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Discovery of 2-[2-(5-nitrofuran-2-yl)vinyl]quinoline derivatives as a novel type of antimetastatic agents

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ABSTRACT

A number of 2-furanylvinylquinoline derivatives were synthesized and evaluated for antiproliferative activities against the growth of four cancer cell lines including non-small cell lung cancer (A549 and H1299), breast cancer (MCF-7 and MDA-MB-231) and normal diploid embryonic lung cell line (MRC-5). Among them, (*E*)-6-methoxy-3-(4-methoxyphenyl)-2-[2-(5-nitrofuran-2-yl)vinyl]quinoline (**10c**) was found low cytotoxic in all cancer cells and normal cell. The aim of this study was to investigate the anti-invasive and anti-metastatic activity of compound **10c** in H1299 human lung cancer cells. In this study, compound **10c** inhibited the migration and invasion of cells in a concentration-dependent manner by wound healing assay and transwell invasion assay. Furthermore, the inhibition of both phosphorylation of Akt and ERK by compound **10c** may be critical for cell migration and this may result in the down-regulation of several factors associated with cellular migration, including β-catenin transcription factor, Bcl-2 and COX-2. Interestingly, the treatment of compound **10c** did not affect the expression level but reduced the activities of the MMP-2 and -9. The phosphorylation level of stress-activated kinase p38 was significantly increased following compound **10c** treatment. To sum up, compound **10c** had potential to suppress the migration and invasion of H1299 cancer cells in vitro and it could serve as a promising drug for the treatment of cancer metastasis.

1. Introduction

Lung cancer is one of leading cause of death worldwide. The human non-small cell lung cancer (NSCLC) accounts for 80–85% of lung cancer cases. More than one of three patients with NSCLC is surgically unavailable.[1] Therefore, chemotherapy is still the major treatment for clinical NSCLC.[2–4] However, although the great improvement of current therapies for lung cancers, both poor prognosis at the advanced stage of NSCLC and chemoresistance caused to low survival rate of NSCLC patients.[4]

Tumor metastasis occurs by a complex series of events including cell adhesion, invasion, proliferation and vessel formation.[5] Degradation of basement membranes and stromal extracellular matrix (ECM) is crucial for invasion and metastasis of malignant cells. The matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases involved in the degradation of the extracellular matrix. The MMPs have been implicated in the processes of tumor growth, invasion and metastasis; are frequently over-expressed in malignant tumors; and have been associated with an aggressive malignant phenotype and adverse prognosis in patients with cancer.[6]

Quinoline ring is found in a wide variety of pharmacologically active compounds and is frequently condensed with various heterocycles.[7–16] Recently, a number of condensed quinoline derivatives have been synthesized and evaluated for their top I inhibitory activities. Camptothecin (CPT), an alkaloid isolated from *Camptotheca acuminata*, and its derivatives such as topotecan and irinotecan are prototypical topo I inhibitors and currently used as anticancer drugs. However, the chemically unstable lactone ring limited their clinical utility. To improve chemical stability and anticancer potency of CPT, we have designed and synthesized certain quinoline derivatives for anticancer evaluation. Among them, 2,3-diarylquinoline (**1**),[12] 6-arylindeno[1,2-*c*]quinoline (**2**),[13] and 3-arylquinolinylchalcone (**3**)[14] were found to be more potent than CPT against the growth of human cancer cell lines. (*E*)-2-[2-(5-Nitrofuran-2-yl)vinyl]quinolin-8-ol (**4**)[15] is another example of quinoline derivative which exhibited potent antiproliferative activ-

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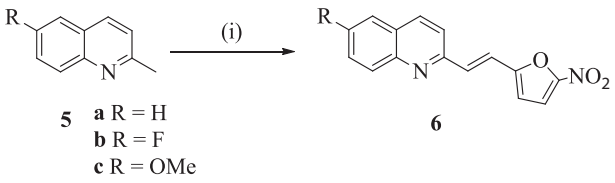
ities with an IC_{50} value of 0.35 and 0.14 μM, respectively, against the growth of LNCaP and PC3 cancer cells. In continuation of our search for potential anticancer drug candidates, the present study describes the synthesis of 3-aryl-2-[2-(5-nitrofuran-2-yl)vinyl]quinoline derivatives (Target compounds, Fig. 1) whose structures resemble compound **1** by the replacement of the 2-phenyl group with a 2-vinylfuran ring. The target compounds can also be considered as hybrids of compounds **3** and **4**. These newly synthesized compounds were evaluated for their antiproliferative and antimetastatic activities.

2. Chemistry

Reaction of 2-methylquinoline (**5a**) and (5-nitrofuran-2-yl)methylene diacetate in acetic anhydride gave (*E*)-2-(2-(5-nitrofuran-2-yl)vinyl)quinoline (**6a**) in 81% yield as described in Scheme 1. Accordingly, compounds **6b** and **6c** were prepared from their respective 2-methylquinoline precursors **5b** and **5c** by the same reaction conditions. Thermal decarboxylation of 3-(4-methoxyphenyl)-2-methylquinoline-4-carboxylic acid (**7a**)[14] gave 3-(4-methoxyphenyl)-2-methylquinoline (**8a**)[14] which was then reacted with 5-nitro-2-furaldehyde diacetate in acetic anhydride to afford (*E*)-3-(4-methoxyphenyl)-2-(2-(5-nitrofuran-2-yl)vinyl)quinoline (**10a**) as described in Scheme 2. Compounds **10b** and **10c** were prepared from their respective 2-methylquinoline precursors **8b** and **8c**[16] by the same reaction conditions. Treatment of **7a** with $POCl_3$ afforded 6-methyl-11*H*-indeno[1,2-*c*]quinolin-11-one (**9a**), which was then reacted 5-nitro-2-furaldehyde diacetate in acetic anhydride to give (*E*)-9-methoxy-6-[2-(5-nitrofuran-2-yl)vinyl]-11*H*-indeno[1,2-*c*]quinolin-11-one (**11a**). Accordingly, compounds **11b** and **11c** were prepared from their respective methyl precursors **9b** and **9c** which in turn were prepared from their respective 4-carboxylic acid precursors **7b** and **7c** with $POCl_3$.



Scheme 1. Reagents and conditions: (i) (5-nitrofuran-2-yl)methylene diacetate, Ac_2O, 150 °C, 3 h.

3. Results and discussion

3.1. Antiproliferative activity

All the newly synthesized compounds were evaluated in vitro against four cancer cell lines (H1299, A549, MCF-7 and MDA-MB-231) using XTT [2,3-bis(2-methyloxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide] assay. The normal human fetal lung fibroblast cell line (MRC-5) was also evaluated since a potential anticancer drug candidate should selectively affect only tumor cells and not somatic cells. The concentration that inhibited the growth of 50% of cells (IC_{50}) was determined from the linear portion of the curve by calculating the concentration of agent that reduced absorbance in treated cells, compared to control cells, by 50%. The results of IC_{50} values are summarized in Table 1. Compound **4** was more active than compounds **6a**–**6c** against all the cancer cells tested indicated 8-hydroxy group is favorable for the cell cytotoxicity. Introduction of 4-methoxyphenyl group at C-3 position of **6b** and **6c** decreased antiproliferative activities in which **10b** and **10c** were inactive against all the cancer cells tested. However, compound **10a** was highly cytotoxic which implied that the substitution at C-6 position of quinoline ring was unfavorable especially the methoxy group. The same structure–activity relationship (SAR) was observed for the tetracyclic indenoquinoline in which the antiproliferative activities decreased in an order **11a** (R = H) >**11b** (R = F) >**11c** (R = OMe). Although compounds **11a**–**11c** exhibited potent inhibitory activities on all the cells tested, their methyl precursors **9a**–**9c** were inactive, indicated that the conjugated nitrofuran moiety is crucial. Among these newly synthesized quinoline derivatives, compound **11a** was the most active, approximately equal potent to the positive daunorubicin.

Although compound **11a** can be further developed as cytotoxic anticancer drug candidates, the inactiveness of **10c** against all the cells attracted our attention due to its structure similarity to combretastatin A4 (CA-4), a known anti-angiogenesis agent. Therefore, compound **10c** was selected for antimetastatic evaluations and the possible signaling pathways in vitro.

3.2. The effect of compound 10c on proliferation of NSCLC cells

To further examine the effect of compound **10c** on cell growth, H1299 cells were treated with compound **10c** at various concentrations (0–200 μM) for 24 h by XTT assay. As results from a representative experiment shown in Figure 2A, compound **10c** at concentrations lower than 100 μM had no cytotoxic effect (>90% cell survival) while at 200 μM it reduced cell viability. There was no significant change in morphology observed between the vehicle control and **10c**-treated (Fig. 2B). It has to be noted that the cell migration and invasion assay were performed with a noncytotoxic

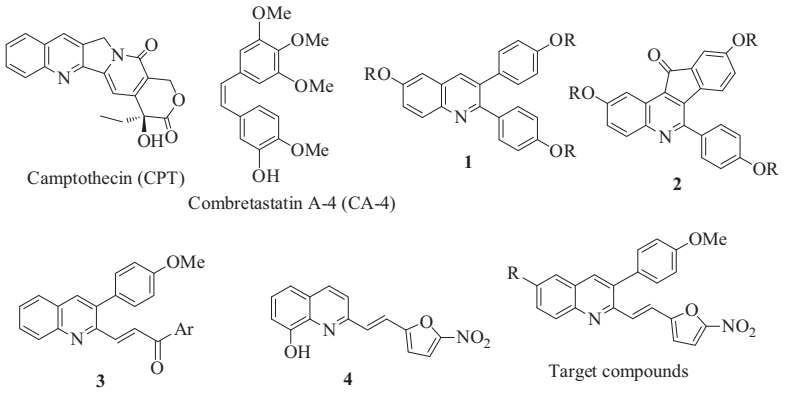


Figure 1. Structures of Camptothecin (CPT), Combrestatin A-4 (CA-4), 2,3-diarylquinoline (**1**), 6-arylindeno[1,2-*c*]quinoline (**2**), 3-arylquinolinylchalcone (**3**), (*E*)-2-[2-(5-nitrofuran-2-yl)vinyl]quinolin-8-ol (**4**) and target compounds.