Boronic Analogues of (R)-6-O-Desmethyantofine as Anticancer Agents

Ziad Omran,*a Ashraf N. Abdalla,a Munjed M. Ibrahim,a Mohammad A. Hossain,a Mohamed Alarja,a Linwei Chen,b Yuxiu Liu,b and Qingmin Wangb

aCollege of Pharmacy, Umm Al-Qura University; Al-Abidiya, 21955 Makkah, Kingdom of Saudi Arabia; and bState Key Laboratory of Elemento-Organic Chemistry, College of Chemistry, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University; Tianjin 300071, P. R. China.

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Phenanthroindolizidines are naturally occurring alkaloids mainly isolated from different species of Asclepiadaceae. These alkaloids are characterized by an excellent anticancer activity against a very wide range of cancerous cell lines including those who are multi drug resistant. Nevertheless, phenanthroindolizidines are associated with sever neurotoxicity that prevented any candidate from this family to pass the clinical trials. A number of boron-based analogues of (R)-6-O-desmethyantofine have been synthesised. Their physicochemical properties were evaluated, same as their in-vitro antiproliferative activity. The pinacol boronate ester derivative (3) showed interesting cytotoxicity against a panel of cancerous cell lines attested by a cancer cell growth-inhibitory potency (GI50) as low as 30nM.

Key words cancer; phenanthroindolizidine; antofine; boronic bioisostere; antitumor activity

Introduction

Phenanthroindolizidines as exemplified by (R)-6-O-desmethyantofine (1), Fig. 1, are a family of alkaloids mainly isolated from different species of Asclepiadaceae. They are characterized by potent anticancer activity with cancer cell growth-inhibitory potency (GI50) in the low nanomolar range for some derivatives.1 Nevertheless, these alkaloids are associated with severe side-effects such as neurotoxicity which prevented any of these agents to pass the clinical trials.2

Although the specific cellular target of these alkaloids is still unidentified, much is known about their cellular activities. Phenanthroindolizidines exert their anticancer activity via a combination of diverse mechanisms which include inhibition of hypoxia-inducible factor-1 (HIF-1),3 inhibition of DNA, RNA and protein synthesis,4–7 inhibition of thymidylate synthase,8–10 inhibition of dihydrofolate reductase,8–10 inhibition of Activator Protein-1, and nuclear factor-kappaB (NF-kB) and down-regulation of cyclooxygen D1.11,12

Boronic acids and esters emerged recently as biocompatible bioisosters and prodrugs of phenolic alcohols with enhanced bioavailability and selectivity.13–16 Jiang et al. showed that boronic derivatives of tamoxifen, the first line therapy in estrogen receptor positive (ER+) breast cancer, inhibit the growth of (ER+) breast cancer cell lines with potencies comparable to or greater than that of the parent drug, 4-hydroxytamoxifen. Moreover, those analogues were taken up by cancerous cells up to 4 times more efficiently than the parent phenol.13

The aim of this work is to develop boronic derivatives of phenanthroindolizidine alkaloids and to evaluate their antiproliferative activity in-vitro. (R)-6-O-Desmethyantofine (1) was chosen as it is characterized by high cytotoxic activity and it possesses an amenable phenol functional group.

Results and Discussion

The alkaloid (1) was synthesised from 3,4-dimethoxybenzaldehyde and 2-(4-hydroxyphenyl)acetic acid in 12 steps as described earlier.17 The boronic derivatives (3–5) were obtained by modifications to the published procedures,13,18,19 Fig. 2. Briefly, the phenol function of compound (1) was transformed to the corresponding triflate (2) by means of its treatment with N-phenyl-bis(trifluoromethanesulfonimide) and excess of sodium carbonate. The so-obtained triflate was then transformed to the pinacol ester (3) by a PdCl2(dppf) catalysed borolation with bis(pinacolato)diboron. Treating pinacol ester (3) by KHF2 in hydromethanolic solution afforded the trifluoroborate derivative (4) quantitatively. The boronic acid (5) was ultimately obtained by hydrolysis of trifluoroborate (4) using trimethylsilyl chloride.

Unfortunately, derivatives (4) and (5) revealed to be unstable, as they were completely transformed to the corresponding phenol within a few days at room temperature in solid state. Nonetheless, boronate ester (3) showed good stability, as less than 5% of (3) was degraded after 3.5h, and less than 50% after one week of incubation in phosphate buffer pH 7.4 at room temperature, Figure S1. The physicochemical properties of boronate ester (3) were then evaluated and compared to the parent alkaloid (1), Table 1. While the water solubility of (R)-6-O-desmethyantofine (1) at pH 7.4 was slightly increased upon the transformation of the phenolic function into its boronic isostere; the logD was increased by one unit. The Blood Brain Barrier Parallel Artificial Membrane Permeability Assay (BBB-PAMPA) predicted that (R)-6-O-desmethyantofine...
**fine (1) would have similar BBB permeability as its boronic analogue (3).**

The cytotoxicities of (R)-6-O-desmethyl antofine (1), the developed ester (3) and doxorubicin as reference were evaluated in resazurin-based assay 20) against six cancerous cell lines: neuroblastoma (SK-N-DZ), human prostate cancer cells (DU145), Burkitt’s lymphoma (Ramos), hepatocarcinoma (HepG2), colorectal adenocarcinoma (DLD-1), and breast adenocarcinoma (MCF7), Table 2.

Both compounds (1) and (3) exhibited potent cytotoxicity to SK-N-DZ, Ramos, HepG2 and DLD-1 cell lines, up to 100 folds more than those of doxorubicin, and weak cytotoxicity to DU-145 and MCF-7 cell lines. Although, the replacement of the phenolic function of (R)-6-O-desmethyl antofine by boronate ester did not result in a significant improvement in the biological activity, this work confirms the biological utility of boronate functional groups.

**Conclusion**

In conclusion, we successfully synthesised boronic derivatives (3–5) of the highly cytotoxic alkaloid (R)-6-O-desmethyl antofine (1). While the trifluoroborate (4) and the boronic ester (5) derivatives revealed to be unstable, the pinacol boronate ester analogue (3) showed good stability and physiochemical properties. The developed ester (3) displayed a potent in-vitro cytotoxicity profile comparable to that of the naturally occurring alkaloid (1).

**Experimental Chemistry**

(R)-2,3-Dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[f,h]-pyrrolo[1,2-b]isoquinolin-6-yl Trifluoromethanesulfonate (2)

To a solution of (R)-6-O-desmethylantofine (2.0 g, 5.72 mmol) and Na2CO3 (3.03 g, 28.6 mmol) in N,N-dimethylformamide (DMF) (40 mL) was added N-phenylbis(trifluoromethanesulphonimide) (3.07 g, 8.59 mmol). The reaction mixture was stirred at room temperature for 3 h and then quenched with water (300 mL). The resulting aqueous solution was extracted with EtOAc (50 mL × 3), and the combined organic phase was washed sequentially with saturated NaHCO3 aqueous and brine, dried over with Na2SO4, filtered, and concentrated under reduced pressure to give the attempted triflate compound (2.33 g, 85%) as a yellow solid.

**Table 1. Physicochemical Properties of (R)-6-O-Desmethyl Antofine (1) and Its Boronic Analogue (3), Values Are the Average of 2 Independent Experiments**

<table>
<thead>
<tr>
<th>Compound</th>
<th>PBS solubility µM Mean ± S.E.</th>
<th>Log D Mean ± S.E.</th>
<th>Permeability Log [10⁻⁶ cm/s] Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>12 ± 0</td>
<td>3.41 ± 0.3</td>
<td>−5.4 ± 0.37</td>
</tr>
<tr>
<td>(3)</td>
<td>62 ± 3</td>
<td>4.48 ± 0.03</td>
<td>−5.3 ± 0.13</td>
</tr>
</tbody>
</table>

**Table 2. Cytotoxicity of (R)-6-O-Desmethyl Antofine (1) and Its Boronic Analogue (3), Values Are the Average of 4 Independent Experiments**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM) Cell lines</th>
<th>SK-N-DZ</th>
<th>DU145</th>
<th>Ramos</th>
<th>HepG2</th>
<th>DLD-1</th>
<th>MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>1.3</td>
<td>1.7</td>
<td>2.6</td>
<td>2.9</td>
<td>15.2</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>(1)</td>
<td>0.007</td>
<td>&gt;15.0</td>
<td>0.03</td>
<td>0.03</td>
<td>0.08</td>
<td>&gt;9.0</td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td>0.07</td>
<td>&gt;15.0</td>
<td>0.04</td>
<td>0.07</td>
<td>0.03</td>
<td>&gt;15.0</td>
<td></td>
</tr>
</tbody>
</table>

To a solution of (R)-6-O-desmethylantofine (2.0 g, 5.72 mmol) and Na2CO3 (3.03 g, 28.6 mmol) in N,N-dimethylformamide (DMF) (40 mL) was added N-phenylbis(trifluoromethanesulphonimide) (3.07 g, 8.59 mmol). The reaction mixture was stirred at room temperature for 3 h and then quenched with water (300 mL). The resulting aqueous solution was extracted with EtOAc (50 mL × 3), and the combined organic phase was washed sequentially with saturated NaHCO3 aqueous and brine, dried over with Na2SO4, filtered, and concentrated under reduced pressure to give the attempted trflate compound (2.33 g, 85%) as a yellow solid.

**mp:** 133–135°C; ¹H-NMR (400 MHz, CDCl3) δ: 8.28 (s, 1H), 7.87 (d, J = 9.0 Hz, 1H), 7.76 (s, 1H), 7.39 (d, J = 8.7 Hz, 1H), 7.26 (s, 1H), 4.63 (d, J = 14.8 Hz, 1H), 4.12 (s, 3H), 4.05 (s, 3H), 3.66 (d, J = 14.8 Hz, 1H), 3.46 (t, J = 8.0 Hz, 1H), 3.29 (d, J = 15.4 Hz, 1H), 2.94–2.80 (m, 1H), 2.54–2.40 (m, 2H), 2.29–2.18 (m, 1H), 2.11–1.85 (m, 2H), 1.83–1.68 (m, 1H); ¹³C-NMR (100 MHz, CDCl3) δ: 150.1, 149.0, 147.2, 129.9, 129.6, 128.5, 127.1, 126.0, 125.1, 123.3, 118.9 (q, J = 320 Hz, CF3), 118.4, 114.8, 103.9, 103.5, 60.0, 56.1, 55.9, 55.0, 53.6, 33.6, 31.2, 21.6.
(R)-2,3-Dimethoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,11,12,13a,14-hexahydrodibenzo[f,h]-pyrrolo[1,2-b]isoquinoline (3)

To a 1,4-dioxane solution of the previously obtained triflate (0.274 g, 0.57 mmol) were added bis(pinacolato) diboron (0.16 g, 0.63 mmol), PdCl2(dppf) (0.025 g, 5% mol) and KOAc (0.14 g, 0.274 g, 0.57 mmol) were added bis(pinacolato) diboron (0.16 g, 0.57 mmol), and the mixture was stirred under an atmosphere of nitrogen at 80°C overnight. After the solution was cooled, the solvent was removed under vacuum, and then CH2Cl2 and water were added. The resulting mixture was extracted with dichloromethane twice, and the combined organic layer was washed with brine and then dried over Na2SO4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH2Cl2 : MeOH = 40 : 1) to give compound 1 (0.16 g, 60%) as a dark solid. mp: 134–136°C; purity 99.4%, enantiomeric excess (ee) 82.4% (by HPLC, CHIRALPAK AD-H, 250 x 4.6 mm, eluent: i-ProH : Hexane = 25 : 75 : 0.1, flow speed: 1 mL/min, wave: 254 nm, tR = 5.795 min); 1H-NMR (400 MHz, CDCl3) δ: 8.86 (s, 1H), 7.94 (s, 1H), 7.89 (d, J = 8.1 Hz, 1H), 7.66 (d, J = 8.3 Hz, 1H), 6.99 (s, 1H), 4.64 (d, J = 15.0 Hz, 1H), 4.08 (s, 3H), 3.93 (s, 3H), 3.62 (d, J = 15.1 Hz, 1H), 3.51–3.42 (m, 2H), 2.58–2.36 (m, 2H), 1.92–1.78 (m, 2H), 1.72–1.57 (m, 1H); 13C-NMR (100 MHz, CDCl3) δ: 149.2, 148.9, 148.1, 131.3, 130.6, 129.7, 128.5, 128.0, 125.7, 124.4, 124.1, 121.6, 103.7, 103.6, 84.1, 60.3, 56.2, 55.8, 54.3, 52.5, 32.1, 30.4, 25.0, 21.1. High resolution (HR) MS electrospray ionization (ESI) calcld for C22H25BNO4 (M + H)⁺ 460.2659, found 460.2662.

(−)-2,3-Dimethoxy-9,11,12,13a,14-hexahydrodibenzo[f,j]pyrrolo[1,2-b]isoquinolin-6-yl) Trifluoroborate (4)

A 100 mL round-bottom flask was charged with boronic acid pinacol ester 3 (0.84 g, 1.83 mmol) and KHF2 (0.57 g, 7.31 mmol). A premixed MeOH/H2O solution (V : V = 75 : 25 : 0.1, flow speed: 1 mL/min, wave: 254 nm, tR = 5.795 min); 1H-NMR (400 MHz, CDCl3) δ: 8.54 (s, 1H), 8.05 (s, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.35 (s, 1H), 4.88 (d, J = 15.3 Hz, 1H), 4.01 (s, 3H), 3.95 (s, 3H), 3.55 (d, J = 15.3 Hz, 1H), 3.34–3.30 (m, 1H), 2.84–2.74 (m, 1H), 2.46–2.28 (m, 2H), 2.19–2.13 (m, 1H), 1.92–1.78 (m, 2H), 1.72–1.57 (m, 1H); 13C-NMR (100 MHz, CDCl3) δ: 148.5, 148.1, 130.3, 127.1, 127.0, 126.8, 125.8, 125.5, 125.4, 123.9, 120.2, 104.2, 103.3, 60.0, 55.4, 55.4, 54.3, 33.0, 30.8, 21.1; HRMS (ESI) calcld for C22H13BF4KO2N2O (M + H)⁺ 440.1411, found 440.1418.

The cells were washed with phosphate buffered saline (PBS) pH 7.4 before they were trypsinized with 1X trypsin in DPBS. The appropriate volume of culture medium was then placed in a flask to stop trypsinization and cells were counted using a counting chamber after being stained with Trypan Blue (Czech). The appropriate volume of the cell solution was introduced into a new falcon tube containing completed DMEM medium to give the desired final concentration. In sterile 384-well black wall clear flat bottom plates, 45 µL of cells suspension were introduced. The concentrations of the suspensions were 1 x 104 cells/mL for DU145, Ramos and SK-N-DZ cells, 2.5 x 104 for MCF-7 and DLD-1, and 5 x 104 for HepG2 cells. Plates with cells were left overnight in humidified atmosphere at 37°C and 5% CO2 for adaptation and adherence. The appropriate concentrations of cells correspond to 20–40% of confluence before incubation with drugs. To points 3-fold serial dilutions in 100% DMSO from 20 mM stock of doxorubicin and from corresponding 5 and 10 mM stocks of test compounds were made. For each concentration point of serial dilution was made 20-fold intermediate dilution in PBS: 5 + 95 µL of PBS. Five microliter doxorubicin and test compounds to a final concentration of doxorubicin from 0.004 to 100.0 µM and test compounds from 0.002 to 50 µM (10 mM stock), from 0.001 to 25 µM (5 mM stock) in quadruplicates were added and incubated for 48h with cells in humidified atmosphere at 37°C and 5% CO2. The final concentration of DMSO in assay was 0.5%. Ten percent of 500 µM resazurin in PBS (5,5′μL) to 50 µM final concentration were added and incubated for 3h in humidified atmosphere at 37°C and 5% CO2. The presence of resorufin was quantified by measuring fluorescence Ex = 555 nm, Em = 585 nm (protocol for Cell viability assay “CellTiter-Blue” fluorescence in SoftMaxPro (Molecular devices)).21,22

Water solubility, logD and PAMPA-BBB were evaluated using the protocols we previously descried.23

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Conflict of Interest  The authors declare no conflict of interest.

Supplementary Materials  The online version of this article contains supplementary materials (spectra for compounds (3–5), and stability figure of boronic ester (3) in aqueous media).

References