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Anti-tumor activity of novel biisoquinoline derivatives against breast cancers

Aruna S. Jaiswal [a], Dimitri Hirsch-Weil [b], Erick R. Proulx [b], Sukwon Hong [b,c,*], Satya Narayan [a,*]

[a] Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL 32610, USA
[b] Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, USA
[c] Research Institute for Solar and Sustainable Energies (RISE), School of Materials Science & Engineering, Gwangju Institute of Science and Technology, 123 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, Republic of Korea

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ABSTRACT

Breast cancer is classified into three groups according to its expression of hormone/growth factor receptors: (i) estrogen receptor (ER) and progesterone receptor (PR)-positive; (ii) human epidermal growth factor receptor 2 (HER2)-positive; and (iii) ER, PR, and HER2-negative (triple-negative). A series of methoxy-substituted biisoquinoline compounds have been synthesized as a potential chemotherapeutic agent for the triple-negative breast cancers which are especially challenging to manage. Structure activity relationship study revealed that rigid 6,6′-dimethoxy biisoquinoline imidazolium compound (**1c**, **DH20931**) exhibited the significant growth inhibitory effects on both triple-positive and triple-negative human breast cancer cell lines with IC_{50} in the range of 0.3–3.9 μM. The **1c** (**DH20931**) is more potent than structurally related noscapine for growth inhibition of MCF7 cell line (IC_{50} = 1.3 vs 57 μM) and MDA-MB231 cell line (IC_{50} = 3.9 vs 64 μM).

Breast cancers exhibit genetic alterations representing an accumulation of mutations, failure of DNA repair, activation of oncogenes, and loss of tumor suppressor function.[1,2] These genetic defects result an inappropriate intracellular signaling pathways that lead to the initiation, progression, and invasion of breast tumorigenesis.[3] Breast cancer development may be associated with the presence or absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor-2 receptor (HER2).[4] A positive receptor status is associated with favorable prognostic features and predicts response to hormonal therapy; however, this is balanced by a higher recurrence rate in subsequent years.[5,6] ER-, PR- and HER2-negative (triple-negative) breast cancers, which are poorly differentiated and generally fall into the basal subgroup of breast cancers, are significantly more aggressive.[7,8] Due to the absence of specific treatment guidelines for triple-negative breast cancers, they are managed with standard treatments; however, such treatments are associated with a high rate of local and systemic relapse.[9–12]

ER, a transcription factor, involved in the development and maintenance of female reproductive organs, drives the tumor growth in ~70% of all cases.[13] Most chemotherapeutic anticancer drugs used in the clinical setup include anti-estrogenic agents that interfere with ER and prevent tumor progression.[14,15] Mitosis and other distinct pathways of apoptosis are considered as a potential target for the development of novel class of drugs that can overcome the limitations of current tubulin-targeted anti-mitotic drugs.[16] Currently, few treatment options are available for the intervention and prevention of early breast cancer (docetaxel, paclitexal and trastuzumab) and for the advanced or metastatic breast cancer (gemcitabine, lapatinab and bevacizumab).[17] Most of these drugs act by inducing tubulin polymerization, forming multi-polar spindles, causing DNA damage, and leading to mitotic arrest.[18] Since the anti-cancer drugs inducing DNA damage tend to become resistant, their therapeutic outcome often becomes limited.[19,20] Thus, there is clearly an urgent need for the development of new therapeutic treatment strategies.

Polymethoxylated phenyl rings are often found in the structure of anti-cancer agents such as steganacin,[21,22] colchicines,[23,24] podophyllotoxin,[25,26] and noscapine[27] (Fig. 1). During the course of our study to develop isoquinoline-based carbene ligands for asymmetric catalysis,[28–30] we were intrigued by the idea that the methoxylated biisoquinoline compounds could provide pharmacophores similar to those of the aforementioned anti-mitotic agents. Furthermore, such methoxylated biisoquinoline compounds can be conveniently prepared in three steps from commercially available starting materials, rendering the lead optimization process facile

* Corresponding authors. Tel.: +82 62 715 2346; fax: +82 62 715 2304 (S.H.); tel.: +1 352 273 8163; fax: +1 352 392 3305 (S.N.).
E-mail addresses: shong@gist.ac.kr (S. Hong), snarayan@ufl.edu (S. Narayan).

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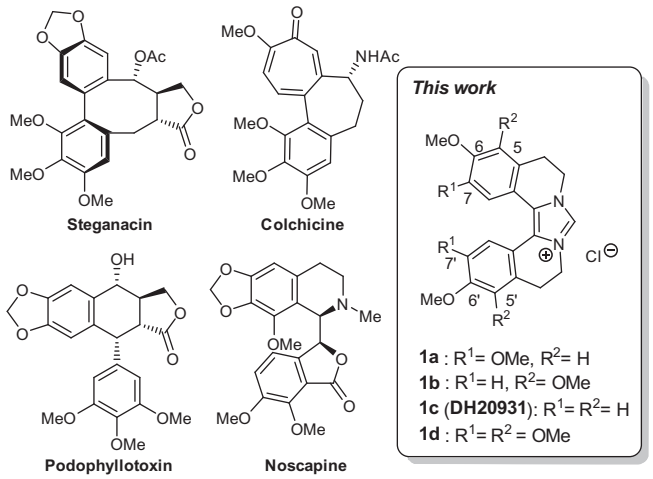
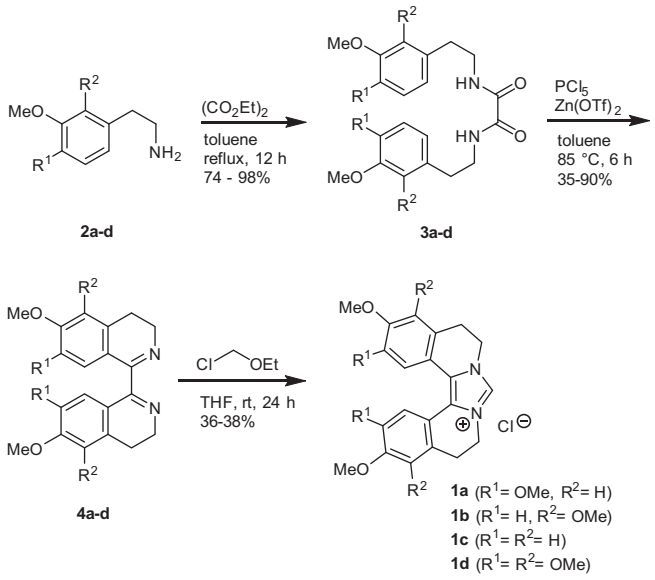


Figure 1. Anti-cancer agents featuring polymethoxylated phenyl rings.

compared to structurally more complex natural products. Herein, we report syntheses of variously methoxylated biisoquinolines derivatives and their highly potent growth inhibitory effects on both triple-positive and triple-negative human breast cancer cell lines.

A series of methoxy-containing biisoquinoline compounds (**1a**–**d**) were prepared according to the previously established method[28–30] (Scheme 1). Variously substituted methoxyphenyl ethanamines (**2a**–**d**) were first transformed into the bisamides (**3a**–**d**) under the standard conditions, and the bisamides were converted to the tetrahydrobiisoquinolines (**4a**–**d**) by Bischler–Napieralski cyclization reaction. Reaction of the tetrahydrobiisoquinolines with chloromethyl ethyl ether successfully afforded the desired polycyclic imidazolium compounds containing tetramethoxy (**1a**,**1b**), dimethoxy (**1c**) and hexamethoxy substituents (**1d**).

Preliminary structure–activity relationship study was conducted with 6,6′,7,7′-tetramethoxy biisoquinoline-imidazolium **1a** as well as its precursor compounds, bisamide **3a** and diimine **4a**, to see if the rigid molecular skeleton is essential (Fig. 2A). Their growth inhibitory effect on triple-negative breast cancer cell line



Scheme 1. Synthesis of methoxylated biisoquinoline compounds.

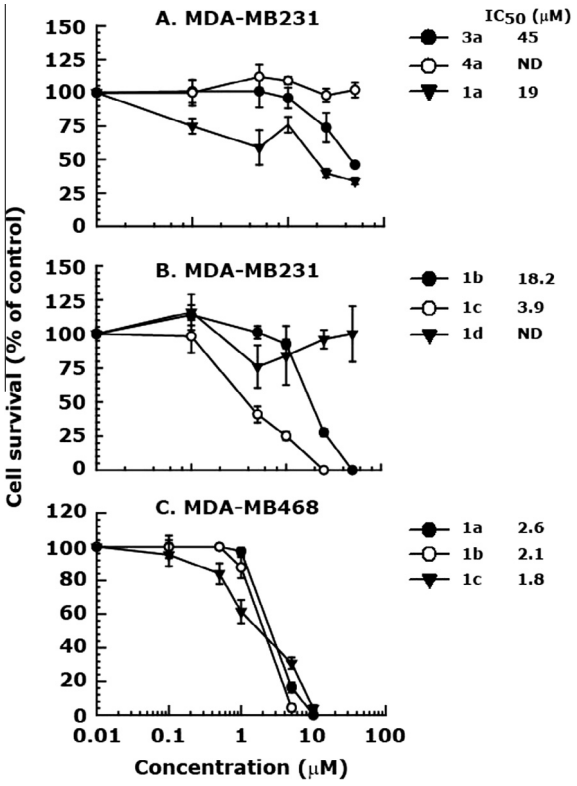


Figure 2. Determination of IC_{50} on MDA-MB231 and MDA-MB468 cell lines. The results were plotted on a common-log scale to determine the IC_{50}. Data are mean ± SE of three different experiments.

(MDA-MB-231) was evaluated by using the clonogenic cell survival assay.[31,32] Interestingly, imidazolium **1a** displayed a notable cytotoxicity (IC_{50} = 19 μM), whereas bisamide **3a** showed less potency (IC_{50} = 45 μM) and diimine **4a** was non-toxic to the MDA-MB231 cells. These initial results seem to suggest that the structural rigidity might be important for the anti-tumor activity. Then, three other methoxylated biisoquinoline-imidazolium compounds (**1b**–**d**) were tested to probe the optimal methoxy substitution pattern (Fig. 2B). It is interesting to note that a higher toxicity was observed with less number of methoxy groups. Thus, 6,6′-dimethoxy compound **1c** (**DH20931**) displayed the lowest IC_{50} value of 3.9 μM among the four compounds tested. Tetramethoxy compounds **1a** and **1b** showed higher IC_{50} values (19 μM and 18.2 μM, respectively), and hexamethoxy compound **1d** did not show any toxicity to the MDA-MB231 cells.

Next, we tested whether the methoxylated biisoquinoline compounds **1a**, **1b**, and **1c** also have the growth inhibitory effect on another triple-negative breast cancer cell line, MDA-MB468. The IC_{50} values of 6,6′,7,7′-tetramethoxy imidazolium **1a**, 5,5′,6,6′-tetramethoxy imidazolium **1b** and 6,6′-dimethoxy imidazolium **1c** (**DH20931**) were 2.6 μM, 2.1 μM and 1.8 μM, respectively, for this cell line (Fig. 2C). From these results it appears that we have successfully identified a potential anti-cancer drug candidate **1c** (**DH20931**), which effectively sensitizes the triple-negative breast cancer cell lines at very low μM concentrations.

The anti-cancer therapeutic efficacy of the biisoquinoline compounds was also evaluated for triple-positive breast cancer cell lines (BT474, MCF7 and T47D). The IC_{50} values of 6,6′,7,7′-tetramethoxy biisoquinoline-imidazolium **1a** for BT474, MCF7 and T47D cell lines were 3.1 μM, 35 μM and 34 μM, respectively (Fig. 3), suggesting that BT474 cells were more sensitive to **1a** than MCF7 or T47D cells. The IC_{50} values of 5,5′,6,6′-tetramethoxy