Brief Report

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# A new boronic acid-based fluorescent sensor for L-dihydroxyphenylalanine

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**ABSTRACT: Catecholamines, such as dopamine and L-dihydroxyphenylalanine (L-DOPA), are associated with different physiological functions and diseases. In our recent studies, a novel water-soluble boronic acid compound 3c was identified as a selective fluorescent sensor for L-DOPA. This compound not only has the ability to interact with dopamine and catechol, but also has no fluorescence intensity change for L-DOPA precursors *in vivo*, such as L-tyrosine.**

***Keywords:*** Boronic acid, fluorescent chemosensor, L-DOPA, dopamine

## 1. Introduction

As a series of endogenous bioactive substances *in vivo*, catecholamine derivatives are generated from L-dihydroxyphenylalanine (L-DOPA) which is converted from L-tyrosine. L-DOPA can be degraded to dopamine by DOPA decarboxylase in the body and then following conversion can release norepinephrine and epinephrine. It is well known that dopamine, norepinephine, and epinephrine exhibit various physiological effects, such as increasing heart rate and blood pressure. In addition, dopamine and its precursor L-DOPA are involved in many diseases including parkinsonism, hypertension, and schizophrenia (*1-8*). For example, significant depletion of dopamine was found in brains of Parkinson's disease victims. Administration of L-DOPA could supply the dopamine *in vivo* and offset its deficient effect (*9,10*). For this reason, it is of great interest for scientists to develop various analytical methodology to detect catecholamine derivatives, such as spectrophotometry, gas chromatography, radioimmunoassay, voltammetric determination, potentiometry, chemiluminescense, and flow injection analysis (FIA) (*11*). Currently, the commonly used simultaneous determination of L-DOPA and dopamine are done using high-performance liquid chromatography (HPLC) (*12,13*). Other methods using nuclear magnetic resonance (NMR) spectroscopy (*14*) and capillary electrophoresis (CE) (*15*) have also been reported.

Recently, some selective fluorometric methods for dopamine have been reported (*16*). In 2004, Akkaya reported a selective fluorescent chemosensor for L-DOPA using a Lucifer yellow scaffold to link a phenyl boronic acid structure. The phenyl boronic acid was used to recognize the catechol moiety in the structure of L-DOPA (*17*). However, this fluorescent sensor did not give a binding constant for other catecholamine derivatives, such as dopamine and catechol. Recently, our group reported compound **1** is a selective fluorescent chemosensor for catechol derivatives (*18*). However, this compound did not show selectivity for catechol derivatives, especially for dopamine and L-DOPA. According to our previous results, amidation of the carboxyl group in compound **1** didn't influence its binding affinity for catechol derivatives. In our on-going study, binding affinity and selectivity could be improved by introducing a second binding site and this strategy has also been successfully used to develop other selective chemosensors for dopamine (*16,19*). This paper describes our recent work focus on introducing a carboxylic acid as a second binding site with different linker, which could help us develop selective fluorescent chemosensor for L-DOPA.

## 2. Materials and Methods

### 2.1. *General methods*

Solvents were reagent grade and were purified and dried using standard methods when necessary. All melting points were determined on a micromelting point apparatus (and are uncorrected). $^{1}$H-NMR and $^{13}$C-NMR spectra were obtained on a Bruker

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Avance-300 instrument in the indicated solvent. Chemical shifts are expressed in delta (δ) units with TMS as internal reference. ESI-MS were determined on an API 4000 spectrometer. All reactions were monitored using TLC on 0.25 mm silica gel plates (60GF-254) and visualized with UV light. Flash column chromatography was performed on a column packed with silica gel 60 (200-300 mesh). Concentration of the reaction solutions involved use of a rotary evaporator at reduced pressure.

### 2.2. *General procedure for the preparation of title compounds (**3a-3c**)*

Compound **1** was refluxed with thionyl chloride to generate the corresponding acyl chloride and then reacted with different ω-aminoalkanoic acid methylesters to yield **2a-2c**. The hydrolysis of methylester will give target compound **3a-3c** (Scheme 1).

### 2.3. *Binding study*

Different concentration of analytes (0.10 mM to 1.0 mM) were added to the solution of the target compound (**3a-3c**) at a concentration of $3.0 \times 10^{-5}$ M in phosphate buffer at pH 7.4. The fluorescent intensity was recorded by a THERMO-LABSYSTEMS VARIOSKAN FLASH Multimode Spectral Scanning Microplate Reader. The apparent association constant (*K*a) was calculated according to the Benesi-Hildebrand equation.

## 3. Results

### 3.1. *Fluorescent intensity changes and associate constants*

The fluorescence intensity of three target compounds decreased significantly after addition of catechol and catecholamine derivatives. For example, the fluorescence intensity decreased almost 65% after addition of 1 mM L-DOPA (Figure 1). According to the apparent association constants (*K*a) of three target compounds (Table 1), L-DOPA showed a two-fold greater affinity than catechol and dopamine for compound **3c**. For compound **3a** and **3b**, the association constants for catechol, dopamine, and L-DOPA are almost the same, which suggests that these two compounds have no selectivity for catecholamine derivatives.

These results indicate that the length of the linker of the target compound has a significant influence on the binding affinity and selectivity for L-DOPA. For example, compounds with the linker of one or three methylene units (compound **3a** and **3b**) have no selectivity for catechol and catecholamine derivatives. While when the linker was extended to five methylene units, such as compound **3c**, the binding affinity and selectivity for L-DOPA could be increased.

### 3.2. *Selectivity of compound **3c** interacting with analytes*

Considering that the origin of L-DOPA is related

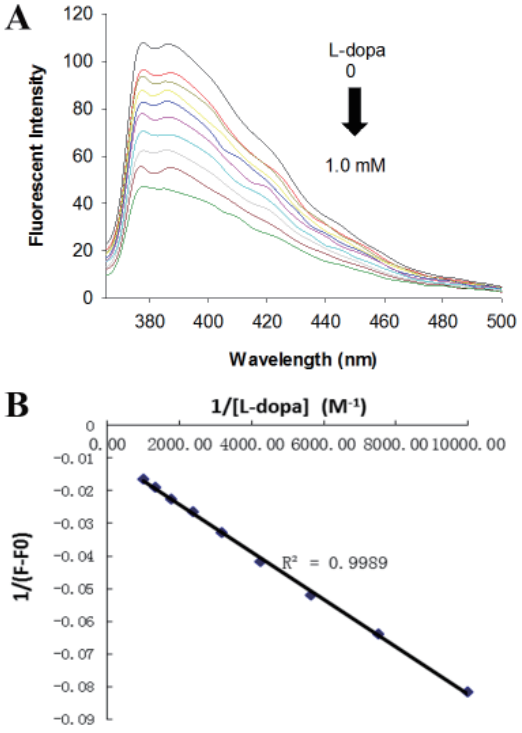
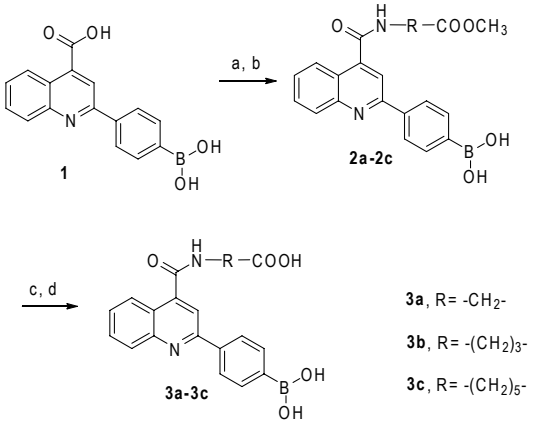



**Figure 1. Fluorescent spectral changes of compound 3c ($3 \times 10^{-5}$ M) upon addition of various concentrations of catechol in 0.1 M phosphate buffer at pH 7.4, $\lambda_{ex}$ = 337 nm, $\lambda_{em}$ = 385 nm. (A)** Fluorescence spectra of **3c** upon addition of catechol; **(B)** Relative fluorescent intensity changes *vs.* concentration of catechol.




**Scheme 1. Synthetic scheme, reagents, and conditions. (a)** $SOCl_2$, reflux; **(b)** $NH_2$-R-$COOCH_3$; **(c)** NaOH; **(d)** HCl.

**Table 1. Apparent association constants (*K*a) of chemosensors ($3 \times 10^{-5}$ M in 0.1 M phosphate buffer, pH 7.4) with catechol, dopamine, and L-DOPA**

| Chemosensors | Catechol | Dopamine | L-DOPA |
|---|---|---|---|
| **3a** | 780 | 674 | 730 |
| **3b** | 837 | 890 | 770 |
| **3c** | 680 | 791 | 1405 |

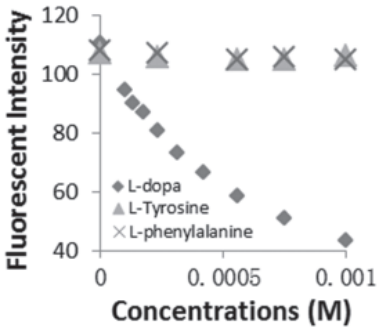



**Figure 2. Fluorescense changes *versus* concentration of various analytes whose structures are related to L-DOPA (L-DOPA, L-tyrosine and L-phenylalaine) added to 3c ($3 \times 10^{-5}$ M) in phosphate buffer (0.1 M, pH 7.4), $\lambda_{ex}$ = 337 nm, $\lambda_{em}$ = 382 nm.**

to L-tyrosine and L-phenylalanine *in vivo*, apparent association constants of **3c** interacting with L-tyrosine and L-phenylalanine were also tested. The results showed that fluorescence intensity had no change after adding them to the buffer of **3c** (Figure 2). The possible reason is that the catechol moiety does not exist in the structure of L-tyrosine and L-phenylalanine, which leads to missing the crucial interaction with phenylboronic acid in compound **3c** which is maybe due to the missing catechol moiety.

## 4. Discussion

L-DOPA has a similar structure to dopamine and possesses an important role in the treatment of parkinsonism. Until now, few chemosensors have been reported to have good selectivity for L-DOPA in the literature. As we know, phenylboronic acid has the unique property to bind to 1,2- or 1,3-diols in aqueous solution. A stronger binding interaction can easily occur for an adjacent rigid *cis* diol in the structure of catechol or carbohydrate. Therefore, boronic acids have been investigated as chemosensors for catechol and carbohydrate in the recent two decades (*16,19,20*). Compound **1** is a new water-soluble fluorescent sensor for carbohydrate and catechol (*18*). This compound shows decreasing fluorescence intensity after addition of catechol and enhancing fluorescence intensity after addition of carbohydrate. This different fluorescence changing properties are no doubt very useful to develop selective chemosensor for catechol derivatives without interference of carbohydrate. In our recent studies, compound **3c** was identified as a novel chemosensor for L-DOPA by coupling with an alkanoic acid with five methylene units. This result indicated that carboxylic acid could be used as the second binding site to interact with the amino group in L-DOPA, which could be helpful to improve binding affinity and selectivity for the chemosensor **3c**. On the other hand, the linker also showed a crucial role in constructing a selective chemosensor. Therefore, further studies could focus on introducing long alaphatic chains or rigid rings as linkers to develop a better chemosensor for catecholamine derivatives.

In conclusion, compound **3c** was found to be a new water-soluble fluorescent chemosensor for L-DOPA under physiological conditions compared with sugars and some phenol derivatives. This demonstrates that structural modifications on carboxyl groups will be helpful for developing new selective chemosensors for bioactive catechol derivatives in the future.

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## Appendix

**Synthesis of compounds 3a-3c.**

*2-(2-(4-Boronophenyl)quinoline-2-carboxamido)butanoic acid (**3a**)*

Compound **1** (0.28 g, 1.0 mmol) was refluxed in thionyl chloride (15 mL) for 2 h and the thionyl chloride was removed *in vacuo* to give the acyl chloride. Then using triethylamine (0.61 g, 6.0 mmol) as the acid neutralizer, the acyl chloride reacted with glycine methyl ester chloride (0.38 g, 3.0 mmol) in anhydrous methylene dichloride to yield amide **2a** (0.24 g). Crude product **2a** was dissovled in 15 mL methanol and 3.0 mL of 2N NaOH solution (6.0 mmol) was added. The mixture solution was stirred for 1 h until the reaction was completed. The methanol was removed the methanol *in vacuo* and filterd to give a yellow solid. The product was purified by chromtography (DCM:MeOH = 15/1) to give a light yellow power **3a** (0.18 g). Yield: 49%. $^{1}$H-NMR (600 Mz, $CD_3OD$) δ 8.38 (m, 1H), 8.12-8.20 (m, 4H), 7.84 (m, 3H), 7.65 (m, 1H), 3.59 (s, 2H); $^{13}$C-NMR (150 Mz, DMSO-$d_6$) δ 170.77, 167.82, 156.24, 148.79, 142.59, 139.87, 135.15 (2C), 130.76, 129.94, 127.70 (2C), 126.59, 125.58, 123.84, 117.31, 41.46; MS (ESI) m/z (%) 351 (M + 1, 100).

*4-(2-(4-Boronophenyl)quinoline-4-carboxamido)butanoic acid (**3b**)*

Yield: 56%. $^{1}$H-NMR (600 Mz, $CD_3OD$) δ 8.40 (m, 1H), 8.15-8.20 (m, 4H), 7.84 (m, 3H), 7.70 (m, 1H), 3.10 (m, 2H), 2.06 (m, 2H); $^{13}$C-NMR (150 Mz, DMSO-$d_6$) δ 170.77, 167.82, 156.24, 148.79, 142.59, 139.87, 135.15 (2C), 130.76, 129.94, 127.70 (2C), 126.59, 125.58, 123.84, 117.31, 40.42, 35.54, 23.40; MS (ESI) m/z (%) 379 (M + 1, 100).

*6-(2-(4-Boronophenyl)quinoline-4-carboxamido)hexanoic acid (**3c**)*

Yield: 52%. $^{1}$H-NMR (600 Mz, $CD_3OD$) δ 8.36 (m, 1H), 8.12-8.20 (m, 4H), 7.84 (m, 3H), 7.65 (m, 1H), 3.06 (m, 2H), 2.05 (m, 2H), 1.30-1.50 (m, 6H); $^{13}$C-NMR (150 Mz, DMSO-$d_6$) δ 170.77, 167.82, 156.24, 148.79, 142.59, 139.87, 135.15 (2C), 130.76, 129.94, 127.70 (2C), 126.59, 125.58, 123.84, 117.31, 41.42, 34.40, 30.54, 24.90, 26.40; MS (ESI) m/z (%) 407 (M + 1, 100).