

**SYNTHESIS AND AROMATASE INHIBITION  
PROPERTIES OF NEW BORONIC ACID  
CHALCONE DERIVATIVES**

**A Thesis Submitted to  
the Graduate School of Engineering and Sciences of  
İzmir Institute of Technology  
in Partial Fulfillment of the Requirements for the Degree of**

**MASTER OF SCIENCE  
in Chemistry**

**by  
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**July 2011  
İZMİR**

## ACKNOWLEDGEMENTS

First of all I would like to offer my respect and gratitude to my research advisor Assist. Prof. Dr. Ali ÇAĞIR for his support, patience, and guidance during the study.

I am honored by the participation of Assoc. Prof. Yusuf BARAN and Assist. Prof. Dr. Mustafa EMRULLAHOĞLU who readily agreed to be my committee members. Also thanks to Prof. Dr. Serdar ÖZÇELİK for his valuable comments during my thesis studies.

I would like to thank Leyla Eral DOĞAN and Özge TUNUSOĞLU who shared their experiences with me. Also I thank to my lab mate İsmail AKÇOK who helped me at first stage of my studies.

I am grateful to Işın ÖZÇELİK and Salih GÜNNAZ for NMR analysis during the study. Next I thank to Pınar BAYDARA for GC-MS analyses.

I would like to express my gratitude to my dear friends Merve DEMİRKURT, Esen DÖNERTAŞ, Banu ÖNEN and Taylan MEŞİN who always gave me encouragement, friendship and support through my most difficult times. It was honor to work with them in the same department.

Finally my deepest thank goes to my parent and brother. It would be impossible to accomplish this study without their endless support and love.

## ABSTRACT

### SYNTHESIS AND AROMATASE INHIBITION PROPERTIES OF NEW BORONIC ACID CHALCONE DERIVATIVES

Chalcones (1,3-diaryl-2-propen-1-ones) are one of the best known naturally occurring biological active compounds belonging to flavonoid family. They possess wide range of biological properties such as; anti-cancer, anti-bacterial, anti-inflammatory, anti-angiogenic. In addition, chalcone boronic acid derivatives have been tested for their anti-cancer properties and saccharide sensors abilities in few of the recent studies. In those studies, it is speculated that boronic acid chalcone derivatives are selectively cytotoxic against cancer cells rather than healthy cells.

In the present study, synthesis of simple chalcones, derivatized by two boronic acids, and 2-naphthyl chalcones having a single boronic acid are reported first time in literature. For this purpose different commercially available acetylphenyl boronic acid and formylphenyl boronic acid derivatives are reacted in alkaline methanol to yield diboronic acid chalcone compounds. Similarly condensation reaction of 2-naphthaldehyde with different acetylphenyl boronic acids yielded the monoboronic acids derivatized 2-naphthyl chalcones. Synthesized diboronic acid chalcone derivatives and 2-naphthyl calcone boronic acid derivatives were tested for their anti-cancer properties against human mammary adenocarcinoma cancer cell lines (MCF-7), and human prostate cancer cell lines (PC3). Their aromatase inhibition potentials were also reported first time in here. In addition, their binding capabilities upon D-fructose, D-galactose and D-glucose of these compounds in physiologic pH were also examined.

## ÖZET

### YENİ BORONİK ASİT ÇALKON TÜREVLERİNİN SENTEZLENMESİ VE AROMATAZ İNHİBİSYONU ÖZELLİKLERİNİN İNCELENMESİ

Çalkonlar (1,3-diaril-2-propen-1-on) flavanoid ailesine dahil olup, bir dizi biyolojik aktiviteye sahip olan bileşikler olarak bilinirler. Çalkon türevleri anti-kanser, anti-bakteriyel, anti-inflamatuvar, anti-anjiogenik gibi geniş bir spektrumda biyolojik aktivite gösterebilmektedir. Buna ek olarak çalkon boronik asit türevlerinin anti-kanser ve karbonhidratlara bağlanma kapasiteleri literatürde yeni çalışılan konulardır. Söz konusu çalışmalarda çalkon boronik asit türevlerinin kanser hücrelerine karşı seçici sitotoksik etkilerinin olduğu rapor edilmiştir.

Bu çalışmada ise literatürde ilk olarak çift boronik asit türevlendirilmiş çalkon türevleri ve 2-naftalin substituentli çalkon boronik asit türevlerinin sentezleri çalışılmıştır. Çift boronik asit çalkon türevlerini elde edebilmek için ticari olarak asetilfenil boronik asit türevleri ve formilfenil boronik asit türevleri bazik metanol içerisinde tepkimeye sokulmuştur. Benzer koşullarda 2-naftilaldehit ile asetilfenil boronik asitlerin kondensasyonundan tek boronik asit fonksiyonellendirilmiş çalkonlar elde edilmiştir. Sentezlenen çift boronik asit çalkon türevleri ve 2-naftil çalkon boronik asit türevlerinin aromataz inhibisyonu potansiyelleri, buna ek olarak meme kanseri hücre hattında (MCF-7) ve prostat kanseri (PC3) hücre hatlarında anti-proliferatif özellikleri test edilmiştir. Ek olarak sentezlenen boronik asit türevlendirilmiş çalkon bileşiklerinin, D-fruktoz, D-glikoz ve D-galaktoz ile bağlanabilme testleri fizyolojik pH'da denenmiştir.

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# CHAPTER 1

## INTRODUCTION

Cancer is the common name used to define more than two hundred diseases having similar characteristics. By the year of 2002, about 11 million cancer cases were reported in worldwide. Future estimations of cancer are incidence in worldwide predicted that there will be an increase and number of cases will be around 17 millions in 2020, and 27 millions in 2050 (Bray and Moller, 2005). These expectations force scientific communities to define or develop new cancer treatment methods. Treatment of a patient diagnosed with cancer can be depend on type of cancer, extent of metathesis, and sensitivity to treatment (Gabriel, 2007).

Cancers can be characterized by unregulated cell proliferation and migration in the body. It is well known fact that; multi-mutations of DNA in mitotic cells are the main reason for such unregulated cell proliferation. So it is clear that only the mutated mitotic cells are susceptible for cancer development.

In human body the tissues can be divided into four main groups; epithelial, connective, muscle and nervous tissues. But not all of these tissues have equal probability to develop cancer because of the differences in their ability to respond to cell loss. In some parts of the body, integrity of the tissues are controlled by proliferation of cells of cells to replace older, damaged or dead cells. In terms of cell replacement capability, tissues can also be derived into three parts; rapidly self renewing tissues (such as; skin, intestine and hematopoietic system), conditionally renewing tissues (such as; liver, breast, prostate and connective tissues), and non-renewing tissues (such as; female germ line, and central nervous system). Among those renewable tissues have the types of cells which can develop cancer (Marshman et al., 2005).

DNA in eukaryotic cells undergoes damage, repair, and resynthesis in a continuous manner. There are some checkpoints in healthy cells and any damage in the DNA can be repaired correctly. But this is not the case in cancer cells. In cancer cells this equilibrium is disturbed, and that allows multi-mutations in the DNA of cell and

causes immortal cell formation and invasion by these uncontrolled cells (Loeb, 2000). Thus cancer is not a localized disease but a disease that can spread other tissues via the lymphatic system and blood stream, creating secondary tumor regions (metastasis) (Gabriel, 2007).

Such mutations in DNA can be formed by genetic factors and environmental factors like smoking, diet, obesity, chemicals, and pollution etc. can result mutations in DNA therefore cancers. In addition, such chronic diseases are much more related with environmental factors compared to genetic factors. Studies have shown that; the connectivity between breast cancer and genetic factors was found to be only one in five in identical twins (Anand et al., 2008).

Since cancer is a fatal disease, there is still a need to develop new and effective anti-cancer agents to take carcinomas under control. To stop or reverse the development of cancer by using natural or synthetic substances is called cancer chemoprevention which was first described by Sporn (Sporn, 1976).

Chemopreventive agents play an important role to control cancer only if they target the genetically mutated cells which are the starting point of cancer. Second important point is chemopreventive agent should be highly effective at low doses. For that reason synthetic modifications of naturally occurring substances might improve the selectivity and potency of that naturally occurring chemopreventive compound.

Chalcones (Figure 1.1, **1**) and its derivatives are a class of natural products belonging to flavanoid family, represent important biological activities, such as anti-inflammatory, anti-oxidant, anti-cancer, and anti-bacterial. Chalcone derivatives are abundant in plants and relatively easy to produce unsubstituted derivatives in synthetic ways, and these properties make chalcones important candidates for chemoprevention. Up to date there are hundreds of chalcone derivatives either isolated from natural products or synthesized in laboratory conditions showing promising biological activities have been reported. Because it is not possible to review all of them in here, a few examples of those will be shown to clarify the importance of chalcones.

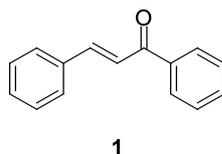


Figure 1.1. Simple chalcone structure.

Boronic acid compounds are promising candidates for prevention of cancer, based on their unique properties: (1) inter-convertibility between the trigonal and tetrahedral forms as carbonyl functional group does (**2** and **3** Figure 1.2), (2) their strong binding ability with diol-functionalized compounds, (3) their Lewis acidity, and (4) beneficial unstability upon neutron bombardment.

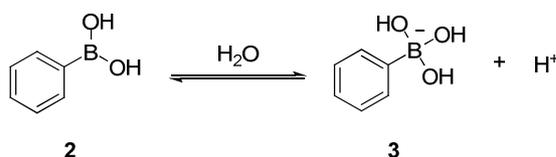


Figure 1.2. Inter-convertibility between the trigonal (**2**) and tetrahedral form (**3**) of phenyl boronic acid in water.

Although, pharmaceutical studies related with chalcones were done for a long time boronic acids are relatively new in pharmacy because of their unknown mode of actions. So, many researchers focus on to develop new boronic acid derivatives to fight with cancer. For this purpose in this study chalcone structure is chosen as a rigid carbon skeleton to arrange distances between two boronic acid moieties

## 1.1. Synthesis

### 1.1.1. Synthesis of Chalcone Derivatives

Chalcones are readily synthesized by Claisen-Schmidt condensation reactions catalyzed by a strong base like NaOH or KOH. Claisen-Schmidt condensation reaction of substituted benzaldehydes and acetophenones favors (*E*)-isomer of chalcone derivatives in good yield. Lawrence et al. synthesized a small library of chalcone derivatives (Figure 1.3) starting from acetophenones and aldehydes having different functional groups in alkaline methanol solution, in reasonable yields (43-70%) (Lawrence et al., 2001).

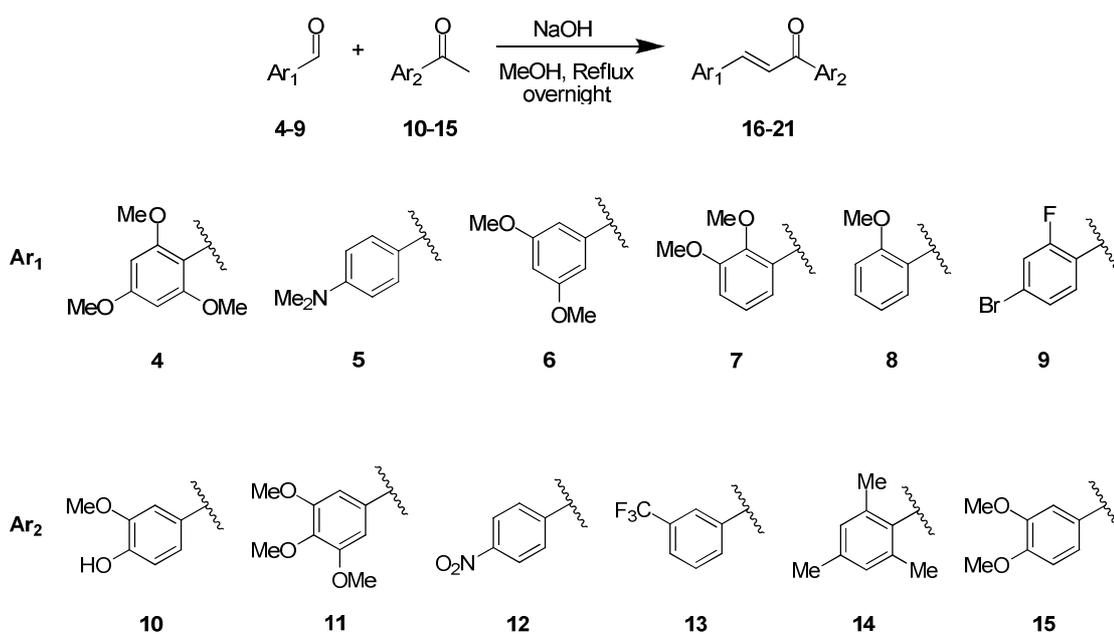
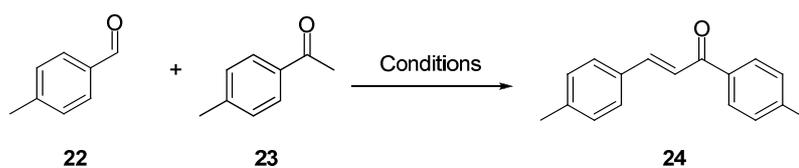


Figure 1.3. Synthesis of chalcone analogues **16-21** in basic solution.

Toda et al. go through a series of chalcone derivatives (Figure 1.4) that were formed up with NaOH in the absence of solvent. Efficient synthesis of chalcone analogues have been reported in high yields in a shorter time compared to the reactions performed in solvent (Toda et al., 1990).



Conditions	Time	Yield(%)
NaOH, Pestle Mortar	5 min.	99%
NaOH, 50% EtOH/Water	5 min.	3%

Figure 1.4. Synthesis of chalcone analog **24** in the absence of solvent.

As an alternative, Eddair et al. reported an influential method for the synthesis of chalcone analogues (Figure 1.5) via Suzuki coupling reaction between benzoyl chlorides **26-31** and phenylvinyl boronic acid (**25**) in anhydrous toluene. In these reactions Mc Charty's Suzuki Coupling conditions (Haddach and McCarthy, 1999) (solvent: anhydrous toluene; catalyst: tetrakis(triphenylphosphine)palladium(0); base: cesium carbonate) was used to produce chalcone analogues in high yields (68-93%) regardless of the types of substituents in phenyl rings (Eddair et al., 2003).

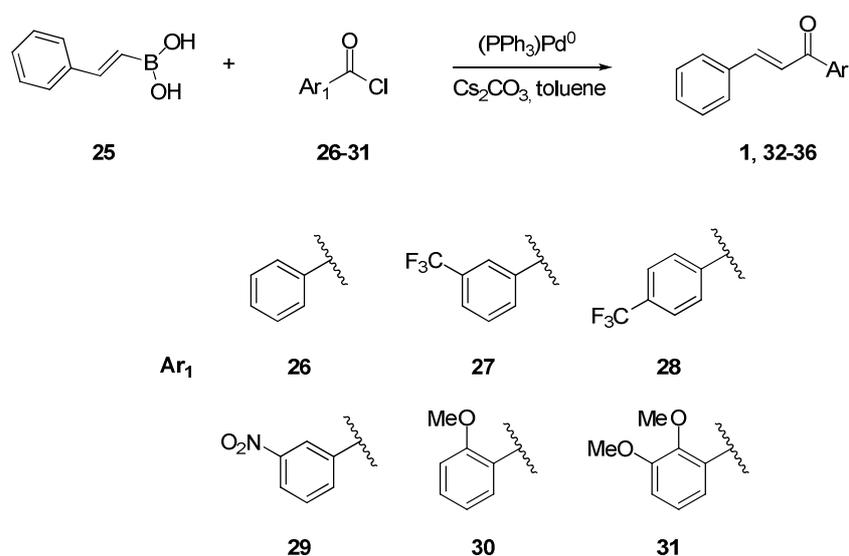


Figure 1.5. Synthesis of chalcone derivatives **1**, **32-36** via Suzuki coupling reaction.

Chalcones can also be synthesized under acidic conditions. Either protic or Lewis acids can be used to perform aldol condensation reactions.  $\text{AlCl}_3$  (Calloway and Green, 1937),  $\text{RuCl}_3$  (Iranpoor and Kazemi, 1998) and  $\text{TiCl}_4$  (Mazza and Guaram, 1980) are just few examples of Lewis acids, which can be used to catalyze the formation of chalcones. Narender and Reddy gave an example of acid catalyzed aldol condensation reaction (Figure 1.6) by using of borontrifluoride-etherate. Synthesis of chalcone analogues **41** and **42** were successfully accomplished at room temperature in reduced reaction times. This method also gave no side reactions and resulted in high yields (81-88%) to produce chalcones having electron withdrawing or donating groups on aryl rings (Narender and Reddy, 2007).

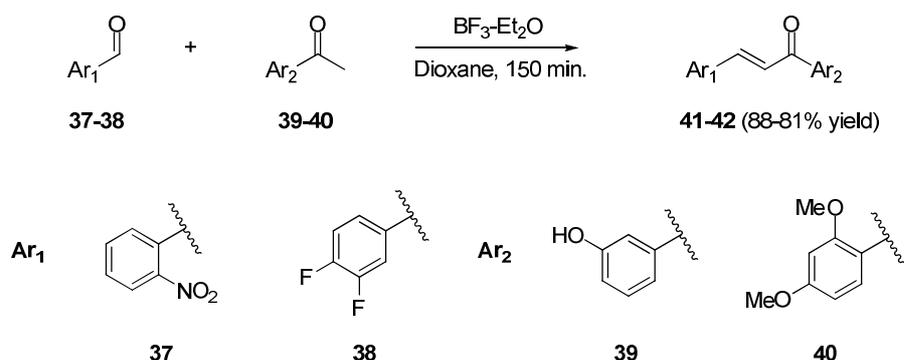


Figure 1.6. Synthesis of chalcone derivatives **41** and **42** by acid catalyzed aldol condensation.

### 1.1.2. Synthesis of Chalcone Boronic Acid Derivatives

Up to date, there are only few reported work dealing with the synthesis of boronic acid derivatized chalcones. In general base catalyzed Claisen-Schmidt reaction were performed in an alcoholic solution. One example for the synthesis of chalcone derivatives having boronic acid substituent **47-51** were reported by Kumar et al. simply from the Claisen-Schmidt condensation of corresponding acetophenone and

benzaldehyde derivatives in KOH-MeOH solution as shown in Figure 1.7 (Kumar et al., 2003).

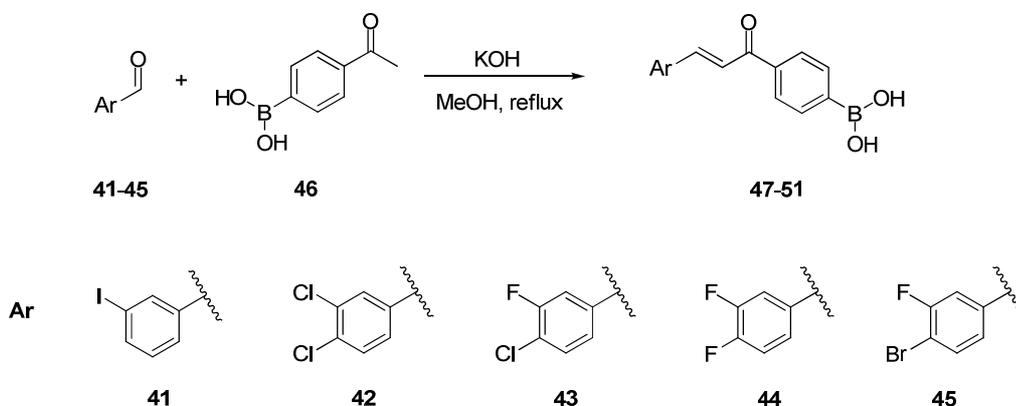


Figure 1.7. Preparation of boronic acid chalcone derivatives **47-51** via Claisen-Schmidt condensation reaction.

## 1.2. Biological Activities

### 1.2.1. Biological Activities of Chalcones

Natural and synthetic chalcone derivatives have a broad range of biological applications, and only few of the biological activities of chalcones will be discussed under this title: anti-inflammatory activity, antibacterial activity, antioxidant activity and anticancer activity with examples.

#### 1.2.1.1. Anti-inflammatory Activity

Chronic inflammation accepted as a risk factor that can lead cancer, since the relationship between tumor and inflammation has been first pioneered by Rudolf Virchow (Smith and Missailidis, 2004). Nitric oxide is an important component of inflammatory responses. Large quantities of free radical nitric oxide (NO) production by activated macrophages in human body (Molina et al., 2001), may lead toxicities and

tumor development (Kaur et al., 1994). Rojas et al. reported two chalcone derivatives **52** and **53** (Figure 1.8) that were found to be effective inhibitors of nitrite production having  $IC_{50}$  values  $0.28 \mu\text{M}$  and  $0.03 \mu\text{M}$  respectively (Rojas et al., 2002).

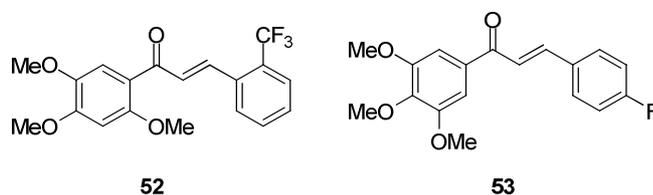


Figure 1.8. Compounds **52** and **53** having anti-inflammatory property.

### 1.2.1.2. Anti-bacterial Activity

Nielsen et al. reported a series of chalcone derivatives (Figure 1.9) showing anti-bacterial effect against the gram-positive bacterium *S. aureus* ATCC 29213. Chalcone derivatives **54** and **55** are highly effective anti-bacterial agents in vitro with minimal inhibition concentrations (MIC) of  $2 \mu\text{M}$  and not shown cytotoxicity (measured by MTT reduction) even at  $100 \mu\text{M}$  concentrations (Nielsen et al., 2004).

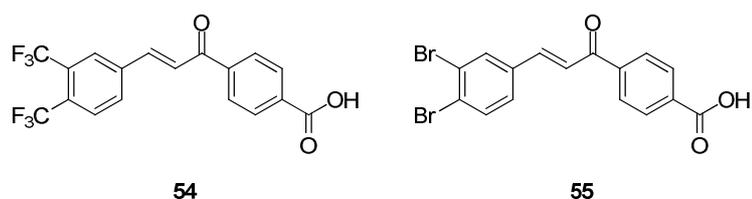


Figure 1.9. Structures of chalcone derivatives **54** and **55** reported having anti-bacterial properties.

### 1.2.1.3. Antioxidant Activity

Forms of reactive oxygen species, such as superoxide anion ( $O_2^-$ ), hydroxy radical (OH $\cdot$ ) and hydrogen peroxide ( $H_2O_2$ ) can produce oxidative stress and give damage to biological macromolecules to lead into serious diseases, even cancer. Antioxidant molecules can react with these highly reactive oxygen species and prevent the formation of any possible damage on biological macromolecules. Flavanoid family of compounds are well known for their anti-oxidant properties. As an example Bandgar et al., reported some simple methoxlated chalcone derivatives as potential anti-oxidation agents. Chalcone derivative **56** (Figure 1.10) has a moderate antioxidant efficiency and bioavailability. Compound **56** have also shown to be non-toxic on healthy cells. (Bandgar et al., 2009).

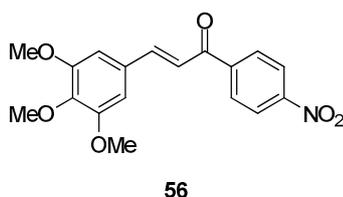


Figure 1.10. Structure of chalcone derivative **56** having anti-oxidant property.

### 1.2.1.4. Anti-cancer Activity

A naturally occurring chalcone derivative licochalcone A (**57** Figure 1.11), is a major phenolic constituent of *Glycyrrhiza inflata* that shows significant anti-tumor activity in human cancer cell lines (Kim et al., 2010).

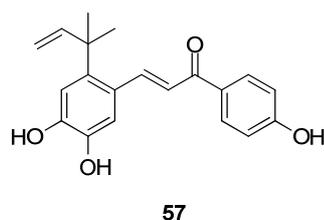


Figure 1.11. Licochalcone A.

Cell cytotoxicity assays are used as a primary screening method for anti-cancer drug development. MTT assay is one of the most common method to determine cytotoxic activity of anti-cancer agent. Lawrence et al. announced highly cytotoxic chalcone analogues **16** and **58** over K 562 (human leukemia) cancer cell line. Compounds **16** and **58** (Figure 1.12) cause to inhibit K 562 cells proliferation 50% at 30 nM and 40 nM respectively determined by MTT assay. (Lawrence et al., 2001)

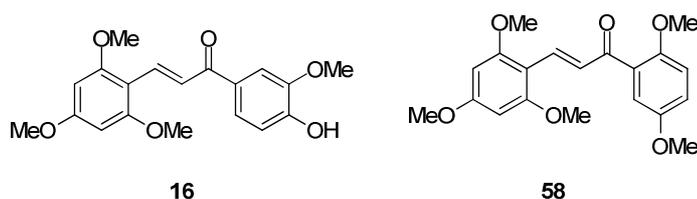


Figure 1.12. Chalcone derivatives **18** and **58** possessing anti-cancer activity.

### 1.2.3. Biological Activities of Boronic Acid Functionalized Molecules

Boronic acid functionalized compounds are important biologically active agents that have been used for various biological applications such as; enzyme inhibition, building sensors and lectin mimics (boronlectins), in transmembrane transporters, in bioconjugations, protein immobilization and boron neutron capture therapy (BNCT) agents (Yang et al., 2005).

### 1.2.3.1. Boronic Acid Derivatives as Enzyme Inhibitors

Thrombin is a serine protease protein involved in blood coagulation cascade, is a target for the development of thrombin inhibitors. Inhibition of thrombin decreases the risk of obstruction of blood vessels by coagulation. DUP 714 (**59**) is a well known boronic acid derivatized peptide that inhibits thrombin effectively ( $K_i = 0.04$  nM). In vivo studies of DUP 714 in laboratory animals showed serious side effects. Fevig et al. reported another boronic acid derivatized peptide **60** (Figure 1.13) that selectively inhibits thrombin ( $K_i = 0.06$  nM). Compared to DUP 714, compound **60** has decreased the side effects in vivo studies (Fevig et al., 1998).

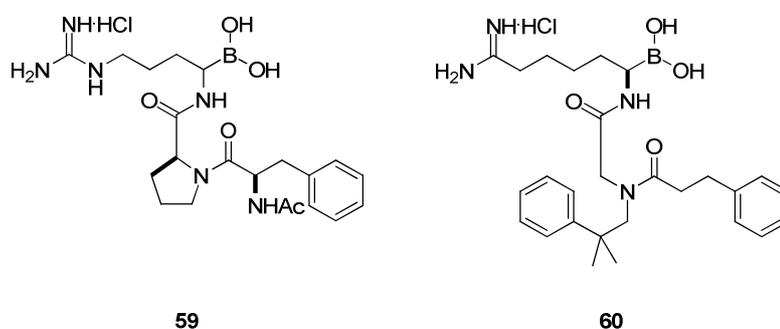


Figure 1.13. Structure of DUP 714 (**59**) and compound **60** that inhibit thrombin activity.

### 1.2.3.2. Boronolectins

Carbohydrates are involved in the metabolic pathways of living organisms; therefore, modification of cell surface carbohydrates are associated with the development and progression of many types of diseases including cancer. Thus, it is crucial to develop carbohydrate sensors under physiologic conditions for early detection of cancer. Recognition of carbohydrates by these sensors mimics the action of lectins so these sensors are called as artificial lectins. Because of these lectin mimics contain boronic acid moieties they are called boronolectins (Yang et al., 2005). Yang et al. reported a boronolectin compound **61** (Figure 1.14) that differentiate sialyl Lewis X

expressing cells from non-sLex expressing cells by high fluorescence intensity change (Yang et al., 2002).

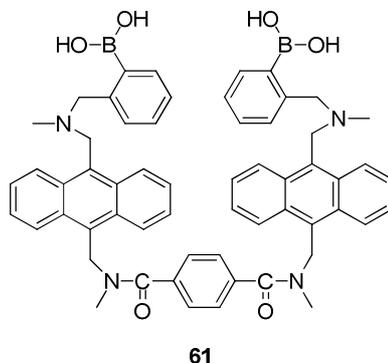


Figure 1.14. A boronolactin compound **61** reported by Yang group.

### 1.2.3.3. BNCT Agents

The unique property of  $^{10}\text{B}$  isotope bombarded by low energy neutrons can be decomposed into  $^7\text{Li}$  ions and  $^4\text{He}$  nuclei thus producing gamma particles without producing other types of ionizing radiation. This unique property of boron makes its derivatives an indispensable candidate for boron neutron capture theory (BNCT) (Yang et al., 2003).

Nemoto et al. reported a water soluble boronic acid derivative, which is uptake by cancer cells rather than healthy cells. p-Boronophenylalanine (BPA) derivative **62** (Figure 1.15) have been tested for its ability to be a BNCT agent. After 18 days of in vivo test, the tumor volume was decreased to approximately 18% by using [ $^{10}\text{B}$ ]BPA-(OH) $_2$  (**62**) (Nemoto et al., 1995).

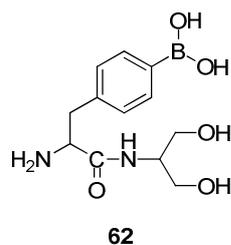


Figure 1.15. An aryl boronic acid derivative **62** as a BNTC agent.

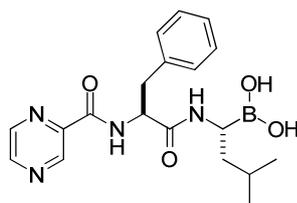
### 1.2.4. Biological Activities of Boronic Acid Chalcone Derivatives

Because of the essential biological properties of chalcones and boronic acids there are just a few literature works for boronic acid derivatized chalcones showing anti-tumor, anti-cancer and anti-angiogenesis activities. Some of the examples of those literature works are summarized under this title.

#### 1.2.4.1. Anti-cancer Activity

As discussed earlier, chalcone derivatives are potent anti-cancer drug candidates that have promising therapeutic efficacy to cope with cancer disease. Kumar et al. hypothesized that chalcone boronic acid analogues were selectively inhibit growth of breast cancer cells, with minimal effect on healthy cells. For this purpose they synthesized new boronic acid substituted chalcone analogues and compared their cytotoxic activities with those of chalcone analogues which are not substituted with boronic acid.

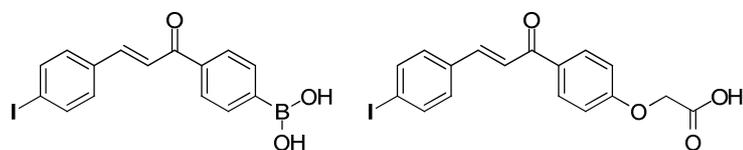
Bortezomib (**63** Figure 1.16) is a boronic acid functionalized peptide derivative which is a perfect example for the use of boronic acid compounds on cancer treatment. It was granted by the US Food and Drug Administration in early 2003, as a single agent for the treatment of multiple myeloma, a bone marrow cancer that affects two to three people per 100.000 (Paramore and Frantz, 2003).



63

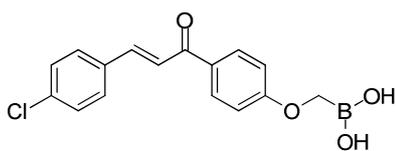
Figure 1.16. Structure of Bortezomib.

Boronic acid substituted chalcone **64** inhibits growth of MDA-MB-231 (Human breast cancer estrogen receptor negative), MCF-10A (Healthy breast epithelial) cell lines with  $IC_{50}$  value of  $8.8 \mu\text{M}$  and  $75 \mu\text{M}$ . Compound **65** is equally toxic to MDA-MB-231 and MCF-10A cell lines with  $IC_{50}$  value of  $44 \mu\text{M}$  (Kumar et al., 2003). Compound **66** having a similar structure compared to compound **65** except of boronic acid moiety and it inhibits proliferation of MDA-MB-231 cancer cell lines with 50% at  $15.1 \mu\text{M}$  concentration (Modzelewska et al., 2006).



64

65



66

Figure 1.17. Boronic acid substituted chalcone derivatives **64**, **65**, and **66** as anti-tumor agents.

A study by Modzelewska et al. reported that chalcone derivatives having two boronic acid moieties (Figure 1.18) exhibited higher in vitro cytotoxic activity compared to single boronic acid substituted chalcones. To show that, single boronic

acid substituted chalcone derivatives and bis-chalcone boronic acid derivatives were prepared and tested for their possible anticancer activities on human breast cancer cell line (MDA-MB-231). Compound **67** is found cytotoxic on MDA-MB-231 cancer cell lines with IC<sub>50</sub> values of 350 nM (Modzelewska et al., 2006).

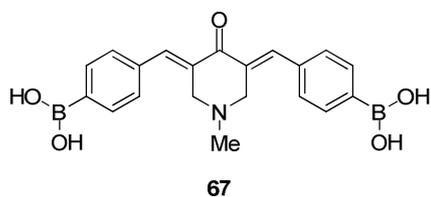


Figure 1.18. Bis-chalcone boronic acid derivative **67** that show high cytotoxic activity against cancer cells.

### 1.2.4.3. Anti-angiogenesis Activity

Angiogenesis, the growth of the new blood vessels to supply food and oxygen, is necessary for cancerous tumors to keep growing and spreading (NCI, 2011). Kong et al. reported that boronic acid chalcone derivative **68**, which inhibits the growth of new blood vessels in HUVEC (human umbilical vein endothelial cells) in 1  $\mu$ M concentration. Also in aortic ring assay compound **68** shows inhibition of angiogenesis in 5  $\mu$ M concentration (Kong et al., 2010).

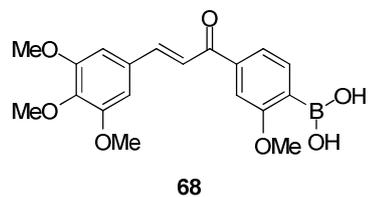


Figure 1.19. Structure of compound **68** showing anti-angiogenesis activity.

### 1.3. Carbohydrate Sensors

Boronic acids are the most commonly used functional group for the construction of sensors for carbohydrates because of their strong interactions with 1,2-diols or 1,3-diols to form five or six membered cyclic esters,. These lectin mimicking compounds are called boronolactins because they all contain boronic acid functionality (Jin et al., 2010).

There are several equilibriums, which should be noticed during the binding of a boronic acid to a diol in water (Figure 1.20). These include:  $K_{trig}$  to describe the equilibrium between the two boronic acid species **2** and **3**;  $K_{tet}$  to describe the equilibrium between the un-complexed **3** and **70**;  $K_{eq}$  to describe the overall binding process between **2**, **3** and the complexed species **69**, **70** (Figure 1.21) (Yan et. al., 2005).

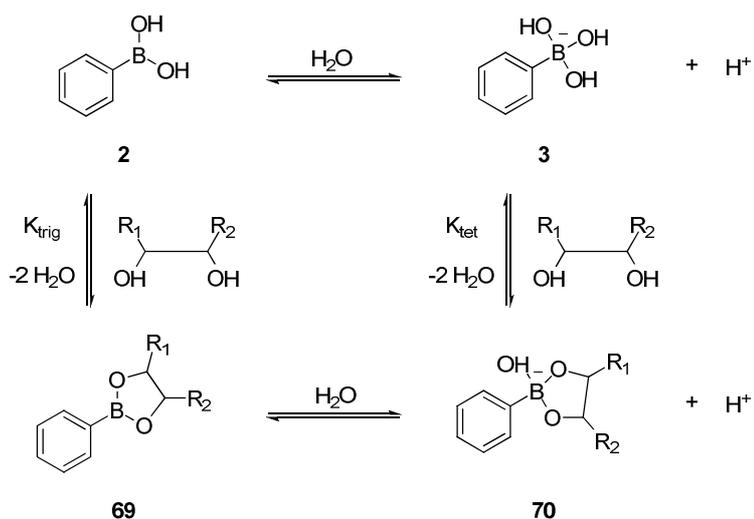


Figure 1.20. Binding equilibrium between phenyl boronic acid and diol in water.

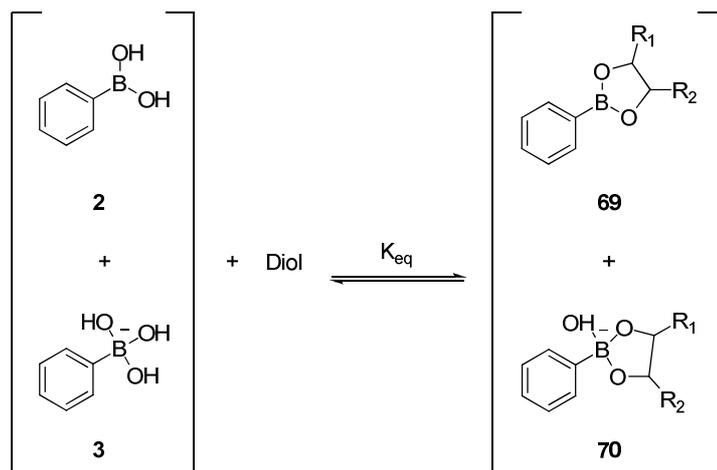


Figure 1.21. Overall binding constant  $K_{\text{eq}}$ .

In literature there are different sensitive methods to determine the binding constant of boronic acids with diols. Early approaches to determine the binding were pH depression and  $^{11}\text{B}$ -NMR method. Long experiment times and requirement for large amount of sample are the disadvantages of these methods. Spectroscopic methods are favorable in recent studies, which are fast, sensitive, easy to use and require small quantity of sample. In such methods to determine binding between a boronic acid and a diol, the binding process has to change the spectroscopic properties of the boronic acid sensor. There are many boronic acid compounds that show significant fluorescent property changes upon diol binding. These boronic acid compounds are called boronic acid fluorescent reporter compounds (Yan et al., 2005). According to their fluorescent properties, ICT (Internal Charge Transfer) sensors and PET (Photoinduced Electron Transfer) sensors occupies a respectable place in literature (James, 2005).

#### 1.4.1. Internal Charge Transfer (ICT) Sensors

DiCesare et al. investigated new boronic acid chalcone derivatives (**71**, **72**: figure 1.22) for the development of fluorescent probes based on the internal charge transfer (ICT) mechanism. The equilibrium between their neutral and anionic form through the binding process to a diol, altered the electron withdrawing property of the boron, thus cause a large change in fluorescence intensities and wavelength shifts.

Compound **71** and **72** produced large fluorescence changes in phosphate buffer (2:1 water/MeOH (v/v) at pH 7.0) in the presence of varying amount of carbohydrates.  $K_d$  values of **71** and **72** for tested carbohydrates are given in Table 1.1 (DiCesare and Lakowicz, 2002).

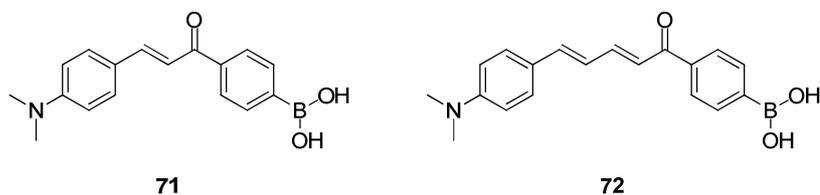


Figure 1.22. Structures of boronic acid chalcones **71** and **72**, used as Internal Charge Transfer (ICT) saccharide sensors.

Table 1.1. Reported binding constants of **71** and **72** with D-fructose, D-galactose and D-glucose.

Carbohydrate	Compound	<b>71</b>	<b>72</b>
	$K_D$		
D-Fructose		2.1	2.5
D-Galactose		14	16
D-Glucose		34	30

Since internal charge transfer (ICT) is an important mechanism for fluorescent signaling, Arimori et al. prepared an ICT fluorescent sensor **73** (Figure 1.23) for saccharides. Compound **73** shows significant fluorescence intensity change and a shift in wavelength with increasing saccharide concentration in 0.1 M potassium dihydrogen phosphate buffer (52.1 wt% MeOH/Water at pH 8.21). In the presence of saccharides the shift in fluorescent wavelength is caused by the transformation of the single unbound form of compound **74** (Figure 1.24(a)) to a single saccharide bound form **75** (Figure 1.23(b)). The appearing binding constant  $K_{app}$  for saccharide complexes of fluorescence sensor **73** are shown in Table 1.2 (Arimori et al., 2001).

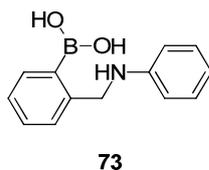


Figure 1.23. Compound **73** as an ICT saccharide sensor.

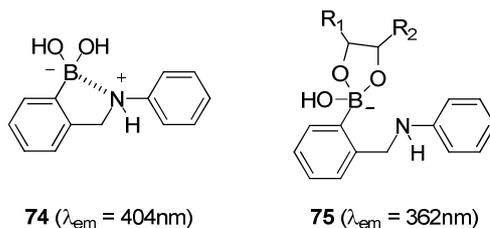


Figure 1.24. The unbound formation **74** and a single saccharide bound formation **75** forms of compound **73**.

Table 1.2. Reported binding constant  $K_{app}$  values of compound **73** with D-fructose, D-galactose and D-glucose.

Carbohydrate	Compound	<b>73</b>
	$K_{app}$ ( $M^{-1}$ )	
<b>D-Fructose</b>	106 ± 7	
<b>D-Galactose</b>	27 ± 4	
<b>D-Glucose</b>	18 ± 6	

### 1.4.2. Photoinduced Electron Transfer (PET) Sensors

Photoinduced electron transfer (PET) has been widely used as the preferred tool in fluorescent sensor design for atomic and molecular species. PET sensors generally consist of a fluorophore and a receptor linked by a spacer. Changes in oxidation/reduction potential of the receptor upon guest binding can affect the PET process, resulting changes in fluorescence (James, 2005).

de Silva et al. figured out a single simple picture (Figure 1.25) summarizes the design of fluorescent PET sensors. The fluorophore, spacer and receptor format is a combination of three components. Electron delocalization from un-bound receptor to excited fluorophore forms the ‘off’ state of PET sensors (Figure 1.26 (a)). Blocked electron delocalization from carbohydrate bound receptor forms the ‘on’ state of PET sensors emitting an energy in the form of fluorescence light (Figure 1.26 (b)) (de Silva et al., 2009).

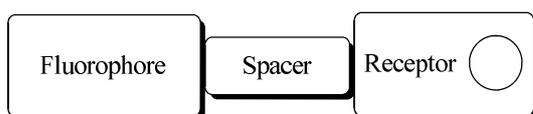


Figure 1.25. The fluorophore-spacer-receptor combination of fluorescent PET sensors. (Source: de Silva et al., 2009)

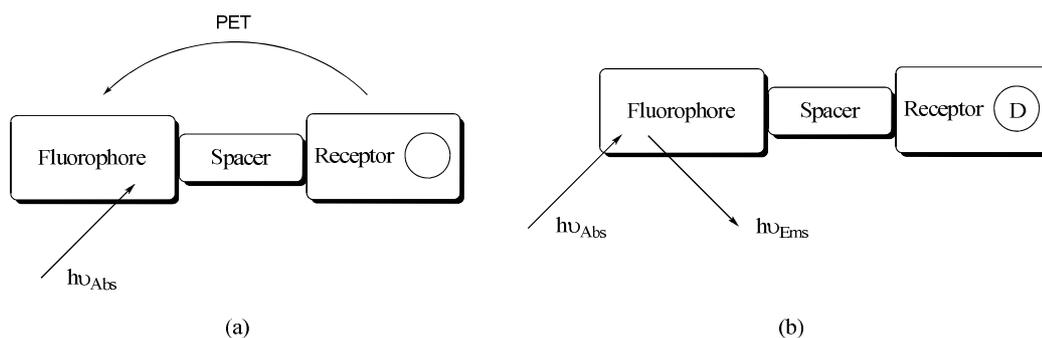


Figure 1.26. PET sensor mechanism. (Source: de Silva et al., 2009)

James et al. improved selectivity of fluorescent PET sensor **76** by introducing a second boronic acid functionality as it's shown by molecule **77**. Due to spacing of the boronic acid moieties the diboronic acid compound **77** was selective for D-glucose over other saccharides including D-fructose in phosphate buffer (33.3 wt% methanol-water at pH 7.77). Binding constants ( $K_{app}$ ) are shown in Table 1.3 (James et al., 1995).

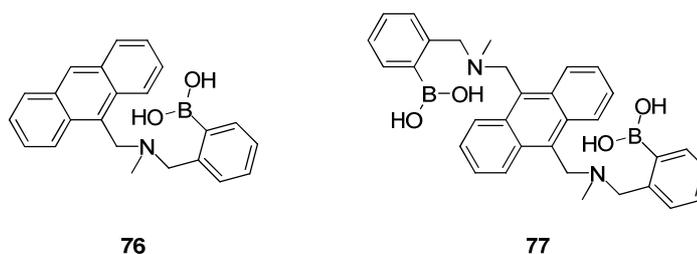


Figure 1.27. Structure of compound **76** and selective PET sensor **77** for glucose reported by James and coworkers.

Table 1.3. Reported  $K_{app}$  (binding constants) values of PET sensors **76** and **77** with D-fructose and D-glucose.

Sugar	Compound	<b>76</b>	<b>77</b>
	$K_{app}$ ( $M^{-1}$ )		
<b>D-Fructose</b>		1000	320
<b>D-Glucose</b>		60	4000

Diboronic acid functionalized compound **78** (Figure 1.28) with a small spacer between the boronic acid groups was synthesized by James et al. and shown to be selective for D-sorbitol in phosphate buffer (300:1 v/v water/methanol at pH 8). Binding constants ( $K_{app}$ ) of compound **78** are given in Table 1.4 (James et al., 1997).

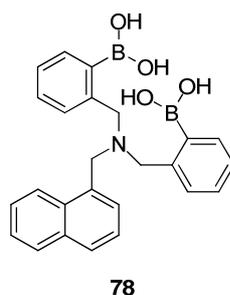


Figure 1.28. Selective D-sorbitol sensor compound **78**.

Table 1.4. Binding constants for compound **78** with D-fructose and D-sorbitol.

Carbohydrate	Compound	<b>78</b>
		$K_{app}$ (M <sup>-1</sup> )
	D-Fructose	330
	D-Sorbitol	350

## 1.4. Aromatase Inhibitors

Aromatase is an enzyme that catalyses the estrogen production. Since estrogen is an essential hormone and high level of estrogen was involved in the development and growth of breast tumors, inhibition of aromatase is vital for the treatment of cancer. Thus, there are several aromatase inhibitors in literature. In general aromatase inhibitors are classified into two main parts: steroidal inhibitors (form a stable bond with the aromatase enzyme complex) and non-steroidal inhibitors (inhibit the enzyme by reversible competition) (Brueggemeier et al., 2005).

### 1.4.1. Steroidal Aromatase Inhibitors

#### 1.4.1.1. Competitive Aromatase Inhibitors

These type of enzyme inhibitors compete with the substrate to bind itself to enzyme by intermolecular attraction forces (noncovalent binding) to reduce the formation of estrogen. Brueggemeier et al. reported a potent steroidal aromatase inhibitor **79** (Figure 1.29). The reported inhibition constant for the reversible binding ( $K_i$ ) of **79** was 18 nM in human placental microsomes (Brueggemeier et al., 1987). In another study Brueggemeier et al. studied aromatase inhibition property of compound **79** in MCF-7 cell culture in a dose-dependent fashion, with an ED<sub>50</sub> (effective dose) of compound **79** was reported as 25.07 nM (Brueggemeier et al., 1978).

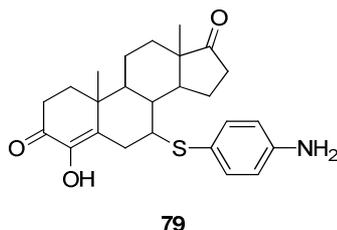


Figure 1.29. Structure of steroidal competitive aromatase inhibitor **79**.

### 1.4.1.1. Enzyme Activated Irreversible Aromatase Inhibitors

Enzyme activated irreversible inhibitors act as a substrate that binds to the enzyme, so inactivation of the enzyme occur. Because of these inhibitors mimic substrate perfectly without causing any other interactions in biological pathways they are unique candidates for enzyme inhibition. Inactivation of the enzyme is described by a half time of inactivation at infinite inhibitor concentration ( $t_{1/2}$ ) and the rate of inactivation ( $K_{inact}$ ) (Brueggemeier et al., 2005).

Brodie et al. indicated a competitive aromatase inhibitor **80**, produces enzyme mediated-inactivation. In enzyme inhibition assays, compound **80** (Figure 1.30) exhibited a half time of inactivation at infinite inhibitor concentration ( $t_{1/2}$ ) of 2.57 min and rate of inactivation ( $K_{inact}$ ) of  $4.50 \times 10^{-4} \text{ sec}^{-1}$  (Brodie et al., 1981).

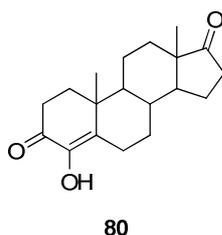


Figure 1.30. Structure of steroidal enzyme activated irreversible aromatase inhibitor, **80**.

## 1.4.2. Non-steroidal Aromatase Inhibitors

### 1.4.2.1. Triazole Based Aromatase Inhibitors

Triazole rings are used as potentially non-steroidal inhibitors of aromatase enzyme. Letrozole (**81** Figure 1.31) is a triazole derivative that causes 50% aromatase enzyme inhibition effectively at 11.5 nM in human placental microsomes (Bhatnagar et al., 1990).

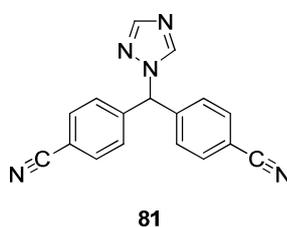


Figure 1.31. Structure of triazole based aromatase inhibitor, **81**.

### 1.4.2.2. Flavanoid Derivatives as Aromatase Inhibitors

The observation of the lower breast cancer ratios of the regions that have abundant nutrients like soy and rye flour lead the hypothesis that the flavonoids, that are abundant in these nutrients, were responsible for lower breast cancer incidences in women. Flavonoids are found to be inhibitory agents for aromatase enzyme thus lowering estrogen levels and decrease the risk factor for the development of cancer. However, biological active flavonoids may be involved in many therapeutic pathways and thus can limit their uses (Brueggemeier et al., 2005).

Pouget et al. showed synthesis and human placental aromatase inhibitory action of novel flavanones (**82** and **83** Figure 1.32). Compound **82**, and **83** inhibit human placental aromatase 50% at 2.5  $\mu$ M and 3.5  $\mu$ M concentrations respectively (Pouget et al, 2002).

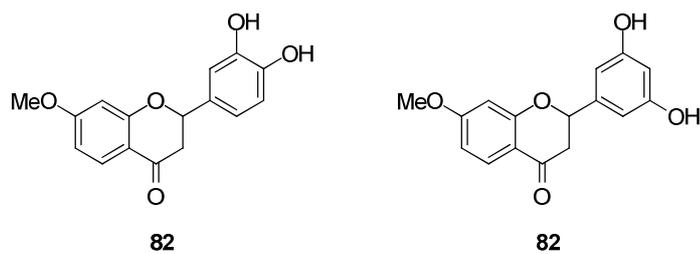


Figure 1.32. Structures of flavanone derivatives **82**, and **83** inhibiting aromatase enzyme.

Le Bail et al. demonstrated for the first time that chalcone derivatives are potent inhibitors of aromatase. They noted that flavones and flavanones generally inhibit aromatase activity more than chalcone derivatives do except with naringenin chalcone (**84**) and eriodictyol chalcone (**85**) (Figure 1.33). Reported  $IC_{50}$  values of those are 2.6  $\mu$ M and 2.8  $\mu$ M respectively (Le Bail et al., 2001).

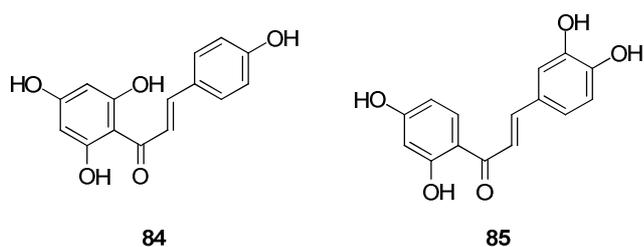


Figure 1.33. Structures of chalcone derivatives **84**, and **85** inhibiting aromatase enzyme.

Another study of aromatase inhibition activity by chalcone and flavanone derivatives was reported by Maiti and coworkers. Maiti group showed that flavanones are much more efficient aromatase inhibitors. Chalcone **86** and flavanone **87** have the same functional groups on their aromatic regions. Chalcone **86** and flavanone **87** inhibit aromatase enzyme by 50% at 82.94  $\mu$ M and 4.08  $\mu$ M concentrations respectively (Maiti et al., 2007).

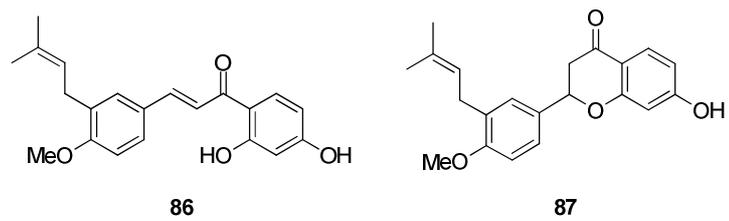


Figure 1.34. Structures of chalcone and flavanone derivatives **86** and **87** exhibiting aromatase inhibition.

## CHAPTER 2

### RESULTS AND DISCUSSIONS

#### 2.1. Synthesis of Boronic Acid Chalcone Derivatives

As discussed in the introduction part, chalcone and boronic acid derivatives are promising drug candidates for chemoprevention of cancer. A recent study also shows that, chalcone derivatives having boronic acid either on its phenolic A or B ring are effective anti-cancer drug candidates. But to the best of our knowledge, diboronic acid chalcone derivatives have never been synthesized or tested for their biological activities in literature.

Because modifications on the cell surface carbohydrates play important role for cancer development, it would be a good choice to synthesize molecules which are targeting or binding carbohydrates. Therefore, in this work it is aimed to synthesize chalcone derivatives substituted by two boronic acids by Claisen-Schmidt reaction starting from corresponding acetylphenyl or formylphenyl boronic acids (Figure 2.1).

Additionally, fluorescent properties of chalcones are well known in literature. Replacement of one of the phenyl ring in chalcone with naphthyl may help to increase fluorescence intensity of from the chalcone and presence of a boronic acid on the other phenyl group of chalcone may convert the chalcone into a carbohydrate sensor. For that reason, it is also aimed to prepare boronic acid naphthyl chalcones substituted with starting from corresponding 2-naphthaldehyde and acetyl phenyl boronic acids (Figure 2.1).

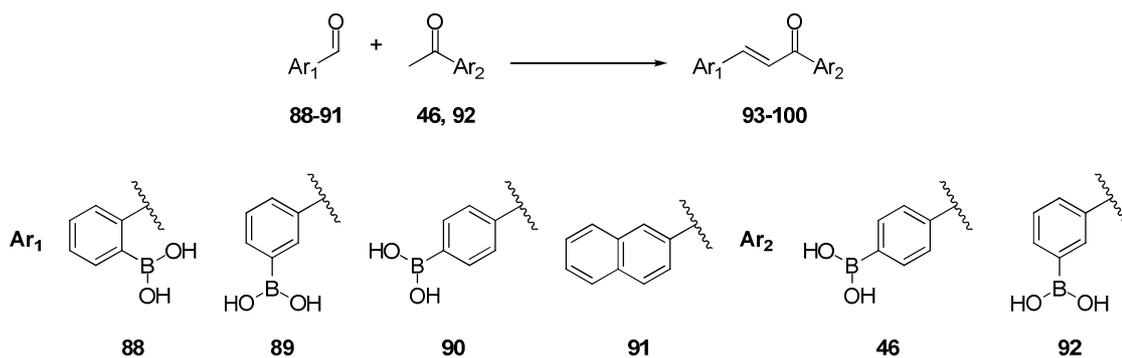
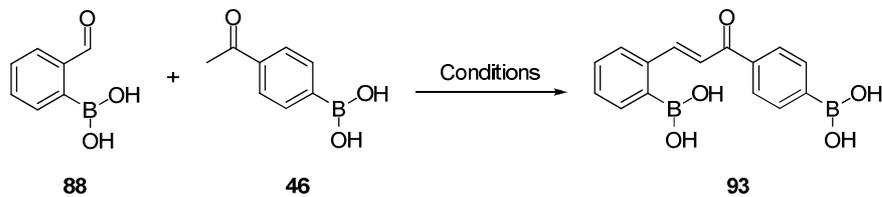


Figure 2.1. Structures of proposed mono and diboronic acid substituted chalcone derivatives **93-100**.

### 2.1.1. Synthesis of Diboronic Acid Chalcone Derivatives

To yield 2,4'-diboronic acid chalcone derivative **93** different reaction conditions were tried. However, all of the reactions carried out between 2-formylphenyl boronic acid (**88**) and 4-acetylphenyl boronic acid (**46**) gave either no reaction or inseparable mixtures of products. In these attempts, both acidic (BF<sub>3</sub>.OEt<sub>2</sub>) and basic (KOH, NaOH, KO<sup>t</sup>Bu) conditions were used to catalyze the reaction in alcohol or dioxane (Table 2.1). Reactions definitely do not yield any product formation at lower temperatures or shorter reaction times under reflux. Purification of boronic acid derivatives in silica gel column is a challenging subject and most of the time 100% recovery is impossible (Hall, 2005).

Table 2.1. Reaction conditions performed for the preparation of compound **93**.



Entry	Conditions				Yield
	Base/Acid	Solvent	Temp.	Time	
<b>1</b>	NaOH	-	RT	20 min.	No Rxn
<b>2</b>	NaOH	EtOH	70 °C	6 h	No Rxn
<b>3</b>	NaOH	EtOH	70 °C	48 h	Inseparable mixture
<b>4</b>	KO <sup>t</sup> Bu	EtOH	reflux	12 h	Inseparable mixture
<b>5</b>	NaOH	EtOH	reflux	24 h	Inseparable mixture
<b>6</b>	KO <sup>t</sup> Bu	MeOH	reflux	12 h	No Rxn
<b>7</b>	KO <sup>t</sup> Bu	MeOH	reflux	24 h	Inseparable mixture
<b>8</b>	NaOH	MeOH	reflux	24 h	No Rnn
<b>9</b>	NaOH	MeOH	reflux	24 h	Inseparable mixture
<b>10</b>	KOH	MeOH	reflü	48 h	Inseparable mixture
<b>11</b>	NaOH	DMSO	Reflux	24 h	Inseparable mixture
<b>12</b>	NaOH	H <sub>2</sub> O	RT	-	No rxn
<b>13</b>	BF <sub>3</sub> ·OEt <sub>2</sub>	Dioxane	RT	6 h	No rxn

Crude products of the reactions was also analyzed by <sup>1</sup>H-NMR to identify the formation of α,β-unsaturated carbonyl functionality. Although there was not any sign for the formation of α,β-unsaturated carbonyls, crude products were applied to flash column chromatography to identify the resulted side products.

Only one of the crude product, reduced form of 2-formylphenyl boronic acid could be identified, which implies the formation of Cannizzaro reaction. Interestingly 2-formylphenyl boronic acid or 4-acetylphenyl boronic acid alone did not give any new product formation under the same reaction conditions.

Although, separation of boronic acid derivatives in silica gel column possible, separation of boronate esters are easier as regarded in literature (Yan et al., 2005). For that reason it might be a good choice to convert the boronic acids to their ethylene glycol esters (Figure 2.2) and then to carry out the Claisen-Schmidt reaction (Figure 2.4). Ethylene glycol esterification of boronic acids were performed at room temperature in the presence of anhydrous  $\text{MgSO}_4$  successfully to produce **101** and **102**. But purification of the products from unreacted ethylene glycol was not possible in our hands. Afterwards crude products of boronate diethyl glycol esters **101** and **102** were reacted under basic condition to produce the corresponding protected diboronic acid chalcone derivative but reaction gave again an inseparable mixture (Figure 2.3).

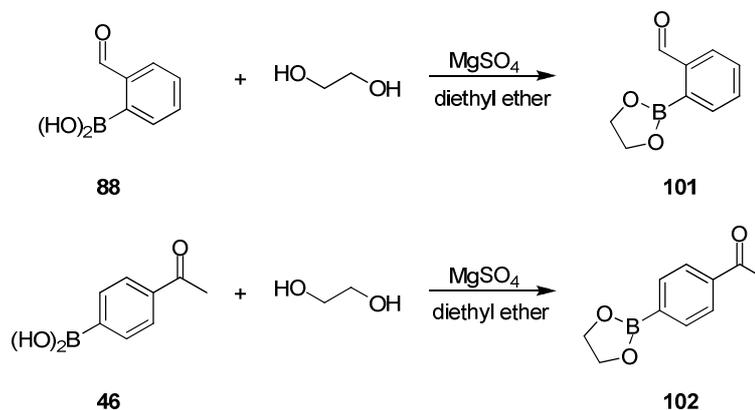


Figure 2.2. Protection of boronic acid functionality with ethylene glycol.

Because all of the attempts toward the preparation of 2,4'-diboronic acid derivatized chalcone was failed, the rest of the study was carried out by 3-formyl and 4-formyl phenyl boronic acids. Synthesis of 2,3'-diboronic acid derivatized chalcone (**94**) was not attempted due to the Cannizzaro reaction of 2-formylphenyl boronic acid.

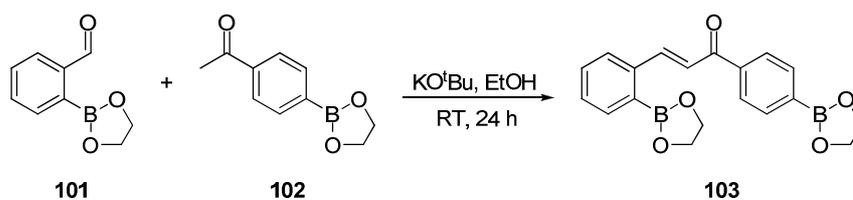
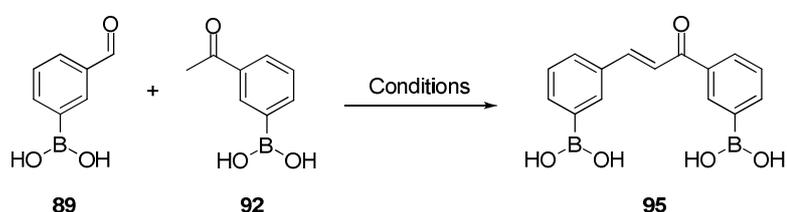


Figure 2.3. Reaction conditions performed for preparation of compound **103**.

For the synthesis of 3,3'-diboronic acid substituted chalcone derivative corresponding boronic acid functionalized benzaldehyde **89** and acetophenone **92** was stirred under acidic or basic conditions (Table 2.2). Synthesis was successfully completed in MeOH in the presence of excess sodium hydroxide under reflux with 84% yield. In other attempts, low concentrations of sodium hydroxide or presence of amine base did not give any successful product formation. Additionally acid catalyzed reaction ( $\text{BF}_3 \cdot \text{OEt}_2$ ) gave 63% yield for the target chalcone product formation.

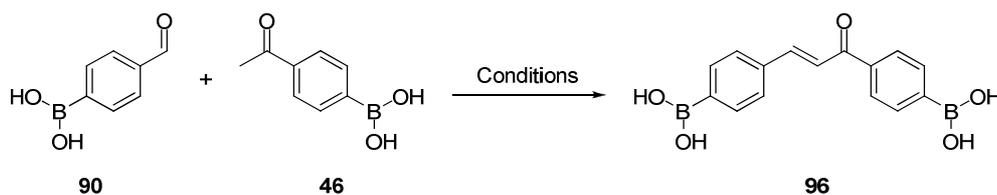
Table 2.2. Reaction conditions performed for the preparation of compound **95**.



Entry	Conditions				Yield
	Base	Solvent	Temp.	Time	
1	NaOH	MeOH	Reflux	24 h	No Rxn
2	$\text{BF}_3 \cdot \text{OEt}_2$	Dioxane	RT	8 h	63%
3	$\text{NEt}_3$	DMSO	RT $\rightarrow$ 60 °C	48 h	No Rxn
4	NaOH / Mol. Sieve	EtOH	RT	24 h	No Rxn
5	NaOH (excess)	MeOH	reflux	4 h	84%

The all reaction attempts between 4-formylphenyl boronic acid and 4-acetylphenyl boronic acid to yield 4,4'-diboronic acid chalcone derivative was performed in alcoholic alkaline solution. In the presence of excess amount of base, reaction proceeded smoothly but the separation of the boronic acid derivatives was a little bit problematic. The difficulty was mainly caused by the different behavior of boronic acid derivatives in TLC and flash column chromatography. By the usage of acetic acid in the eluent system of column chromatography, the tailing effect of boronic acid compounds was obstructed. For entries 3, 4 and 5 during the purification of product by flash column chromatography, acetic acid was used at 0.1%, 0.5% and 1% concentrations respectively. The purification of the product was best performed by the use of 1% acetic acid in eluent system in flash chromatography to give 94% yield (Table 2.3).

Table 2.3. Reaction conditions performed for the preparation of compound **96**.



Entry	Conditions				Yield
	Base	Solvent	Temp.	Time	
1	NaOH	MeOH	Reflux	24 h	No rxn
2	NaOH	MeOH	Reflux	24 h	Inseparable mixture
3	NaOH (excess)	MeOH	Reflux	24 h	45%
4	NaOH (excess)	MeOH	Reflux	24 h	74%
5	NaOH (excess)	MeOH	Reflux	24 h	94%

Last two of the diboronic acid chalcone derivatives 3,4'- (**97**) and 4,3'-diboronic acid chalcone (**98**) were prepared under the same reaction conditions, performed to synthesize previous diboronic acid chalcones (Figure 2.4, Figure 2.5). But purification of these two diboronic acid chalcone derivatives was a little bit problematic compared to that of compound **95** and **96**. Although 1% acetic acid was used in the eluent system of flash column, totally purification of **97** and **98** from the starting materials was not possible. Only 44% and 34% of the products **97** and **98** were fully separated from reaction mixtures.

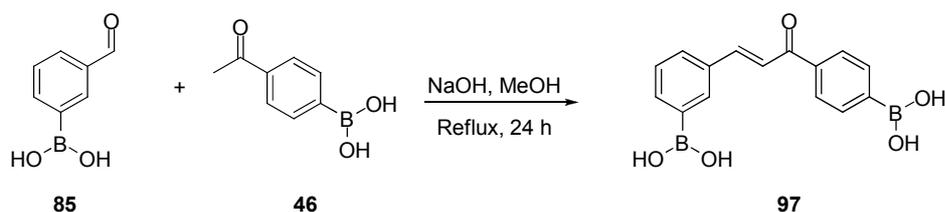


Figure 2.4. Reaction conditions performed for preparation of compound **97**.

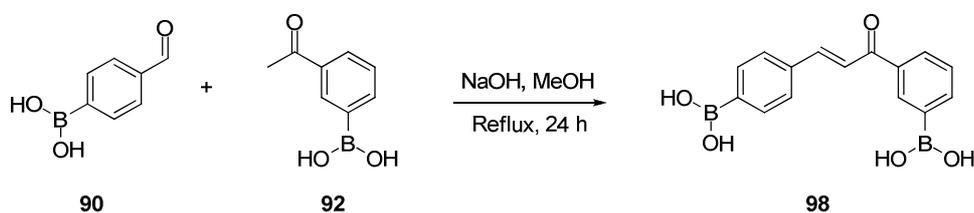
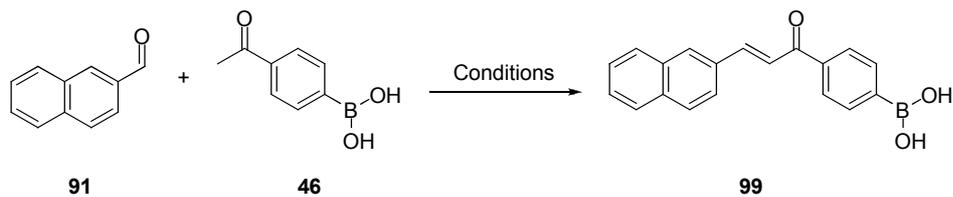


Figure 2.5. Reaction conditions performed for preparation of compound **98**.

### 2.1.2. Synthesis of 2-Naphthyl Chalcone Boronic Acid Derivatives

Claisen-Schmidt condensation reaction between 2-naphthaldehyde and acetylphenyl boronic acid derivatives was performed under basic conditions (Table 2.4). Reaction of 2-naphthaldehyde with 4-acetylphenyl boronic acid gave product formation in all attempts but the purification of those are quite challenging because of the close  $R_f$  values of products with the starting material (4-acetylphenyl boronic acid **46**).

Table 2.4. Reaction conditions performed for preparation of compound **99**.



Entry	Conditions				Yield
	Base	Solvent	Temp.	Time	
1	KO <sup>t</sup> Bu	<sup>t</sup> BuOH	70 °C	24 h	Inseparable mixture
2	NaOH	MeOH	Reflux	24 h	Inseparable mixture
3	KOH	H <sub>2</sub> O	RT	-	Inseparable mixture
4	KOH	MeOH/H <sub>2</sub> O	Reflux	48 h	Inseparable mixture
5	NaOH	MeOH/H <sub>2</sub> O	Reflux	24 h	44%

To overcome this problem two different approaches was tried. In the first case the mixture obtained from column chromatography was subjected to crystallization. This approach gave 44% of product in pure form. In the second case, boronic acid derivatized chalcone (**99**) and 4-acetylphenyl boronic acid mixture was converted into its pinacol ester to create a  $R_f$  difference, but ester forms of those boronic acids have also similar  $R_f$  values, so column chromatography was failed (Figure 2.6).



Figure 2.6. Esterification of boronic acid moieties on 2-naphthyl chalcone boronic acid with pinacol.

As an alternative to the second approach, esterification of 4-acetyl phenyl boronic acid (**46**) with pinacol was performed before the Claisen-Schmidt reaction. Although esterification reaction was proceeded smoothly, again formation of inseparable mixture from Claisen-Schmidt reaction observed. (Figure 2.7).

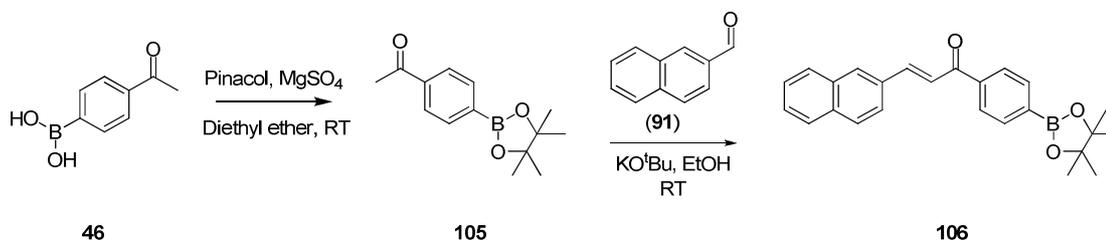


Figure 2.7. Esterification of 4-acetylphenyl boronic acid (**46**) with pinacol and reaction of pinaco ester form of acetophenone (**105**) and 2-naphthaldehyde (**91**).

At last, 2-naphthaldehyde was reacted with 3-acetylphenyl boronic acid to form chalcone **100** under alcoholic alkaline solution and product was purified by the same procedure explained for chalcone **99** (Figure 2.8).

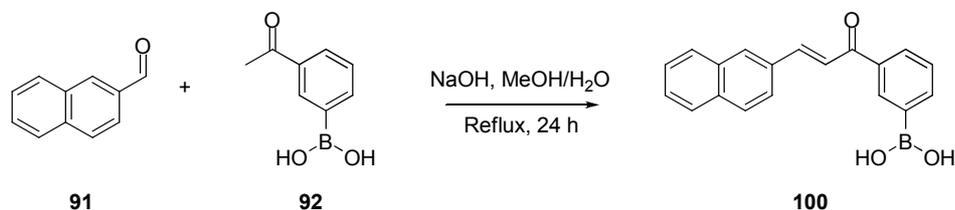


Figure 2.8. Reaction conditions performed for preparation of compound **100**.

## 2.2. Carbohydrate Binding Tests for Boronic Acid Chalcone Derivatives

Synthesized mono and diboronic acid chalcone derivatives were tested for their binding capability with D-fructose, D-galactose and D-glucose (Figure 2.9). Fluorescent studies of diboronic acid functionalized chalcones were performed in 0.1 M phosphate

buffer at pH 7.4 containing 1% DMSO. All of the diboronic acid chalcones are excited between 320-325 nm wavelengths and gave no remarkable fluorescence emissions. Therefore, diboronic acid chalcones may not be used as carbohydrate sensors at physiological pH.

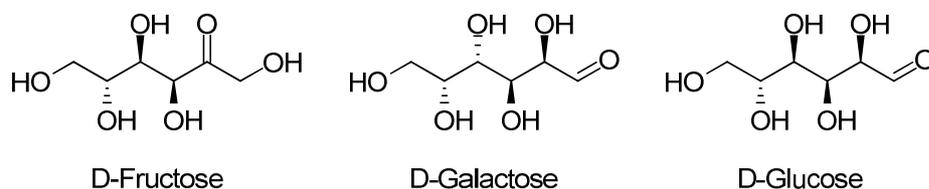


Figure 2.9. Structures of D-fructose, D-galactose and D-glucose.

Although fluorescence properties of diboronic acid chalcone derivatives did not change in the presence of carbohydrates at physiological pH, it does not mean the lack of interactions between carbohydrates and diboronic acid chalcones. Because, requirement for fluorescence intensity change is the complexation of both boronic acids in tetrahedral form with carbohydrates at the same time (**DBAC 5**, figure 2.10). Binding of an only one boronic acid moiety with carbohydrate maybe lacks of fluorophore which should cause fluorescence emission.

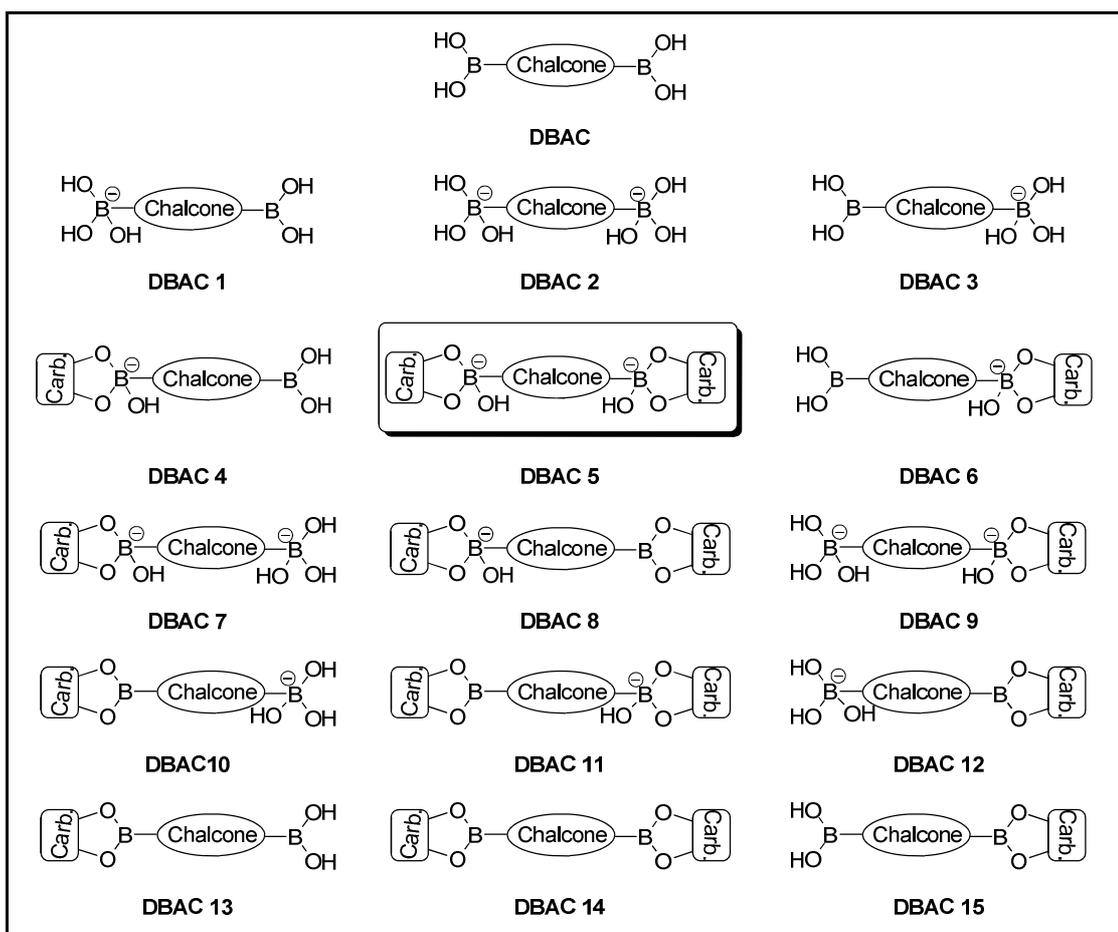


Figure 2.10. Possible diboronic acid chalcone (**DBAC**) forms in 0.1 M phosphate buffer at pH 7.4 containing 1% DMSO.

On the other hand, 2-naphthyl chalcone boronic acids have stronger fluorescence responses itself. Both compounds were excited at 340 nm and a fluorescence emission maximum were observed at 523 nm. At physiological pH, fluorescence intensity of compound **99** increases with increasing concentrations of carbohydrates. In the presence of 100 mM D-fructose compound **99** gave a maximum which is 73 fold stronger compared to chalcone itself. Similarly 15 fold and 5 fold increases are observed in the fluorescence intensity with D-galactose and D-glucose respectively. Changes in the fluorescence spectrum of compound **99** in varying concentrations of D-fructose is shown in figure 2.11. Relative fluorescence intensity changes of compound **99** with different concentrations of D-fructose, D-galactose and D-glucose was summarized in figure 2.12. The appearing binding constants ( $K_{app}$ ) are calculated according to Benesi-

Hildebrand method, assuming 1:1 binding was occurred. A sample  $K_{app}$  calculation for compound **99** upon binding to fructose was shown in figure 2.13.

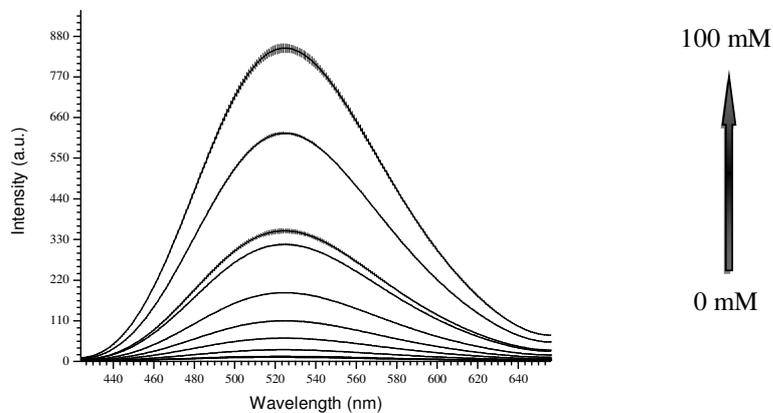


Figure 2.11. Fluorescence intensity change of compound **99** in the presence of varying concentrations of D-fructose.

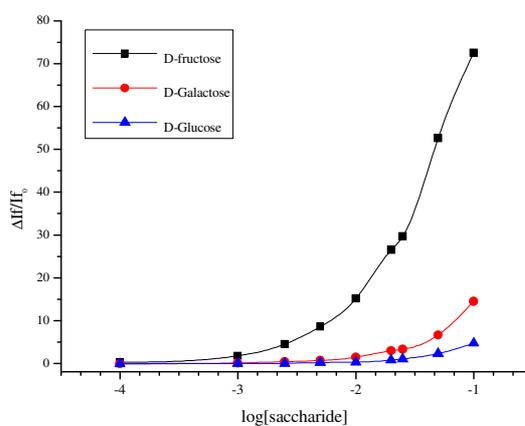
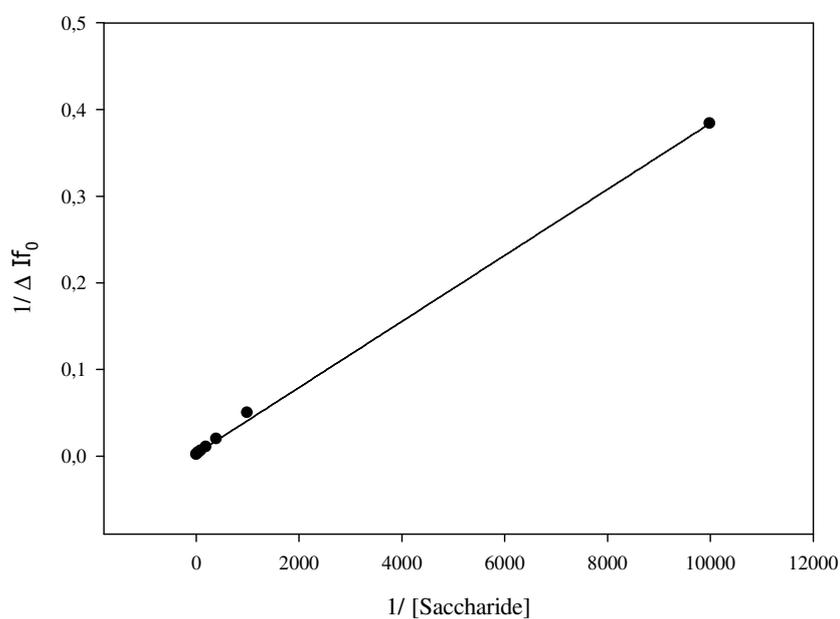


Figure 2.12. The plot for normalized fluorescence intensity to  $\log[\text{saccharide}]$  in the presence of D-fructose  $\blacksquare$ , D-galactose  $\bullet$ , D-glucose  $\blacktriangle$  for compound **99** ( $3.30 \times 10^{-5}$  M) in 0.1 M phosphate buffer at pH 7.4  $\lambda_{ex} = 340$  nm and  $\lambda_{em} = 523$  nm.

If	If <sub>0</sub>	If-If <sub>0</sub>	[D-Fructose] (M)	1/saccharide	1/ Δ If	slope	intercept	K <sub>app</sub> (M <sup>-1</sup> )
14.14034		2.61	0.0001	10000	0.383162			
31.77098		20.24	0.001	1000	0.049406			
63.28095		51.75	0.0025	400	0.019323			
110.1764		98.65	0.005	200	0.010137			
186.2089	11.53	174.68	0.01	100	0.005725	3.81E-05	0.0029	76,1
316.7682		305.24	0.02	50	0.003276			
353.3921		341.86	0.025	40	0.002925			
617.7861		606.26	0.05	20	0.001649			
847.9275		836.40	0.1	10	0.001196			



**Equation**  $y = 3.81E-05x + 0.0029$

**If** = Fluorescence intensity of complexed species.

**If<sub>0</sub>** = Fluorescence intensity of compound **99** itself.

**D-Fructose (M)** = Concentrations of fructose.

**Slope** = Slope of the plot calculated.

**Intercept** = Intercept of the plot calculated.

**K<sub>app</sub>** = Intercept/Slope

Figure 2.13.  $K_{app}$  calculation for compound **99** upon binding to fructose.

Appearing binding constants of compound **99** with D-fructose, D-galactose and D-glucose are calculated as  $76.1 \text{ M}^{-1}$  and  $23 \text{ M}^{-1}$  respectively (Table 2.5).

Table 2.5. Calculated  $K_{app}$  binding constants and  $r^2$  values for various saccharides of compound **99**.

Compound	<b>101</b>	
	$K_{app} (\text{M}^{-1})$	$r^2$
<b>D-Fructose</b>	76.1	0.99
<b>D-Galactose</b>	23	0.98
<b>D-Glucose</b>	NA	NA

Similarly fluorescence intensity of compound **100** increases with the concentrations of carbohydrates present in the buffer solution. Fluorescence spectra of compound **100** gave a maximum at 524 nm when it is excited at 340 nm. In the presence of 100 mM D-fructose compound **100** gave an emission intensity maximum which is 33 fold stronger than chalcone **100** in the absence of D-fructose. Similarly 8 fold and 4 fold fluorescence intensity changes was observed for D-galactose and D-glucose correspondingly. Changes in the fluorescence spectrum of compound **100** in varying concentrations of D-fructose is shown in figure 2.14. Relative fluorescence intensity changes of compound **100** in different concentrations of D-fructose, D-galactose and D-glucose are shown in figure 2.15. Calculated appearing binding constants of compound **100** upon binding to D-fructose, D-galactose and D-glucose are  $27.5 \text{ M}^{-1}$ ,  $0.7 \text{ M}^{-1}$  and  $1.3 \text{ M}^{-1}$  respectively (Table 2.6).

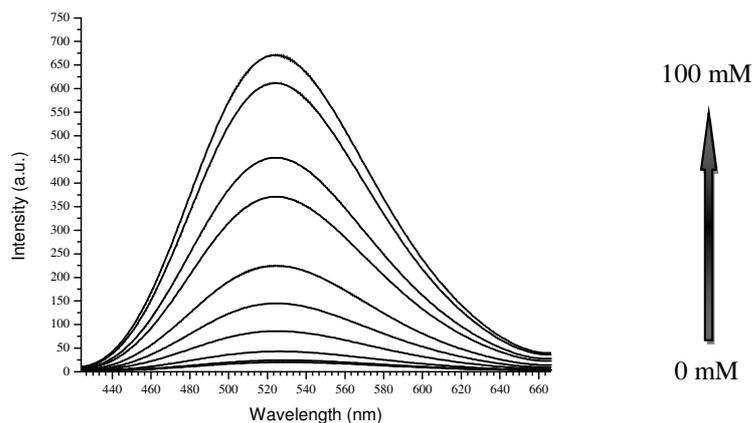


Figure 2.14. Fluorescence intensity change of compound **100** in the presence 0 to 100 mM of D-fructose **100** ( $3 \times 10^{-5}$  M) in 0.1 M phosphate buffer at pH 7.4  $\lambda_{ex} = 340$  nm  $\lambda_{em} = 524$  nm.

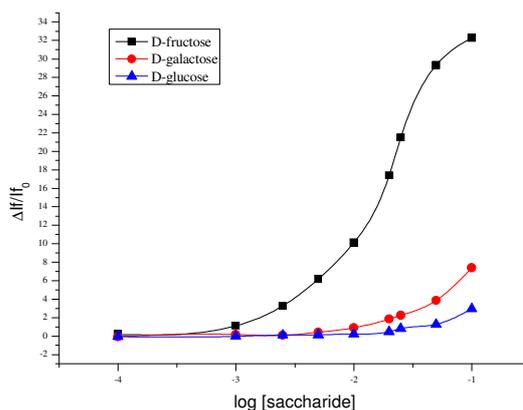


Figure 2.15. The plot for normalized fluorescence intensity to log [saccharide] in the presence of varying concentrations of D-fructose ■, D-galactose ●, D-glucose ▲ for compound **100** ( $3 \times 10^{-5}$  M) in 0.1 M phosphate buffer at pH 7.4  $\lambda_{ex} = 340$  nm and  $\lambda_{em} = 524$  nm.

Table 2.6. Calculated  $K_{app}$  binding constants and  $r^2$  values for various saccharides of compound **100**.

Compound	<b>100</b>	
	$K_{app}$ ( $M^{-1}$ )	$r^2$
<b>Carbohydrate</b>		
<b>D-Fructose</b>	27.5	0.99
<b>D-Galactose</b>	0.7	0.99
<b>D-Glucose</b>	1.3	0.98

These results imply that binding ability of 2-naphthyl chalcone boronic acids with carbohydrates is altered by changing the place of boronic acid substitution at the chalcone structure. Previously it was shown that binding constants of aryl boronic acids to carbohydrates increases in the presence of an electron withdrawing groups on the same phenyl ring (Mulla et al., 2004).

Boron atom has a special property because of its unfilled in neutral form. In such case boron atom behaves as a Lewis acid and electron deficient group. When the resonance structures of compound **99** and **100** are shown in figure 2.16. It can be seen that none of the resonance structures of compound **100** have a positive charge next to electron deficient boron atom. Resonance structures of compound **100** should be relatively less energetic compared to the resonance structures of compound **99** having a positive charge next to boron atom (**99-b**). Such resonance structure may force compound **99** to bind strongly to the carbohydrates.

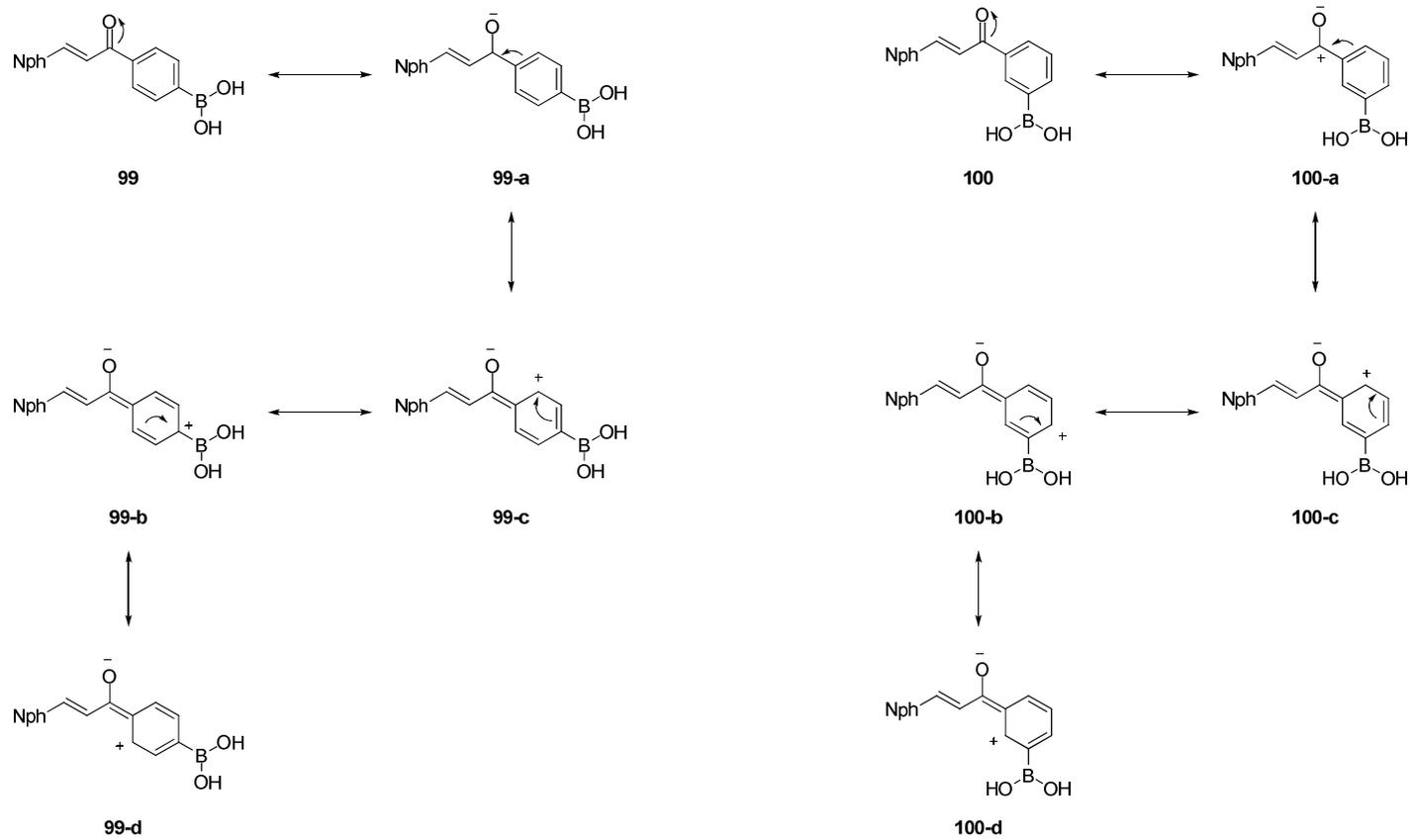
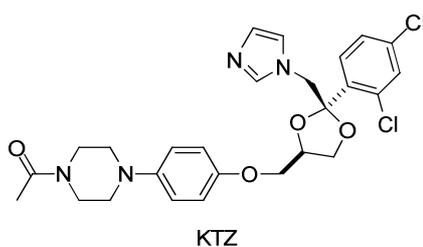


Figure 2.16. Resonance structures of compound **99** and **100**

### 2.3. Aromatase Inhibition Properties of Diboronic Acid Chalcone Compounds

Up to date in the best of our knowledge, there are not any reported boronic acid derivatized aromatase inhibitors. Because aromatase inhibitors are valuable for the fight with cancer, mono and diboronic acid chalcones (**95-100**) were tested for their aromatase inhibition properties. For this purpose, commercially available CYP19/MFC high throughput aromatase inhibition kit was used Ketoconazole (KTZ, figure 2.17) was used as a positive control for aromatase inhibition. Primary results showed that only three of the diboronic acid chalcones (**95**, **97** and **98**) inhibit aromatase activity 50% at 169  $\mu\text{M}$ , 146  $\mu\text{M}$  and 149  $\mu\text{M}$  respectively. Percent aromatase inhibition values for KTZ, **95**, **97** and **98** at different concentrations are given in figure 2.18. As shown in figures, all of the non linear regression analysis result  $r^2$  values higher than 0.9. On the other side, aromatase inhibition properties of other mono- and diboronic acid chalcones **96**, **99** and **100** could not be detected because of a possible contamination emitted a fluorescence that interferes with the fluorescence of the product of MFC. It is also possible that these chalcones may have an against activity on aromatase enzyme can lead the formation of higher amount of product. To clarify the subject more experimentation is necessary.



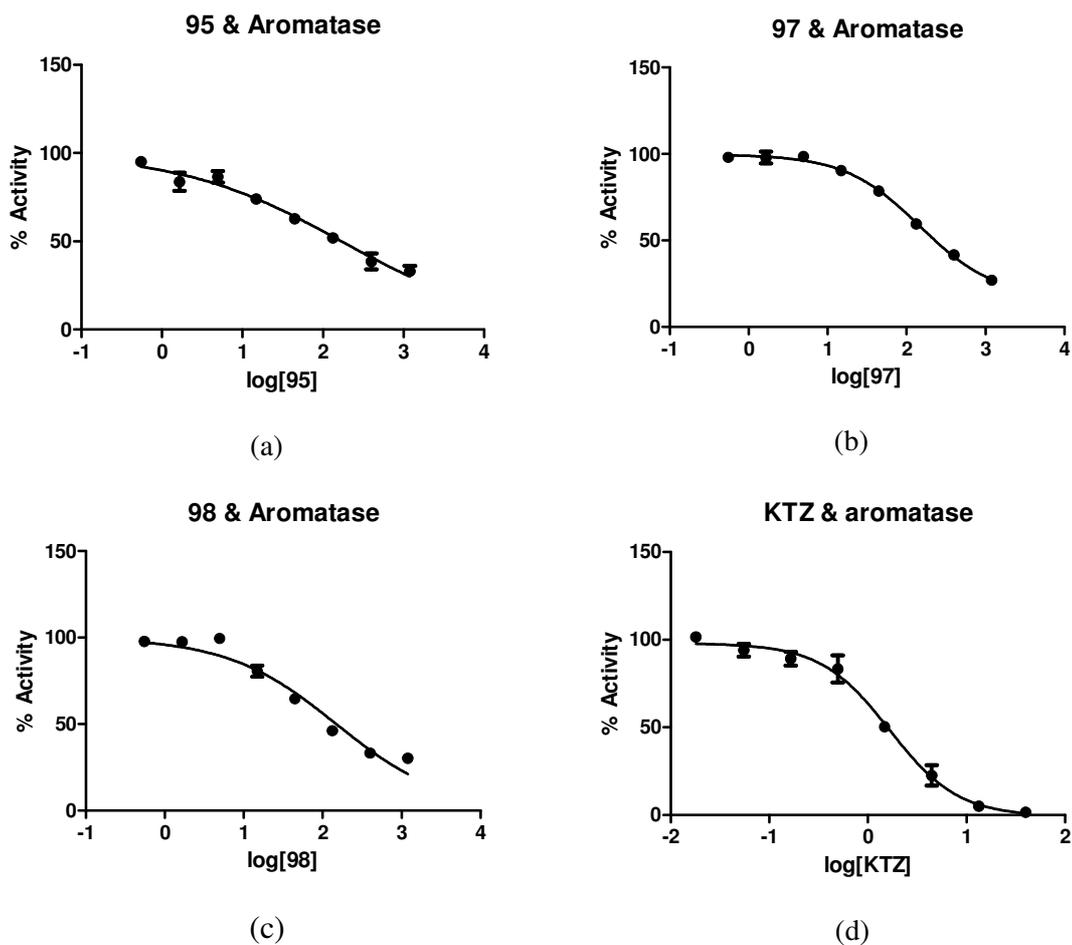


Figure 2.18. Aromatase inhibition curves of diboronic acid chalcone derivatives (a) **95**, (b) **97** and (c) **98** and (d) KTZ.

## 2.4. Cytotoxic Properties of Diboronic Acid Chalcone Derivatives

Cytotoxic properties of chalcone boronic acids were examined in MCF-7 and PC3 cancer cell lines by MTT assay. As preliminary studies were shown in figure 2.23, compounds **96**, **97** and **98** were found cytotoxic against MCF-7 cells equally with  $IC_{50}$  values of 45  $\mu$ M. On the other hand monoboronic acid derivatized naphthyl chalcones **99** and **100** are found more cytotoxic. They inhibit MCF-7 cell proliferation 50% at 16.3  $\mu$ M and 14.3  $\mu$ M respectively (Figure 2.19).

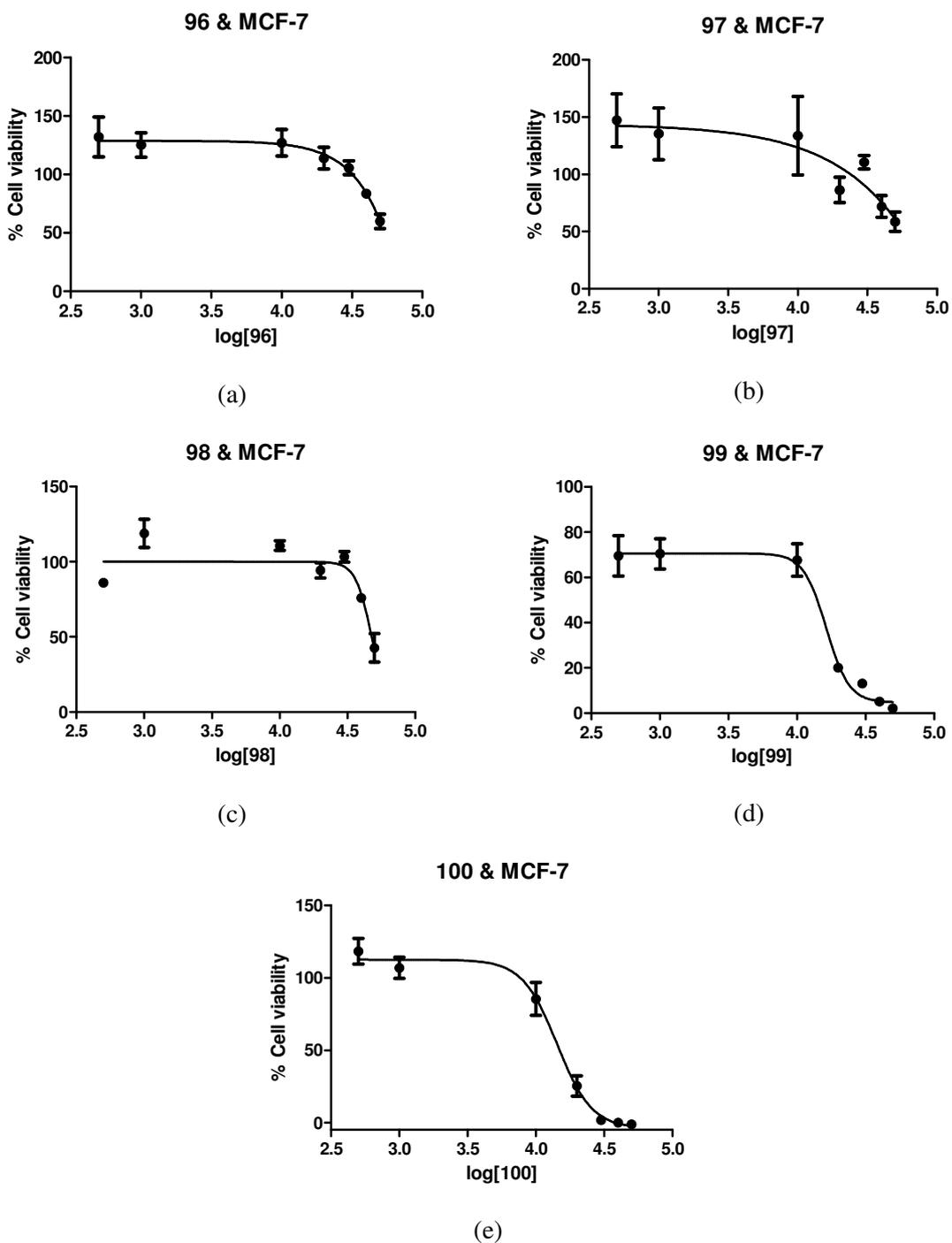


Figure 2.19. Cytotoxic activity profiles of diboronic acid chalcone derivatives against MCF-7 cancer cell lines (a) **96**, (b) **97**, (c) **98**, (d) **99** and (e) **100**.

Similar trend was observed in PC3 cancer cells for mono and diboronic acid derivatized chalcones. 2-Naphthyl chalcone boronic acids are found more cytotoxic than disubstituted ones. Calculated IC<sub>50</sub> values for compounds **96**, **97**, **99** and **100** are found 45  $\mu$ M, 47  $\mu$ M, 31.2  $\mu$ M and 22  $\mu$ M respectively (Figure 2.20).

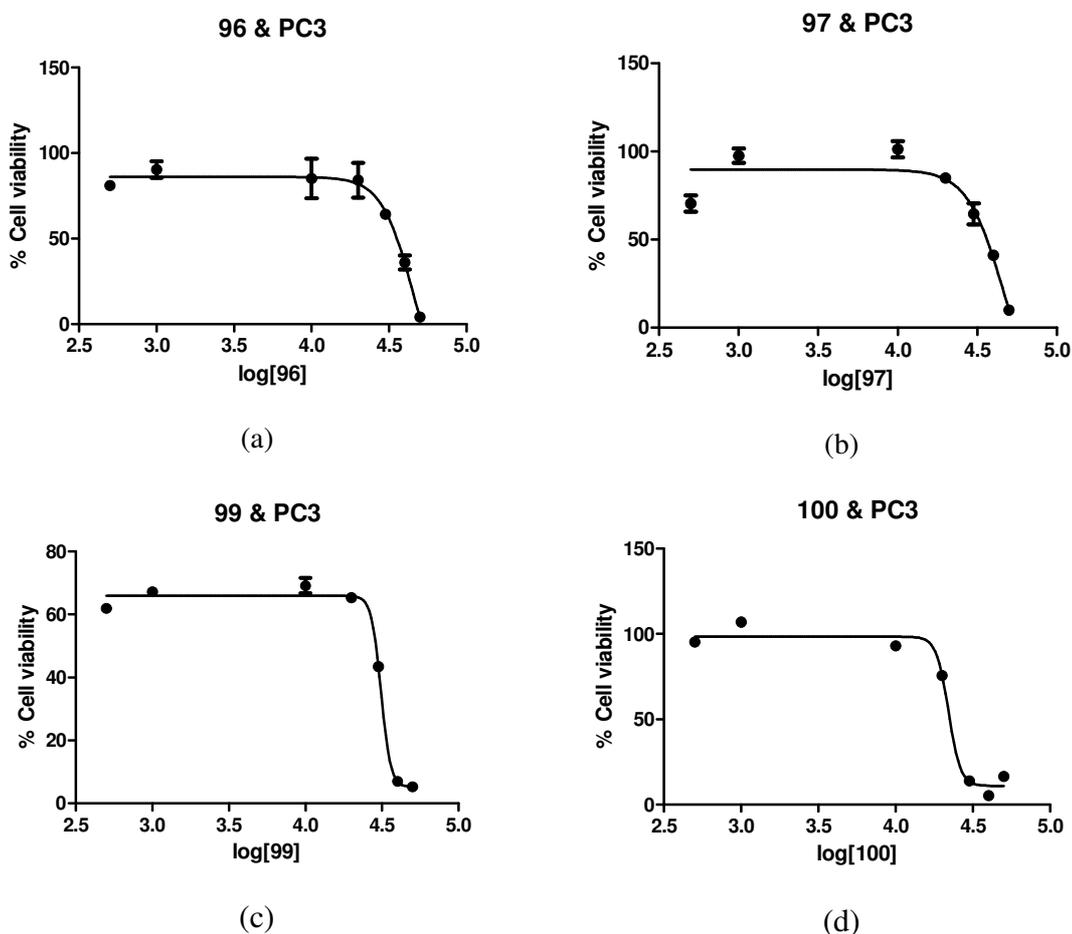
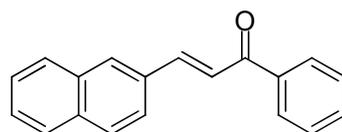


Figure 2.20. Cytotoxic activity profiles of diboronic acid chalcone derivatives against PC3 cancer cell lines (a) **96**, (b) **97**, (c) **99**, (d) **100**.

Cytotoxic activity of simple 2-naphthyl chalcone (Figure 2.21, **107**) have previously reported by Brodie et al., that inhibited SVR cell proliferation 79% at 3 $\mu$ g/ml (11  $\mu$ M) which is quite close to those of the monoboronic acid derivatized 2-naphthyl chalcones over MCF-7 (Brodie et al., 2005). Thus implies that boronic acid substitution did not help to improve the cytotoxic activity of the naphthyl chalcones against cancer cells. Additionally tests are underway to confirm the findings.



**107**

Figure 2.21. Structure of 2-naphthyl chalcone (**107**).

## CHAPTER 3

### EXPERIMENTAL

#### 3.1. Chemistry

##### 3.1.1. General Methods

All reagents and solvents were commercially purchased from Sigma-Aldrich and Riedel (Extra pure grade), and were used as supplied. MERC TLC (Silica Gel 60 F 254) plates were used for monitoring reactions. Boronic acids were screened with UV lamp in 254 nm and 365 nm wavelength and 2% (w/v) solution of diphenylcarbazone ethanol in TLC. For chromatographic purification of the products were performed by flash column using 70-230 mesh sized silica gel.

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were acquired by Varian-400-MR (400 MHz) spectrometer. Data processed by ACD/NMR Processor Academic Edition Version: 12.01. MeOD-d<sub>4</sub> and DMSO-d<sub>6</sub> were used as NMR solvent and chemical shifts reported in  $\delta$  (ppm).

Carbohydrate binding studies were performed with Varian Cary Eclipse fluorescence spectrophotometer. UV-VIS absorption data carried out with Varian Cary 50 Scan UV-Vis spectrophotometer. In all UV-VIS and fluorescence studies QS 1100 quartz cuvettes were used. Ultra pure water was used for boronic acid carbohydrate binding studies purified with Milli-Q filtration system. pH adjusted with Inolab WTW pH 720 pH meter.

MTT assay and aromatase inhibition assay were performed by Özgür Yılmaz ÇAKMAK and Burcu ALAÇAM.

### 3.1.5. Synthesis of Boronic Acid Chalcone Derivatives

#### 3.1.3.4. (E)-3-(3-boronic acid phenyl)-1-(3-boronic acid phenyl)prop-2-en-1-one (95)

In a two neckled flask 218.17 mg of 3-acetylphenyl boronic acid (1.3 mmol, 1 eq.) and 400 mg of 3-formylphenyl boronic acid (2.6 mmol, 2 eq.) were placed and dissolved in 7 mL of methanol and was stirred under reflux. Then 520 mg of NaOH (13 mmol, 10 eq.) was directly added into mixture. After 4 hours, mixture was cooled down and acidified with 1M HCl to adjust pH 4 and then methanol was removed under reduced pressure. Crude product was extracted with ethyl acetate (3x60 mL) and the combined organic phase was dried over anhydrous MgSO<sub>4</sub> and filtered with millipore system. After removal of solvent of the crude product, purification by column chromatography (MeOH/HOAc/DCM; 2:0.5:97.5 to 5:0.5:94.5) resulted desired product in 84% yield. <sup>1</sup>H NMR (400 MHz, MeOD-d<sub>4</sub>) δ = 8.46 (br. s., 1H), 7.65 - 8.17 (m, 7H), 7.49 (t, J=7.24 Hz, 1H), 7.40 (t, J=7.24 Hz, 1H); <sup>13</sup>C NMR (100 MHz, MeOD-d<sub>4</sub>) δ = 192.89, 146.65, 139.71, 138.58, 135.34, 135.05, 131.33, 129.37, 129.13, 122.91.

#### 3.1.3.3. (E)-3-(4-boronic acid phenyl)-1-(4-boronic acid phenyl)prop-2-en-1-one (96)

In a two neckled flask 218.17 mg of 3-acetylphenyl boronic acid (1.34 mmol, 1 eq.) and 400 mg of 3-formylphenyl boronic acid (2.66 mmol, 2 eq.) was placed and dissolved in 5 mL of methanol. Mixture was stirred under reflux until reagents were fully dissolved. After that; 520 mg of NaOH (13 mmol, 10 eq.) in 5 mL of methanol was added drop wise into reaction mixture. After 24 hours reflux, reaction mixture was cooled down and acidified with 1 M HCl to adjust pH 4, and then methanol was removed under reduced pressure. Crude product was extracted with ethyl acetate (3x60 mL) and then the combined organic phase was dried over anhydrous MgSO<sub>4</sub>. Organic layer was filtered with millipore system, and removed under reduced pressure to yield crude product. Purification by column chromatography (MeOH/HOAc/DCM; 3:1:96) resulted desired product in 94% yield; <sup>1</sup>H NMR (400 MHz, MeOD-d<sub>4</sub>) δ = 7.97 (d,

J=8.20 Hz, 2H), 7.84 (d, J=6.65 Hz, 2H), 7.80-7.60 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz, MeOD- $d_4$ )  $\delta$  = 192.67, 146.29, 140.27, 137.56, 135.51, 135.17, 128.84, 128.59, 123.48.

### **3.1.3.5. (E)-3-(3-boronic acid phenyl)-1-(4-boronic acid phenyl)prop-2-en-1-one (97)**

Into a two neckled flask 400 mg of 3-formylphenyl boronic acid (2.6 mmol, 2 eq.) and 218.17 mg of 4-acetylphenyl boronic acid (1.3 mmol, 1 eq.) were placed and solved in 3 mL of methanol under reflux. Then solution of 520 mg of NaOH (13 mmol, 10 eq.) in 7 mL methanol was added into mixture drop wise. A day after reaction mixture was cooled down and acidified to adjust pH 4 with 1 M HCl. Afterwards methanol was evaporated, and remaining aqueous mixture extracted with ethyl acetate (3 x 60 mL). Organic layer was dried over  $\text{MgSO}_4$  and then removed under reduced pressure to yield crude product. Purification by column chromatography (MeOH/HOAc/DCM 3:1:9) resulted desired product in 34% yield.  $^1\text{H}$  NMR (400 MHz, MeOD- $d_4$ )  $\delta$  = 8.06 (br. s., 1 H), 7.99 (d, J=7.83 Hz, 2 H), 7.85 (d, J=7.04 Hz, 2 H), 7.80 - 7.66 (m, 4 H), 7.39 (t, J=7.43 Hz, 1 H);  $^{13}\text{C}$  NMR (100 MHz, MeOD- $d_4$ )  $\delta$  = 192.74, 146.75, 135.39, 135.30, 135.15, 129.43, 128.59, 122.89.

### **3.1.3.6. (E)-3-(4-boronic acid phenyl)-1-(3-boronic acid phenyl)prop-2-en-1-one (98)**

Into a two neckled flask 400 mg of 4-formylphenyl boronic acid (2.66 mmol 2 eq) and 218.17 mg of 3-acetylphenyl boronic acid (1.3 mmol 1 eq) were placed and solved in 3 mL of methanol and stirred under reflux. Afterwards a solution of 520 mg of NaOH (13 mmol, 10 eq.) in 7 mL methanol was added drop wise into mixture. After 24 hours reflux, reaction mixture cooled down and acidified to adjust pH 4 with 1 M HCl. Then methanol was evaporated under reduced pressure. Remaining aqueous mixture was extracted with ethyl acetate (3 x 60 mL). The combined organic layer was dried over  $\text{MgSO}_4$  and concentrated under reduced pressure to yield crude product. Purification by column chromatography (MeOH/HOAc/DCM; 1:1:98 to 4:1:95) resulted desired product in 44% yield.  $^1\text{H}$  NMR (400 MHz, MeOD- $d_4$ )  $\delta$  =  $^1\text{H}$  NMR (400 MHz, METHANOL- $d_4$ )  $\delta$  ppm 8.41 (br. s., 1 H), 8.05 (dt, J=7.83, 1.56 Hz, 1 H), 7.99 -

7.89 (m, 1 H), 7.82 - 7.59 (m, 6 H), 7.48 (t,  $J=7.43$  Hz, 1 H);  $^{13}\text{C}$  NMR (100 MHz, MeOD- $d_4$ )  $\delta$  = 192.72, 146.13, 139.74, 138.52, 137.60, 135.49, 135.06, 131.23, 129.17, 129.01, 128.82, 127.13, 123.52.

### **3.1.3.1. (E)-1-(4-boronic acid phenyl)-3-(naphthalen-2-yl)prop-2-en-1-one (99)**

Into a two necked flask 312.36 mg of 2-naphthaldehyde (2 mmol, 1 eq.) and 327.94 mg of 4-acetylphenyl boronic acid (2 mmol, 1 eq.) were placed and solved in 5 ml of methanol, and then stirred for 5 minutes under reflux. Afterwards 0.6 mL of 40% NaOH (6 mmol, 3 eq.) solution in water was added into reaction mixture drop wise. A day after, reaction mixture was cooled down and diluted with 1 M HCl to adjust pH 4. Methanol was evaporated under reduced pressure and remaining aqueous mixture was extracted with ethyl acetate (3x50 mL). The combined organic phase was dried over anhydrous  $\text{MgSO}_4$  and filtered with millipore system. Then filtered organic layer was concentrated under reduced pressure to yield crude product. Purification of the crude product by column chromatography (EtOH/DCM; 2:98), followed by recrystallization in ethyl acetate methanol mixture (10:1) yielded yellow crystals in 44% yield.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 8.40-8.29 (m, 2H), 8.26-8.05 (m, 4H), 8.03-7.87 (m, 5H), 7.64-7.51 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  = 189.36, 144.06, 138.69, 134.40, 133.96, 132.97, 132.36, 130.78, 128.57, 128.49, 127.75, 127.48, 127.40, 124.48, 122.43.

### **3.1.3.2. (E)-1-(3-boronic acid phenyl)-3-(naphthalen-2-yl)prop-2-en-1-one (100)**

To a refluxed solution of 95 mg of 2-naphthaldehyde (0.6 mmol, 2 eq.) in 4 mL methanol was added 3-acetylphenyl boronic acid (0.6 mmol, 1 eq.) and the mixture was stirred for 5 minutes under reflux. Then 3.6 mL of 40% NaOH (1.8 mmol, 3 eq.) solution in water was added into reaction mixture drop wise. After 24 hours, reaction mixture was cooled down and acidified with 1M HCl to adjust pH 4. Reaction solvent was evaporated under reduced pressure and remaining aqueous mixture was extracted with ethyl acetate (3x50 mL). Combined organic layer was dried over anhydrous

MgSO<sub>4</sub> and filtered with millipore filtration system. Then organic phase was concentrated under reduced pressure to yield crude product. Purification by column chromatography (EtOH/DCM 2:98), followed by recrystallization in ethyl acetate methanol mixture (10:1) yielded yellow crystals in 41% yield. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ = 8.60 (s, 1 H), 8.38-8.28 (m, 3 H), 8.21 (d, *J*=7.83 Hz, 1 H), 8.14 – 7.87 (m, 7 H), 7.64 - 7.52 (m, 3 H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ = 189.48, 143.79, 138.77, 136.80, 134.10, 133.90, 132.95, 132.32, 130.61, 130.11, 128.49, 127.71, 127.43, 126.80, 124.26, 122.50. MS (EI, m/z): 257.2 ([M-B(OH)<sub>2</sub>]<sup>+</sup>) [Calculated: 302,11 for C<sub>19</sub>H<sub>15</sub>BO<sub>3</sub>].

### 3.1.2. Carbohydrate Binding

All carbohydrate binding studies were carried out as following procedure; 3.30 x 10<sup>-5</sup> M solution of boronic acid chalcone derivative in 0.1 M sodium phosphate monobasic buffer having 1% DMSO were prepared and then pH was adjusted to 7.4 ± 0.01 with 5 M NaOH. To prepare 1% DMSO buffer solution, 6.60 x 10<sup>-3</sup> M of chalcone boronic acid derivative was prepared with 10 mL DMSO and then 5 mL of this stock solution was added into 500 mL of 0.1 M sodium phosphate monobasic buffer. Then pH was adjusted with 5 M NaOH if necessary (stock 1). To alternate carbohydrate concentrations a 200 mM stock carbohydrate solution (stock 2) was prepared by using stock 1. Stock 2 was stored in room temperature for 15 minutes to be sure for the binding of boronic acid to carbohydrates. Then stock 2 was diluted into 50 mL falcon tubes to form 9 different carbohydrate concentrations between 0,1 mM and 100 mM (0.1 mM, 1 mM, 2,5 mM, 5 mM, 10 mM, 20 mM, 25 mM, 50 mM, 100 mM) with stock 1. After 15 minutes fluorescence measurements were carried out with 3 mL of solutions in quartz cuvettes. All measurements were done in 3 replicates. Samples were excited between 315 nm and 340 nm. Binding constants of boronic acids with carbohydrates were calculated by Benesi-Hildebrand method, by assuming 1:1 binding of boronic acids with carbohydrates.

### 3.1.3. MTT Assay

Human Prostat Cancer (PC-3) cell line was kindly provided by Associate Professor Kemal Sami Korkmaz (Ege University, Engineering Faculty, Department of Bioengineering), human breast cancer (MCF-7) cell line was obtained from Şap Institute. PC3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS), 1 µg/mL streptomycin/100 IU/mL penicillin, MCF7 cell line was maintained in Roswell Park Memorial Institute-1640 (RPMI-1640) containing 15% FBS (BIO-IND), 1 µg/mL streptomycin/100 IU/mL penicillin incubated at 37 °C in the dark with 5% CO<sub>2</sub> humidified incubator and passaged when they reached 80-85% confluency. Cells used in experiments were maintained from 10-20<sup>th</sup> passages.

To investigate the cytotoxic activity of the compounds, 95 µL of cell suspension was inoculated into 96-well microculture plates at  $1 \times 10^4$  cells density per well in culture media containing FBS, penicillin/streptomycin. Compounds were dissolved in dimethyl sulfoxide (DMSO) (Sigma Chemical Co.), filter sterilized, diluted at the appropriate concentrations with the culture medium. In all well, 1% DMSO concentration was fixed. Dilutions of compounds were freshly prepared before each experiment. After 24h cultivation for cell attachment, compounds were added at final concentration 50.0, 40.0, 30.0, 20.0, 10.0, 1.0, and 0.5 µM for triplicate assay. Cells were treated with the compounds for 48 hours and cytotoxic effects were determined by tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma Chemical Co.) based colorimetric assay. This method depends on the cleavage of tetrazolium salt to purple formazan crystals by mitochondrial enzymes of metabolically active cells (Ciapetti, et al. 1993). Briefly, 4 hours before the end of incubation period, medium of the cells was removed and wells were washed by pre-warmed phosphate-buffered saline (PBS) to remove any trace of compounds and to prevent color interference while optical density determination. MTT stock solution (5 mg/mL) was diluted at 1:10 ratio into complete culture media, 100 µL of MTT dilution was added into each well and incubated. After 3.5 hours plates were centrifuged at 1800 rpm for 10 minute at room temperatures to avoid accidental removal of formazan crystals. Crystals were dissolved with 100 µL

DMSO. The absorbance was determined at 540 nm. Results were represented as percentage cell viability.

### **3.1.4. Aromatase Inhibition Assay**

CYP19/MFC high-throughput screening kit (BD Biosciences, Oxford, UK) was used for aromatase inhibition assays. According to the manufacturer's protocol, amount of the conversion of 7-methoxy-4-trifluoromethyl coumarin (MFC) substrate into fluorescent 7-hydroxy-4-trifluoromethyl coumarin (HFC) product was measured in the presence of varying concentrations of compounds to be tested in acetonitrile solutions in 96-well plates. Fluorescence of HFC was detected by employing an excitation wavelength of 405 nm and emission wavelength of 520 nm. Concentrations of CYP19 and substrate were fixed to 7.5 nM and 25  $\mu$ M respectively. Potential aromatase inhibitors were applied at 1200.00, 400.00, 133.33, 44.44, 14.81, 4.94, 1.65, and 0.55  $\mu$ M concentrations. Ketoconazole (KTZ) was used as a positive standard. Background fluorescence was subtracted from the fluorescence of the all reactions to find the fluorescence emitted only from HFC products. All reactions were carried out in a parallel duplicate.

## CHAPTER 4

### CONCLUSION

Boronic acid chalcone molecules are selective anticancer drug candidates and fluorescent probes for saccharide detection. In this study, mono- and diboronic acid chalcone derivatives were designed and synthesized to increase the cytotoxic activity of the chalcone boronic acid derivatives against cancer cells. First time in literature, synthesized boronic acid chalcone derivatives were tested for possible aromatase inhibition properties, besides their cytotoxic activity. In addition binding studies of boronic acid chalcone derivatives with various carbohydrates were performed at physiological pH.

Compounds are synthesized via Claisen-Schmidt condensation reaction in the presence of an excess amount of strong base in methanol. Synthesis of diboronic acid chalcone analogues and 2-naphthyl chalcone boronic acid derivatives were successfully accomplished except ortho substituted (2,3'- and 2,4'-) diboronic acid chalcone derivatives.

Diboronic acid chalcone derivatives were tested to show their aromatase inhibition and cytotoxic properties against MCF-7 and PC3 cell lines. Our investigation showed that addition of a second boronic acid moiety to chalcone structure did not cause an increase in the cytotoxic activity of boronic acid chalcone derivatives against cancer cell lines. Unexpectedly, cytotoxic activities of diboronic acid chalcones are lower than those of boronic acid modified chalcones in literature. Boronic acid derivatized 2-naphthyl chalcones are cytotoxic against both cancer cell lines MCF-7 and PC3. They were found more cytotoxic compared to diboronic acid derivatives. However, comparison of their  $IC_{50}$  values with those of unsubstituted 2-naphthyl boronic acid chalcones implies that boronic acid substitution does not have any effect on the cytotoxic profiles of 2-naphthyl chalcones.

Additionally, even three of the diboronic acid functionalized chalcones show aromatase inhibition activity,  $IC_{50}$  values of those are much more higher compared to the known flavonoids showing aromatase inhibition in literature

Finally, carbohydrate binding studies resulted that diboronic acid chalcones cannot be used at physiological pH to detect carbohydrate because of the lack of their fluorescence responses in carbohydrate solutions. On the other hand fluorescence responses of 2-naphthyl chalcone boronic acids proportionally increase with the concentration of carbohydrates especially in D-fructose as expected.

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## APPENDIX A

### **$^1\text{H}$ NMR AND $^{13}\text{C}$ NMR SPECTRUM OF COMPOUNDS 96 AND 100**

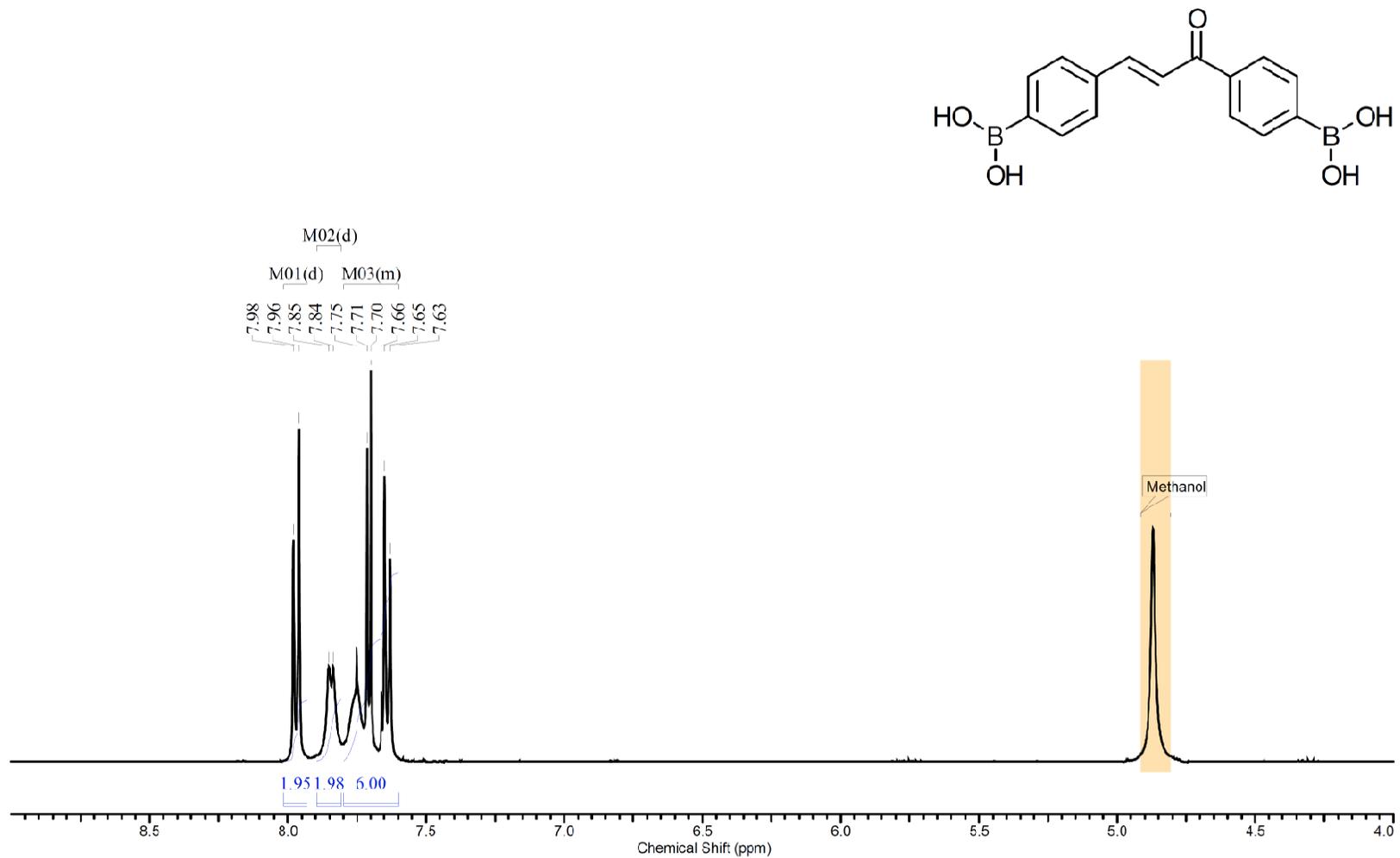


Figure A.1. <sup>1</sup>H NMR spectrum of (E)-3-(4-boronic acid phenyl)-1-(4-boronic acid phenyl)prop-2-en-1-one (**96**).

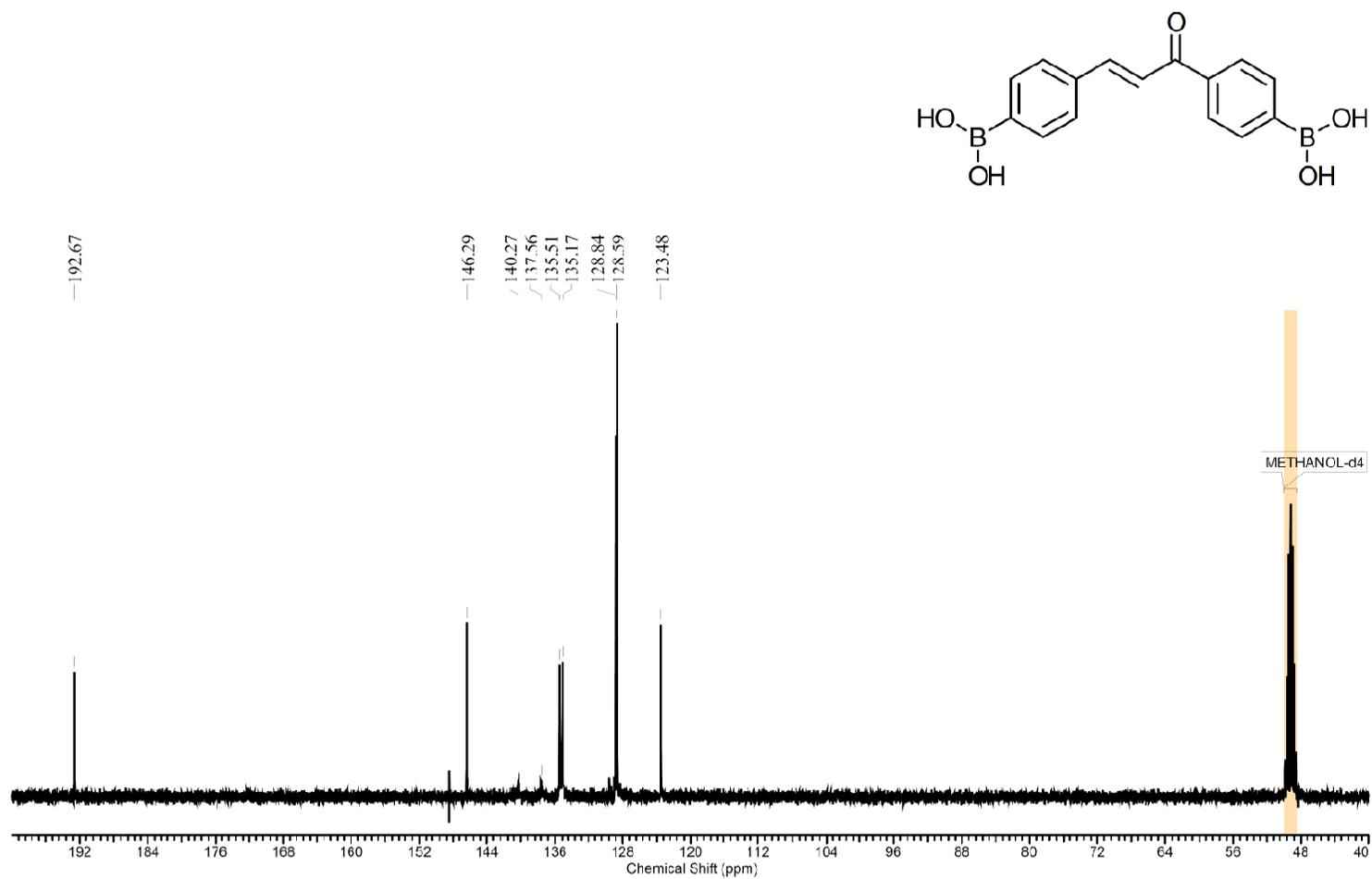


Figure A.2.  $^{13}\text{C}$  NMR spectrum of (E)-3-(4-boronic acid phenyl)-1-(4-boronic acid phenyl)prop-2-en-1-one (96).

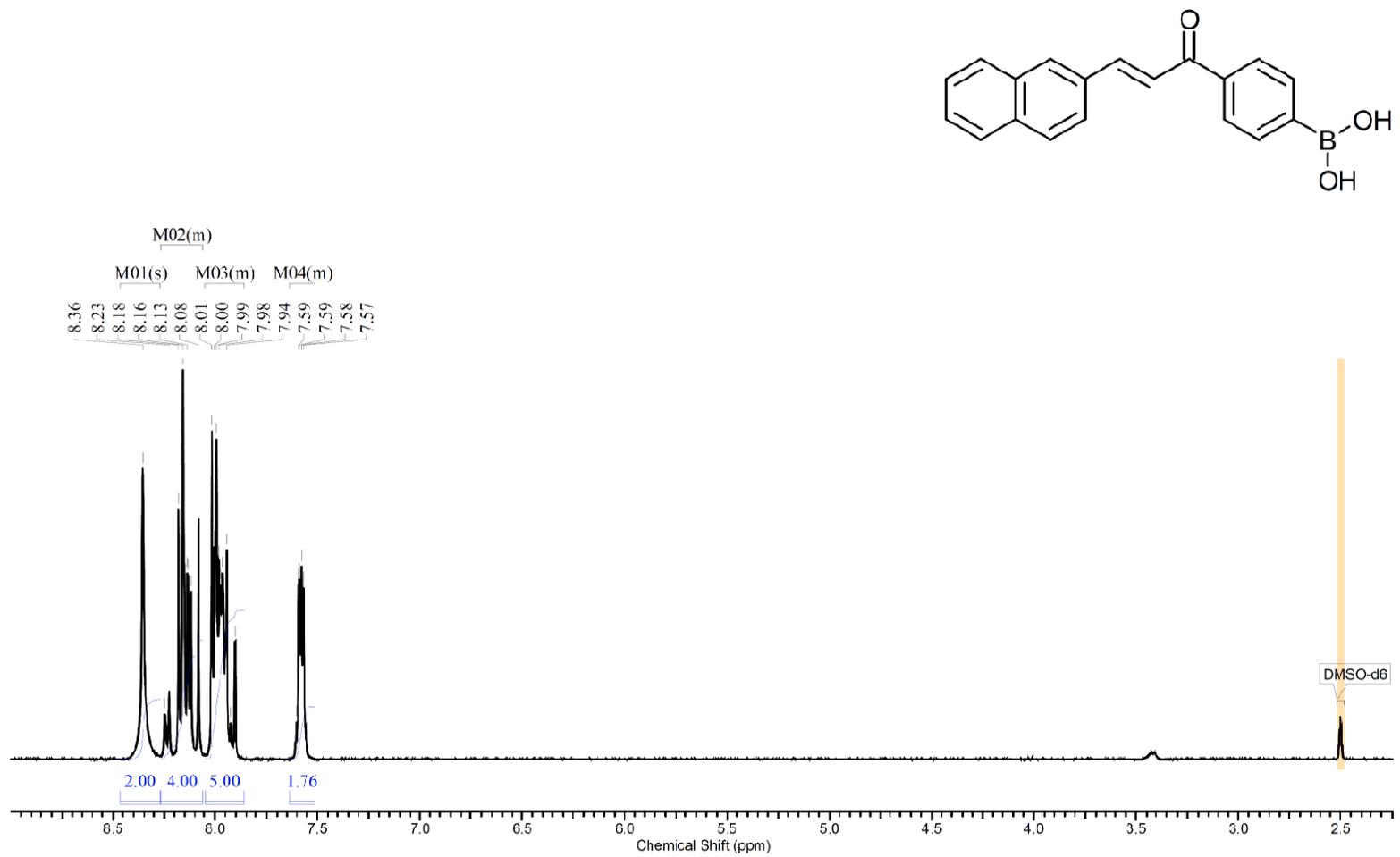


Figure A.3. <sup>1</sup>H NMR spectrum of (E)-1-(4-boronic acid phenyl)-3-(naphthalen-2-yl)prop-2-en-1-one (**99**)

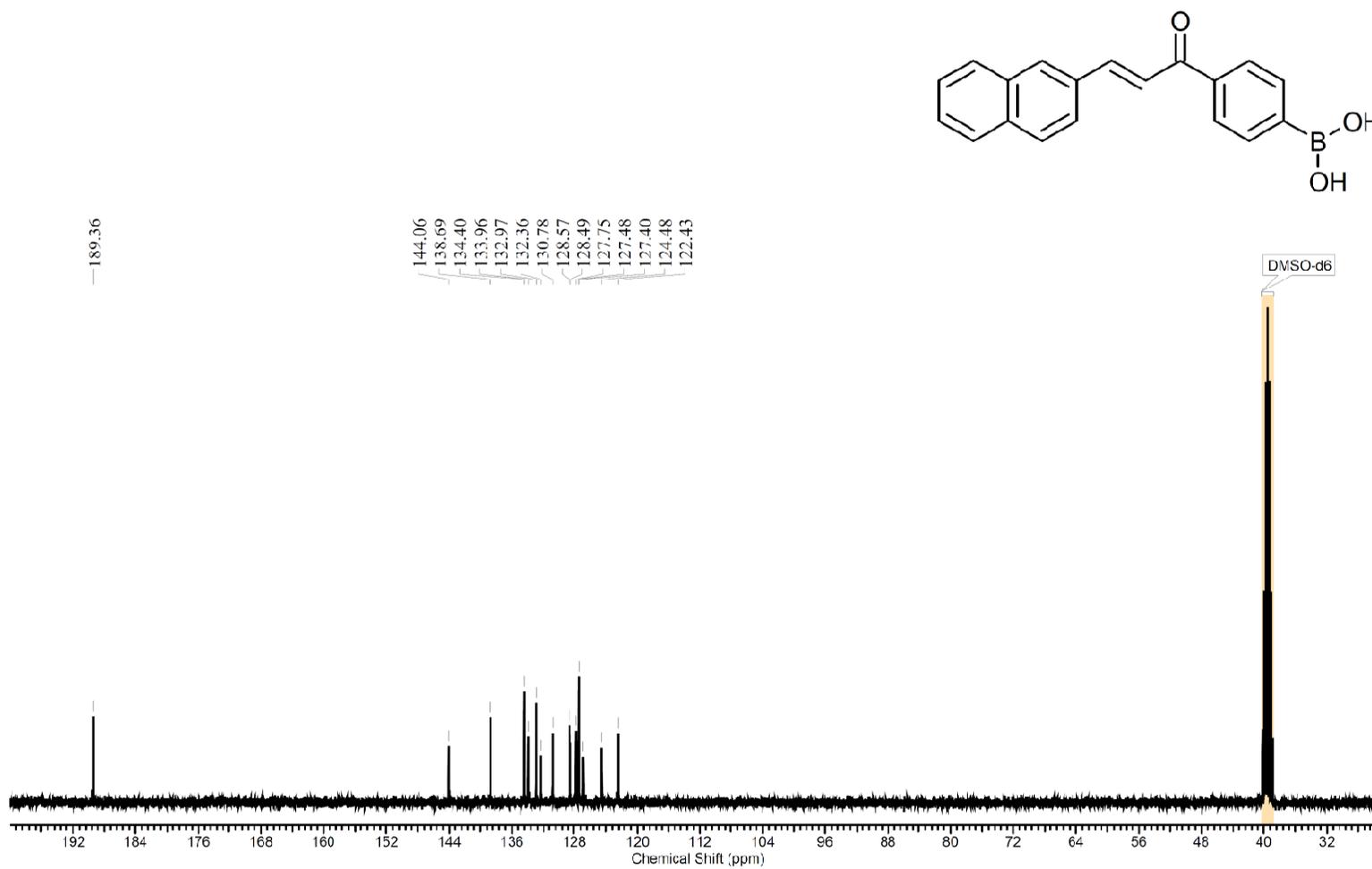


Figure A.4. <sup>13</sup>C NMR spectrum of (E)-1-(4-boronic acid phenyl)-3-(naphthalen-2-yl)prop-2-en-1-one (99)