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Synthesis and bio-evaluation of novel quinolino-stilbene derivatives as potential anticancer agents

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ABSTRACT

A series of 25 novel quinolino-stilbene derivatives were designed, synthesized and evaluated for their potential as anticancer agents. Three of them not only displayed quite potent antiproliferative activity with IC_{50} values <4 μM but also showed approximately twofold selectivity against cancer cells, compared to non-cancerous cells. Three other compounds exhibited comparatively good activity with IC_{50} values in the range of 4-10 μM, and the rest was moderately active or inactive. One of these viz. 3-[*E*-(4-fluorostyryl)]-2-chloroquinoline (compound **7B**) caused substantial DNA damage and arrested cell cycle in S phase. Interestingly, **7B** was very active against MDA-MB468 (IC_{50} = 0.12 μM), but not against other cell lines examined. Compound 3-[*Z*-(3-(trifluoromethyl)styryl)]-2-chloroquinoline (**12A**), the most effective against all cancer cell lines examined, caused prolonged cell cycle arrest at mitosis and eventually apoptosis. Data from an in vitro study showed that compound **12A** inhibited microtubule polymerization in a similar fashion to nocodazole. Further study using in silico molecular modeling revealed that **12A** causes the impediment of microtubule polymerization by binding to tubulin at the same cavity where podophyllotoxin binds.

1. Introduction

Cancer is the second most common cause of death in the United States, and the American Cancer Society estimates 1,658,370 new cases and 583,430 cancer-related deaths in 2015.[1] Although the development of early detection methods and timely intervention have resulted in the substantial improvement of survival and quality of life in certain cancer populations, effective cancer treatments are still difficult to achieve. One crucial limitation of current cancer therapies is that a curable dose often cannot be given due to severe side effects. Moreover, cancer curability is further hampered by the emergence of tumor cells that are resistant to therapeutic agents.[2] Therefore, there is a great need of developing new anticancer therapeutics with better pharmacological properties including increased selectivity for tumor cells. As a part of achieving this goal, we previously synthesized and characterized quinoline-based anticancer agents, some of which showed substantial promises.[3–6] Quinoline, a heterocyclic aromatic compound present in a wide range of natural and synthetic pharmaceuticals, is a privileged skeleton in drug discovery. Quinoline derivatives possess many different biological and pharmacological activities including antimicrobial, antimalarial, antifungal, anti-inflammatory, analgesic, antiviral, antiprotozoal, cardiovascular, CNS effective and antineoplastic.[7,8] Several well-known anticancer agents including camptothecin, topotecan and irinotecan contain a quinoline moiety.[9] It is also well-known that quinoline analogs often inhibit tubulin polymerization, DNA repair, tyrosine kinase activity, proteasome, histone acetyl-transferases (HATs) and histone deacetylase (HDACs), all of which are considered to be effective cancer therapeutic targets.[10]

Stilbenes (1,2 diarylethylene, Fig. 1) attract considerable interest because of their wide range of biological activities and potential therapeutic values, especially against cancer.[11–15] E-Resveratrol (Fig. 1) is a phytoalexin stilbene found in berries, grapes, peanuts and red wine. Resveratrol and its analogs exhibit various biological and pharmacological activities including anticancer.[16–18] Combretastatin A4 (Fig. 1), isolated from *Combretum caffrum*, is another natural stilbene analog possessing potent tubulin inhibition activity.[9,19] CA4P (combretastatin-4,3-O phosphate), a prodrug of CA4, significantly reduces blood flow to the tumor cells, leading to extensive tumor necrosis. Several other stilbene derivatives have been synthesized previously in an attempt to develop effective anticancer drugs.[20–23] Tamoxifen (Fig. 1), one of the stilbene

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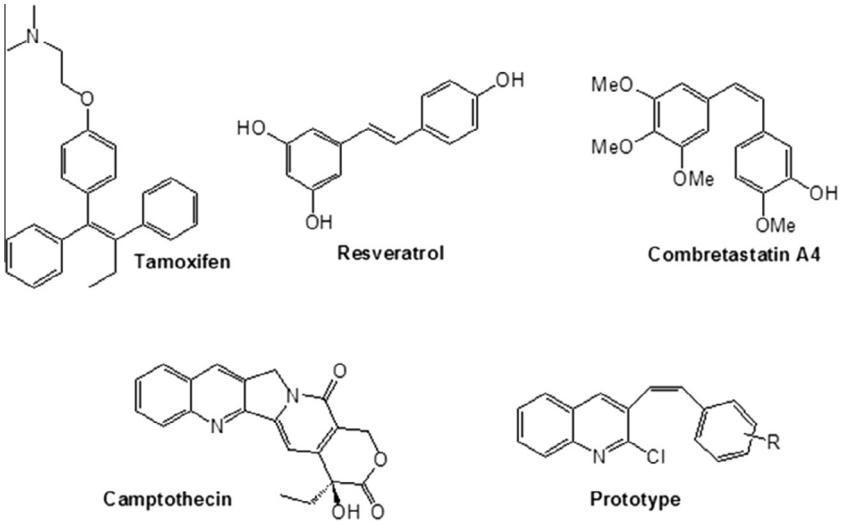

Figure 1. Prototype of quinoline-based stilbene derivatives. Shown are various stilbene derivatives being used as anticancer agents (tamoxifen, resveratrol and combretastatin A4) and the quinoline-based anticancer agent camptothesin.

derivatives, shows selective inhibition activity against estrogen receptor and is currently used as chemotherapeutic agent for breast cancer treatment.[24,25]

The introduction of fluorine to bioactive molecules may alter their physiological properties and bioactivities. For instance, a fluorinated taxane is more active than the parental taxane against several cancer cell lines.[26] High electronegativity, chemical reactivity and the small size of fluorine all contribute to the enhancement of binding affinity, metabolic stability and selective target reactivity.[27] As the number of drug candidates with one or more fluorine atoms continues to increase in recent years, several groups have reviewed the role of fluorine in medicinal chemistry.[27–30]

To extend our ongoing efforts to develop effective and safe anticancer agents, we designed and synthesized a series of novel stilbene derivatives that are composed of a substituted quinoline moiety as ring A and a fluorine- or trifluoromethyl-substituted phenyl group as ring B. Subsequently, we examined their anticancer activities and cancer cell selectivity using four different cancer cell lines and a matching non-cancer cell line.

2. Result and discussion

2.1. Chemistry

All the quinolino-stilbene derivatives were synthesized by performing Wittig reactions on substituted quinoline-3-carbaldehyles (**5a**–**5d**), Wittig salts (**2**) using dimethyl sulfoxide as polar aprotic solvent, and sodium hydroxide as the base (Scheme 1).[31] Quinoline-3-carbaldehydes (**5a**–**d**; Scheme 1) were either synthesized starting with substituted acetanilide (**4**) via Vilsmeier–Haack reaction using dimethylformamide (DMF) and phosphorus oxychloride ($POCl_3$) at 85–90 °C as described previously[32] or purchased from sigma. Benzyl triphenylphosphene bromide (Wittig salts, **2**) were synthesized by the reaction of corresponding benzyl bromides with triphenylphosphine and toluene under refluxing conditions. All of the final products were characterized by ^1H, ^{13}C NMR and mass spectroscopy, which were found to be consistent with the assigned structures (Table 1).

cis (**6A**–**21A**) and *trans* (**6B**–**20B**) isomers, the major and minor products of the Wittig reaction respectively, were purified by column chromatography. We sometimes encountered difficulties in separating these two stereoisomers due to their close retention factors (RFs), resulting in medium to low yield of pure isomers along with the mixture of isomers. In some cases only *cis* products were isolated in the pure chemical form. The geometry of stilbenes (*cis* and *trans*) was identify by ^1H NMR on the basis of H–H coupling constant (J). For some *cis* derivatives (for instance **12A**), we unexpectedly got only one signal for two ethylene protons when spectra were run in $CDCl_3$. On the other hand, two signals (one doublet, J = 15 Hz, and one merged doublet) for two ethylene protons were found for *trans* isomer (**12B**). The resultant mass spectra of both the isomers were in agreement with expected structures. To confirm geometry of *cis* isomer, we ran the sample **12A** in acetone d_6, by which we could clearly resolve doublets (J = 12.15 Hz) for two ethylene protons.

2.2. Biological activity

2.2.1. In vitro cytotoxicity and structural activity relationship (SAR)

We examined the novel compounds for their antiproliferative activities against four cancer cell lines: HeLa (cervical carcinoma), MDA-MB231 (ER-negative undifferentiated metastatic breast cancer), MCF7 (ER-positive well-differentiated breast cancer), and MDA-MB468 (PTEN mutated, intermediately differentiated breast cancer). To determine differential effects between cancer and non-cancer cells, we also examined the effect of these compounds on 184B5, a non-cancer breast epithelial cell line. Data from sulforhodamine B (SRB)-based assays[33] showed that the stilbene analogs displayed cell line-dependent IC_{50} values (Table 2). Three of the compounds examined (**12A**, **13A** and **21A**) showed IC_{50} values in the range of 2.6–4.0 μM against all four cancer cell lines. Furthermore, they were approximately twofold more effective on cancer cells than non-cancerous cells (Table 2). Three of the derivatives (**6A**, **14A** and **15A**) were also relatively active with IC_{50} values in the range of 4–10 μM. The rest was generally not very active with IC_{50} of 10 μM or higher.

When considered in the context of SAR, the results suggest that stereochemistry and the positions of different functional groups are likely to play important roles in their activities. We found that the *cis* configuration of stilbene derivatives is generally more favorable for higher activity and better cancer selectivity than *trans*, except for **7B**. This data is in line with previous report indicating that s-*trans* conformation of chalcones possess better anti-invasive