_2O (4:2:2), (C) n-BuOH-AcOH-H_2O (2:1:1), (D) $CHCl_3$-MeOH-AcOH (90:10:5), (E) $CHCl_3$-MeOH (30:1), (F) $CHCl_3$-MeOH (4:1), (G) $CHCl_3$-MeOH (9:1). All synthesized protected peptides were homogeneous on TLC. Qualitative amino acid analyses of the hydrolyzates of the compounds were

accomplished on TLC in solvent B. The detection was by UV and ninhydrin. Mass spectra were recorded on Biflex III Bruker MALDI-TOF mass spectrometer.

The following abbreviations were applied: Boc – *tert*-butyloxycarbonyl-, DMF – dimethylformamide, DPPA – diphenyl azidophosphate, Lys – lysine, Pro – proline, Arg – arginine, Thr – threonine.

(*Z*)-2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-phenylamine (9). This compound was synthesized by method described [19]. Yield 38 %; oil; ^{1}H-NMR ($CDCl_3$): δ 3.68 (s, 6H), 3.80 (s, 3H), 3.81 (s, 3H), 6.34 (d, J = 12.0 Hz, 1H), 6.43 (d, J = 12.0 Hz, 1H), 6.56 (s, 2H), 6.65 (s, 2H), 6.67 (s, 1H).

General procedure for the preparation (12a-e). To a stirred solution of tuftsin derivatives **10a-e** (1 mmol) (Scheme 1) in anhydrous DMF (5 ml) cooled to 0 ºC, DPPA (1.1 mmol) and amino-combretastatin **9** (1.1 mmol) were added. The mixture was stirred at 0 ºC for 3 h and then for 24 h at room temperature. After evaporation of the solvent the reaction mixture was purified using preparative TLC in solvent A to obtain compounds **11a-e**. The conjugate (**11a-e**) was dissolved in MeOH and HCl-dioxane was added to this solution. After 40 min, the mixture was evaporated to dryness. Purification by preparative TLC ($MeOH/CH_2Cl_2$) gave the pure product **12a-e**.

3'-*N*-(Arg(NO_2)-Pro-Lys-Thr)-amino-CA-4x2HCl (12a). Anal. Calcd. for ($C_{39}H_{60}Cl_2N_{10}O_{11}$): C, 51.15; H, 6.60; N, 15.29. Found: C, 51.29; H, 6.89; N, 15.09.

3'-*N*-(Thr-Lys-Pro-Arg(NO_2))-amino-CA-4x2HCl (12b). Anal. Calcd. for ($C_{39}H_{60}Cl_2N_{10}O_{11}$): C, 51.15; H, 6.60; N, 15.29. Found: C, 51.33; H, 6.92; N, 15.43.

3'-*N*-(Arg(NO_2)-Pro-Lys(Ala)-Thr)-amino-CA-4x2HCl (12c). Anal. Calcd. for ($C_{42}H_{65}Cl_2N_{11}O_{12}$): C, 51.11; H, 6.64; N, 15.61. Found: C, 51.29; H, 6.87; N, 15.38.

3'-*N*-(Arg(NO_2)-Pro-Lys(Val)-Thr)-amino-CA-4x2HCl (12d). Anal. Calcd. for ($C_{44}H_{69}Cl_2N_{11}O_{12}$): C, 52.07; H, 6.85; N, 15.18. Found: C, 52.18; H, 7.12; N, 14.99.

3'-*N*-(Thr-Lys(Ala)-Pro-Arg(NO_2))-amino-CA-4x2HCl (12e). Anal. Calcd. for ($C_{42}H_{65}Cl_2N_{11}O_{12}$): C, 51.11; H, 6.64; N, 15.61. Found: C, 51.31; H, 6.88; N, 15.76.

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Table 1. Protected compounds **11a-e**

Comp.	R	Yield (%)	MS $[M+H]^+$ Calcd	Found
11a	Boc-Thr-Lys(Boc)-Pro-Arg(NO_2)-	37	1043.54	1043.6
11b	Boc-Arg(NO_2)-Pro-Lys(Boc)-Thr-	31	1043.54	1043.5
11c	Boc-Thr-Lys(BocAla)-Pro-Arg(NO_2)-	35	1114.58	1114.4
11d	Boc-Thr-Lys(BocVal)-Pro-Arg(NO_2)-	32	1142.61	1142.8
11e	Boc-Arg(NO_2)-Pro-Lys(BocAla)-Thr-	29	1114.58	1114.7

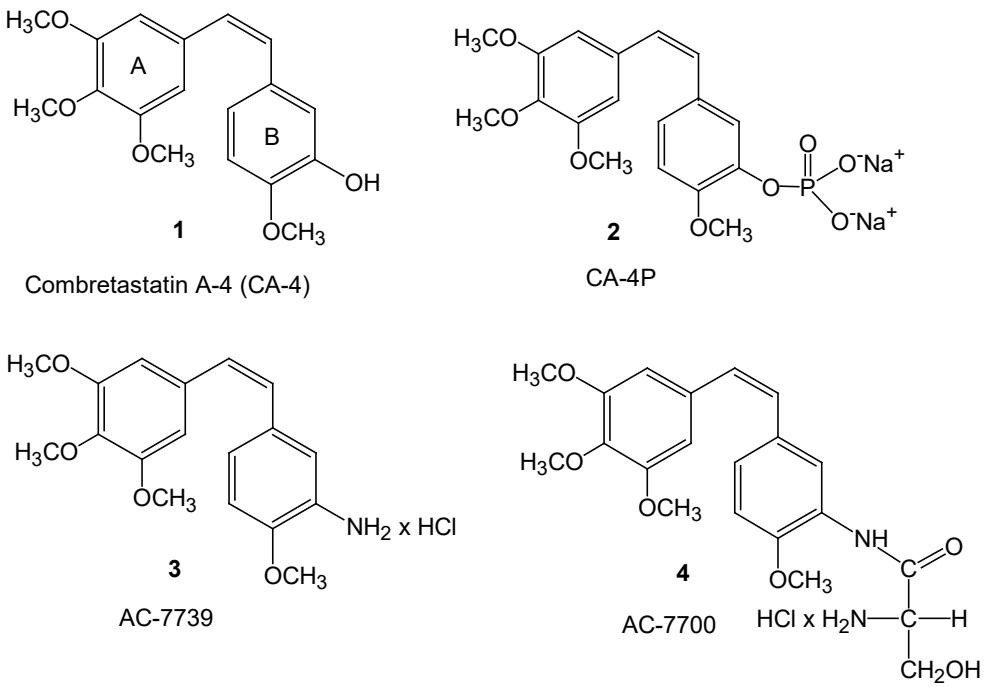
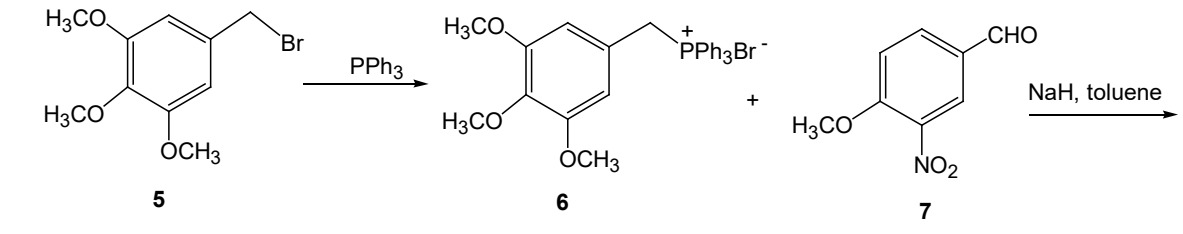
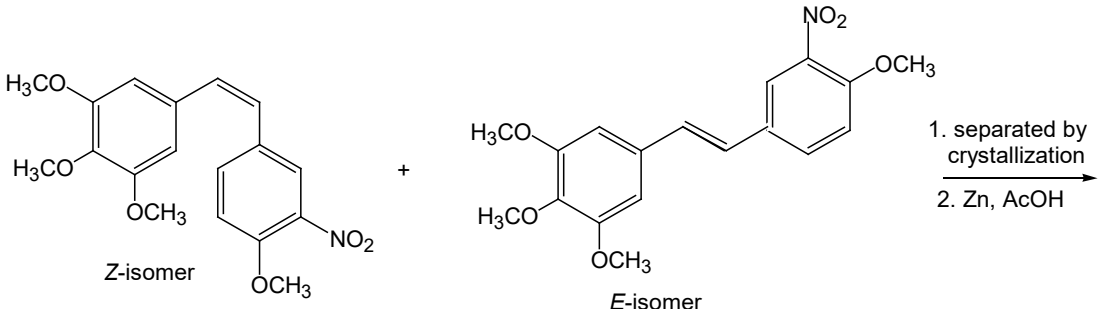


Figure 1

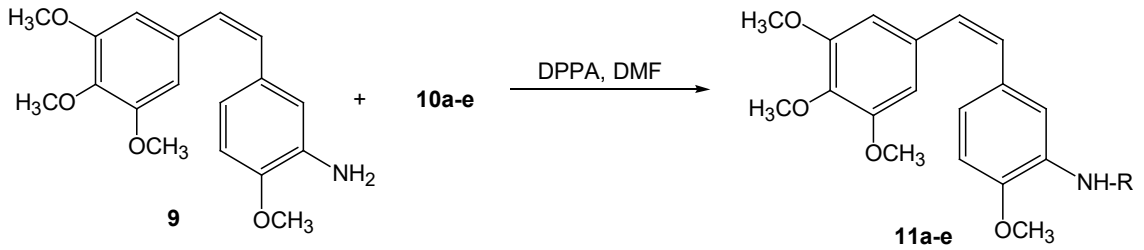
Scheme 1



5 **6** **7**

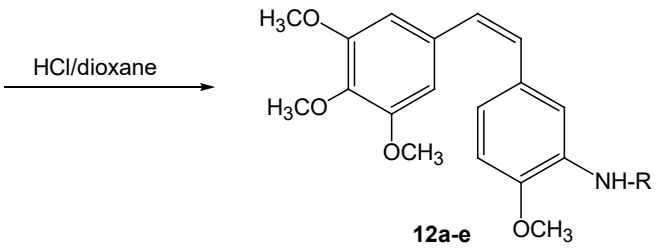


Z-isomer **8a** + *E*-isomer **8b**



9 Amino-Combretastatin A-4 **11a-e**

11a: R = Boc-Thr-Lys(Boc)-Pro-Arg(NO_2)-
11b: R = Boc-Arg(NO_2)-Pro-Lys(Boc)-Thr-
11c: R = Boc-Thr-Lys(BocAla)-Pro-Arg(NO_2)-
11d: R = Boc-Thr-Lys(BocVal)-Pro-Arg(NO_2)-
11e: R = Boc-Arg(NO_2)-Pro-Lys(BocAla)-Thr-



12a-e

12a: R = 2HClxThr-Lys-Pro-Arg(NO_2)-
12b: R = 2HClxArg(NO_2)-Pro-Lys-Thr-
12c: R = 2HClxThr-Lys(Ala)-Pro-Arg(NO_2)-
12d: R = 2HClxThr-Lys(Val)-Pro-Arg(NO_2)-
12e: R = 2HClxArg(NO_2)-Pro-Lys(Ala)-Thr-

10a: Boc-Thr-Lys(Boc)-Pro-Arg(NO_2)-OH
10b: Boc-Arg(NO_2)-Pro-Lys(Boc)-Thr-OH
10c: Boc-Thr-Lys(BocAla)-Pro-Arg(NO_2)-OH
10d: Boc-Thr-Lys(BocVal)-Pro-Arg(NO_2)-OH
10e: Boc-Arg(NO_2)-Pro-Lys(BocAla)-Thr-OH