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Anticancer activities of novel chalcone and bis-chalcone derivatives

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Abstract—A series of novel chalcones and bis-chalcones containing boronic acid moieties has been synthesized and evaluated for antitumor activity against the human breast cancer MDA-MB-231 (estrogen receptor-negative) and MCF7 (estrogen receptor-positive) cell lines and against two normal breast epithelial cell lines, MCF-10A and MCF-12A. These molecules inhibited the growth of the human breast cancer cell lines at low micromolar to nanomolar concentrations, with five of them (**1**–**4**, **9**) showing preferential inhibition of the human breast cancer cell lines. Furthermore, bis-chalcone **8** exhibited a more potent inhibition of colon cancer cells expressing wild-type p53 than of an isogenic cell line that was p53-null.

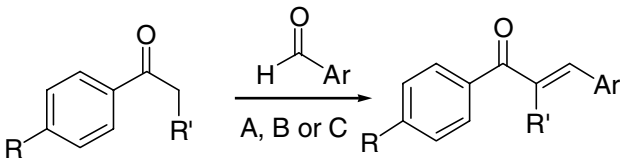
1. Introduction

Despite recent advances in our understanding of the biological processes leading to the development of cancer, there is still a need for new and effective agents to help bring this disease under control. Among the currently identified antitumor agents, chalcones represent an important class of molecules that are abundant in edible plants. Chemically, they are open-chained molecules bearing two aromatic rings linked by a three-carbon enone fragment. Many of these molecules exhibit beneficial biological activities, including anti-inflammatory,[1–3] antipyretic,[4] and anti-invasive[5,6] properties.

Recent reports have focused on the antiproliferative,[7,8] and tumor-reducing[9] activities of some chalcones, causing renewed interest in this class of molecules. These broad antitumor properties have prompted us to synthesize a series of novel boronic chalcones and to evaluate these compounds at the molecular level. We have previously shown[10] that a set of chalcones containing the boronic acid moiety possesses selective antiproliferative activity against breast cancer cell lines when compared to normal epithelial cell lines. In addition, the boronic acid moiety has already shown promise in potential pharmaceutical agents such as enzyme inhibitors,[11] saccharide sensors,[12] and boron neutron capture therapy (BNCT) agents.[13]

In the present study, we have further demonstrated the ability of these novel chalcones, appended to a boronic acid group, to effectively inhibit breast cancer cell lines. Biological evaluation of these molecules led us to examine an unusual class of synthetic molecules containing a pair of α,β-unsaturated groups; in these studies, we found that two boronic acid moieties exhibited a higher in vitro efficacy than did a single boronic acid group. Although similar compounds featuring dienone groups have been described in the literature, information about their biological activity is relatively scarce.[14,15]

A series of novel chalcone derivatives were synthesized for use in the present study. Compounds **1**–**11** (Scheme 1)



Scheme 1. Procedure A = KOH(aq), EtOH, reflux. Procedure B = KOH, EtOH, RT. Procedure C = (1) KOH, EtOH, reflux (2) KH, Et_2O, $BrCH_2B(OR)_2$.

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were synthesized according to the Claisen–Schmidt condensation protocol.[16]

2. Results and discussion

2.1. Chemistry

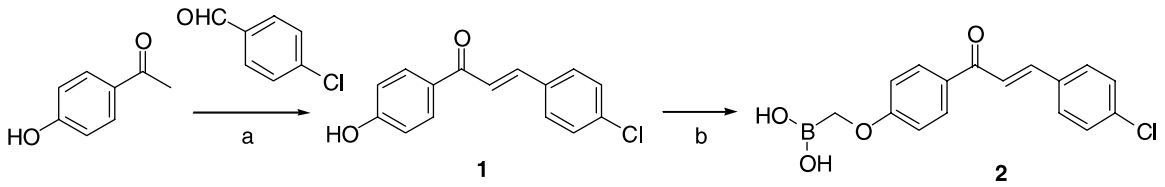
Compounds **1**, **3**, **4**, and **5** (Table 1) were prepared from substituted acetophenones and appropriate aldehydes in the presence of ethanolic KOH. After a standard work-up (dilution with water, acidification with 1 M HCl to pH 3, and extraction with ethyl acetate), the resulting solid was purified by column chromatography to yield the desired product.

Compound **2** was prepared in a two-step process that involved formation of chalcone **1** and then incorporation of the boronic acid moiety into the molecule. Compound **1** was treated with pinacol (bromomethyl)boronate in the presence of KOH and a catalytic amount of 18-crown-6. After a standard workup, the product obtained was purified by crystallization (Scheme 2).

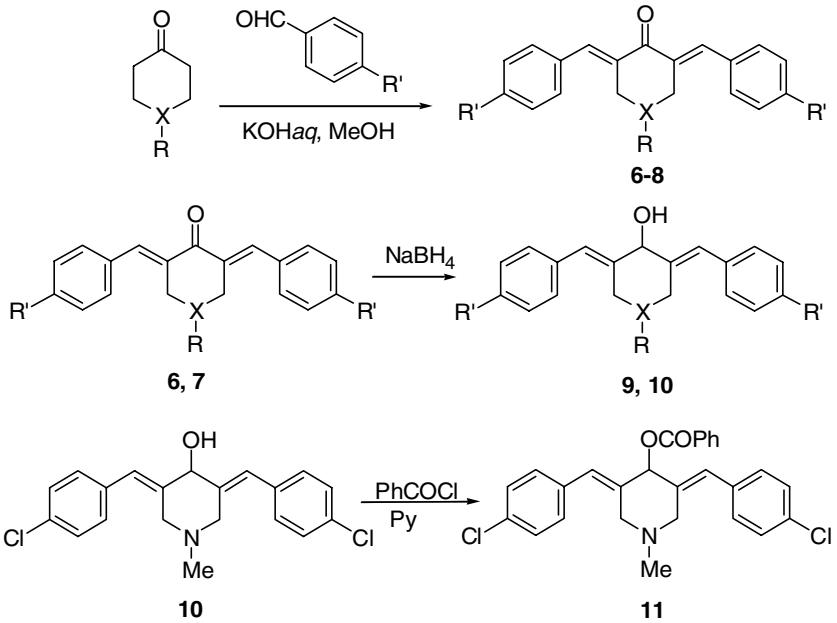
Synthesis of compounds **6**, **7**, and **8** (Scheme 3, Table 2) required the use of 3 equiv of the appropriate aldehydes and 4-*t*-Bu-cyclohexanone or *N*-methylpiperidine-4-one in the presence of an ethanolic solution of KOH. The residual solid was purified by crystallization. Treatment of two of the bis-chalcones, one bearing an N-Me unit (**7**) and the other containing a 4-*t*-Bu group (**6**), with $NaBH_4$ generated bis-α,β-unsaturated alcohols **9** and **10**. Compound **10** was further esterified using benzoyl chloride to afford compound **11**. The whole series was synthesized and evaluated to address the question of whether higher potency and specificity could be achieved by introducing multiple boronic acid fragments into a

Table 1. Novel chalcone derivatives (**1**–**5**)

Compound	R	R′	Ar	Procedure	Yield (%)
1	OH	H	PhCl	A	92
2	$OCH_2B(OH)_2$	H	PhCl	C	81
3	$B(OH)_2$	H	Thiophene	B	98
4	$B(OH)_2$	H	Benzothiophene	B	97
5	Cl	COOH	PhOH	A	85



Scheme 2. Reagents and condition: (a) KOH, MeOH, reflux; (b) KH, pinacol (bromomethyl)boronate, Et_2O, 18-crown-6.



Scheme 3.

Table 2. Novel chalcone derivatives (**6**–**11**)

Compound	X	R	R′	Yield (%)
6	C	*t*-Bu	$B(OH)_2$	87
7	N	Me	Cl	96
8	N	Me	$B(OH)_2$	93
9	C	*t*-Bu	$B(OH)_2$	85
10	N	Me	Cl	78
11	N	Me	Cl	90

molecule. A study regarding the importance of the enone unit was performed by converting the dienone to a dienol and subsequently masking the free hydroxyl group as an ester.

2.2. Biology

Chalcones **1**–**11** were then evaluated against the breast cancer cell lines MCF-7 and MDA-MB-231. The normal breast cell lines MCF-10A and MCF-12A were included in the panel as controls. The cell lines were incubated with each of the chalcones **1**–**11** for 96 h, and an MTT assay was performed to determine the IC_{50} for each chalcone against the panel of cell lines (Table 3).

2.2.1. Growth inhibition studies. IC_{50} values were used to determine growth inhibition in the presence of the chalcone derivatives. Of particular interest were the compounds that were able to preferentially inhibit the growth of human breast cancer cell lines over the normal breast epithelial cell lines. Compounds **1** and **2** inhibited the cancer cell lines at low micromolar concentrations, whereas the normal breast cell lines were 4- to 6-fold less sensitive to these chalcones. In the presence of compounds **3** and **4**, growth of the human breast cancer cell lines MDA-MB-231 and MCF7 was inhibited, with low IC_{50} values ranging from 3.3 to 15.1 μM. Cell growth in the normal breast epithelial cell lines MCF-10A and MCF-12A appeared unaffected at the maximum concentration used in this study (25 μM). Chalcone **5** was toxic only to MCF7; it had no effect on either MBA-MD-231 or normal breast cells (MCI-10A and -12A).

In a related series, bis-chalcones **6**, **7**, and **8** were found to inhibit all the cell lines at low micromolar to nanomolar concentrations. Reduction of the carbonyl group (**9**, **10**) led to a higher selectivity for the malignant over the normal cell lines. Compound **9** was completely selective for the cancer cell lines, inhibiting only the MCF7 and MDA-MB-231 cell lines, with IC_{50} values of 16.5 and 15, respectively. However, conversion of the free alcohol to an ester caused a complete lack of sensitivity at 25 μM, with none of the treated cell lines reaching IC_{50}s.

Table 4. Inhibition of colon cancer cell lines by bis-chalcone **8**[a]

Cell line	IC_{50}	SD	Replicates (*n*)
HCT116 p53+/+	2.8	0.32	3
HCT116 p53−/−	5.5	0.23	3

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

To explore the potential growth inhibitory effect of these chalcones on colon cancer cell lines and to determine whether the growth inhibition was p53-dependent, we evaluated the effect of bis-chalcone **8** on a pair of isogenic HCT116 colon cancer cell lines that differed only in p53 expression. An initial screening suggested that cells with wild-type p53 (HCT116 p53+/+) were more sensitive to the cytotoxic effects of bis-chalcone **8** than were cells that lacked p53 (HCT116 p53−/−) (Table 4). Further studies addressing these considerations are underway.

3. Conclusions

In summary, we have prepared and characterized a series of chalcones and bis-chalcones that inhibit the growth of human breast and colon cancer cells in culture at low micromolar to nanomolar concentrations. Some of them are particularly exciting, as they preferentially inhibited the growth of human breast cancer cell lines, as compared to normal epithelial cells.

The long-term objective of these studies is to develop highly potent and selective inhibitors of the MDM2-p53 interaction for use as anticancer drugs. Our goal in this specific proposal is to develop and characterize

Table 3. Inhibition of human breast cell lines by chalcones (**1**–**11**)[a]

Compound	Wt-MCF7	SD	MDA-MB-231	SD	MCF-10A	SD	MCF-12A	SD
1	2.3	0.76	3.8	1.06	25.0	1.41	21.2	2.54
2	3.1	1.65	2.0	1.15	13.0	8.0	22.5	2.08
3	7.0	3.18	15.1	2.18	—[b]	0	—[b]	0
4	3.3	1.61	5.5	1.0	—[b]	3.17	—[b]	0
5	22.0	2.12	—[b]	0	—[b]	0	—[b]	0
6	1.3	0.94	0.7	0.25	7.0	2.75	8.0	1.77
7	2.0	2.12	0.4	0.14	2.5	1.87	4.8	2.40
8	0.35	3.33	0.35	1.05	1.5	0.58	4.0	2.42
9	16.5	1.06	15	1.06	—[b]	0.35	—[b]	0.35
10	20	3.89	20.3	2.12	22.3	0.35	—[b]	0
11	—[b]	0	—[b]	0	—[b]	0	—[b]	0

[a] IC_{50} values expressed in μM; see Section 2.2 for details of the MTT assay.
[b] $IC_{50} > 25$ μM.

novel boronic chalcone compounds that can prevent the interaction between the tumor-suppressor p53 and the oncogene MDM2, as a means of activating p53 tumor-suppressor activity in tumors. Therefore, we have used computer-aided drug design techniques followed by virtual screening to design and evaluate a preliminary series of novel boronic chalcones. Molecular modeling studies have suggested that boronic acid efficiently binds to lysine residues K51 and/or K94 of MDM2 oncoprotein. These boronic-chalcone derivatives can therefore serve as potential platforms for the design of clinically useful MDM2 antagonists. Structure–activity relationship studies of this class of compounds are continuing and will be reported in due course.

4. Experimental

4.1. Biology

The human breast cancer cell lines MDA-MB-231 (estrogen receptor-negative) and MCF7 (estrogen receptor-positive) were maintained in DMEM supplemented with 5% fetal bovine serum, 2 mM glutamine, and 100 units/mL penicillin/streptomycin. The normal human mammary epithelial cell lines MCF-10A and MCF-12A were maintained in 5% and 10% horse serum, 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.[17] Cells were plated in 96-well plates and allowed to attach for 24 h. Chalcone derivatives were dissolved in DMSO at 10 mM. Cells in quadruplicate wells were exposed to the individual chalcone derivatives at 0.5–25 μM for 96 h. After 96 h, the medium was aspirated, and 100 μL of 1 mg/mL MTT solution (Sigma Chemical Co.) diluted in serum-free medium was added to each well. After 4 h of incubation, the MTT solution was removed, and 200 μL of a 1:1 (v/v) solution of DMSO/ethanol was added to each well to dissolve the formazan crystals. The absorbance at A_{540nm} 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.

HCT116 p53+/+ and HCT116 p53−/− colon cancer cells (kindly provided by Dr. Bert Vogelstein, Johns Hopkins University, Baltimore) were maintained in McCoy's 5A medium containing 10% FBS. Cell growth inhibition was evaluated using the MTT assay. The p53+/+ and p53−/− cells were plated in 96-well plates at the density of 1500 cells/well. The cells were allowed to attach overnight and then treated with various concentrations of bis-chalcone **8** (0–10 μM) in triplicate. At the end of 72 h, 50 μl of 3 mg/ml of MTT was added to each well and mixed. The plates were incubated for a further 4 h. The supernatant from each well was then carefully removed, and 200 μl of DMSO was added to each well and mixed thoroughly. The plate was read with a 540-nm filter using a Multiskan Ascent plate reader (Thermo Electron, WI). The growth inhibition in cells treated with bis-chalcone **8** was plotted as a percentage of the value obtained for untreated control cells, and the IC_{50} values (or the concentration of the drug that caused a 50% inhibition in cell growth) were calculated for the two cell lines.

4.2. Chemistry

4.2.1. General method for syntheses of chalcones

4.2.1.1. Procedure A. To a solution of substituted acetophenone in ethanol (10 ml/mmol) was added the appropriate aldehyde (1.5 equiv). The mixture was stirred for 15 min, followed by the addition of an aqueous solution of KOH (3 equiv) at 0 °C. The mixture was refluxed overnight. After the reaction reached completion, it was quenched with water (20 ml). The resulting mixture was concentrated in vacuo, diluted with water (50 ml), acidified with 1 M HCl to pH 3, and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residual solid was purified by column chromatography to yield the desired product.

4.2.1.2. Procedure B. To a solution of substituted acetophenone in ethanol (10 ml/mmol) was added the appropriate aldehyde (3 equiv). The mixture was stirred for 15 min, followed by the addition of an ethanolic solution of KOH (6 equiv) at 0 °C. The mixture was stirred at room temperature overnight. After the reaction reached completion, it was quenched with water (20 ml). The resulting mixture was concentrated in vacuo, diluted with water (50 ml), acidified with 1 M HCl to pH 3, and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residual solid was purified by crystallization to yield the desired product.

4.2.1.3. Procedure C. An ethereal solution of compound **1** was added dropwise to a cooled solution of KH (1.8 equiv) in diethyl ether, and the reaction mixture was stirred at room temperature for 40 min. The mixture was cooled to 0 °C, and a solution of pinacol (bromomethyl)boronate in diethyl ether was added dropwise, followed by a catalytic amount of 18-crown-6. The mixture was stirred at room temperature overnight. After a standard workup, the product obtained was purified by crystallization.

4.2.1.4. 3-(4-Chloro-phenyl)-1-(4-hydroxy-phenyl)-propenone (1). *Procedure A*: yield 92%; ^{1}H NMR ($CDCl_3$) δ 8.00 (d, 2H, J = 8 Hz), 7.76 (d, 1H, J = 16 Hz), 7.57 (d, 2H, J = 8 Hz), 7.50 (d, 1H, J = 16 Hz), 7.39 (d, 2H, J = 8 Hz), 6.93 (d, 2H, J = 8 Hz); ^{13}C NMR (MeOD) σ 191.2, 164.9, 144.4, 138.0, 136.1, 133.3, 133.2, 131.4, 130.6, 124.5, 117.9; HRMS (FAB-MS) m/z 258.04476 (M^+).

4.2.1.5. 3-(4-Chloro-phenyl)-1-(phenyl-4-hydroxymethylboronic acid)-propenone (2). *Procedure C*: yield 81%; ^{1}H NMR ($CDCl_3$) δ 8.04 (d, 2H, J = 8.8 Hz), 7.76 (d, 1H, J = 16 Hz), 7.57 (m, 3H), 7.40 (d, 1H, J = 8 Hz), 7.07 (d, 2H, J = 8.8 Hz), 3.89 (s, 2H); ^{13}C NMR (MeOD) σ 190.6, 166.0, 144.0, 137.2, 135.2,

132.4, 132.2, 131.0, 130.0, 124.0, 75.1; MS (ESI) *m/z* 316.9 (M^+).

4.2.1.6. 1-(Phenyl-4-boronic acid)-3-thiophen-2-yl-propenone (3). *Procedure B*: yield 98%; ^{1}H NMR (MeOD) δ 8.0 (br d, 2H), 7.95 (d, 1H, J = 15.2 Hz), 7.77 (br d, 2H), 7.62 (d, 1H, J = 5.2 Hz), 7.52 (d, 1H, J = 3.2 Hz), 7.48 (d, 1H, J = 15.2 Hz), 7.16 (dd, 1H, J = 4.2, 3.6 Hz); ^{13}C NMR (MeOD) δ 192.0, 141.6, 138.9, 135.2, 134.1, 133.4, 131.0, 130.6, 130.3, 128.5, 121.3; MS (ESI) *m/z* 258.9 (MH^+).

4.2.1.7. 3-Benzo[*b*]thiophen-2-yl-1-(phenyl-4-boronic acid)-propenone (4). *Procedure B*: yield 97%; ^{1}H NMR (MeOD) δ 8.06 (m, 3H), 7.87 (m, 2H), 7.81 (br d, 2H), 7.76 (s, 1H), 7.52 (d, 1H, J = 15.2 Hz), 7.42 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 191.5, 141.8, 141.4, 141.2, 139.1, 134.6, 131.7, 131.4, 128.5, 128.2, 127.3, 126.5, 125.7, 124.4, 123.5; MS (ESI) *m/z* 308.9 (MH^+).

4.2.1.8. 2-(4-Chloro-benzoyl)-3-(4-hydroxy-phenyl)-acrylic acid (5). *Procedure A*: yield 85%; ^{1}H NMR (MeOD) δ 7.94 (d, 2H, J = 8.8 Hz), 7.88 (s, 1H), 7.50 (d, 2H, J = 8.8 Hz), 7.22 (d, 2H, J = 8.8 Hz), 6.68 (d, 2H, J = 8.8 Hz); ^{13}C NMR (MeOD) δ 197.5, 168.1, 141.5, 136.2, 134.1, 133.0, 132.0, 130.1, 128.9, 125.4, 117.3, 116.2; HRMS (FAB-MS) *m/z* 302.03459 (M^+).

4.2.1.9. 4-*tert*-Butyl-2,6-bis-(4-boronic acid-benzylidene)-cyclohexanone (6). *Procedure B*: yield 77%; ^{1}H NMR (MeOD) δ 7.81 (s, 2H), 7.69 (br d, 4H), 7.48 (br d, 4H), 3.18 (d, 4H, J = 15.6 Hz), 2.52 (t, 4H, J = 16.0 Hz), 0.94 (s, 9H); ^{13}C NMR (MeOD) δ 192.6, 138.8, 138.6, 138.5, 135.5, 131.4, 130.6, 46.1, 33.9, 28.9, 27.6; MS (ESI) *m/z* 419.1 (MH^+).

4.2.1.10. 3,5-Bis-(4-chloro-benzylidene)-1-methyl-piperidin-4-one (7). *Procedure B*: yield 96%; ^{1}H NMR ($CDCl_3$) δ 7.77 (br s, 2H), 7.43 (d, 4H, J = 8 Hz), 7.34 (d, 4H, J = 8 Hz), 3.75 (br d, 4H, J = 16 Hz), 2.49 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 193.8, 133.6, 133.4, 132.5, 131.5, 129.3, 128.5, 57.0, 45.9; HRMS (FAB-MS) *m/z* 357.06775 (M^+).

4.2.1.11. 3,5-Bis-(4-boronic acid-benzylidene)-1-methyl-piperidin-4-one (8). *Procedure B*: yield 83%; ^{1}H NMR (MeOD) δ 7.81 (s, 2H), 7.72 (br d, 4H), 7.47 (br d, 4H), 3.89 (s, 4H), 2.5 (s, 3H); ^{13}C NMR (MeOD) δ 185.2, 135.2, 133.9, 133.0, 132.1, 129.1, 128.2, 57.2, 42.1; MS (ESI) *m/z* 378.0 (MH^+).

4.2.1.12. 4-*tert*-Butyl-2,6-bis-(4-boronic acid-benzylidene)-cyclohexanol (9). Yield 85%; ^{1}H NMR (MeOD) δ 7.60 (m, 4H), 7.25–7.40 (m, 4H), 6.38 (br s, 2H), 4.78 (s, 1H), 3.62 (m, 2H), 3.33 (m, 2H), 0.85 (s, 9H); ^{13}C NMR (MeOD) δ 144.6, 140.9, 135.8, 135.0, 130.5, 129.2, 80.8, 40.7, 33.8, 20.8, 20.7; MS (ESI) *m/z* 419.1 (MH^+).

4.2.1.13. 3,5-Bis-(4-chloro-benzylidene)-1-methyl-piperidin-4-ol (10). Yield 78%; ^{1}H NMR (MeOD) δ 7.40 (d, 4H, J = 9 Hz), 7.25 (d, 4H, J = 9 Hz), 6.97 (s, 2H), 4.89 (s, 1H), 4.01 (d, 2H, J = 16 Hz), 3.86 (d, 2H, J = 16 Hz), 2.50 (s, 3H); ^{13}C NMR (MeOD) δ 136.1, 135.6, 134.7, 133.1, 131.2, 130.3, 75.5, 53.4, 42.6; HRMS (FAB-MS) *m/z* 359.08437 (M^+).

4.2.1.14. Benzoic acid 3,5-bis-(4-chloro-benzylidene)-1-methyl-piperidin-4-yl ester (11). Yield 90%; ^{1}H NMR ($CDCl_3$) δ 7.30–7.40 (m, 13H), 6.38 (br s, 2H), 5.22 (s, 1H), 3.32–3.50 (m, 2H), 2.70–3.10 (m, 2H), 2.34 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.0, 137.5, 133.0, 132.8, 130.5, 129.7, 128.8, 128.4, 127.6, 119.2, 84.8, 54.0, 39.8; HRMS (FAB-MS) *m/z* 463.11058 (M^+).

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