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Research article

# Investigation of interaction between boronic acids and sugar: effect of structural change of sugars on binding affinity using steady state and time resolved fluorescence spectroscopy and molecular docking

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ABSTRACT

Binding interactions of boronic acid derivatives viz. 2-Methylphenylboronic acid (B1) and 3-Methoxyphenylboronic acid (B2) with mono saccharides (arabinose, fructose and galactose) and disaccharides (sucrose, lactose and maltose) in aqueous condition at pH 7.4 by means of fluorescence spectroscopy is reported in the present investigation. Sugar sensing as well as continuous glucose monitoring (CGM) plays a significant role in diabetes regulation. Sugar sensors mediated through enzymes have their own drawbacks, which led to encouragement to search for designing new sensors through alternate approaches. Among many, fluorescence-based sensors are drawing more attention. Boronic acid-based fluorescence sensors have the capacity to bind reversibly with diols, which makes their demand high in applications. Addition of sugar reduces fluorescence intensities. Change in intensities is associated to cleavage of intermolecular hydrogen bonding which leads in reduced stability of boronate ester. Lineweaver-Burk and Benesi-Hildebrand equation is used for analysing data. Mono sugars are estimated to have higher binding constants. Mutarotation leads to structural changes in saccharides which play a key role in binding interactions. Sugars in furanose form are found to be highly favoured for binding. Molecular docking of B1 and B2 with proteins with PDB ID: 2IPL and 2IPM being periplasmic was done with the help of Schrodinger Maestro 11.2 version. GLIDE scores terms are used for expressing binding affinity.

## 1. Introduction

Boronic acids are the important building blocks in carbon-carbon bond formation in organic synthesis. In addition to their application in synthetic organic chemistry, they also found application in recognition of nucleic acids, adenosine triphosphate (ATP) and fluoride ions [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. Though they usually exist in neutral trigonal planar form, can comfortably change into tetrahedral anionic form at pH value being higher the pKa. The reason behind it is the behaviour of boron as Lewis acid and the fact that it accepts a pair of electrons from donors forming covalent bonds. Functional groups in boronic acid form strong complexes along with compounds like cis-diols i.e. compounds having two adjacent nucleophiles. Boronic acids are continuing to gain interest in scaffold designing of sensors for carbohydrate cognition because of their high sugar intrinsic affinity and reversible rapid equilibrium. Even in simple cells, carbohydrates are found to be endogenous and vital in many of the biochemical processes [14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47]. Also, they are often linked to proteins that affect activity and cell surfaces. As an example, blood type is determined by chain of carbohydrates in red blood cell. Modified forms of saccharide chains have been detected in many diseases that are degenerative, and glycosylated proteins are found to take part in cancer metastasis and communication between cell to cell. Boronic acids are abundantly found

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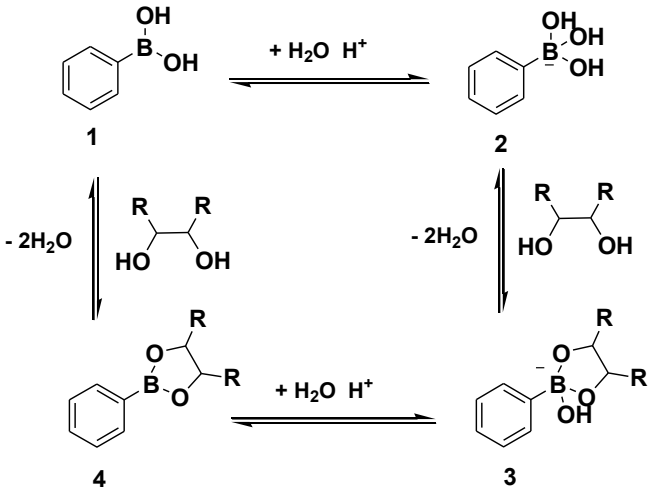
in metabolic processes so, they are focused in large number for sensing of fructose and glucose. Still, it is equally important to detect other carbohydrates too. So, for specificity to sugars, the synthesis and design of boronic acids is important. The application of boronic acids also includes recognition of carbohydrates on cell surface and in sensing various types of saccharides. Boronic acid ligands are more stable, of low cost and are easy to handle, so they are being used in replacing enzymes. Due to their ability of binding tightly with diols which are found in sugar molecules, boronic acids are used for designing chemo sensors. It has a covalent reaction with 1,2 or 1,3-diols and forms cyclic esters of five or six members in aqueous solution which is a reversible complexation, and that makes it an ideal interaction in designing of sensors [44, 45, 46, 47]. For determining compounds that contain diol, efforts based on interaction of boronic acid and diol are being made to focus on developing fluorometric and colorimetric sensors [11, 12, 13, 14, 15, 16, 17, 18]. There are special advantages with fluorescence-based systems which includes - (a) extremely sensitive technique, (b) host system is not damages due to fluorescence measurements, (c) better results due to fluorescence decay time measurements, (d) the information on microenvironment of the molecule and about the structure is obtainable. For studying the interaction between sugars and boronic acid, it is desired to know general steps involved in bonding process and also about the structural properties of boronic acid. Boronic acids being Lewis acids, their reaction with water yields tetrahedral boronate. There is a release of proton during the process (Scheme 1). The tetrahedral form is found to be anionic and trigonal form is generally neutral. Boronic acids have an ability to bind reversibly with Lewis bases and diols due to their open shell structure. Like boronic acid, boronic ester also reacts with water, forms tetrahedral boronic ester. In reaction Scheme 1, 1 refers to boronic acid, 2 represents anionic boronate having tetrahedral geometry, 3 refers to boronate ester, finally 4 represents boronate ester having tetrahedral geometry [19, 20, 21, 22, 23, 24]. Two esters are produced as a result of diol binding in tetrahedral or trigonal form. Various factors like concentration and nature of buffer, pH of medium, temperature, dihedral angle of diol, the and pKa value of formed ester, influences the process of binding [25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47]. To have a quantitative study of process of binding, calculation of binding constant, here association constant (Ka) is important.




**Scheme 1.** Binding mechanism between a diol and phenylboronic acid.

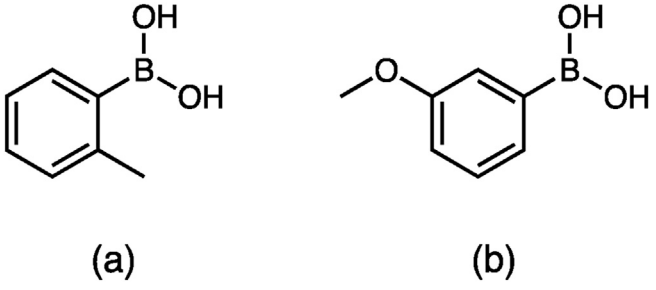



**Figure 1.** Molecular structures: (a). 2-Methylphenyl boronic acid B1, and (b). 3-Methoxy phenyl boronic acid B2.

## 2. Theory

Boronic acids have intrinsic affinity for carbohydrates, their bonding with mono-saccharide and disaccharides changes the intensity of fluorescence and have reversible and rapid equilibrium which is why they are considered to be good fluorescence sensors. Studies for the same are performed under physiological conditions and in aqueous solution. One of our group's long-term goal is to create a library of scaffolds of boronic acids to design chemosensors for identifying some specific biomarkers. Calculated values of Ka were used for determining the binding affinity and binding strength of boronic acid with sugars. For calculating the values of Ka, an equation was derived which correlates change in fluorescence intensity to sugar concentration. Selection of wavelength to measure fluorescence was done on the basis of maximum intensity observed at particular wavelength shown in Figure 3. Fluorescence intensity measurement at maximum emission wavelength on adding sugar was done to estimate the boronic acid binding constants. Ka calculation was done by deriving equation which correlates concentration of sugar to the changes in fluorescence intensity. The calculation of Ka value for boronate ester is done using Eq. (1) [37].

### 2.1. Benesi-Hildebrand equation

At equilibrium, [BQ] is the sugar-boronic acid complex concentration, [B]-[BQ] is boronic acid concentration, and [Q]-[BQ] gives sugar concentration. We are assuming the sugar concentration to be greater than the concentrations of boronic acids as well as sugar -boronic acid complex and $[B] = mI_0$ and $[BQ] = n\Delta I$. Now from the derivation, Ka is calculated by using linear equation as shown below:

$$\underset{[B]}{\text{Boronic acid}} + \underset{[Q]}{\text{sugar}} \rightleftarrows \underset{[BQ]}{\text{Boronate ester}}$$

Assume *[B]* = Boronic acid concentration, *[Q]* = Sugar concentration, *[BQ]* = Boronate ester concentration

Now *[Q]* is varying whereas *[B]* is set to be constant. Assuming *[B]* << *[Q]*, binding constant can be defined by,

$$K_a = \frac{[BQ]}{[B][Q]} \quad (1)$$

Again defining $[B]_0$ = Total *B* [ Un-complexed + Complexed] = *[B ]*+ *[BQ]*

And $[Q]_0$ = Total *Q* [Un-complexed + Complexed] = *[Q]* + *[BQ]*

$$K_a = \frac{[BQ]}{\{[B]_0 - [BQ]\}\{[Q]_0 - [BQ]\}}$$

Since *[B]* <<< *[Q ]*, $[Q]_0 - [BQ] \approx [Q] \approx [Q]_0$ and fluorescence intensity before is $I_0$ and after adding sugar, change in intensity is $\Delta I$, then

**Table 1.** Docking studies for B1 and B2 periplasmic protein (PDB ID: 2IPM and 2IPL).

| Boronic acid derivatives | G-Scores | |
|---|---|---|
| | 2IPM | 2IPL |
| B1 | -6.3 | -5.4 |
| B2 | -4.3 | -4.5 |

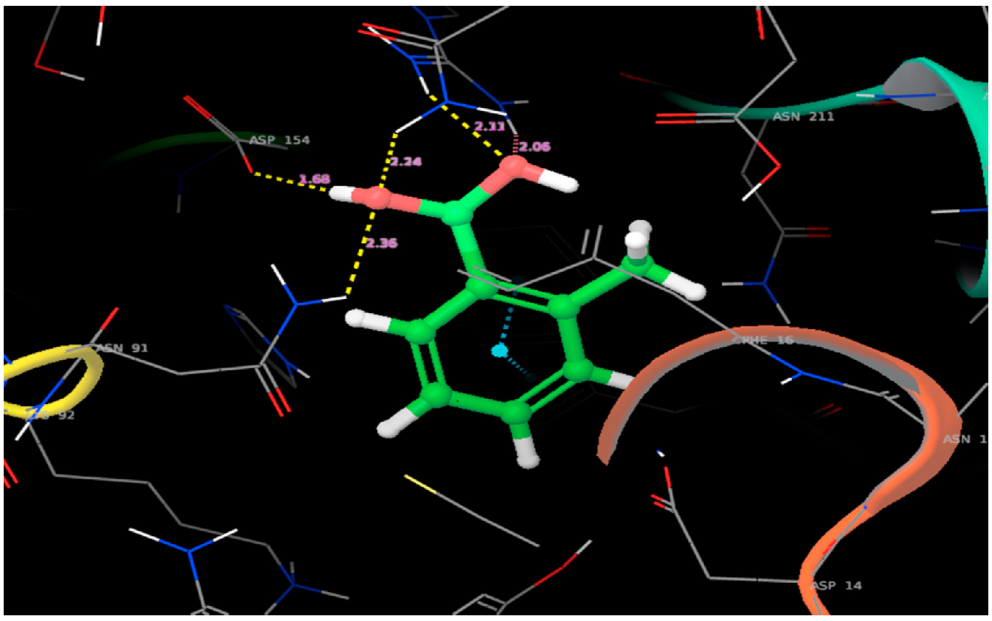



**Figure 2.** Docked image of compound B1 with 2-IPM protein.

$[B] \propto I_0 \Rightarrow [B] = mI_0 \quad [BQ] \propto \Delta I \Rightarrow [BQ] = n\Delta I$

here $n$, $m$ are constants depending on [boronic acid], temperature and path length.

$$\frac{n\Delta I}{I_0} = \frac{m[Q]K_a}{1+[Q]K_a} \Rightarrow \frac{I_0}{n\Delta I} = \frac{1+[Q]K_a}{m[Q]K_a}$$

$$\frac{I_0}{\Delta I} = \frac{n}{mK_a} \times \frac{1}{[Q]} + \frac{n}{m} \tag{2}$$

The above equation is linear. It was initially obtained by Benesi-Hildebrand for studying absorption; and then used for studying fluorescence [37, 38, 39, 40]. Binding constant $K_a$ can be calculated using the graph of $(I_0/\Delta I)$ vs $[Q]$ ($K_a$ = intercept/slope). Dissociation constant ($K_D$) is the reciprocal of $K_a$.

### 2.2. Experimental methods

Boronic acids used in our study are 2-Methylphenyl boronic acids (B1), 3-Methoxyphenyl boronic acid (B2) were purchased from Combi-Blocks, USA Figure 1. Monosaccharides (arabinose, fructose, galactose) and disaccharides (sucrose, lactose, galtose) are purchased from Thomas Baker, Mumbai, India. Samples were prepared by dilution method using double distilled water. Boronic acid solutions were prepared in distilled water, concentrations were adjusted to $1 \times 10^{-4}$M. The required buffers were prepared by dissolving $NaH_2PO_4$ (0.023M) and $Na_2HPO_4$ (0.077 M) in 1 L of double distilled water, also pH is adjusted to 7.4 by using saturated NaOH and 0.1M HCl. Measurement of pH values for all samples is done by Elico LI-120 pH meter which is portable. Further, buffer solvents are used for diluting the boronic acids dissolved in water. Phosphate buffer is commonly used since phosphate ions does not have any

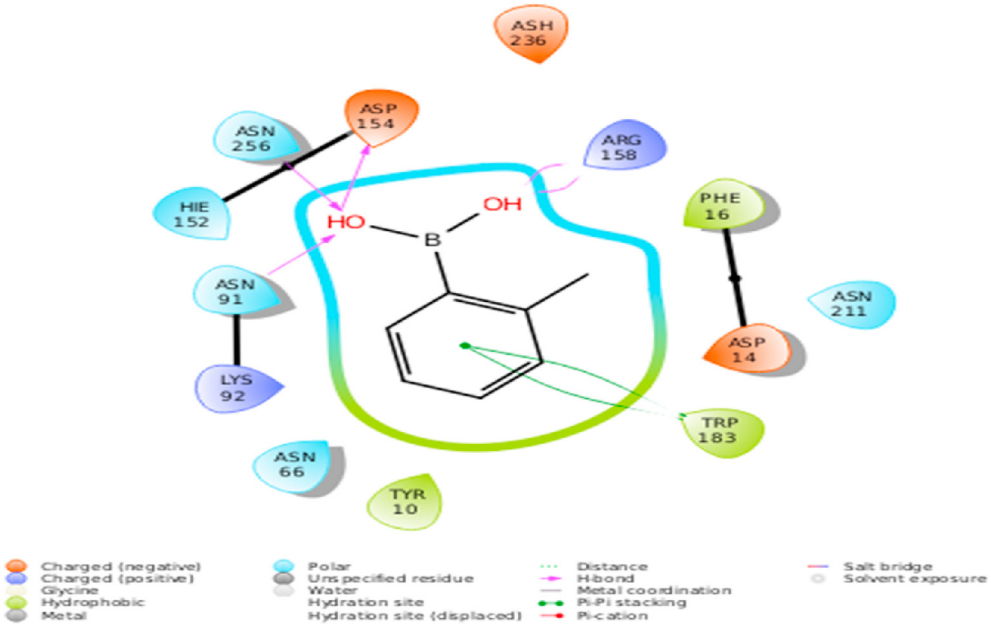



**Figure 3.** Interaction of compound B1 with amino acid residues of 2-IPL protein.

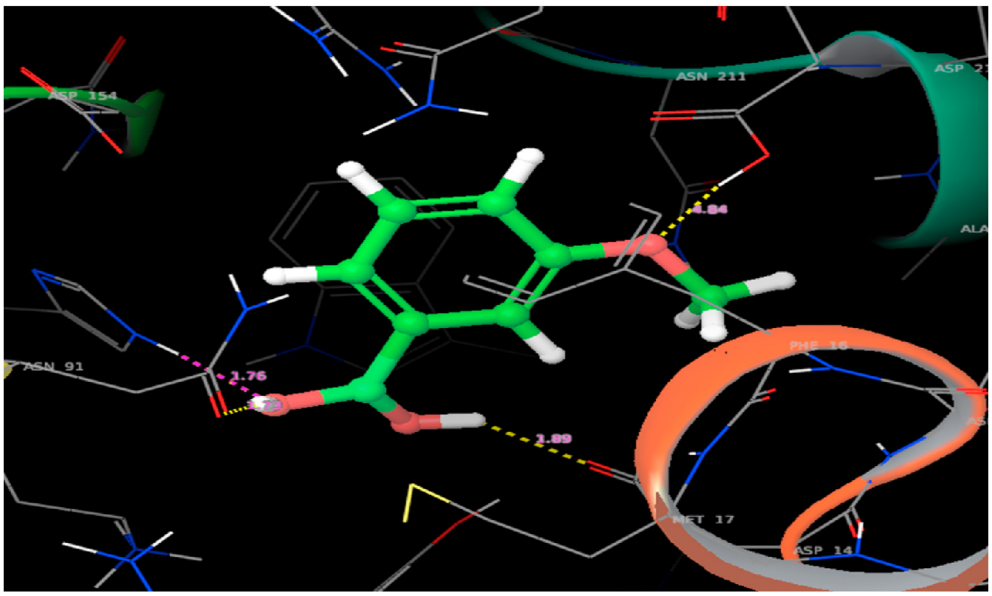



**Figure 4.** Docked image of compound B2 with 2-IPM protein.

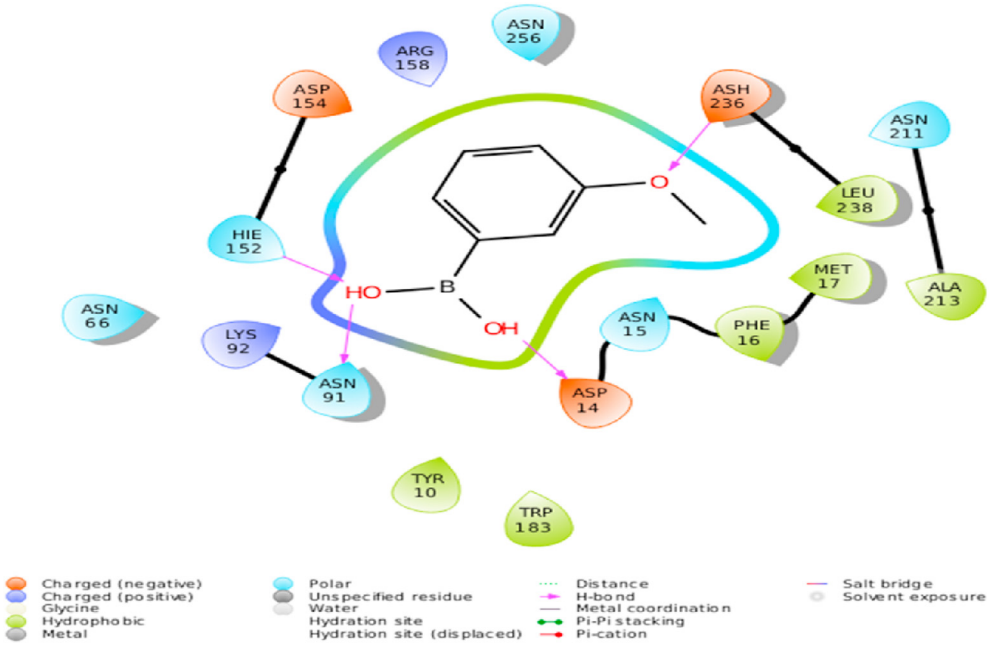



**Figure 5.** Interaction of compound B2 with amino acid residues of 2-IPM protein.

**Table 2.** Fluorescence intensities of B1 upon mixing of sugars in various sugar concentrations [Q].

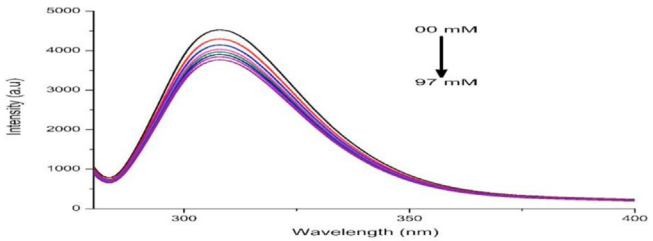
| [Q] mM | Fluorescence Intensities (a. u) | | | | | |
|---|---|---|---|---|---|---|
| | Sucrose | Lactose | Maltose | Arabinose | Fructose | Galactose |
| 0 | 4530 | 4530 | 4530 | 4530 | 4530 | 4530 |
| 9.8 | 4395 | 4070 | 4252 | 4298 | 4443 | 3625 |
| 19.2 | 4283 | 3965 | 4115 | 4148 | 4371 | 3279 |
| 37 | 4113 | 3870 | 3958 | 4035 | 4313 | 3010 |
| 53.6 | 3911 | 3811 | 3884 | 3969 | 4279 | 2905 |
| 69 | 3778 | 3791 | 3872 | 3905 | 4258 | 2850 |
| 83.33 | 3613 | 3766 | 3809 | 3844 | 4250 | 2699 |
| 96.77 | 3542 | 3743 | 3360 | 3768 | 4235 | 2587 |

**Table 3.** Fluorescence intensities of B2 upon mixing of sugars at various sugar concentrations [Q].

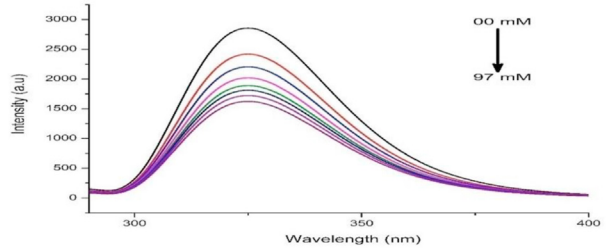
| [Q] mM | Fluorescence Intensities (a. u) | | | | | |
|---|---|---|---|---|---|---|
| | Sucrose | Lactose | Maltose | Arabinose | Fructose | Galactose |
| 0 | 2857 | 2857 | 2857 | 2857 | 2857 | 2857 |
| 9.8 | 2309 | 2371 | 2787 | 2419 | 2390 | 2445 |
| 19.2 | 2250 | 2265 | 2730 | 2207 | 2171 | 2158 |
| 37 | 2217 | 2173 | 2630 | 2020 | 2000 | 1914 |
| 53.6 | 2198 | 2158 | 2463 | 1890 | 1940 | 1757 |
| 69 | 2180 | 2143 | 2257 | 1811 | 1873 | 1583 |
| 83.33 | 2178 | 2130 | 2229 | 1722 | 1859 | 1370 |
| 96.77 | 2166 | 2105 | 2220 | 1625 | 1796 | 1289 |

influence on intensity of fluorescence [19]. Stock solutions (0.5M) of all sugars were prepared in distilled water. Using these stock solutions, solutions of different concentrations viz 97mM, 83.3mM, 699nM, 53.5mM, 37.0mM, 19.3mM and 9.8mM were prepared using dilution method. Sugar concentration was maintained approximately 100–1000 times more than boronic acids. Spectra of absorption of boronic acid solutions with and without sugar were recorded using uv-visible spectrometer in the range 250–600nm (Shimazdu UV-1800 spectrometer). The fluorescence spectra were recorded at 285nm (excitation wavelength) in quartz cuvettes of perpendicular geometry using fluorescence spectrometer (Hitachi F-2700). Operating voltage was set to 400V and the slit width 5 nm. After the addition of sugar, the fluorescence spectra were recorded within 30 min.

*In-silico* Maestro software 11.2 version was used in molecular docking of both compounds B1 and B2. From protein data bank, retrieving the crystal structure of galactose binding periplasmic protein (PDB ID: 2IPL and 2IPM) was performed. Using a protein preparation wizard, protein was pre-processed and preparation for docking. Also, refinement of their bond orders, formal charges and missing hydrogen atoms, topologies, incomplete and terminal amide groups was done. Elimination of Water molecules of more than 3Å was done. Protein structures with possible ionization states was generated to choose the best stable state among them. Corrections were carried out in orientations of retained water molecules and hydrogen bonds was assigned. For selection of ligand most

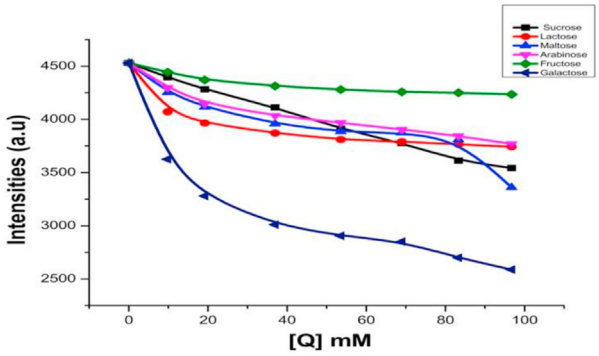



(a)

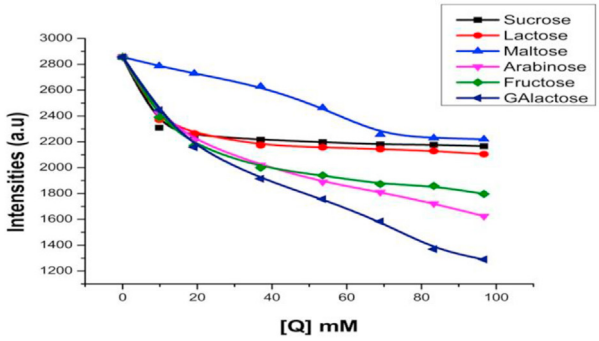



(b)

**Figure 6.** Spectra of emission for compounds without and with different concentration of sugar in galactose (a) B1 (b) B2.

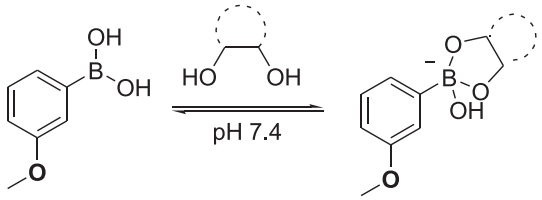



(a)




(b)

**Figure 7.** Variation of fluorescence intensity with increase in concentration of sugars (a) B1 (b) B2.




**Scheme 2.** Formation of boronic ester with sugars.

probable binding site on the surface of protein, protein receptor grid generation was performed. With the use of ligand docking module, docking was performed. Results analysis was done with the help of extra precession visualizer (XP visualizer) [5, 6, 7, 8, 9, 10].

## 3. Results and discussions

### 3.1. Molecular docking

Protein interaction of compounds 2IPL and 2IPM with glide scores are illustrated in Table 1. 2IPL interaction with B1 has shown G score to be -5.4 kcal/mol with five Hydrogen-bonds with Asp154, Asn256, Asn91 and Pi-Pi stacking interaction with Trp183 where as B2 has shown G score of -4.5 kcal/mol with four Hydrogen-bonds with His152, Asn91, Asp14 and Asp236. The interaction of 2IPM with B1 has shown -6.3 kcal/mol G score with four H-bonds with Asn211, Asp236 twice, Arg158 and stacking interaction Pi-Pi with Trp183 whereas B2 has shown G score -4.3 kcal/mol with four H-bonds with His152, Asn91, Asp14, Asp236 [5]. Results denote that both the compounds have good binding affinity. Molecular docking results were compared using the two proteins and both of them gave similar results with good agreements. Best affinity mode for B1 which is the docked compound with proteins 2-IPL and 2-IPM is illustrated in Figures 2 and 3 whereas Figures 4 and 5 indicates that of docked compound B2 with 2-IPL and 2-IPM proteins.

### 3.2. Change in fluorescence intensities

On adding sugar, the determination of binding constant is done by measuring the intensity of fluorescence of the emission peak. Intensities corresponding to the maxima of emission at different concentrations of sugar level are recorded in Tables 2 and 3. It is observed that the fluorescence intensity decreases with increase in concentration of sugar in all cases. Figure 6 shows the emission spectra of B1 and B2 in the aqueous medium after addition of galactose. Figure 7 shows the fluorescence intensity vs sugar concentration [S] plot. Decrease in the fluorescence intensity shows the binding of sugar with boronic acids is highly effective leading to the formation of boronate ester. It is found to be 21% decrement in arabinose, 23% in galactose, 29% in fructose, 17% in sucrose, 25% in maltose and 27% in lactose. Explanation for the decrement in intensity is, there is an opportunity for an intramolecular hydrogen bonding between O-methyl group and hydrogen atoms of boronic acid. There is a high stability of boronic acid because of the hydrogen bond between oxygen and free boronic acid. The fluorescence intensity is higher as a result of hydrogen bonding in the form of ester, none of the hydrogen atoms are linked to oxygen atoms of boronic acid; so that results to absence of any hydrogen bonding. We can also predict that after adding sugar, the breaking of hydrogen bond might play a key role in decreasing the intensity of fluorescence [22]. Scheme 2 shows formation of boronic ester.

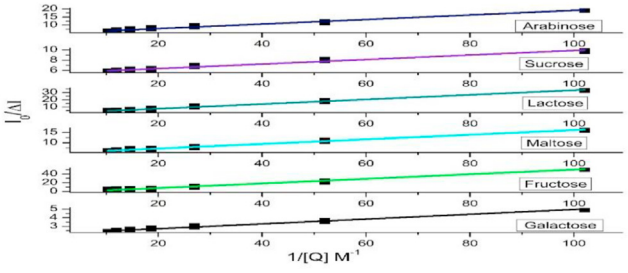
### 3.3. Binding constants ($K_a$) or determination of association and dissociation constants ($K_D$)

Benesi-Hildebrand plots are constructed using Eq. (2) by taking into account the intensities matching to emission peak for all different concentrations of sugar. A linear variation is observed in the co-efficient which is as shown in Figure 8. The Lineweaver-Burk equation is used to analyze fluorescence data [38]:

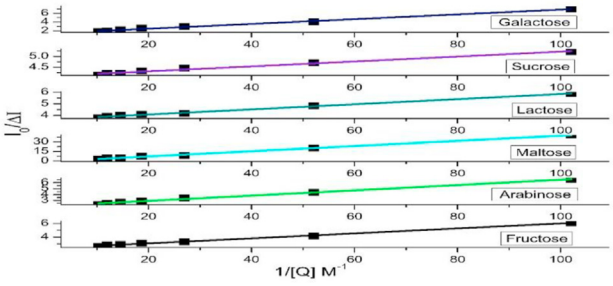
$$\frac{1}{\Delta I} = \frac{1}{fK_a[Q]} + \frac{1}{f}$$

$\Delta I$ is change in fluorescence intensity, [Q] is sugar concentration and $f$ is a constant.

Figure 9 shows $\Delta I^{-1}$ versus $[Q]^{-1}$ i.e. double reciprocal plots which is observed to have a linear relation. These plots are used for estimating $K_a$ and $K_D$ values using the slope and y-intercept. Tables 4 and 5 lists the determined $K_a$ and $K_D$ values with R i.e. correlation co-efficient values. L-B plots and B–H plots are used for calculating binding constants which is nearly same. In the case of disaccharides and monosaccharides the

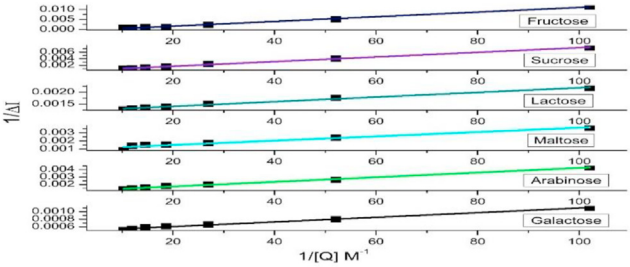



(a)

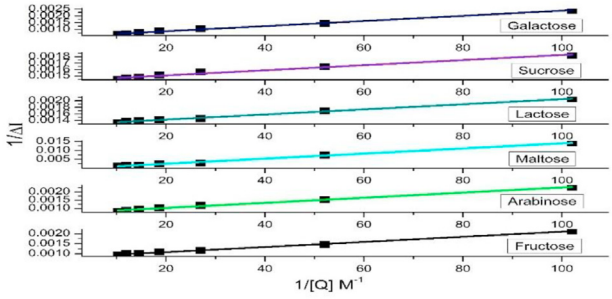



(b)

**Figure 8.** B–H equation plots in various sugars (a) B1 (b) B2.




(a)




(b)

**Figure 9.** L-B equation plots in various sugars (a) B1 (b) B2.

**Table 4.** Values of Ka, $K_D$ and R obtained from various plots of B1.

| Sugars | B–H Plot | | | L-B Plot | | |
|---|---|---|---|---|---|---|
| | $K_a$ ($M^{-1}$) | $K_D$ (mM) | R | $K_a$ ($M^{-1}$) | $K_D$ (mM) | R |
| Sucrose | 124.7 | 0.008 | 0.98 | 123.7 | 0.009 | 0.98 |
| Lactose | 4.8 | 0.205 | 0.99 | 4.81 | 0.205 | 0.99 |
| Maltose | 44.5 | 0.022 | 0.99 | 44.3 | 0.023 | 0.99 |
| Arabinose | 35.3 | 0.028 | 0.99 | 35.1 | 0.027 | 0.99 |
| Fructose | 26.4 | 0.037 | 0.99 | 26.1 | 0.036 | 0.99 |
| Galactose | 80.0 | 0.012 | 0.99 | 80.1 | 0.013 | 0.99 |

**Table 5.** Values of Ka, $K_D$ and R obtained from various plots of B2.

| Sugars | B–H Plot | | | L-B Plot | | |
|---|---|---|---|---|---|---|
| | $K_a$ ($M^{-1}$) | $K_D$ (mM) | R | $K_a$ ($M^{-1}$) | $K_D$ (mM) | R |
| Sucrose | 370.9 | 0.002 | 0.98 | 369.9 | 0.003 | 0.98 |
| Lactose | 163.6 | 0.006 | 0.99 | 163.4 | 0.007 | 0.99 |
| Maltose | 0.2 | 4.402 | 0.99 | 0.22 | 4.400 | 0.99 |
| Arabinose | 51.2 | 0.019 | 0.99 | 51.0 | 0.018 | 0.99 |
| Fructose | 65.2 | 0.015 | 0.99 | 65.1 | 0.016 | 0.99 |
| Galactose | 26.1 | 0.038 | 0.99 | 26.3 | 0.039 | 0.99 |

estimated binding constant is observed to be high. The findings were 124.7 for sucrose and 80.0 for galactose (B–H plot). The corresponding values of dissociation constant are 0.008 mM for sucrose and 0.012 mM for galactose (B–H plot). The binding constant values being in the moderate clinical range, the studied boronic acids are capable of sensing sucrose and galactose in an efficient way. Moreover, selectivity to mono and disaccharides by B1and B2 can be attributed to changes in structure in saccharides and hydroxyl group availability and relevant explanation is given as follows. Similarly, same results obtained from L-B plot.

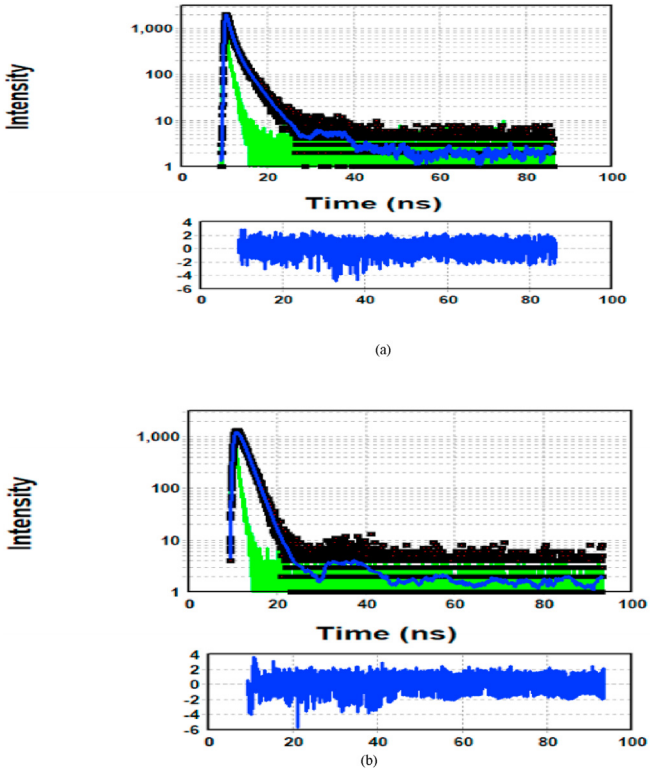



**Figure 10.** . Fluorescence life time decay curve without sugar concentration (a) B1 (b) B2.

Literature survey makes it evident that, there are various factors that affect binding interaction and complex formation between boronic acids and sugar. One of the key factors responsible for complex formation is the mutarotation of sugar which changes the structure. When sugar is dissolved in water, there is generation of α - and β -furanose and α- and β -pyranose forms because of opening and closing of ring. The furanose and

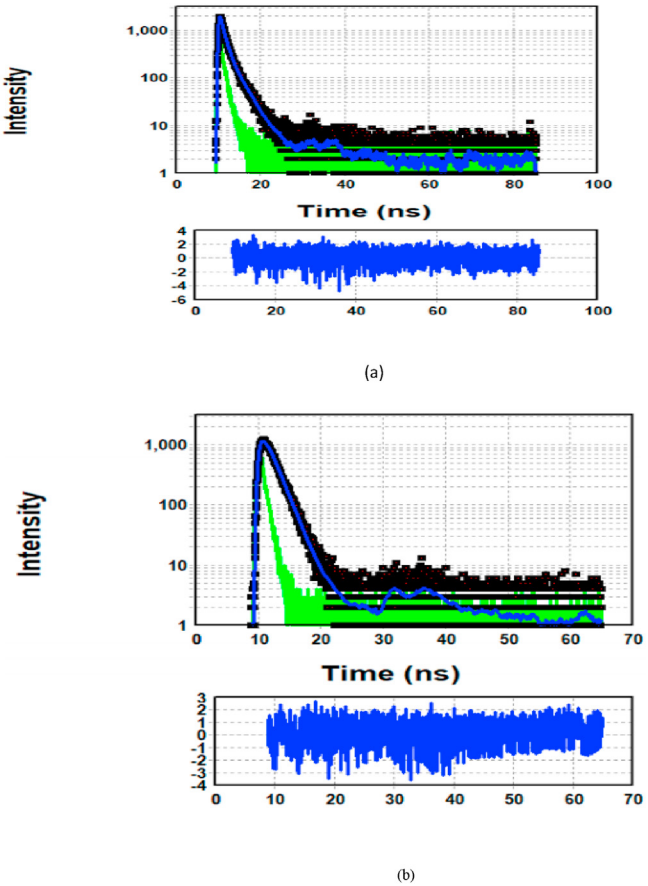



**Figure 11.** Lifetime decay with sugar concentration (a) B1 (b) B2.

pyranose forms having configurations α and β are specific forms of anomeric ring present in sugar. Convincing evidence was found for α-D-glucofuranose formation with boronic acids rather than α-D-glucopyranose complexes from NMR investigations by Norrild and Eggert [28]. The preference of boronic acids for diol of furanose form was pointed out by James et al [29] and suggested that binding constant is high if there is higher proportion of both forms of furanose. Identification of both forms of α- and β- furanose of mono and disaccharides aqueous solutions was done. Equilibrium composition in aqueous medium at 25 °C in sucrose is 6% β-furanose and 10% α-furanose, in D-galactose it is 8.4% α-furanose and 15.5% β-furanose. The only significant forms in sucrose are α and β pyranose forms whereas α and β furanose forms in water are only about 0.08% at 31 °C 30, 44, 45, 46, 47]. Our study also shows the effect of these changes in structure on the binding process. The proportion of α and β furanose forms in sucrose and D-galactose, is more so, binding constants are higher. However, $K_a$ value of D-glucose is comparatively low and is assigned to a low proportion of α and β furanose present in dextrose. Thus, sucrose and lactose are disaccharides which on hydrolysis, gives the mixture of two different mono saccharides. Sucrose results in to dextrose and fructose whereas lactose yields dextrose and galactose. The reason behind it might point towards presence of dextrose which has lower percentage value for α and β furanose form, also binding constant values in disaccharides are not comparatively higher than sucrose and D-galactose. So, the above observations point to the fact that intrinsic structural features of sugar are the factors that govern the binding of boronic acid to sugar. Upon adding glucose there is a 40% of decrement in the mean lifetime. Fluorescence lifetime decay curves without addition of sugar and with 97mM sugar concentrations are given for both B1 and B2 in Figures 10 and 11. The fluorescence lifetime decay curves are in good agreement with experimental values. The results show that B1 and B2 when labelled with suitable fluorophores, serves as protein sensor for glucose. So, for the increment in binding affinity and selectivity to sucrose and D-galactose, it is a necessity for a more selective recognition element to be built into the system.

## 4. Conclusions

It is concluded that in both boronic acids, the decrement in fluorescence intensity caused due to increment in concentration of sugar shows that binding interaction between sugar and boronic acid is highly effective and the decrease is attributed to the intramolecular hydrogen bond cleavage on ester formation. Also, values estimated for larger $K_a$ by using L-B plot and B–H plot suggests that compounds under study are more favorable to sugars like sucrose and galactose. Higher values of $K_a$ in these two sugars are as a result of high percentage of their furanose forms. Molecular docking study revealed that both the boronic acid derivatives B1 and B2 showed best suitable binding pattern at the active site by interacting non-covalently with amino acid residues of proteins.

## Declarations

### *Author contribution statement*

Raveendra Melavanki: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

N R Patil: Conceived and designed the experiments; Wrote the paper.

Raviraj Kusanur: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Kishor Kumar Sadasivuni, Diksha Singh: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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### *Competing interest statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

## References

[1] Chris D. Geddes, Joseph R. Lakowich, Topics in Fluorescence Spectrosopy Volume 11 Glucose Sensing, Springer Science Business, 2006.
[2] Sarah R. Laughlin, Arylboronic Acids with Strong Fluorescence Intensity Changes Upon Sugar Binding, Thesis, Georgia State University, 2011.
[3] Al-Omary, M. Raja, Ali Abdel-Rahman, El-EmamMay, R Raj Muhamed, Haitham Al Rabiah, M. Raja, Fatmah, S. Muthu, Abdul-Malek Al-Tamimi, Molecular structure, vibrational spectra, NBO, Fukui function, HOMO-LUMO analysis and molecular docking study of 6-[(2-methylphenyl)sulfanyl]-5-propylpyrimidine-2,4(1H,3H)-dione, Maced. J. Chem. Chem. Eng. 36 (1) (2017).
[4] J.R. Lakowicz, Topics in fluorescence spectroscopy, in: Probe Design and Chemical Sensing Vol. 4, Springer Science & Business Media, 1994 Nov 30.
[5] Chan Chong Ma, et al., Molecular structure, vibrational spectra, NBO, Fukui function, HOMO-LUMO analysis and molecular docking study of 6-[(2-methylphenyl)sulfanyl]-5-propylpyrimidine-2,4(1H,3H)-dione, Eur. J. Med. Chem. 111635 (15 November 2019) 182.
[6] Vellasamy Shanmugaiah, Vediamenn Padmini, Rajendra Sribalan, Govindharasu Banuppriya, Biological evaluation and molecular docking studies of new curcuminoid derivatives: synthesis and characterization, Bioorg. Med. Chem. Lett. 26 (7) (1 April 2016).
[7] S. Sankar, M. Pandiselvi, B. Gowramma, R. Kalirajan, Molecular docking studies and insilico ADMET screening of some novel chalcone substituted 9-anilinoacridines as topoisomerase II inhibitors, S F J. Pharmaceut. Anal. Chem. 1 (2018) first ed., Article 1004, Science Forecast Publications LLC.
[8] Olaf J. Rolinski, Nicholas D. Evans, John C. Pickup, David J.S. Birch, Faeiza Hussain, Biosensors and Bioelectronics, 2005.
[9] Anthony K. Mittermaier, Alexander S. Wahba, Nicolas Moitessier, Naela Janmamode, Caroline Dufresne, Jessica Plescia, Discovery of covalent prolyl oligopeptidase boronic ester inhibitors, Eur. J. Med. Chem. 185 (2019).
[10] V.C. Bulgrin, J.M. Conner, Equilibria between borate ion and some polyols in aqueous solution, J. Inorg. Nucl. Chem. 29 (1967) 1953–1961.
[11] B.S. Sumerlin, J.N. Cambre, Biomedical applications of boronic acid polymers, Polymer 52 (2011) 4631–4643.
[12] E.A. Theodorakis, D. Lichlyter, T.P. Brady, M.A. Haidekker, Effects of solvent polarity and solvent viscosity on the fluorescent properties of molecular rotors and related probes, Bioorg. Chem. 33 (2005) 415–435.
[13] N. Engl, J. Med, The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus, Diabetes Contr. Compil. Trial Res. Group 329 (1993) 977–986.
[14] R.M. Melavanki, P. Bhavya, D. Nagaraja, R.A. Kusanur, H.S. Geethanjali, Binding of boronic acids with sugars in aqueous solution at physiological pH-Estimation of association and dissociation constants using spectroscopic method, J. Mol. Liq. 227 (2017) 37–43.
[15] B. Wang, G. Kaur, H. Fang, Progress in boronic acid-based fluorescent glucose sensors, J. Fluoresc. 14 (2004) 481–489.
[16] P. Bhavya, Raveendra Melavanki, C.K. Narayanappa, Raviraj Kusanur, U. Meghana, B. Suma, Binding interaction between boronic acid derivatives with monosaccharaides: effect of structural change of monosaccharaides upon binding using S–V plots, Macromol. Symp. 392 (2020) 2000166.
[17] Raveendra Melavanki, Kalpana Sharma, Basappa ChanabasapaYallur, Raviraj Kusanur, Kishor Kumar Sadasivuni, Diksha Singh, Smita Mane, Kariyappa Katagi, Shridhar V. Pattar, Understanding the binding interaction between phenyl boronic acid P1 and sugars: determination of association and dissociation constants using S-V plots, steady-state spectroscopic methods and molecular docking, Luminescence (2020) 1–6. Luminescence Wiley.
[18] R.A. Kusanur, H.S. Geethanjali, D. Nagaraja, M.N. Manjunatha, R.M. Melavanki, P. Bhavya, Binding interaction between 2-methoxy-5-fluoro phenyl boronic acid and sugars: Effect of structural change of sugars on binding affinity, Can. J. Phys. 94 (12) (2016) 1384–1389.
[19] Joseph R. Lakowich, Chris D. Geddes, Topics in fluorescence spectroscopy, Glucose Sens. 11 (2006).
[20] H. Van Bekkum, A.P.G. Kieboom, J.G. Batelaan, J.A. Peters, J. Van Haveren, pH dependence of the stability of boric acid and borate esters of amino acids and amino diols in aqueous medium as studied by 1B and 1H NMR spectroscopy, Recl. Trav. Chim. Pays-Bas 108 (1989) 179–184.
[21] H. Van Bekkum, A.P.G. Kieboom, J.A. Peters, M. Van Duin, Studies on borate esters 1 : The ph dependence of the stability of esters of boric acid and borate in aqueous medium as studied by 11B NMR, Tetrahedron 40 (1984) 2901–2911.
[22] R. Sarah, Laughlin, Arylboronic Acids with strong Fluorescence Intensity Changes upon Sugar Binding, Thesis, Georgia State University, 2011.
[23] T.A. Houston, M.J. Kiefel, B. Pappin, Boron-carbohydrate interactions, INTECH Open Access Pub. (November 2012).

[24] Binghe Wang, Nanting Ni, Yingji Wang, Sarah Laughlin, Arylboronic acids with strong fluorescence intensity changes upon sugar binding, Heterocycl. Commun. 18 (1) (2012) 23–28.
[25] Spencer D. Dreher, Sarah LJ. Trice, Gary A. Molander, Direct boronic acid synthesis from aryl chlorides: A simplified route to diverse boronate ester derivatives, Chem. Soc. 132 (50) (2010) 17701–17703.
[26] S. Shinkai, M. Philips, T. James, The importance of pyranose to furanose interconversion. In boronic acids in saccharide recognition, RSC (2006) 77–82. Cambridge, UK.
[27] H. Eggert, J.C. Norrild, Evidence for mono- and bisdentate boronate complexes of glucose in the furanose form. Application of 1JC-C coupling constants as a structural probe, Chem. Soc. 117 (1995) 1479–1484.
[28] Hari Mohan, K.I. Priyadarsini, Barik Alok, Photophysical studies on binding of curcumin to bovine serum albumin, Photochem. Photobiol. 77 (6) (2003) 597–603.
[29] Nasseem Ahmad El- Khatatneh, Maryam Gilandoust, Shamantha Kumar, B.H. Doreswamy, M. Mahendra, N.R. Bhavya, Computational and spectroscopic investigations on boronic acid based fluorescent carbohydrate sensor in aqueous solution at physiological pH 7.5, J. Mol. Struct. 1194 (15 October 2019) 304–319.
[30] Guo-wen Xing, Zhang-wei Ning, Guang-jian Liu, Xiao-tai Zhang, Boronic acid-based chemical sensors for saccharides, Carbohydr. Res. 452 (27 November 2017) 129–148.
[31] Ronald E. Wrolstad, Food Carbohydrate Chemistry, frist ed., John Wiley & Sons, 2012.
[32] Jens Christian Norril, Thomas Hoeg-Jensen, Johannes Fabritius Petersen, Jorn Bolstad Christensen, Jon Stefan Hansen, Arylboronic acids: A diabetic eye on glucose sensing, Sensor. Actuator. B Chem. 161 (1) (January 2012) 45–79.
[33] Emil C Buruiana, Tinca Buruiana, Violeta Melinte, Andreea L. Chibac, Fluorescent polymeric sensors containing boronic acid derivatives for sugars and dopamine detection. Sensing characteristics enhancement by Au NPs, Sensor. Actuator. B Chem. 253 (December 2017) 987–998.
[34] D. Burk, H. Lineweaver, The determination of enzyme dissociation constants, J. Am. Chem. Soc. 56 (3) (1934) 658–666.
[35] Todd A Houston, Milton J Kiefel, Brighid Pappin, Boron-carbohydrate Interactions: chap. 3
[36] J.H. Hildebrand, H.A. Benesi, A spectrophotometric investigation of the interaction of iodine with aromatic hydrocarbons, J. Am. Chem. Soc. 71 (1949) 2703–2707.
[37] Sarat K. Swain, S.K. Basiruddin, Phenylboronic acid functionalized reduced graphene oxide based fluorescence nano sensor for glucose sensing, Mater. Sci. Eng. C 58 (Jan 2016) 103–109.
[38] P Bhavya, Raveendra Melavanki, C K Narayanappa, Raviraj Kusanur, Meghana U and Suma B., Binding interaction between boronic acid derivatives with monosaccharaides: effect of structural change of monosaccharaides upon binding using S–V Plots, Macromolecular Symposia.
[39] Piyush A. Gediya, Sharanappa Nembenna, Shanmugasundar Saravanakumar, Manjunath D. Ghate, Nikum D. Sitwala, Raviraj Kusanur, Dayanand Patagar, Synthesis of novel 4-substituted coumarins, docking studies, and DHODH inhibitory activity, J. Heterocycl. Chem. 56 (10) (2019) 2761–2771.
[40] Emil C Buruiana, MelinteTinc Buruiana, Andreea L. Chibac, Fluorescent polymeric sensors containing boronic acid derivatives for sugars and dopamine detection. Sensing characteristics enhancement by Au NPs, Sensor. Actuator. B Chem. 253 (Dec 2017) 987–998.
[41] Yufang Liu, Hua Zhang, Xiaopeng Xuan, Tony D. James, Ge Wang, Yafu Wang, Yuhuan Li, Yonggang Yang, Yuzhu Wang, Yuehua Chan, A molecular-logic gate for COX-2 and NAT based on conformational and structural changes: visualizing the progression of liver disease, Chem. Sci. 11 (2020) 6209–6216 (Edge Article).
[42] Nasseem Ahmad El-Khatatneh, Mariyam Gilandoust, Shamantha Kumar, B.H. Doreswamy, M. Mahendra, N.R. Bhavya, Computational and spectroscopic investigations on boronic acid based fluorescent carbohydrate sensor in aqueous solution at physiological pH 7.5, J. Mol. Struct. 1194 (Oct 2019) 305–319.
[43] Mehdi Salami-Kalajahi, Mahdi Salami Hosseini, Behrouz Dehghani, Neutral pH monosaccharide receptor based on boronic acid decorated poly(2-hydroxyethyl methacrylate): Spectral Methods for determination of glucose-binding and ionization constants, Microchem. J. 157 (Sept 2020) 105112.
[44] Eduardo Nieto-Sepulveda, Andrew D. Bage, Louise A. Evans, Thomas A. Hunt, Andrew G. Leach, Stephen P. Thomas, Guy C. Lloyd-Jones, Kinetics and mechanism of the arase-hoshi R2BH-catalyzed alkyne hydroboration: alkenylboronate generation via B–H/C–B metathesis, J. Am. Chem. Soc. 141 (46) (2019) 18600–18611.
[45] Suying Xu, Adam C. Sedgwick, Souad A. Elfeky, Wenbo Chen, Ashley S. Jones, George T. Williams, A.Toby A. Jenkins, Steven D. Bull, John S. Fossey, Tony D. James, A boronic acid-based fluorescent hydrogel for monosaccharide detection, Front. Chem. Sci. Eng. 14 (2020) 112–116.
[46] Qingxian Wang, Mian Fu, Ying Guan, Tony D. James, Yongjun Zhang, Mechanistic insights into the novel glucose-sensitive behavior of P(NIPAM-co-2-AAPBA), Sci. China Chem. 63 (2020) 377–385.
[47] Veronica Forcina, Andrés García-Domínguez, C. Guy, Lloyd-Jones, Kinetics of initiation of the third generation grubbs metathesis catalyst: convergent associative and dissociative pathways, Faraday Discuss 220 (2019) 179–195.