Physical and Chemical Properties of Boronic Acids: Formulation Implications

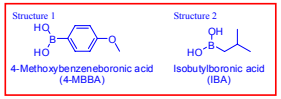
William A. Marinaro and Valentino J. Stella

Department of Pharmaceutical Chemistry, The University of Kansas

Purpose

The objective of the following research is to determine the effect of various polyols on the physical and chemical properties of two model boronic acids; 4-methoxybenzeneboronic acid (4-MBBA) shown in structure 1, and isobutylboronic acid (IBA) illustrated in structure 2. The research will quantify the ability of the polyols, first, to form boronate esters with the model compounds. Second, to determine the effect that this formation has on the pKa and solubility of the boronate compound. This information should provide crucial insights to the formulation chemist when developing boronic acid containing pharmaceuticals.

Structure 1



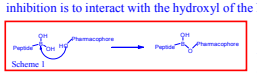
4-Methoxybenzeneboronic acid (4-MBBA)

Structure 2

Isobutylboronic acid (IBA)

Background

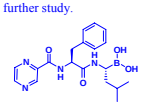
Boronic acid compounds are the focus of increasing interest as therapeutic agents due to their ability to inhibit enzyme activity, among other characteristics. Many peptide boronic acid compounds have been investigated as enzyme inhibitors with implications in pathologies as varied as Alzheimer's disease, cervical cancer, blood clotting disorders, hepatitis C, etc. (1). In 2003 the drug Velcade® (bortezomib), figure 1, was approved by the FDA as a second-line treatment for multiple myeloma, it acts via inhibition of the 26S proteasome in mammalian cells (2). As shown in scheme 1, the role of the boronic acid moiety in this inhibition is to interact with the hydroxyl of the N-terminal threonine of the β-subunit of the proteasome (3).



Scheme 1

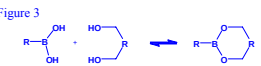
While the pharmacologic properties of these compounds appears to be impressive, there are obstacles to their formulation into suitable dosage forms. One obstacle is their apparent low solubility. Workers have observed solubility values for boronic acid containing compounds well below that of the respective non-boronic acid containing analog (4). It was also noted that solubility could be increased by adding monosaccharides to the aqueous solutions (4). Further, the formulators of Velcade® observed an increase in the solubility of the drug when it was lyophilized in the presence of mannitol (5). They attributed this to the formation of boronic acid esters, shown in green, and the simultaneous avoidance of forming the less soluble trimeric boroxine species shown in orange (Figure 2). A further explanation of this increased solubility is a pKa lowering effect that polyols impart to boronic acids (Figure 3). The mechanism by which polyols help solubilize boronic acid containing compounds is not completely understood and deserves further study.

Figure 1



Structure of Velcade® (bortezomib)

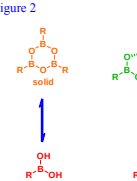
Figure 3



boronic acid pKa: 9-10

boronate ester pKa: ~6

Figure 2



< 1mg/mL solution

6 mg/mL solution

Solubility

Figure 4

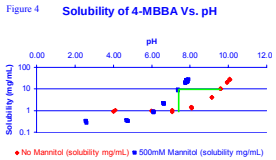
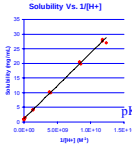


Figure 5

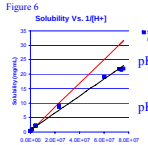


Equation 1

$$S = [HA]_0 + \frac{K_a[HA]_0}{[H^+]}$$

pKa': 8.69

Figure 6



pKa': 5.87

pKa': 6.39

Figure 4 demonstrates the large effect mannitol has on the solubility of 4-MBBA. Highlighted in green is the effect at physiologic pH, a 10-fold increase in solubility. Figures 5 and 6 illustrate the direct effect on the pKa as determined from the solubility pH profile, as described by equation 1. Through figure 6 one can see that the ratio of mannitol to boronic acid has an effect on solubility through pKa.

Effect of Polyols on Boronic Acid pKa Values

A large change in the UV spectra of 4-MBBA is observed at 235nm, this was utilized in pKa determination by UV spectrophotometric titrations (Figure 7).

Figure 7

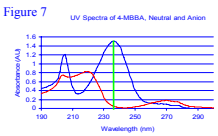
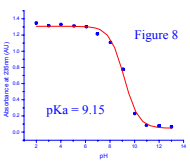


Figure 8



The titration of 4-MBBA is shown (Figure 8). The curve is fit to a modified Henderson-Hasselbach equation.

Potentiometric titrations were also performed using a Mettler-Toledo DL-53 autotitrator and the pKa values were calculated as described by Albert and Serjeant (6). Figure 9 shows the type of data generated, in this case 4-MBBA alone yields a pKa of 9.31±0.01.

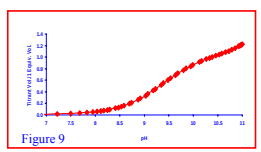


Figure 9

Figure 10

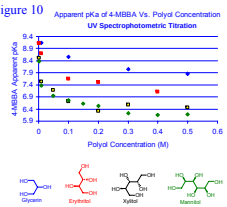


Figure 10 demonstrates the correlation between polyol size and pKa lowering effect. As the number of hydroxyls on the polyol increases, the effect on 4-MBBA increases. The greatest effect comes from mannitol and xylitol, as the pKa lowering effect plateaus at around pKa 6.2, almost a full 3 units below the original pKa of ~9.2.

Figure 11

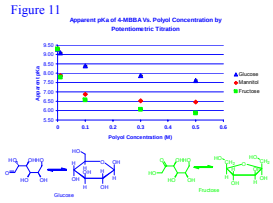


Figure 11 illustrates the utility of the potentiometric technique, described above, in studying the effect of glucose and fructose on the apparent pKa of 4-MBBA. The UV spectrophotometric technique could not be used due to spectral interference, most likely the carbonyl group. The mannitol experiment confirms that both the UV spectrophotometric and potentiometric titration techniques yield the same results, when accounting for the different concentrations used in the experiments. Fructose decreased the apparent pKa of 4-MBBA slightly greater than did mannitol, and much greater than did glucose.

Figure 12

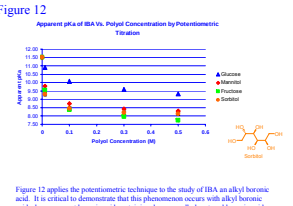


Figure 12 applies the potentiometric technique to the study of IBA an alkyl boronic acid. It is critical to demonstrate that this phenomenon occurs with alkyl boronic acids, because most boronic acid containing drugs are alkyl, not aryl boronic acids. First, this series of experiments shows that a similar drop in pKa occurs, roughly 3 units or greater for the highest binding polyols, when alkyl or aryl boronic acids form boronate esters. Second, the rank order of the polyols' effect is maintained.

Isothermal Titration Calorimetry

Figure 13

ITC Instrumental Diagram

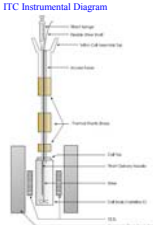
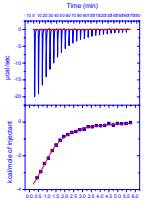


Figure from User's Manual Revision 12705, Calorimetry Sciences Corp., Lindon, UT

Isothermal titration calorimetry or ITC, is a calorimetric analytical techniques which measures the heat evolved or absorbed upon titrating a solution of ligand into a solution of receptor. The instrumental setup is shown in figure 13. As the mole ratio increases during the titration and there is less free receptor, the total heat per injection decreases, as shown in figure 14. This profile is described, for a molecule with a single set of receptors, by equation 2. Thus, the data may be fit, as in figure 15, and one may solve for the variables: N, K, ΔH, and ΔS

Figure 14



Equation 2

$$Q = V \cdot \Delta H \cdot \left[[L] + \frac{1 + [M] \cdot n \cdot K - \sqrt{(1 + [M] \cdot n \cdot K - [L] \cdot K)^2 + 4 \cdot K \cdot [L]}}{2 \cdot K} \right]$$

Where:
Q is the total heat
ΔH is the enthalpy of binding
K is the binding constant
n is the number of binding sites
V is the volume of the reaction cell
[L] is the ligand concentration
[M] is the receptor concentration

Figure 15

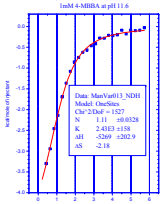


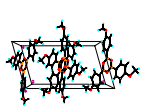
Figure 15

Interaction of various polyols with 4-methoxybenzene boronic acid as the receptor in ITC experiments

Ligand:	mannitol	sorbitol	erythritol	glycerol	2,3-butanediol
K (M^{-1}) ±SD	2767±39	6254±605	118±NA	23.7±0.7	13.2±0.8
n ±SD	1.05±0.00	1.07±0.08	0.84±NA	NA	NA
ΔH (kJ/mol) ±SD	-21.5±1.9	-26.7±0.6	-24.3±NA	NA	NA

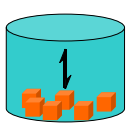
The ITC data confirms the rank order of polyol effects observed during pKa lowering studies. Further, the binding constants observed are on the same order as those predicted from the pKa lowering studies.

Future Work



•The solid state of boronic acids and boronate esters will be studied, with a particular emphasis on how to exploit the formulation of boronic acid containing drugs with polyols in order to maximize the binding phenomena.

•The solubility and dissolution of boronates will be explored in greater detail to further elucidate the mechanism of boronate ester and boroxine dissolution to yield free boronic acids.



Conclusions

•pKa determination by UV spectroscopic and potentiometric titrations have identified that some high binding polyols decrease the pKa of a boronate by over three units. The high binding polyols include some common pharmaceutical excipients.

•This effect occurs in both aryl and alkyl boronic acids, and is maintained regardless of the starting pKa of the unbound boronic acid. Further, the rank order of polyol effect is maintained from alkyl to aryl boronic acids.

•Isothermal titration calorimetry confirms that a boronic acid-polyol binding correlates with this pKa lowering effect.

•Solubility studies show that pKa lowering of boronic acids by polyols has a large effect on the solubility of these molecules, especially near physiologic pH.

Acknowledgements

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