Letters

Design, Synthesis, and Evaluation of Novel Boronic-Chalcone Derivatives as Antitumor Agents

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Abstract: A series of boronic-chalcone derivatives were synthesized and tested for antitumor activity against human breast cancer cell lines. The results show the boronic-chalcones are more toxic to breast cancer cells compared to normal breast cells than other known chalcones.

Introduction. Breast cancer is expected to account for 203 500 new cancer cases and 39 600 deaths in 2002.[1] Although major advances have been made in early detection, prevention, and treatment, the need for more effective therapy in the fight against late-stage breast cancer continues. Currently there is no curative treatment for women with metastatic breast cancer once they have failed adjuvant therapies. New, effective cytotoxic agents with novel mechanisms of action are therefore urgently needed for the treatment of women with metastatic breast cancer.

The mouse double minute 2 (MDM2) oncogene has been suggested as a target for breast cancer therapy.[2,3] It is amplified or overexpressed in human breast cancer. The oncoprotein MDM2 inhibits the tumor suppressor protein p53 by binding to the p53 transactivation domain. The p53 gene is inactivated in human cancer either by mutations or by binding to oncogenic proteins such as MDM2.[4−7] In breast tumors, overexpression of MDM2 inactivates an otherwise intact p53, disabling the genome integrity checkpoint and allowing cell cycle progression of defective cells.[8] Studies comparing MDM2 overexpression and p53 mutation concluded that these are mutually exclusive events, supporting the notion that the primary impact of MDM2 amplification in cancer cells is the inactivation of the endogenous wild-type p53.[7] It has been shown recently that a peptide homologue of p53 is sufficient to induce p53-dependent death of cells overexpressing MDM2.[9] This result provides clear evidence that disruption of the p53/MDM2 complex might be effective in cancer therapy. It has been shown that MDM2 additionally has a role in tumor growth p53-independent mechanisms.[10−15]

Chalcones are a class of anticancer agents that have shown promising therapeutic efficacy for the management of human cancers. Chalcones, considered as the precursor of flavonoids and isoflavonoids, are abundant in edible plants. Chemically they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system. Licochalcone-A, a chalcone derivative found in the licorice root, has been associated with a wide variety of anticancer effects.[16] Chalcones inhibit the proliferation of both established and primary ovarian cancer cells.[17] In vivo, chalcones have been demonstrated to be effective as antitumor agents in skin carcinogenesis[18,19] and chemopreventive agents in several experimental models.[20−22] Recent studies have shown that these chalcones induce apoptosis in variety of cell types, including breast cancers.[23−26] Biochemical experiments have shown that these compounds could disrupt the MDM2/p53 protein complex, releasing p53 from both the p53/MDM2 and DNA-bound p53/MDM2 complexes.[27] However, carboxylic acid analogues of chalcone **3g** and **9** reported in the literature[27] are equally toxic to both normal and malignant breast epithelial cells. The toxicity to normal breast cells may be due to MDM2/p53-independent mechanisms. Therefore, a chalcone derivative that could bind strongly and irreversibly to disrupt MDM2 protein complexes may be selectively toxic to MDM2 overexpressing breast cancer cells. A novel boronic-chalcone strategy outlined in this paper should overcome the limiting lack of specificity of carboxylic acid analogues of chalcones.

Boronic chalcone analogues have been previously described. These compounds have been used as fluorescent probes that may be useful for detection of fluorides[28] and saccharides such as glucose that may be applicable to the design of biosensors for diabetes.[28] Previous studies[27,29] on the binding modes of carboxylic acid analogues of chalcones **3g** and **9** with MDM2 revealed that the carboxylic acid group could be placed near the base of lysine51 (K51), which is found in a salt bridge interaction with glutamic acid25 (E25). It was presumed that the acid group of the chalcone forms a salt bridge with K51 and simultaneously breaks the salt bridge with E25 of the MDM2. We envisioned that the boronic acid analogue might form a stronger salt bridge with K51 of MDM2 than the corresponding carboxylic acid analogue of chalcones.

Here we investigate the potential value of MDM2 as a drug target for breast cancer therapy by inhibiting MDM2 expression with specific boronic-chalcone analogues. We hypothesize that anti-MDM2 boronic-chalcone analogues will selectively inhibit growth of breast cancer cells. To test our hypothesis, we systematically designed a set of boronic acid-chalcone derivatives and tested their ability to selectively kill breast cancer vs normal breast epithelial cells. To our knowledge, there are no reports investigating the anticancer activity of boronic-chalcones on different cancer cell lines. Thus the identification of new boronic-chalcone analogues will be important in the continued development of this class of agents as anticancer drugs.

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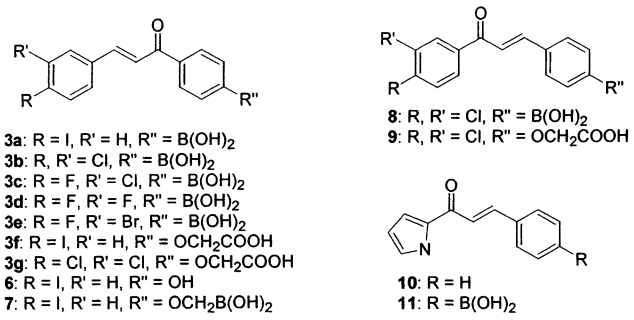
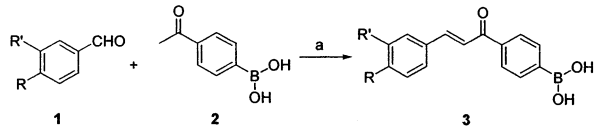
Chart 1. Structures of Boronic Chalcones Used in This Work

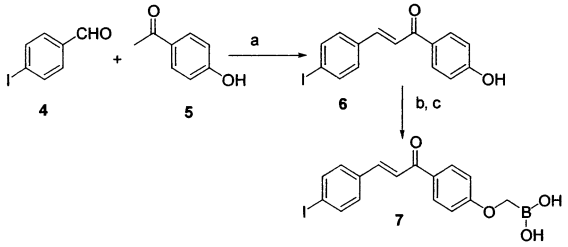


Scheme 1[a]



[a] Reagents: (a) KOH, MeOH, reflux.

Scheme 2[b]



[b] Reagents: (a) KOH, MeOH, reflux. (b) NaH, pinacol (bromomethyl)boronate, THF. (c) NaOH, H_2O.

Boronic acids are Lewis acids and isosteres of carboxylic acid. The pK_a's of boronic acids are ~9−10, and therefore at physiological pH, boronic acids remain unionized.[30] Thus, a coordinate covalent bond (boron−nitrogen) can be formed between a electron-deficient boronic acid moiety and electron-donating amino group, which may strongly enhance binding of boronic-chalcones with the lysine51 of MDM2 at neutral pH when compared to the corresponding carboxylic acid analogue of chalcones. Here we report the design and synthesis of a series of potent antitumor boronic-chalcone derivatives. Compound **7** displays the highest activity for the breast cancer cell lines that we tested. This compound has been shown to be 6−9-fold less toxic to normal MCF-12A cell lines.

Chemistry. Chart 1 shows the compound series used in this study. Compounds **3a**−**g**, **6**, **8**, **9**, **10**, and **11** were synthesized according to standard Claisen−Schmidt aldol condensation protocols as previously published[31,32] (Scheme 1). Compound **7** was prepared by treating compound **6** with pinacol (bromomethyl)boronate in the presence of sodium hydride in DMSO and further deprotection in alkaline condition[33] (Scheme 2).

Biology. Human breast cancer MDA-MB-231 (estrogen receptor negative) and wtMCF7 (estrogen receptor positive) cells were maintained in DMEM medium supplemented with 5% fetal bovine serum, 2 mM glutamine, and 100 units/mL penicillin/streptomycin. MDA-MB-435 (estrogen receptor negative human breast cancer cells) was maintained in IMEM medium supplemented with 5% fetal bovine serum and 2 mM glutamine.

Table 1. Chalcones Inhibit Growth of Human Breast Cell Lines[a]

compd	MDA-MB-435	MDA-MB-231	Wt-MCF7	MCF-10A	MCF-12A
3a	10	8.8	7.0	75	63
3b	3.5	9.5	5.0	18	11
3c	16	8.5	6.0	25	22
3d	8.8	8.8	7.8	18	39
3e	8.8	9.5	8.5	17	38
3f	18	44	9	44	38
3g	9	9	13	13	15
6	4.5	8	7	15	30
7	18	11	9.5	38	100
8	4	8	5.5	18	15
9	13	18	15	12	28
10	15	15	9	63	38
11	15	23	19	38	60

[a] IC_{50} values expressed in μM; see biology section for details of the MTT assay.

Normal breast epithelial cell lines, MCF-10A and MCF-12A, were maintained in 5% and 10% horse serum in DMEM:Ham's F12 media, respectively, supplemented with 2 mM glutamine, 100 units/mL penicillin/streptomycin, 0.02 μg/mL EGF, 0.01 mg/mL insulin, and 0.1 μg/mL cholera toxin.

Cells were incubated at 37 °C in a 5% CO_2 atmosphere. The MTT colorimetric assay was used to determine growth inhibition.[34] Cells were plated in 96-well plates and allowed to attach for 24 h. Chalcone derivatives were dissolved in DMSO at 10 mM concentrations. Cells were expose in quadruplicate well to chalcone concentrations of 0.5−100 μM for 96 h. After 96 h the media was aspirated, and 100 μL of 1 mg/mL MTT solution (Sigma Chemical Co.) diluted in serum free media was added to each well. After 4 h of incubation, the MTT solution was removed and 200 μL of 1:1 (v/v) solution of DMSO:ethanol was added to each well to dissolve formazan crystals. The absorbance at $A_{540\ nm}$ was determined on a plate reader. IC_{50} values were determined from log plots of percent of control vs concentration. Each compound was assayed twice in quadruplicate.

Results and Discussion. IC_{50} values were used to determine growth inhibition in the presence of chalcone derivatives. Of particular interest are compounds able to differentially inhibit growth such that human breast cancer cell lines are inhibited, but normal breast epithelial cells are significantly less inhibited. Compound **3a**, **3d**, **3e**, **7**, **8**, **10**, and **11** are 5−10-folds more toxic to human breast cancer cell lines compared to normal breast epithelial cell lines (Table 1). In the presence of these compounds, cell growth in the human breast cancer cell lines MDA-MB-435, MDA-MB-231, and wt-MCF7 is inhibited, indicated by the range of low IC_{50} values from 3.5 to 23. Cell growth in the normal breast epithelial cell lines MCF-10A and MCF-12A is less inhibited, shown by higher IC_{50} values ranging from 11 to 75.

Conclusions. Here we describe a series of boronic-chalcones that inhibit growth of human breast cell lines at micromolar concentrations. Several are particularly interesting as they preferentially inhibit growth of human breast cancer cell lines. The results show the boronic-chalcones are more toxic to breast cancer cells compared to normal breast cells than other known chalcones. These antitumor compounds will now be tested in various other cancer cell lines. Structure−

activity relationship studies of this new class of compounds are continuing and will be reported in due course.

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Supporting Information Available: ^{1}H NMR (400 MHz) and mass spectral (EI mode) data for the compounds **3a**−**g**, **6**, **7**, **8**, **9**, **10**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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