Chalcone-Benzoxaborole Hybrids as Novel Anticancer Agents

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ABSTRACT

In this study, we report the synthesis of a series of chalcone-benzoxaborole hybrid molecules and the evaluation of their anticancer activity. Their anticancer potency and toxicity were tested on three human cancer cell lines and two normal cell lines. The 4-fluoro compound **15** was found to be the most potent compound with an IC₅₀ value of 1.4 μM on SKOV3 cells. The 4-Iodo compound **18** and 3-methoxy-4-amino compound **47** showed good potency on SKOV3 cells while exhibiting low toxicity on normal cells. This work extended the application of benzoxaboroles to the field of anticancer research.

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Cancer, as one of the major causes of death, seriously endangers human health.¹ In 2015 alone, an estimated 2.8 and 0.6 million cancer deaths occurred in China² and United States,³ respectively. While chemotherapy remains the main treatment, clinically used anticancer drugs are generally limited by toxicity, side effects, and resistance.^{4,5} Consequently, it remains urgent to develop novel anti-cancer agents. During the past decade, boron-containing compounds has started to attract much attention from medicinal chemists.^{6,7} The boronic acid-containing drug bortezomib (Velcade) (Figure 1), a proteasome inhibitor,⁸ was approved in 2003 to treat multiple myeloma.⁹ An interesting class of cyclic boron-containing compounds, benzoxaboroles, were first synthesized in 1957¹⁰ while its medicinal usage had remained unexplored for fifty years until tavaborole (Kerydin) (Figure 1) was approved in 2014 for the treatment of fungal infection of the toenails.^{11,12} To date, benzoxaboroles have been investigated for their antifungal, antibacterial, anti-parasite, antiviral, and anti-inflammatory properties.¹¹⁻¹⁹ However, an investigation of their anticancer activity has been lacking. Although Kunar and coworkers tested a few benzoxaboroles against cancer cell line, their compounds failed to show any significant inhibitory activity.²⁰ Li and coworkers reported benzoxaborole-containing phenylalanine analogues which have potential applications in boron neutron capture therapy (BNCT), but subsequent study was not reported.²¹

In order to develop novel anticancer agents and extend the range of application of benzoxaboroles, we screened a series of

Figure 1. Structures of bortezomib and tavaborole.

benzoxaboroles against three human cancer cell lines, SKOV3 (ovarian carcinoma), MDA-MB231 (breast cancer), and HCT116 (colon colorectal carcinoma), and two human normal cell lines, MCF-10A (human mammary epithelial cells) and WI-38 (lung fibroblast) by MTT assay.²² The chalcone-benzoxaborole hybrid molecule **8** showed inhibitory activity against SKOV3, MDA-MB231, and HCT116 cell lines with IC₅₀ value of 3.4, 2.3, and 6.1 μM, respectively. It also showed an 8-fold selectivity between human cancer cells (MDA-MB231) and normal cells (WI-38). Further investigation of the derivatives of compound **8** led to the discovery of anticancer agents with IC₅₀ value as low as 1.4 μM (compound **15**) and compounds **18** and **47** which possess both high potency and good selectivity.

The compounds were synthesized according to our previously reported methods.²³ As shown in Scheme 1, 2,6-dimethylbromobenzene (**1**) was oxidized to 2-bromoterephthalic acid **2** which was subsequently converted to methyl ester **3**. Reduction of ester **3** gave benzyl alcohol **4** which underwent protection and treatment with *n*-BuLi and triisopropyl borate, followed by deprotection and cyclization in 6 M HCl to give

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benzoxaborole **6**. Oxidation of alcohol **6** gave aldehyde **7** which underwent aldol condensation to give compounds **8-45**. Compound **46** was obtained by alkali hydrolysis from ester **27**. Compound **47** was obtained from nitro compound **45** by SnCl₂ reduction.

Scheme 1. Reagents and conditions: (a) KMnO₄, *t*-BuOH, H₂O, 70 °C; (b) SOCl₂, reflux; (c) MeOH, Et₃N; (d) LiBH₄, MeOH, THF; (e) DHP, *p*-TsOH, DMF; (f) *n*-BuLi, B(*i*-PrO)₃, THF, -78 °C; (g) 6 M HCl; (h) PCC, Celite, DCM; (i) NaOH, EtOH, H₂O; (j) CH₃COCl, EtOH.

Scheme 2. Reagents and conditions: (a) NaOH, EtOH, H₂O; (b) SnCl₂·2H₂O, EtOH.

First, we explored the effect of different aromatic rings on anticancer activity. As shown in Table 1, bulky aromatic rings such as naphthyl (**9**) and biphenyl (**10**) showed activity comparable to compound **8**. However, heterocycles including furanyl (**11**), thiophenyl (**12**), and pyridinyl (**13**) gave significantly reduced inhibitory activity. In addition, the

Table 1. The effect of different aromatic A rings.

Compound	R	^a IC ₅₀ (μM)				
		SKOV3	MDA-MB231	HCT116	MCF-10A	WI-38
8		3.4	2.3	6.1	15.9	19.1
9		2.7	3.6	5.0	13.4	18.8
10		4.9	6.4	9.5	20.7	18.3
11		20.7	7.3	24.1	7.4	59.0
12		10.4	4.6	14.2	13.8	26.0
13		18.5	11.4	20.5	34.4	76.2
14		93.6	>100	>100	>100	>100
Doxorubicin		0.42	0.07	0.31	0.06	9.0

Cisplatin	12.8	41.8	21.7	11.0	>100
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^a IC₅₀ indicates compound concentration required to inhibit cell viability by 50%. Values are expressed as the mean of triplicate experiments.

N-methyl-pyrrolyl compound **14** showed completely diminished activity. Therefore, we next continued to investigate the substituted phenyl compounds for their anticancer activity.

As shown in Table 2, the anticancer activity of *para*-substituted phenyl compounds is discussed. Compared to unsubstituted phenyl compound **8**, *p*-fluoro compound **15** showed a 2-fold increase of potency, while *p*-chloro, -bromo, and -iodo compounds **16-18** showed moderately decreased potency. It is worth mentioning that the *p*-iodo compound **18** showed more than 25-fold of selectivity between cancerous cells (SKOV3) and normal cells (MCF-10A and WI-38). The *p*-methyl, -ethyl, -methylthio, and -benzyloxy compounds **19-22** showed moderate decrease of activity. Introduction of hydrogen bond donors, including hydroxyl (**23**) and amino (**24**), gave significantly reduced potency. Electron withdrawing groups, including trifluoromethyl (**25**), nitro (**26**), and ester (**27**) resulted in moderate decrease of potency, while the carboxylic acid **46** completely lost inhibitory potency.

Table 2. The effect of different *para*-substitutions.

Compound	R	^a IC ₅₀ (μM)				
		SKOV3	MDA-MB231	HCT116	MCF-10A	WI-38
15		1.4	1.6	2.8	11.8	7.6
16		3.7	7.9	9.3	12.1	24.7
17		4.5	9.5	9.9	27.8	36.7
18		4.2	14.9	11.6	>100	>100
19		3.7	6.5	10.5	8.3	22.2
20		4.4	9.8	10.9	8.8	20.6
21		4.1	9.8	10.3	7.4	54.3
22		6.0	5.3	12.1	23.3	>100
23		13.0	25.6	24.1	24.8	>100
24		20.2	25.8	26.1	19.9	25.4
25		3.7	9.2	6.3	22.3	22.6
26		7.9	11.2	9.1	17.5	39.3
27		7.1	13.0	7.6	12.9	47.2
46		>100	>100	>100	>100	>100

^a IC₅₀ indicates compound concentration required to inhibit cell viability by 50%. Values are expressed as the mean of triplicate experiments.

We also explored more substitution patterns on the phenyl group as shown in Table 3. The *o*- and *m*-fluoro compounds **28-29**, dichloro compound **30**, and methyl compounds **31-32** showed

potency comparable to parent compound **8**. Although the phenols **23** and **33-34** all showed significantly decreased or even abolished activity, the *m*-OH compound **35** showed activity comparable to compound **8**. Similarly, the *m*-NH₂ compound **36** also showed good activity although its *p*-NH₂ analog **24** gave poor activity. Introduction of single methoxy group (**37-38**) gave inhibitory activity comparable to compound **8**, while multiple methoxy or alkoxy groups gave moderately reduced activity (**39-44**). Introduction of an electron withdrawing group and an electron donating group simultaneously (**45**) gave good inhibitory potency against SKOV3 cells (IC₅₀ = 1.7 μM), however it also exhibited strong toxicity to human normal MCF-10A cells (IC₅₀ = 3.6 μM). However, its amino analog **47** retained the high anticancer activity while showed low toxicity on human normal WI-38 cells.

Table 3. The effect of different phenyl substitutions.

Compound	R	^a IC ₅₀ (μM)				
		SKOV3	MDA-MB231	HCT116	MCF-10A	WI-38
28		3.7	2.2	4.2	25.4	23.6
29		4.8	3.0	6.7	15.8	26.7
30		1.8	3.4	3.2	14.9	12.5
31		2.9	4.5	6.7	15.9	16.7
32		1.9	5.3	6.1	4.7	22.0
33		48.8	16.9	77.9	53.7	>100
34		>100	67.6	>100	>100	>100
35		3.8	2.7	6.5	25.3	26.4
36		7.4	3.2	4.5	19.6	25.3
37		2.6	3.1	5.0	9.1	21.7
38		2.6	2.1	5.0	7.4	14.6
39		6.1	11.2	16.2	12.2	86.3
40		4.8	13.2	12.2	13.5	33.5
41		5.7	10.6	7.9	48.5	18.5
42		6.5	10.2	10.2	11.7	27.0
43		11.7	4.5	13.3	48.1	33.2
44		7.3	7.9	10.3	11.7	30.2
45		1.7	11.4	4.1	3.6	26.0

47		1.7	24.3	27.9	17.6	>100
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^a IC₅₀ indicates compound concentration required to inhibit cell viability by 50%. Values are expressed as the mean of triplicates experiments.

In summary, we reported the anticancer activity of a series of chalcone-benzoxaborole hybrid compounds. We have shown that the terminal phenyl group can tolerate bulky aromatic rings, and it is sensitive to the substitution pattern and the electronic properties of the substituents. Iodo- and amino-groups are beneficial for selectivity between cancerous and normal cells. Compound **15** showed both high potency on cancer cells and high toxicity on normal cells. Compounds **18** and **47** showed high potency on cancer cells with low toxicity on normal cells. This structure-activity relationship provided a basis for further optimization. To date, despite of their application in antifungal, antibacterial, antiviral, and antiparasitic research, benzoxaboroles have not been reported for any significant anticancer activity. This work showed that bezoxaboroles may serve as a potentially useful class of anticancer agents. However, their mechanism of action remains to be elucidated. In previous reports, their biological activities were mainly assessed by phenotypic screening while the cellular targets remained unknown. Much effort has been made to reveal their cellular targets, such as the successful identification of an antifungal target leucyl-tRNA synthetases (LeuRS).¹² Although LeuRS was also suggested as an anticancer target²⁴ our chalcone-benzoxaboroles did not show any significant inhibition of it. In addition, it has been reported that specific benzoxaboroles inhibited β -lactamases¹⁵ and HCV NS3 protease.¹⁸ But these targets are not involved in anticancer activity. We are currently making efforts to investigate the cellular targets of these molecules in cancer cells and will report our progress in due time.

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22. Cells were seeded into 96-well plates and treated with compounds at various concentrations for 48 h. MTT (20 μ L, 5 mg/mL) was added to each well. After incubation for 4 h, MTT solution was removed and 150 μ L DMSO was added to each well. Optical density (OD) was determined at 550 nm on a plate reader (Thermo Varioskan Flash). Data were analyzed with GraphPad Prism 5 to obtain IC₅₀ curves. Each compound was tested in triplicate.
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Graphical Abstract

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<p>Chalcone-Benzoxaborole Hybrids as Novel Anticancer Agents</p>	<p>Leave this area blank for abstract info.</p>
<p>Jiong Zhang, Fei Yang, Zhitao Qiao, Mingyan Zhu, Huchen Zhou</p> <div><div> <p>$IC_{50}(SKOV3):$ $4.2\ \mu M$ $IC_{50}(WI-38):$ $> 100\ \mu M$</p></div><div> <p>18</p></div><div> <p>47</p></div></div> <div><div>$IC_{50}(SKOV3):$ $4.2\ \mu M$ $IC_{50}(WI-38):$ $> 100\ \mu M$</div><div>$1.7\ \mu M$ $> 100\ \mu M$</div></div>	