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Synthesis and *Topoisomerase I* inhibitory properties of klavuzon derivatives

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ABSTRACT

Klavuzon is a naphthalen-1-yl substituted α,β-unsaturated δ-lactone derivative, and is one of the anti-proliferative members of this class of compounds. Asymmetric and racemic syntheses of novel α,β-unsaturated δ-lactone derivatives are important to investigate their potential for the treatment of cancer. In this study, asymmetric and racemic syntheses of heteroatom-substituted klavuzon derivatives are reported. The syntheses were completed by a well-known three-step procedure. Anti-proliferative activity of seven novel racemic klavuzon derivatives were reported against MCF-7, PC3, HCT116 p53+/+ and HCT116 p53−/− cancer cell lines. *Topoisomerase I* inhibitory properties of 5,6-dihydro-2H-pyran-2-one derivatives were also studied.

1. Introduction

Reactive electrophilic compounds, such as nitrogen mustards, are still in use for chemotherapy of cancer patients [1,2]. Their therapeutic effect is explained by the formation of an irreversible covalent bond between mustards and DNA bases [3]. Similarly, a compound that covalently binds to the active site of proteins may inhibit the enzymatic activity of that protein irreversibly. For example, it was reported that vicinal cysteine 504 and 505 residues are crucial for the catalytic mechanism of human *topoisomerase I* (Topo I) enzyme, a well-known target for anti-cancer drugs [4], and thiol reactive electrophilic compounds may inhibit the human Topo I activity [5,6].

Similarly, it is believed that α,β-unsaturated δ-lactone derivatives can covalently bind to the nucleophilic sites of proteins through a Michael addition reaction, and this is the reason for the resulting biological activity [7]. In the literature, hundreds of compounds-either synthesized in the laboratory or isolated from the natural resources- that have a functional group for this type of reaction have been reported. Styryl lactones bear an α,β-unsaturated δ-lactone pharmacophore in their structures and goniothalamin (GTN, **1**) is a promising example of this class of compounds because of its reported selective anti-proliferative activity on cancer cells [8,9].

To date many syntheses and SAR studies have been carried out for goniothalamin derivatives. The presence of an α,β-unsaturated carbonyl group and a *trans* C=C double bond are essential for the biological activity [10,11]. The (*R*)-isomer is more potent and triggers apoptosis, while the (*S*)-isomer causes autophagy in the cell [12]. In another work, it has been shown that racemic goniothalamin induces TP53 transcription-dependent and -independent apoptosis in hepatocellular carcinoma (HCC)-derived p53 (TP53)-positive and -negative cells [13]. A heteroatom substitution, that donates a pairs of electrons to the benzene ring (**2**–**4**), decreases anti-proliferative activity of goniothalamin. In the same study, compounds having LogP values between +4.4 and +4.7 were more potent [14]. Recently, Barcelos et al. reported that a 2,4-dimethoxy analog (**5**) was approximately twice as potent as goniothalamin (Fig. 1) [15].

Compound **6**, conformationally constrained analog of (*R*)-goniothalamin, and klavuzon (**7**) are α,β-unsaturated δ-lactone derivatives that were first described by our group in 2008. Conformationally constrained analog (**6**) has similar anti-proliferative activity compared to (*R*)-goniothalamin, while klavuzon (**7**) is more anti-proliferative than goniothalamin. Presence of a methyl substitution in the naphthalene-1-yl group (**8** and **9**) further increases its potency (Fig. 1) [16,17].

In this study, asymmetric and racemic preparations of novel derivatives of klavuzon (**10**–**15**) and goniothalamin (**16**) were

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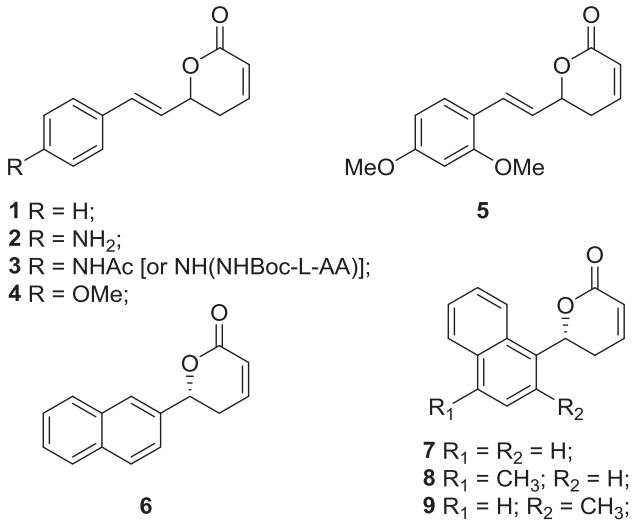


Fig. 1. Structures of heteroatom-substituted goniothalamin (**2**–**5**) and 6-bicycloaryl substituted (*R*)-5,6-dihydro-2H-pyran-2-one (**6**, **9**) derivatives.

studied (Fig. 2). The effects of the heteroatom substitutions on the biological activity of klavuzon were also investigated.

2. Results and discussions

2.1. Chemistry

The syntheses of novel 5,6-dihydro-*2H*-pyran-2-one derivatives (**10**–**16**) were planned by a well-known three-step procedure. Asymmetric or racemic allylation of aldehydes was followed by acrylate aster formation and ring-closing metathesis.

Asymmetric allylation of the 4-dimethylamino-1-naphthaldehyde (**17**) was performed by allyltrimethoxysilane in the presence of *R*-Tol-BINAP.AgF complex at −20 °C to produce *R*-homoallylic alcohol **18** with 69% yield [18]. Chiral HPLC studies with two separate chiral columns were performed to determine the ee% of the product and it showed a single peak, which might be the result of formation of a single enantiomer or poor resolution of enantiomers in the columns. To check the enantiopurity of the formed homoallylic alcohol **(*R*)-18**, it was reacted with acryloyl chloride to form its acrylate ester. Transformation of the obtained alcohol to acrylate ester (***R*-19**) was monitored by TLC [16]. Although the formation of the product was observed in TLC, acrylate ester was highly unstable in silica gel column chromatography.

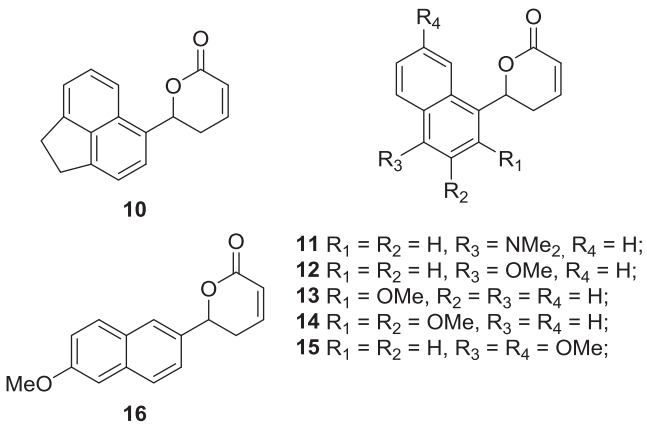


Fig. 2. Proposed klavuzon (**10**–**15**) and goniothalamin (**16**) derivatives.

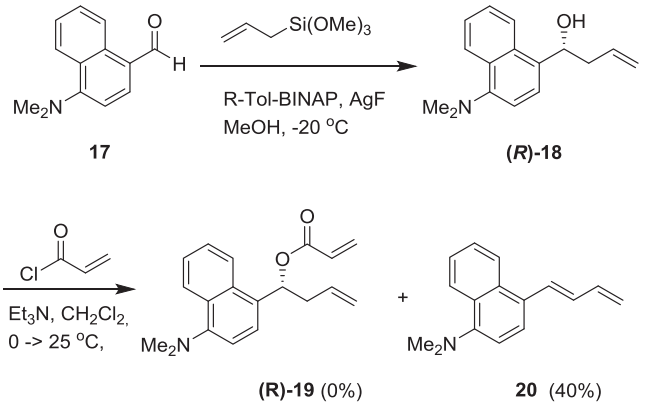
Purification of the crude product gave only elimination product **20** with 40% yield, so the enantiomeric purity of the formed alcohol (***R*-18**) could not be determined. It is likely that donation of the lone pair of electrons from nitrogen atom to the naphthalene was the driving force of this elimination (Scheme 1).

Similarly asymmetric allylation of 4-methyl-1-naphthaldehyde at −20 °C gave compound (***R*)-22** with 56% yield and 82% ee. When the same reaction was performed at room temperature, the enantiomeric excess of the reaction did not change (Scheme 2). All further asymmetric allylation reactions were performed both at −20 °C and room temperature to show the applicability of asymmetric allylation of 1-napthaldehydes at room temperature. Asymmetric allylation of 4-methoxy-1-naphthaldehyde furnished compound **(*R*)-24** with almost the same enantiomeric excess at −20 °C and room temperature (91% and 90% respectively). However, the yields of the products were low compared to the yield of racemic allylation by a TBAT-CuCl mixture [19] (Scheme 3).

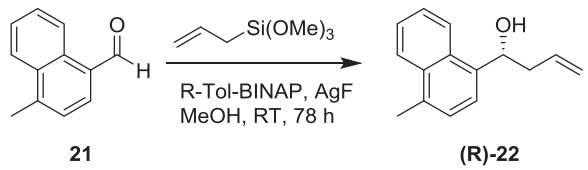
Asymmetric allylation of 2-methoxy-1-napthaldehyde and of 4,7-dimethoxy-1-naphthaldehyde gave similar results. The yields of the asymmetric allylation are low compared to racemic allylation and the enantiomeric excess of the reactions were quite similar at −20 °C and room temperature (50% and 55%, respectively, for compound **(*R*)-27**, and 87% and 84%, respectively, for compound **(*R*)-30**). The low enantiomeric excess for compound **(*R*)-27** can be explained by the proximity of the methoxy substituent to the reaction center (Scheme 3 and 4). Surprisingly, it appears that there was no effect of temperature on the enantiomeric excess of the reactions.

To show the asymmetric synthesis of heteroatom-substituted klavuzon derivatives, two chiral homoallylic alcohols, **(*R*)-24** and **(*R*)-27**, were transformed to their acrylate esters and then converted to the lactones by ring-closing metathesis using a first generation Grubbs' catalyst [20]. To minimize the possible elimination reaction, esters were used without purification to produce α,β-unsaturated δ-lactones (Scheme 3).

Finally, racemic syntheses of compounds **10**–**16** were completed (Scheme 5). Briefly, racemic allylation of aldehydes (**17**,



Scheme 1. Attempts towards the asymmetric synthesis of 4′-dimethylaminoklavuzon.



Scheme 2. Asymmetric allylation of 4-methyl-1-naphthaldehyde at room temperature.