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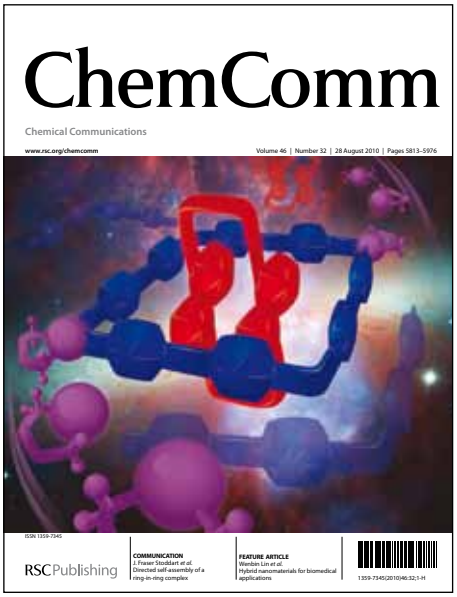

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ARTICLE TYPE

Different Cytotoxicities and Cellular Localizations of Novel Quindoline Derivatives With or Without Boronic Acid Modifications in Cancer Cells

Ruijuan Yin[†], Meng Zhang[†], Cui Hao[†], Wei Wang, Peiju Qiu, Shengbiao Wan, Lijuan Zhang[*], Tao Jiang*

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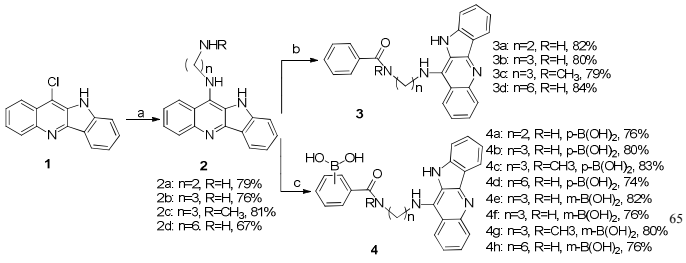
The synthesis of 4x4 series of novel quindoline derivatives with or without boronic acid modifications and their cytotoxicities, cellular localizations, and implications on cancer cells are presented and discussed.

Chemically synthesized boronic acid derivatives[1] have been developed into drugs[2] or biomarker identification reagents[3] that either work inside of cells or at cell surface due to unique carbohydrate interactions[4] and Lewis acidity[2] of the boronic acid moiety at physiological conditions. However, it is unknown if boronic acid modification has any impact on the cellular localizations and biological activities of its parental compounds. Quindoline has intrinsic fluorescence, penetrates cells, interacts with DNAs, and kills cancer cells by three established mechanisms[5]. Thus, we designed and synthesized 4x4 series of novel quindoline derivatives without (**2a-2d** and **3a-3d)** or with boronic acid modifications (**4a-4d** and **4e-4h)** as shown in **Scheme 1**. These derivatives were then used to evaluate the impact of boronic acid modifications on the cellular localizations and cancer cell killing properties by taking advantage of the unique physical and biological activities of the quindoline.

To make the quindoline derivatives, the key intermediate of 11-chloroquindoline (**1**) was prepared following the procedure reported by Bierer[6]. The substitution reaction of compound (**1**) with the commercial aliphatic diamines produced the quindoline derivatives containing 4 different types of alkylamine side-chains at the C-11 position (**2a-2d**). The benzoic acid (**3a-3d**) as well as boronic acid modified benzoic acid derivatives (**4a-4d** and **4e-4h)** were obtained by amidation of 11-animo-10*H*-indolo[3,2-*b*]quinoline (**2a-2d**) with benzoic acid or 4-carboxyphenylboronic acid, or 3-carboxyphenylboronic acid, respectively. The reagents, conditions, and yield are shown in Scheme 1.



Scheme 1. Reagents, conditions and yields: (a) $RNH(CH_2)_nNH_2$, $CH_3CH_2OCH_2CH_2OH$, reflux, 17h; (b) benzoic acid, DMT-MM, $CH_3CH_2OCH_2CH_2OH$, r.t., 17h; (c) 4-Carboxyphenylboronic acid or 3-Carboxyphenylboronic acid, DMT-MM, $CH_3CH_2OCH_2CH_2OH$, r.t., 17h;

Since cell surfaces are covered by a variety of carbohydrates, we hypothesized that introducing boronic acid modification to quindoline derivatives might reduce the number of the derivatives entering the cell due to increased interactions between boronic acid and cell surface carbohydrates based on boronolectin studies[1] . As fewer boronic acid modified quindoline derivatives get inside the cells, less cell killing should be observed. Thus, we used two human colon cancer cell lines (HT29 and HCT116) and two human lung cancer cell lines (H1299 and A549) to test the hypothesis.

We first measured the percentage of viable cancer cells after 48 h exposure to the 16 quindoline derivatives (**2a-2d**, **3a-3d, 4a-4d,** and **4e-4h**) at a concentration of 10 μM as compared to the compound-free control (100% viability) (**Fig. 1**). The results supported our hypothesis in that all boronic acid modified quindoline derivatives (**4a-4d** and **4e-4h)** were less toxic to cancer cells than the boronic acid-free quindoline derivatives (**2a-2d and 3a-3d**) in all cancer cell lines tested.

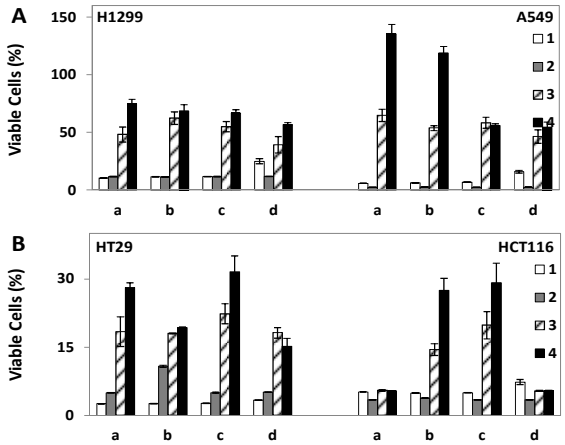


Fig.1. Percentage of viable cancer cells after 48h exposure to the quindoline derivatives **2a-2d**, **3a-3d**, **4a-4d and 4e-4h** at a concentration of 10 μM compared to the compound-free control (100% viability). The derivatives were formulated initially in DMSO and then diluted in complete growth media

(Details in Experimental Protocols) (□, **2a-2d**; ■, **3a-3d**; , **4a-4d**; ■, **4e-4h**). (**A**) Effect on lung cancer cells, H1299 and A549. (**B**) Effect on colon cancer cells, HT29 and HCT116

To confirm this observation, each compound was tested in a series of concentrations to calculate the IC_{50} values in each of the four cell lines (**Table 1**). Interestingly, all aliphatic diamine-modified (**2a-2d)** plus benzoic acid modified (**3a-3d**) quindoline derivatives showed almost the same cytotoxicities in each cell line tested, indicating that different aliphatic chain length (**2a, 2b, and 2d**) and structure (**2c**) plus benzoic acid modifications to quindoline had almost no impact towards observed cytotoxicities. In contrast, introducing a single boronic acid at either 3 (**4e-4h)** or 4 (**4a-4d)** position of the benzoic acid of the derivatives **3a-3d** resulted in greatly reduced cytotoxicities reflecting 3 to 10-fold higher IC_{50} values (**4a-4d** and **4e-4h)** in all four cancer cell lines tested. Furthermore, the quindoline derivatives with 4-boronic acid modification (**4a-4d**) had lower IC_{50} values than that of the derivatives with 3-boronic acid modification (**4e-4h**), suggesting that the location of boronic acid in the quindoline derivatives played a role in the observed cytotoxicities as well (**Fig. 1** and **Table 1**).

Table 1. IC_{50} of quindoline derivatives (**2a-2d**, **3a-3d**, **4a-4h)** in cancer cells HT29, HCT116, A549 and H299 after 48 h exposure to the derivatives.

	IC_{50} (μm)[a]			
Comp.	HT29	HCT116	A549	H1299
2a	0.7±0.14	0.6±0.14	2.1±0.26	1.3±0.66
2b	0.3±0.07	0.3±0.09	1.6±0.89	1.0±0.19
2c	0.4±0.15	0.3±0.11	1.0±0.16	0.62±0.24
2d	1.0±0.40	0.9±0.36	2.1±0.55	0.90±0.06
3a	0.3±0.06	0.8±0.32	1.3±0.24	0.8±0.30
3b	1.3±0.23	1.0±0.32	2.3±0.4	2.1±0.08
3c	0.8±0.24	0.9±0.17	2.4±0.23	2.8±0.15
3d	0.9±0.31	1.2±0.38	3.5±0.48	2.9±0.12
4a	3.7±0.83	2.6±0.56	17.2±2.05	14.1±3.08
4b	2.0±0.01	1.3±0.18	7.8±1.33	6.6±2.26
4c	3.3±0.25	2.1±0.31	14.2±1.18	6.8±1.16
4d	1.6±0.53	1.2±0.30	16.7±6.63	4.2±1.44
4e	5.1±0.36	3.9±0.30	29.1±1.59	12.2±3.56
4f	2.5±0.82	2.0±0.85	14.8±1.49	11.0±3.62
4g	11.9± 4.0	15.7±0.75	23.8±3.43	17.1±3.45
4h	1.7±0.58	1.3±0.74	18.6±5.44	3.4±1.26

a. Each IC_{50} value was calculated from 3 independent experiments performed in triplicate.

Data in **Fig. 1** and **Table 1** showed that all quindoline derivatives (**2a-2d**, **3a-3d, 4a-4d,** and **4e-4h**) were more toxic to the two colon cancer cell lines (HT29 and HCT116) than to the two lung cancer cell lines (A549 and H1299), suggesting that more quindoline derivatives might be associated with colon cancer cells than with lung cancer cells due to different genetic background, such as P-glycoprotein expressing levels[7]. To test this idea, we employed flow cytometry analysis. The logic of this assay is that cancer cells do not have any fluorescence; only cell-associated quindoline derivatives produce fluorescence and the intensity of fluorescence might be in proportion to the amount of quindoline derivatives associated with the cancer cells.

To test this idea, we incubated the same numbers of colon cancer cells (HT29) and lung cancer cells (A549) without (quindoline derivative-free) or with the same amount of the derivatives **2a**, **4a** and **4e** (10 μM) at identical cell culture conditions (24 h at 37 ºC). The cells were then washed three times with PBS followed by flow cytometry analysis. As shown in **Fig. 2A**, the quindoline derivative-free cancer cells indeed showed low auto-fluorescent background (HT29:12.58 ± 0.82 au vs. A549:7.02±2.93 au). In both HT29 and A549 cells, the fluorescent intensity was decreased in the order of the derivatives: **2a** (HT29: 45.82 ± 5.91 au vs. A549: 30.11±7.63 au) > **4a** (HT29: 23.695±1.85 au vs. A549: 11.27±2.68au) > **4e** (HT29: 21.195±1.69au vs. A549: 8.995±4.79au) > the quindoline derivative-free control (HT29: 12.58 ± 0.82 au vs. A549:7.02±2.93 au). After subtracting the auto-fluorescent background, the fluorescent intensities in **2a**, **4a** and **4e** treated HT29 cells were consistently higher than that in **2a**, **4a** and **4e** treated A549 cells. The higher fluorescent intensities correlated perfectly with the higher cytotoxicities (**Fig. 1** and **Table 1)**. These results indicate that the amount of the derivatives associated with the cancer cells was the key to explain the cytotoxicities of the quindoline derivatives.

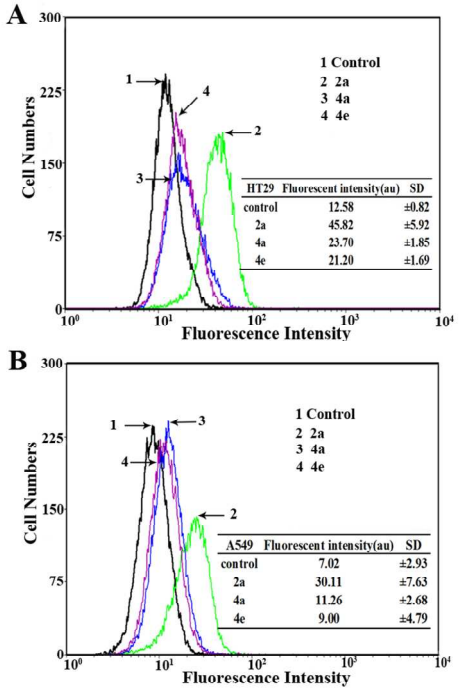


Fig.2. The amount of the derivatives **2a**, **4a** and **4e** associated with the cancer cells. HT29 (**A**) or A549 (**B**) cells were incubated with or without the derivatives **2a**, **4a** or **4e** at a concentration of 10 μM. After incubation for 24h at 37ºC, the cells were harvested, washed and then analyzed by flow cytometry. Ten thousand events were counted for each sample. The result shown is a representative of three independent experiments. Control: cells cultured in complete growth medium; 2: **2a** treated cells; 3: **4a** treated cells; 4: **4e** treated cells.

The results in **Fig. 2** showed that the derivatives **2a**, **4a** and **4e** were associated with HT29 and A549 cells. However, flow cytometry analysis could not precisely determine the location of the derivatives associated with the cancer cells. Therefore, we used confocal imaging analysis to further explore the issue. To

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this end, HT29 and A549 cells were incubated with the derivatives **2a**, **3a**, **4a** and **4e** for 24 h at 37 ºC. The cancer cells were then washed three times before confocal imaging. As shown in **Fig. 3A**, high levels of green fluorescence were detected in the **2a** and **3a** treated HT29 cells, and the fluorescence was mostly found around or in the cell nucleus. In contrast, the **4a** and **4e** treated HT29 cells exhibited less fluorescent intensities than that in the **2a** and **3a** treated HT29 cells, and the observed fluorescence was closer to the cell membranes (**Fig. 3A**).

We also investigated the localization of the derivatives **2a**, **3a**, **4a** and **4e** in A549 cells. The data in **Fig. 3B** showed that the derivative **2a** and **3a** was mostly found around or in the cell nucleus of the A549 cells, but the nuclear localization of **2a** and **3a** in the A549 cells was less than that in the HT29 cells (**Fig. 3A**), which was consistent with the reduced cytotoxicities and cell association of **2a** and **3a** in the A549 cells than in the HT29 cells (**Fig. 1**, **Table 1**, and **Fig. 2**). In agreement with the cytotoxicities and cell association data shown in **Fig. 1**, **Table 1** and **Fig. 2**, the fluorescence observed in the **4a** and **4e** treated A549 cells was less than that in the **2a** and **3a** treated A549 cells, which was consistent with the decreased cytotoxicities in the order of **2a**, **3a, 4a** and **4e** shown in **Fig. 1**, **Table 1**, and **Fig. 2**. Again, the observed fluorescence of the **4a** and **4e** in the A549 cell was closer to the membrane (**Fig. 3B**).

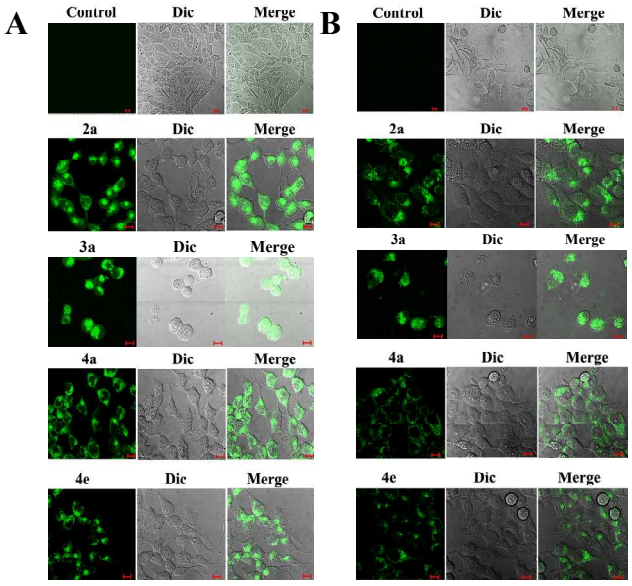

Fig. 3. Distribution of the derivatives **2a**, **3a, 4a** and **4e** in HT29 and A549 cells. Cells were incubated with or without the derivatives **2a**, **3a, 4a** or **4e** at a concentration of 10 μM for 24h at 37ºC. After which, the cells were washed three times with PBS before confocal imaging. (**A**) HT29 cells, (**B**) A549 cells. Scale bar represents 10 μm. Control: non-derivative treated cells.

Conclusions

In summary, boronic acid modifications reduced quindoline's cytotoxicities mainly through decreased cell penetration and nuclear localization that we attributed to the increased cell surface carbohydrate interactions and/or increased difficulties in penetrating cell membranes due to the presence of boronic acid modification in the quindoline derivatives. This study provides useful information about the influence of boronic acid on the cytotoxicities and cellular localizations of the quindoline derivatives, which has important implications in research and development of boronic acid modified drugs.

Notes and references

†These authors contributed equally to this study.
*School of Medicine and Pharmacy, Ocean University of China, 5 Yushan Road, Qingdao, China. Fax: 0086-532-82033054;
E-mails: jiangtao@ouc.edu.cn and lijuanzhang@ouc.edu.cn.

† Electronic Supplementary Information (ESI) available: Experimental procedure, compound characterization, copies of NMR. See DOI: 10.1039/b000000x/

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