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Original article

# Design and synthesis of biotinylated dimethylation of alkannin oxime derivatives

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ABSTRACT

DMAKO-05, a novel dimethylation of alkannin oxime derivative, exhibits remarkable anticancer activity as well as excellent cellular selectivity and thus has been considered as a promising antineoplastic agent for colorectal carcinoma and melanoma. However, its potent cytotoxicity is not closely associated with reactive oxygen species (ROS) and bioreductive alkylation. Its specific antitumor target(s) has still remained elusive. To recognize the molecular target(s) of DMAKO-05 and its analogs, four biotinylated DMAKO derivatives were designed and prepared. The biotin moiety was successfully introduced in the molecule through a modified Mitsunobu reaction, which kept its anticancer activity. Moreover, the cell-based investigation demonstrated that replacement of the linker $C_4$ chain with another alkyl chain ($C_6$ or $C_8$) gave rise to the enhancement of cytotoxicity. Among these biotinyl derivatives, both compound **16** and **8c** exhibited more potent anticancer activity than DMAKO-05 against MCF-7 cells and were comparatively effective to alkannin toward HCT-15 cells. As expected, they might be thought as ideal chemical probes. Collectively, our present work could provide an available approach for the identification of the potential antineoplastic target(s) of DMAKO derivatives.

## 1. Introduction

Alkannin and shikonin (A/S, Fig. 1), an enantiomeric pair of naturally occurring isohexenylnaphthazarins, were isolated and identified from the roots of *Alkanna tinctoria* in Europe and *Lithospermum erythrorhizonin* in the Orient, respectively [1]. A/S and their derivatives exhibited a broad spectrum of biological activities [1–4] including wound healing, anti-inflammatory and immunoregulatory effects, while their potent antitumor activity mediated by multiple mechanisms [5–15] has aroused growing interest recently. The naphthazarin skeleton was found to be the crucial pharmacophore of the A/S, capable of generating ROS and covalently binding to cellular nucleophiles [14,15], resulting in the apoptotic death of normal cells along with cancer cells.

Intriguingly, our previous investigations demonstrated that dimethylated A/S oxime (DMA/SKO) derivatives displayed more potent inhibitory effects than the parent compounds against cancer cell lines along with no apparent toxicity toward normal cells [16,17]. Our continuous research found that DMAKO-05, displayed more potent antitumor activity and less toxicity in comparison to 5-FU both *in vitro* and *in vivo*, which was not a naphthoquinone prodrug due to its metabolism in rat liver microsome through hydrolysis of its side chain and oxidation of its naphthoquinone scaffold, rather than oxidation of hydroxyimine to ketone [18]. More recently, we have further reported that DMAKO-05, a potential candidate compound for melanoma, displayed potent cytotoxicity toward B16F0 cells by inhibiting Akt activation, inducing G1 arrest and further promoting B16F0 cell apoptosis [19]. Moreover, another hallmark molecular DMAKO-20 showed more excellent antitumor activities in a mouse xenograft model (unpublished data). A noteworthy finding was that their potent anti-proliferation effects were not closely associated with ROS and bioreductive alkylation [15,17]. Meanwhile, the mode of action and mechanism of their antitumor activities remained unclear, which significantly hamper the further drug development of DMAKO derivatives.

Molecular probes, selectively bound biomolecular target(s), were widely utilized in molecular biology. In this article, more attention has been paid to the preparation of biotin-coupled activity based-probes (ABPs) of alkannin oxime derivatives incorporating a biotin moiety for avidin affinity "pull down" of

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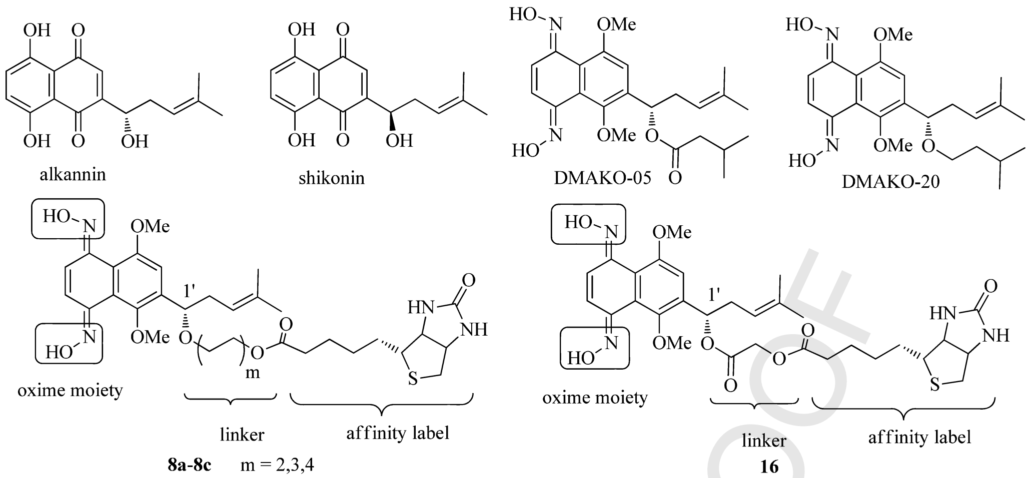


**Fig. 1.** The chemical structures of A/S and biotinylated DMAKO derivatives.

covalently tagged target protein(s) [20,21]. For effective labeling of target proteins, the anticancer activity of biotin probe should be retained. Meanwhile, the appropriate linker connecting the alkannin oxime and the affinity label should be taken into consideration in order to provide enough space for biotin to interact with avidin. Our preliminarily research concerning the structure-activity relationship of A/S oxime derivatives [17] revealed that the hydroxyl of oxime was a conserved functional group and thus was not suitable for incorporation of biotinylated linker. Nevertheless, modification of the 1′-OH on the side chain of A/S was conducive to enhancing anticancer activity [22–24]. Accordingly, the 1′-OH was a feasible position for introduction of biotin and linker. Based on these results, we designed and synthesized biotinylated DMAKO derivatives containing ether bond and ester group at 1′-position on the side chain (Fig. 1).

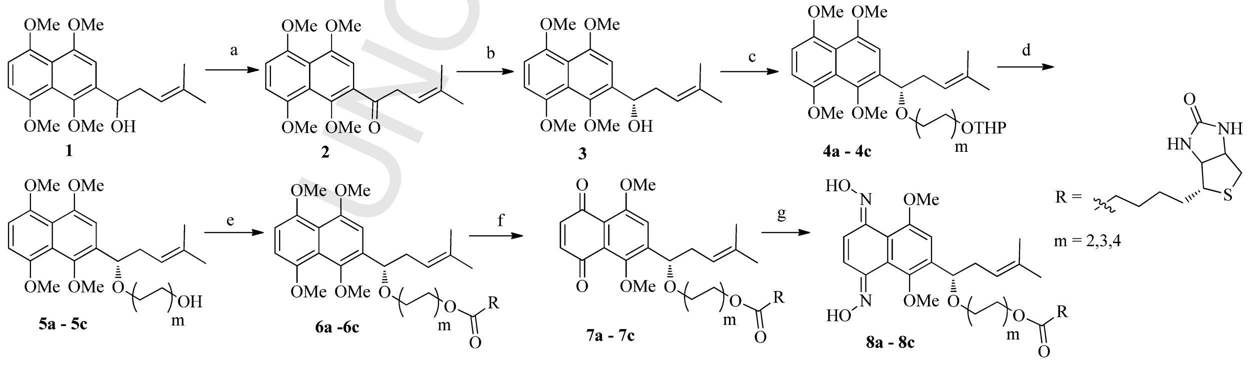
## 2. Results and discussion

### 2.1. Chemistry

The general synthesis strategy for chemical probes **8a**–**8c** is illustrated in Scheme 1. 4-Methyl-1-(1,4,5,8-tetramethoxynaphthalen-2-yl)pent-3-en-1-ol (**1**) was prepared with 1,4,5,8-tetramethoxy-2-naphthaldehyde as the starting material according to the procedures previously reported by our research group [22]. The ketone **2** was obtained by the oxidation of alcohol **1** with Dess-Martin periodinane (DMP) and was then reduced by (−)-DIP-Cl to give (*S*)-4-methyl-1-(1,4,5,8-tetramethoxynaphthalen-2-yl)pent-3-en-1-ol (**3**) in relatively high yield [17]. Etherification of **3** with (m-bromoalkyl)tetrahydro-2*H*-pyran ($Br(CH_2)_mOTHP$) through nucleophilic substitution provided **4a**–**4c** under strong basic conditions. Subsequently, removal of THP protection quantitatively afforded alcohol **5a**–**5c** in the presence of hydrochloric acid. However, succesive condensation of compound **5a**–**5c** and d-biotin by treatment with 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) provided ester **6a**–**6c** in relatively low yield. Therefore, considerable efforts were taken to augument the yield of the key intermediate **6a**–**6c**.

Compound **5a** was used as model substrate to probe the reaction conditions. Initially, treatment of **5a** with EDCI/DMAP in different solvents (DCM, DMF, DMSO and DCM/DMF) afforded **6a** in 15–27% isolated yields (Table 1, entries 1–5). Subsequent replacement of DMAP with 1-hydroxybenzotriazole (HOBt) resulted in no apparent change in isolated yield (entries 6 and 7). Compared with EDCI, alcohol **5a** coupled with *N*, *N*-diisopropylcarbodiimide (DIC) led to slightly higher yield (entry 8). Changing the coupling reagents from DIC to 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylururonium hexafluorophosphate (HATU) and using *N*,*N*-diisopropylethylamine (DIPEA) as a solvent, we found that the yield of **6a** was also no significantly improved (entry 9 and 10). Whereas the coupling reagent HATU was replaced by DCC and the reaction time was prolonged from 48 h to 72 h, it was surprising that this condensation reaction provided **6a** in 53% isolated yield (entries 10 and 11). More importantly, a gratifying observation was that **6a** was also obtained by a modified Mitsunobu reaction in 82% isolated yield (entry 12).

When compounds **6a**–**6c** were successfully obtained through Mitsunobu reaction, subsequent oxidation of them with cerium



**Scheme 1.** Synthesis of chemical probes **8a**–**8c**. Reagents and conditions: (a) DMP, −10 °C, 25 min; (b) (−)-DIP-Cl, THF, −20 °C, 1 h; (c) 60% NaH, DMF, 0 °C, 0.5 h, then $Br(CH_2CH_2)_mOTHP$, KI, 60 °C, 10 h; (d) 12 N HCl, MeOH, 15 min; (e) $Ph_3P$, DEAD, biotin **11**, THF, r. t., 48 h; (f) CAN, $CH_3CN$-EtOAc, 0 °C, 8 min; (g) $NH_2OH.HCl$, pyridine, reflux, 16 h.

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