DOI: 10.1002/cbic.200700663

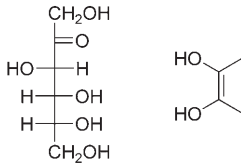
Computer-Based De Novo Design, Synthesis, and Evaluation of Boronic Acid-Based Artificial Receptors for Selective Recognition of Dopamine

Shan Jin, Minyong Li, Chunyuan Zhu, ViLinh Tran, and Binghe Wang*[a]

Dopamine is an important neurotransmitter that plays important roles in various physiological and pathological processes, such as Parkinson's disease. Chemosensors for dopamine have a number of potential applications. On the basis both of the strong and reversible complexation between the boronic acid moiety and a diol functional group and computational chemistry studies, we have designed a series of four compounds for selective three-point recognition of dopamine, which include boronic acid–diol complexation, aromatic–hydrophobic interactions, and ionic interactions between a carboxylate and a protonated amino group. These compounds were synthesized in seven or eight linear steps and showed dopamine selectivity of up to tenfold over epinephrine. NMR spectroscopy experiments were conducted to probe the structures of the receptor–dopamine complexes. These receptors are the first to show such significant selectivity for dopamine over epinephrine in aqueous solution under near physiological conditions.

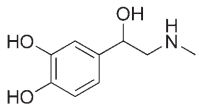
Introduction

Various catecholamines are very important biological compounds, especially as neurotransmitters[1,2] and hormones,[3,4] Therefore, there is a great deal of interest in the detection and concentration determination of such catecholamines in biological fluids.[5–13] Past efforts in this area have been focused on electrochemical detection, because of the presence of the electrochemically active catechol structural moiety.[5–7,9,13] Chemosensors for catecholamines, however, also have applications in

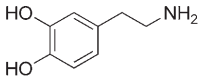


D-fructose

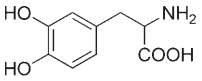
catechol



epinephrine
(adrenaline)

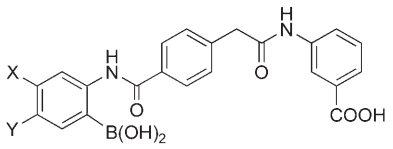


dopamine



L-DOPA

situations that cannot be adequately addressed with electrochemical methods.[8,10–12,14] For example, chemosensors offer the potential to allow for real-time monitoring with minimal disturbance of the normal physiological/pathological processes.[10,11] Among all the catecholamines, dopamine is especially important in neurochemistry. Dopaminergic functions are implicated in Parkinson's disease,[15–18] mood modification,[19–21] and cocaine addiction,[22–25] and so recognition and detection of dopamine have been identified as important issues.[5,8,9] However, chemosensor work for the selective recognition of dopamine has been very limited.[8,10,11] A major challenge in the development of dopamine receptors is to achieve selective recognition between the different catecholamines. In this paper, we report for the first time on boronic acid-based artificial receptors that show up to tenfold selectivity for dopamine over epinephrine, which is unprecedented, and that are functional in an aqueous environment under near physiological conditions. The general structures of such receptor compounds are shown in Scheme 1.



1a: X=H, Y=H;
1b: X=CN, Y=H;
1c: X=H, Y=F;
1d: X=$CONH_2$, Y=H

Scheme 1. Structures of designed boronic acid-based receptors for dopamine.

[a] *S. Jin, Dr. M. Li, C. Zhu, V. Tran, Prof. B. Wang*
Department of Chemistry and Center for Biotechnology and Drug Design
Georgia State University
Atlanta, GA 30302-4098 (USA)
Fax: (+1) 4044135543
E-mail: wang@gsu.edu

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

Results and Discussion

Design of dopamine sensors

Dopamine has three important structural components that should allow for strong interactions in an aqueous environment: 1) a catechol unit, which should interact strongly with the boronic acid functional group,[26–40] 2) a central aromatic ring, which should allow for strong hydrophobic interactions, and 3) an amino group, which upon protonation should afford strong interactions with an anionic functional group. The plan was to use a vector-based approach for the design of artificial receptors with the appropriate relative functional group orientation and spacing.[41]

We chose to start with the crystal structure of dopamine, assuming that it represents or is close to a high population conformation in solution. We calculated possible binding vectors of the dopamine–boronic acid complex from dopamine's X-ray crystal structure, using ab initio/6-31G optimization in the polarizable continuum water model (PCM) integrated in the Gaussian 03 program.[42] The calculated ideal vector arrangements are shown in Figure 1. From the optimized vector arrangements, several structural features were identified as important for the design of dopamine receptors. A 2D virtual library of compounds containing three structural components—a boronic acid, a carboxylic acid, and a hydrophobic linker—was developed with the aid of the lilib diverse 1.02 program[43,44] and was then converted into 3D structures by use of the CORINA program.[45] Finally, ISIS/BASE was used for database search against this 3D virtual library and certain defined chemical features to give candidate structures. The building blocks of the linker component included amino acids, aromatic rings, and aliphatic chains ($C < 4$), while the boronic acid building blocks only included arylboronic acids. Figure 1 shows the calculation results with defined "ideal" distances and orientation of receptors for optimal binding.

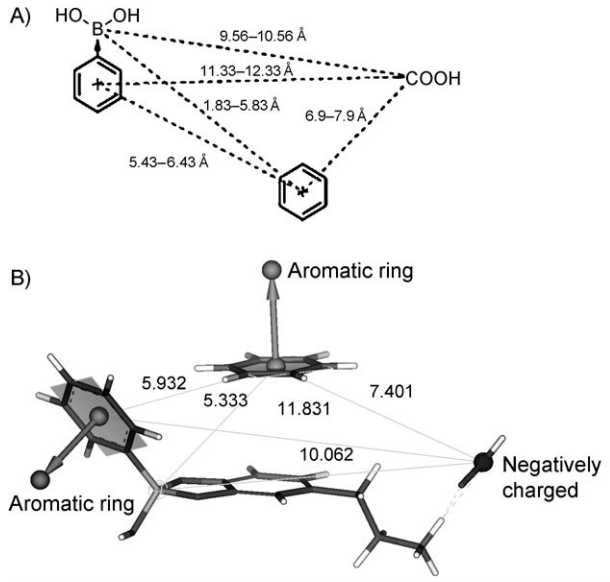


Figure 1. A) Calculated distances between the three binding parts, and B) proposed ideal vector arrangement of the three binding components of the target receptors based on computational results.

Compounds **1a–d** (Scheme 1) were among those identified as possible dopamine receptors with the putative binding mode presented in Figure 2.

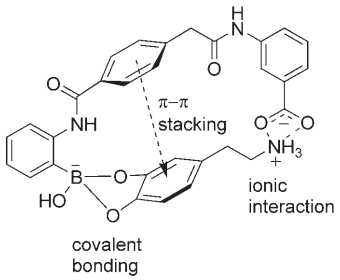


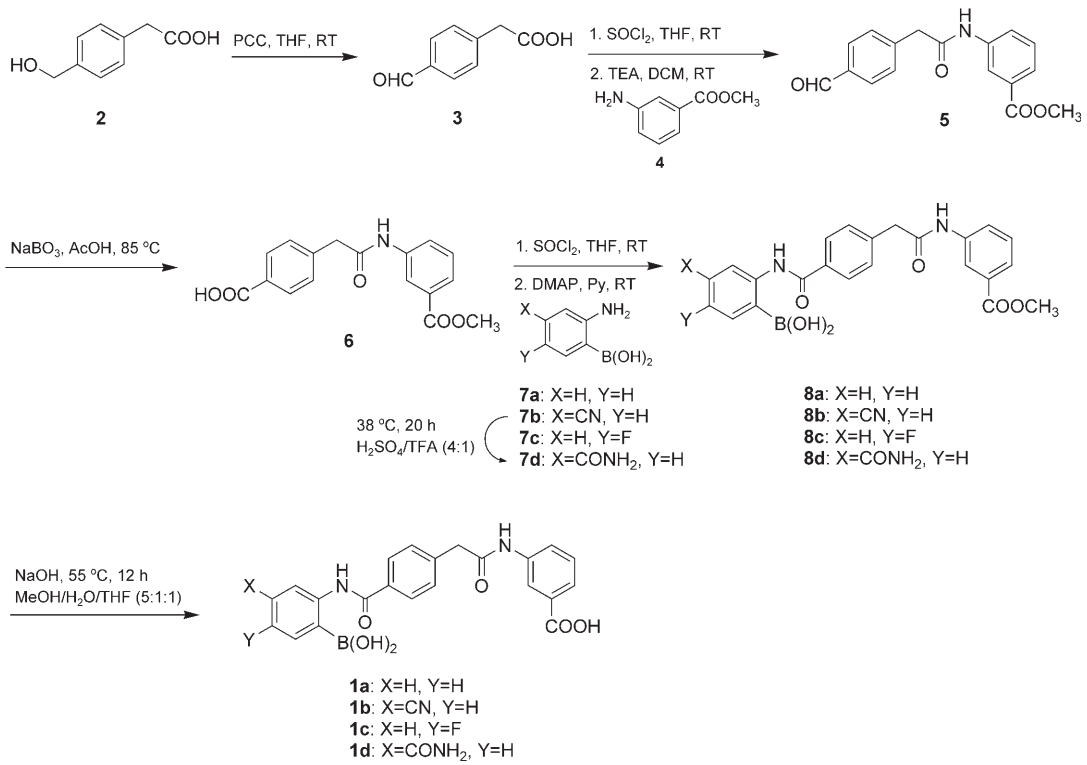
Figure 2. Proposed binding mode of the designed compounds.

Synthesis of compounds 1a–d

As shown in Scheme 2, the identified structures were then synthesized, starting from [4-(hydroxymethyl)phenyl]acetic acid (**2**). Pyridinium chlorochromate (PCC) was used to oxidize the hydroxy group of compound **1** to give the corresponding aldehyde **3** (57%). The (4-formylphenyl)acetic acid (**3**) was then treated with thionyl chloride to afford the corresponding acid chloride. Compound **5** (73%) was obtained by treating the acid chloride with methyl 3-aminobenzoate (**4**) in dichloromethane at room temperature. Oxidation of the aldehyde group to a carboxylic acid group was then achieved by treating compound **5** with sodium perborate tetrahydrate in acetic acid at 85 °C for 24 h (79%).[46] Following treatment of carboxylic acid **6** with thionyl chloride, the second amide bond was formed by addition of the formed acid chloride to a dichloromethane solution of a 2-aminophenylboronic acid (**7a–d**). Compounds **7a–c** were commercially available, while compound **7d** was obtained from **7b** by hydrolysis of the cyano group. Finally, removal of the methyl ester groups from compounds **8a–d** gave the boronic acid end-products **1a–d**.

Binding studies

The abilities of the target dopamine receptors to bind dopamine were evaluated by use of a three-component system with alizarin Red S (ARS) as the reporter compound.[31,32] The binding studies of boronic acid **1a** are presented as an example. Firstly, the binding constant between **1a** and ARS was determined. As expected, binding of ARS with the boronic acid compound gave concentration-dependent fluorescent intensity increases (Figure 3A). The results were consistent with 1:1 binding. Binding constants (K_a) were calculated from Equation (1),[47] where I_0 is initial fluorescent intensity, b is the path length of absorption, and ΔI_{max} is the maximal fluorescent intensity change. The apparent binding constant between **1a** and ARS was found to be 1403 M^{-1} (Figure 3B).



Scheme 2. Synthesis of potential dopamine receptors.

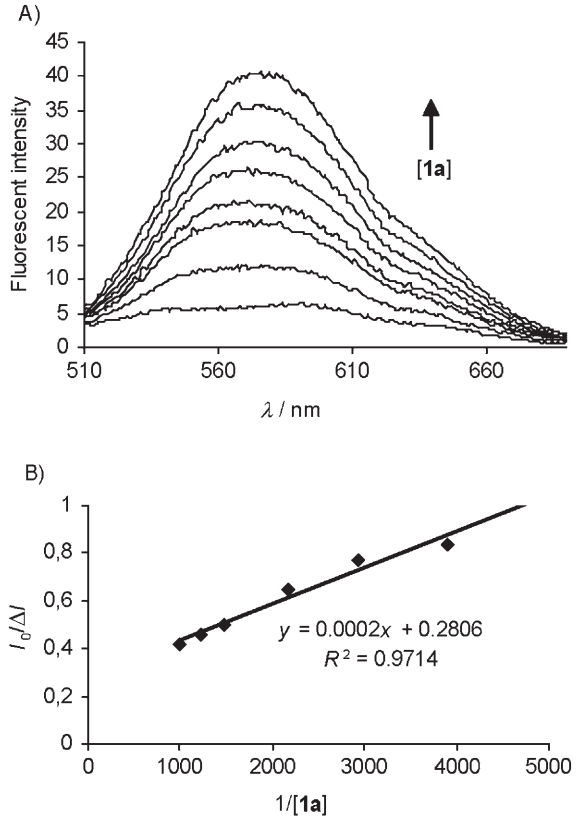


Figure 3. Fluorescence binding studies between ARS (1×10^{-4} M) and boronic acid **1a** (0–1 mM) in phosphate buffer (0.1 M) at pH 7.4; $\lambda_{ex}=495$ nm, $\lambda_{em}=570$ nm. A) Fluorescence spectra of ARS upon addition of **1a**; B) binding constant calculation of **1a** with ARS based on a 1:1 binding model; all experiments were duplicated.

$$([\mathbf{1a}]b)/\Delta I = \frac{I_0}{\Delta I_{max}[ARS]K_a} + \frac{I_0}{\Delta I_{max}} \quad (1)$$

The binding constants between boronic acid **1a** and various diols were then determined by titration of the diol compound at different concentrations into the boronic acid–ARS mixture. Briefly, boronic acid **1a** (1×10^{-3} M) was added into a solution of ARS (1×10^{-4} M in 0.1 M phosphate buffer at pH 7.4) to obtain solution A, in which about 20% of the ARS was in free form (as measured by fluorescence testing). The diol was then added to a portion of solution A to generate solution B, with about 80% ARS in the free form. Solution B was titrated into solution A to make a series of solutions with a constant concentration of ARS and the boronic acid and a range of different concentrations of the diol. After standing for 1 min, each mixture (0.7 mL) was transferred into a 1 cm quartz cuvette, and the fluorescence intensity was recorded.[31,48] When a diol was added to the ARS–boronic acid mixture, a concentration-dependent fluorescent intensity decrease was observed (Figure 4A, for the case of dopamine), which allowed for binding constant determination (Figure 4B).[31,32,47] By using this method, the binding constants of **1a**–**d** with dopamine were determined (Table 1). For comparison, we also determined the binding constants with adrenaline, catechol, and fructose (Table 1 and Figure 5).

As designed, all compounds showed significant binding with, and selectivity for, dopamine (Figure 5). The apparent binding constants with dopamine were in the range of 520–940 M^{-1} (Table 1). In comparison, the binding constants with catechol ranged from 120–600 M^{-1}, while those with adrenaline

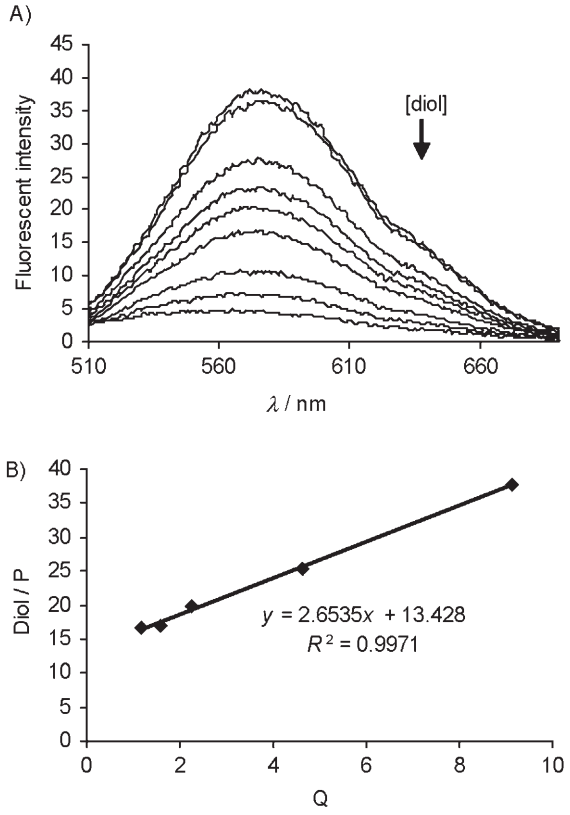


Figure 4. Fluorescence spectral changes of the ARS–**1a** solution upon addition of dopamine (0–50 mM) and binding constant determination in phosphate buffer (0.1 M) at pH 7.4; $\lambda_{ex}=495$ nm, $\lambda_{em}=570$ nm; [**1a**] $=1\times10^{-3}$ M; [ARS] $=1\times10^{-4}$ M. A) Fluorescence spectral changes associated with binding of **1a** with dopamine; B) binding constant calculation between **1a** and dopamine; all experiments were duplicated.

Table 1. Apparent association constants (K_a) of the boronic acid receptors **1a–d** with dopamine and reference compounds.

K_a [M^{-1}]	Fructose	Catechol	Adrenaline	Dopamine
1a	22±7	121±3	51±5	538±34
1b	71±6	597±22	228±1	944±38
1c	59±6	224±22	190±6	524±18
1d	46±2	345±19	95±4	757±42

[Boronic acids] $=1\times10^{-3}$ M, [ARS] $=1\times10^{-4}$ M in phosphate buffer solution (0.1 M) at pH 7.4.

ranged from 50–230 M^{-1}. Compound **1b** showed the highest affinity (940 M^{-1}), but only less than twofold selectivity over catechol and about fourfold selectivity over epinephrine. Compound **1a** and **1c** showed the lowest affinity for dopamine, but compound **1a** showed the highest selectivity over catechol (fourfold) and epinephrine (about tenfold). Compound **1d** showed significant selectivity over epinephrine (about eightfold) as well, and the second highest binding constant with dopamine (760 M^{-1}). All compounds, as expected, showed good selectivity over fructose; this indicates that the binding is not merely through simple diol–boronic acid interactions. The results indicate that the two analogues (**1b**, **1d**) with electron-

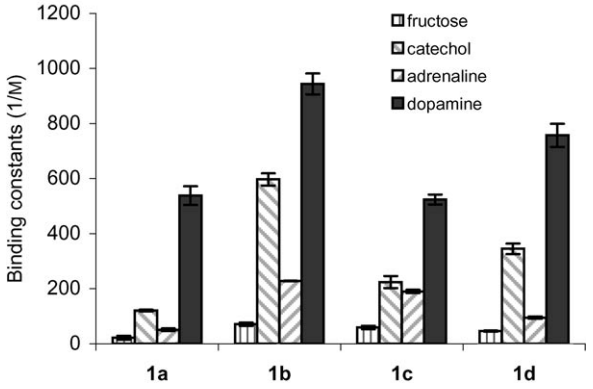


Figure 5. Comparison of the apparent binding constants of **1a–d** with different diols (0–50 mM) in phosphate buffer (0.1 M) at pH 7.4; $\lambda_{ex}=495$ nm, $\lambda_{em}=570$ nm; [boronic acids] $=1\times10^{-3}$ M; [ARS] $=1\times10^{-4}$ M; all experiments were duplicated.

withdrawing groups (cyano and amido) at the *para*-positions of their phenylboronic acid units have the highest affinities. This could be attributable to increased Lewis acidity of the boronic acid units, which is known to increase binding affinity at neutral pH,[32,36] or to conformational effects of these two substituents, or both. One unique feature of our receptors is the higher selectivity of **1a–d** for dopamine over epinephrine than over catechol, despite the fact that epinephrine is structurally more analogous to dopamine than catechol is. Such results indicate the significance of using a hydrophobic linker, which could help to bias against epinephrine because of the presence of an extra hydrophilic hydroxy group on the epinephrine side chain. When a linear alkyl chain was used, in the sensors reported by Glass[11] and Yoon,[8,12] the selectivity over epinephrine was nonexistent or very small. Such information should be very useful for the future design of other receptors for other catecholamines.

As a further control experiment to study the effect of the carboxylate–protonated amine interactions (Figure 2), we were interested to test the binding of the ester precursor (**8a**) of **1a** with dopamine. However, because **8a** requires 30% methanol for solubilization, the binding studies were conducted in a mixture of PBS buffer and methanol (30%); under such conditions, **8a** showed similar binding affinities for dopamine (192 M^{-1}) and catechol (124 M^{-1}). Such results are expected and further demonstrate the importance of the ionic interactions between the carboxylate group in **1a** and the protonated amino group in dopamine. As an additional control, we studied the binding of **1a** with L-3,4-dihydroxyphenylalanine (L-DOPA), which has an extra carboxylate group relative to dopamine. Again, solubility was an issue for L-DOPA, which required the addition of 30% methanol for solubilization. Sensor **1a** showed a binding constant of 258 M^{-1} for dopamine, which is twice that of L-DOPA (115 M^{-1}). Such results are consistent with the experiments with epinephrine, which indicate that an added polar/ionizable group on the side chain does not favor binding. The lowered affinity of **1a** for dopamine in 30% methanol is consistent with decreased hydrophobic interactions resulting from an increased proportion of organic solvent in the test solution.

These results also further indicate the importance of the hydrophobic interactions through the central phenyl ring in the receptor (**1a**).

Though all the binding studies lend support to a three-point binding model, as proposed in Figure 2, we were interested in probing structural evidence for such interactions. Therefore, we conducted ^{1}H NMR spectroscopy experiments with sensor **1a** as a model. The ^{1}H NMR spectra of **1a** were collected in the presence of 0, 1, and 10 equiv dopamine in 20% D_2O in H_2O phosphate buffer at neutral pH (Figure 6A–C). Peak assignments were made on the basis of chemical shifts and COSY studies. Upon addition of dopamine, the proton NMR peaks of **1a** showed concentration-dependent shifts; this indicates binding. In order to facilitate peak assignment of the dopamine–sensor complex, we also conducted the NMR experiments at pH 9 (Figure 6D), which is known to increase boronic acid–diol interactions.[36] Under such conditions, complete complex formation was observed when 1 equivalent of dopamine was added. It should be noted that dopamine has a pK_a of 10.6.[49] Therefore, at pH 9, dopamine should still exist in the protonated form. The NMR studies showed very prominent shifts of peaks for the protons on the central phenyl ring of **1a** upon binding to dopamine. For example, protons "a" were shifted upfield by 0.17 ppm upon binding. The peak corresponding to protons "d" on the central phenyl ring of **1a** were also shifted upfield, but only by about 0.05 ppm. Such results are consistent with the involvement of the central phenyl ring in binding, presumably through stacking/hydrophobic interactions, and further support our proposed three-point binding model (Figure 2). Some of the most prominent shifts occurred on the phenyl ring that bears the boronic acid moiety (Figure 6). For example, the peaks corresponding to protons "f_1", "f_2", and "g" were shifted upfield by 0.5–0.8 ppm. Such results are to be expected, since the boronic acid moiety should change from the neutral trigonal form before binding to an anionic tetrahedral form after binding.[36] This change in charge states by the boron atom should increase the electron density on the phenyl ring and thus significantly influence the chemical shifts of protons on the same ring. The changes in chemical shifts for the protons on the phenyl ring bearing the carboxylate group are relatively small. For example, no major changes were observed with protons "c", "d2", and "e". The only noticeable change was observed with proton "b", which shifted by about 0.02 ppm. Such results are also understandable, since there is no change in charge states for the carboxylate before and after binding. The only issue is what the counterion is. Before binding, the counterion is probably sodium, and after binding it is a protonated amine. Such a minor alteration would not be expected to cause as much change in the electronic environment of the phenyl ring as stacking with another aromatic ring by the center phenyl ring of **1a**, or a change in the ionization state of the boron atom for the ring that bears the boronic acid moiety. Overall, the NMR results are consistent with the proposed three-point binding model (Figure 2).

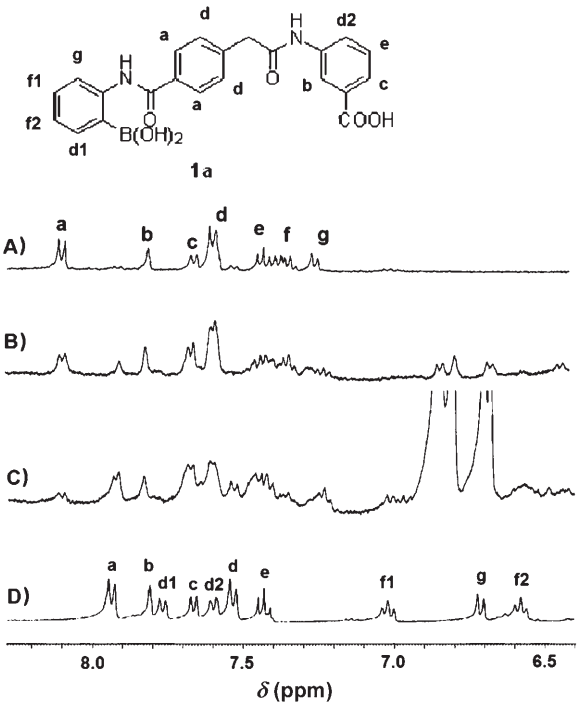


Figure 6. ^{1}H NMR spectra of **1a** (2 mM) in the presence of A) 0, B) 1, and C) 10 equiv dopamine in D_2O (20%) in phosphate H_2O buffer (0.1 M) at near neutral pH (pH 7.4–7.8), and D) at high pH (pH 9).

Several chemosensors[8,10–12] for catecholamines, with binding constants that range from 180 to 10780 M^{-1}, have been reported. However, none of these sensors displays significant selectivity for dopamine over epinephrine (adrenaline) under near physiological conditions. Glass and co-workers, for instance, have reported a chemosensor that had an apparent association constant of 3400 M^{-1} with dopamine, but its association constant with epinephrine is even greater at 5000 M^{-1}.[11] In work by Thomas and co-workers, the apparent binding constant of the best compound with dopamine was 630 M^{-1}, which was similar to that with epinephrine (550 M^{-1}).[10] Yoon and co-workers reported a boronic acid sensor with an affinity for dopamine (K_a = 10780 M^{-1}) two times higher than that for epinephrine (5050 M^{-1}).[8] However, such results were obtained in 50% MeOH/0.05 M HEPES buffer at pH 7.4 because of solubility problems in aqueous solution, which makes it hard to make a meaningful comparison. It is known that the binding constants of various sensors, including boronic acid-based sensors, are much greater in organic solvents than in aqueous environments. The lack of selectivity between catecholamines for most sensors (dopamine and epinephrine in this case) is very understandable, since the differences are very subtle and are mostly only in the side chain. The major difference between our design and those described in the literature is in the introduction of a rigid and hydrophobic phenyl linker rather than flexible linear linkers; this has allowed for improved selectivity for dopamine. Such results suggest that future design should further exploit the hydrophilic and hydrogen-bond-forming hydroxy group of the epinephrine side chain as a way to differentiate dopamine and epinephrine. The dopamine receptors described in this paper represent the first dopamine-selective receptors functional in an aqueous solution.

Conclusions

A series of boronic acid-based receptors for dopamine have been designed and synthesized. The final products have good water solubility because of the boronic acid and carboxylic acid functional groups. Fluorescent binding studies have shown that all sensors display preferential recognition of the target, dopamine. In addition, the attachment of electron-withdrawing groups to the phenyl ring that bears the boronic acid group increased the binding affinity. These chemosensors are the first that show significant selectivity for dopamine over its catecholamine analogue epinephrine under near physiological conditions. Our studies also point to exploitation of the linker moiety as a way to improve selectivity among catecholamines further. A three-point binding model has been proposed. NMR spectroscopy studies indeed support this proposed model.

Experimental Section

General methods and materials: ^{1}H and ^{13}C NMR spectra were recorded by using a Bruker 400 MHz NMR spectrometer in deuterated chloroform ($CDCl_3$) or [D_6]DMSO ($(CD_3)_2SO$) with either tetramethylsilane (TMS; 0.00 ppm) or the NMR solvent as internal reference, unless otherwise specified. HPLC purification was carried out with a Shimadzu LC-10AT VP system and a Zobax C18 reversed-phase column (4.6 mm×25 cm). Fluorescence spectra were recorded by using a Shimadzu RF-5301 PC spectrofluorimeter. Absorption spectra were recorded on a Shimadzu UV-1700 UV/Vis spectrophotometer. Quartz cuvettes were used in all fluorescence and UV studies. All pH values were determined with a UB-10 Ultra Basic Benchtop pH meter (Denver Instrument). Analytical thin-layer chromatography (TLC) was performed by using a Merck silica gel 60 plates (0.25 mm thickness with F-254 indicator). Alizarin Red S (ARS) was purchased from Acros and used as received. Boronic acids (**7a**–**c**) were obtained from Frontier Scientific and Combi-Blocks, Inc. Sugars, buffer ingredients, and diols were bought from Aldrich or Acros and were used as received. Water used for the binding studies was doubly distilled and further purified with a Milli-Q filtration system. Solvents for extraction and chromatography were used as received. Dry solvents (DMF, DMSO) were purchased from Acros.

Syntheses

*(4-Formylphenyl)acetic acid (**3**)*: Dry THF (60 mL) was added to a mixture of compound **2** (2.5 g, 15 mmol, 1.0 equiv) and PCC (4.0 g, 18.6 mmol, 1.2 equiv). The mixture was stirred at room temperature for 2 h. Celite (5 g) was added as filtering agent, and then the mixture was filtered. The solvent was then evaporated under vacuum. The solid product was dissolved in HCl solution (10%, 20 mL) and extracted with dichloromethane (3×20 mL) and ethyl acetate (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and then evaporated under vacuum. Column chromatography (silica gel, hexanes/ethyl acetate, 3:2) gave **3** (1.43 g, 57%) as white crystals. TLC (hexanes/ethyl acetate, 3:1) R_f=0.50; ^{1}H NMR ($CDCl_3$, 400 MHz): δ=10.03 (s, 1H), 7.89 (d, J=8.0 Hz, 2H), 7.49 (d, J=8.0 Hz, 2H), 3.77 ppm (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz): δ=191.9, 176.5, 140.0, 135.5, 130.2, 41.3 ppm; MS (ESI−): *m/e* (relative intensity): 162.9 $[M-1]^-$.

*Methyl 3-[2-(4-formylphenyl)acetylamino]benzoate (**5**)*: Compound **3** (1.18 g, 7.2 mmol, 1.0 equiv) was dissolved in dry THF (10 mL), and a dry THF solution (5 mL) of $SOCl_2$ (1.03 mL, 14.3 mmol, 2.0 equiv) was added dropwise under nitrogen. The mixture was stirred at room temperature under nitrogen for 3 h, and the solvent was then evaporated under vacuum. The solid product was dissolved in dry dichloromethane (10 mL), and a dichloromethane solution (10 mL) of methyl 3-aminobenzoate (**4**; 1.08 g, 7.2 mmol, 1.0 equiv) and triethylamine (2.0 mL, 14.3 mmol, 2.2 equiv) was added dropwise under nitrogen. The mixture was stirred under nitrogen at room temperature for 2 h. The solution was diluted with dichloromethane (50 mL) and washed with water (2×5 mL). The organic phase was dried over anhydrous Na_2SO_4, and the solvent was evaporated under vacuum. Column chromatography (silica gel, dichloromethane/methanol, 100:1) and recrystallization by dichloromethane gave **5** (1.55 g, 73%) as a white solid. TLC (dichloromethane/methanol, 50:1) R_f=0.40; ^{1}H NMR (acetone, 400 MHz): δ=10.05 (s, 1H), 9.63 (s, 1H), 8.33 (d, J=7.6 Hz, 1H), 7.94 (q, J=3.2 Hz, 1H), 7.91 (d, J=8.4 Hz, 2H), 7.72 (d, J=7.6 Hz, 1H), 7.64 (d, J=8.0 Hz, 2H), 7.45 (t, J=8.0 Hz, 1H), 3.89 (s, 2H), 3.88 ppm (s, 3H); ^{13}C NMR (acetone, 400 MHz): δ=191.7, 168.4, 166.1, 142.6, 139.6, 135.5, 130.8, 130.1, 129.5, 129.0, 124.3, 123.5, 120.0, 51.5, 43.7 ppm; MS (ESI+): *m/e* (relative intensity): 298 $[M-1]^+$.

*Methyl 3-[2-(4-carboxyphenyl)acetylamino]benzoate (**6**)*: Acetic acid (60 mL) was added to a mixture of **5** (1.5 g, 5 mmol, 1.0 equiv) and sodium perborate tetrahydrate (7.7 g, 50 mmol, 10 equiv). The mixture was heated at 85 °C and stirred for 24 h; the solvent was then evaporated under vacuum. The solid residue was washed with water (2×5 mL) and dichloromethane (3×20 mL). Another part of the solid was obtained from recrystallization of the organic phase (dichloromethane). Combination of the solid products gave compound **6** (1.24 g, 79%). TLC (hexanes/ethyl acetate, 1:1) R_f=0.30; ^{1}H NMR (acetone, 400 MHz): δ=9.59 (s, 1H), 8.33 (s, 1H), 8.02 (d, J=8.0 Hz, 2H), 7.94 (d, J=9.2 Hz, 1H), 7.71 (d, J=8.8 Hz, 1H), 7.55 (d, J=8.4 Hz, 2H), 7.45 (t, J=8.0 Hz, 1H), 3.88 (s, 3H), 3.86 ppm (s, 2H); ^{13}C NMR (acetone, 400 MHz): δ=210.2, 166.1, 141.0, 130.8, 129.7, 129.4, 129.1, 129.0, 124.2, 123.5, 120.0, 51.5, 43.6 ppm; MS (ESI−): *m/e* (relative intensity): 312.4 $[M-1]^-$.

*2-Amino-4-carbamoylphenylboronic acid (**7d**)*: Trifluoroacetic acid (4.0 mL) and concentrated sulfuric acid (1.0 mL) were added to compound **7b** (198 mg, 1.0 mmol, 1.0 equiv) in a 20 mL vial. The mixture was heated at 38 °C and stirred for 40 h, and ice-cold DI water (4 mL) was then added. The vial was incubated in an ice bath for 30 min. White solid precipitated and was filtered off and washed with ice-cold DI water (2 mL) and then dried under vacuum. Another part of the solid was obtained from precipitation of the filtrate by adjusting the pH to 7. Recombination of the solid product gave compound **7d** (167 mg, 93%). TLC (ethyl acetate) R_f=0.27; ^{1}H NMR (CD_3OD): δ=7.963 (s, 2H), 7.906 ppm (s, 1H); ^{13}C NMR (CD_3OD): δ=168.9, 136.9, 135.8, 135.6, 126.5, 122.4 ppm; MS (ESI+): *m/e* (relative intensity): 181.1 $[M+1]^+$, 195.1 $[M+CH_3OH-H_2O]^+$

*General procedure for compounds **8***: Compound **5** (1.0 mmol, 1.0 equiv) was dissolved in dry THF (20 mL), and a dry THF (20 mL) solution of $SOCl_2$ (20 mmol, 20 equiv) was added dropwise under nitrogen. The mixture was stirred at room temperature under nitrogen for 48 h to give compound **6**, and the solvent and excess $SOCl_2$ were then evaporated under vacuum. Compound **7** (0.9 mmol, 0.9 equiv) and DMAP (0.08 mmol, 0.08 equiv) were dissolved in anhydrous pyridine (20 mL), and a dichloromethane solution (20 mL) of compound **6** was added dropwise under nitrogen. The mixture was stirred at room temperature under nitrogen for 48 h, and the solvent was then evaporated under vacuum. The crude product was purified by column chromatography (hexanes/ethyl acetate 1:1 to ethyl acetate/methanol 10:1) and further puri-

fied by HPLC (C18 RP column, 290 nm). Elution conditions: CH_3CN/MeOH (0.1% TFA in both elutions, flow rate = 1.5 mL min^{-1}), 0–10 min (CH_3CN 0–100%), 10–25 min (CH_3CN 100%), 25–30 min (CH_3CN 100%-50%), 30–200 min (CH_3CN 50%).

*2-(4-{[3-(Methoxycarbonyl)phenylcarbamoyl]methyl}benzoylamino)-phenylboronic acid (**8a**)*: HPLC t_R = 40 min; compound **8a** (26%) was obtained as a yellow powder. ^{1}H NMR (CD_3OD): δ = 8.29 (s, 1H), 8.18 (d, J = 8.4 Hz, 2H), 7.85 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 6.8 Hz, 1H), 7.45 (t, J = 8.0 Hz, 1H), 7.35 (m, 3H), 3.92 (s, 3H), 3.89 ppm (s, 2H); ^{13}C NMR (CD_3OD): δ = 169.8, 166.8, 166.2, 142.2, 138.8, 137.8, 131.6, 130.7, 129.8, 128.7, 128.2, 127.9, 127.8, 126.7, 124.8, 124.2, 120.6, 116.3, 106.8, 51.3, 43.0 ppm; MS (ESI−): *m/e* (relative intensity): 431.3 [$M-1$]$^-$, 432.4 [M]$^-$.

*4-Cyano-2-(4-{[3-(methoxycarbonyl)phenylcarbamoyl]methyl}benzoylamino)phenylboronic acid (**8b**)*: HPLC t_R = 39 min; compound **8b** (29%) was obtained as a yellow powder. ^{1}H NMR (CD_3OD): δ = 8.28 (s, 1H), 8.20 (d, J = 8.4 Hz, 2H), 8.11 (d, J = 7.6 Hz, 2H), 7.83 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.69 (m, J = 5.2 Hz, 2H), 7.63 (d, J = 7.6 Hz, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H), 3.91 (s, 3H), 3.90 ppm (s, 2H); ^{13}C NMR (CD_3OD): δ = 169.7, 167.3, 166.8, 142.9, 138.8, 138.6, 132.9, 130.6, 130.0, 129.4, 128.7, 128.5, 127.4, 127.3, 124.8, 124.2, 120.6, 119.7, 118.0, 111.4, 106.8, 51.3, 43.0 ppm; MS (ESI−): *m/e* (relative intensity): 456.2 [$M-1$]$^-$, 457.2 [M]$^-$.

*5-Fluoro-2-(4-{[3-(methoxycarbonyl)phenylcarbamoyl]methyl}benzoylamino)phenylboronic acid (**8c**)*: HPLC t_R = 39 min; compound **8c** (33%) was obtained as a yellow powder. ^{1}H NMR (CD_3OD): δ = 8.28 (s, 1H), 8.18 (d, J = 8.4 Hz, 2H), 7.84 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.67 (m, J = 8.4 Hz, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.39 (m, 1H), 7.28 (t, J = 8.6 Hz, 1H), 7.20 (q, J = 8.4 Hz, 1H), 7.10 (t, J = 8.6 Hz, 1H), 3.92 (s, 3H), 3.89 ppm (s, 2H); ^{13}C NMR (CD_3OD): δ = 169.9, 166.8, 165.9, 160.7, 142.3, 138.8, 130.6, 129.8, 128.7, 128.3, 127.6, 124.8, 124.2, 121.8, 121.6, 120.7, 117.1, 116.9, 114.6, 106.8, 51.3, 43.0 ppm; MS (ESI−): *m/e* (relative intensity): 449.4 [$M-1$]$^-$, 450.4 [M]$^-$.

*4-Carbamoyl-2-(4-{[3-(methoxycarbonyl)phenylcarbamoyl]methyl}benzoylamino)phenylboronic acid (**8d**)*: HPLC t_R = 39 min; compound **8d** (29%) was obtained as a yellow powder. ^{1}H NMR (CD_3OD): δ = 8.29 (s, 1H), 8.20 (d, J = 8.4 Hz, 2H), 7.96 (s, 1H), 7.93 (s, 1H), 7.87 (s, 1H), 7.78 (t, J = 8.0 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 7.6 Hz, 1H), 7.45 (t, J = 8.0 Hz, 1H), 3.91 (s, 3H), 3.90 ppm (s, 2H); ^{13}C NMR (CD_3OD): δ = 170.4, 169.8, 166.8, 142.5, 138.8, 135.9, 131.9, 130.6, 129.9, 128.7, 128.4, 125.5, 125.0, 124.8, 124.2, 121.6, 120.6, 116.1, 113.2, 51.3, 43.0 ppm; MS (ESI−): *m/e* (relative intensity): 474.1 [$M-1$]$^-$, 488.1 [$M+CH_2$]$^-$, 502.1 [$M+2CH_2$]$^-$, 516.1 [$M+3CH_2$]$^-$.

*General procedure for compounds **1***: A H_2O/THF/MeOH (1:1:5) solution (7 mL) of NaOH (2.5 mmol, 10.0 equiv) was added to a compound **8** (0.25 mmol, 1.0 equiv) in a 20 mL vial. The mixture was stirred under nitrogen at 55 °C for 12 h. The solution was diluted with methanol (50 mL) and the pH was adjusted to 4 by addition of HCl (1 M in methanol). The solvent was then evaporated under vacuum. The crude product was further purified by HPLC (C18 RP column, 290 nm). Elution conditions: CH_3CN/MeOH (0.1% TFA in both elutions, flow rate = 1.5 mL min^{-1}), 0–10 min (CH_3CN 0–100%), 10–25 min (CH_3CN 100%), 25–30 min (CH_3CN 100–50%), 30–200 min (CH_3CN 50%).

*2-{4-[(3-Carboxyphenylcarbamoyl)methyl]benzoylamino}phenylboronic acid (**1a**)*: HPLC t_R = 38 min; compound **1a** (53%) was obtained as a yellow powder. ^{1}H NMR (CD_3OD): δ = 8.26 (s, 1H), 8.18 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 7.2 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 8.0 Hz, 2H), 7.54 (d, J = 6.4 Hz, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.34 (m, 3H), 3.89 ppm (s, 2H); ^{13}C NMR (CD_3OD): δ = 169.8, 168.0, 166.2, 142.2, 138.7, 137.8, 131.6, 131.3, 129.8, 128.6, 128.2, 127.8, 126.7, 125.1, 124.1, 120.9, 116.4, 43.0 ppm; MS (ESI−): *m/e* (relative intensity): 399.1 [$M-H_2O-H$]$^-$, 389.1 [$M-B=O$]$^-$; HRMS: calcd for $C_{22}H_{16}{}^{11}BN_2O_5$: 399.1152; found: 399.1223.

*2-{4-[(3-Carboxyphenylcarbamoyl)methyl]benzoylamino}-4-cyanophenylboronic acid (**1b**)*: HPLC t_R = 38 min; compound **1b** (41%) was obtained as a yellow powder. ^{1}H NMR (CD_3OD): δ = 8.26 (s, 1H), 8.21 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 7.2 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.67 (m, 5H), 7.45 (t, J = 8.0 Hz, 1H), 7.34 (m, 3H), 3.91 ppm (s, 2H); ^{13}C NMR (CD_3OD): δ = 169.7, 168.0, 167.4, 142.9, 138.7, 138.5, 132.9, 131.3, 129.9, 128.6, 128.5, 128.4, 127.3, 126.7, 125.1, 124.0, 120.9, 119.7, 118.0, 111.4, 43.0 ppm; MS (ESI−): *m/e* (relative intensity): 424.1 [$M-H_2O-H$]$^-$, 425.1 [$M-H_2O$]$^-$. HRMS: calcd for $C_{23}H_{15}{}^{11}BN_3O_5$: 424.1105; found: 424.1103.

*2-{4-[(3-Carboxyphenylcarbamoyl)methyl]benzoylamino}-5-fluorophenylboronic acid (**1c**)*: HPLC t_R = 38 min; compound **1c** (54%) was obtained as a yellow powder. ^{1}H NMR (CD_3OD): δ = 8.27 (s, 1H), 8.18 (d, J = 8.0 Hz, 2H), 7.86 (d, J = 7.2 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.67 (d, J = 7.6 Hz, 2H), 7.41 (m, 2H), 7.19 (d, J = 6.8 Hz, 1H), 7.10 (t, J = 8.0 Hz, 1H), 3.89 ppm (s, 2H); ^{13}C NMR (CD_3OD): δ = 169.8, 168.0, 165.9, 142.3, 138.7, 134.0, 131.3, 129.8, 128.6, 128.2, 127.6, 125.1, 124.1, 120.9, 118.7, 118.6, 117.1, 116.9, 114.8, 114.6, 43.0 ppm; MS (ESI−): *m/e* (relative intensity): 417.1 [$M-H_2O-H$]$^-$, 418.1 [$M-H_2O$]$^-$. HRMS: calcd for $C_{22}H_{15}BN_2O_5F$: 417.1058; found: 417.1039.

*4-Carbamoyl-2-{4-[(3-carboxyphenylcarbamoyl)methyl]benzoylamino}-phenylboronic acid (**1d**)*: HPLC t_R = 38 min; compound **1d** (51%) was obtained as a yellow powder. ^{1}H NMR (CD_3OD): δ = 8.26 (s, 1H), 8.20 (d, J = 8.4 Hz, 2H), 7.87 (s, 1H), 7.79 (d, J = 7.6 Hz, 2H), 7.68 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 7.6 Hz, 2H), 7.44 (t, J = 8.0 Hz, 1H), 3.90 ppm (s, 2H); ^{13}C NMR (CD_3OD): δ = 170.4, 169.8, 168.0, 166.8, 142.5, 138.7, 138.2, 135.9, 133.8, 131.9, 131.3, 129.9, 128.6, 128.4, 128.1, 127.6, 125.8, 125.1, 124.1, 121.9, 120.9, 116.1, 43.0 ppm; MS (ESI−): *m/e* (relative intensity): 442.1 [$M-H_2O-H$]$^-$, 443.1 [$M-H_2O$]$^-$, 456.1 [$M-2H_2O+CH_3OH$]$^-$. HRMS: calcd for $C_{23}H_{17}{}^{11}BN_3O_6$: 442.1210; found: 442.1231.

Procedures for the binding studies (1a binding with dopamine as an example): Solutions of ARS (1×10^{-4} M) and of ARS (1×10^{-4} M) with **1a** (1×10^{-3} M) were prepared in phosphate buffer (0.1 M) at pH 7.40. These two solutions were mixed in a 1 cm cuvette. In the solution, the ratio of boronic acid + ARS was increased gradually. After being shaken, the solution was used to test the fluorescence intensity. Six to eight points were collected for the calculation of the apparent binding constant of the boronic acid (**1a**)–ARS complex with the assumption of a 1:1 complex formation mechanism.

In a similar method, two solutions of boronic acid **1a** (1×10^{-3} M)–ARS (1×10^{-4} M) complex and boronic acid (**1a**)–ARS complex with dopamine (50 mM) were prepared in phosphate buffer (0.1 M) at pH 7.40. The binding constant (K_a) of the boronic acid–dopamine complex was obtained by addition of different diols (0–50 mM) in phosphate buffer (0.1 M) to the boronic acid–ARS mixtures; the ratio of boronic acid (**1a**)–ARS + dopamine was increased gradually.

Acknowledgements

Financial support from the Georgia Research Alliance and Georgia Cancer Coalition is gratefully acknowledged. The authors would also like to thank Inte:Ligand Software-Entwicklungs und Consulting GmbH for the evaluation copy of the ilib Diverse program, and Frontier Scientific, Inc. for free boronic acids.

Keywords: boronic acids · catecholamines · chemosensors · dopamines · receptors

[1] C. K. Erickson, *Alcohol Alcohol. Suppl. 1* **1996**, *31*, 5.
[2] E. D. Huey, K. T. Putnam, J. Grafman, *Neurology* **2006**, *66*, 17.
[3] G. Ch Beck, P. Brinkkoetter, C. Hanusch, J. Schulte, K. van Ackern, F. J. van der Woude, B. A. Yard, *Crit. Care* **2004**, *8*, 485.
[4] D. B. Rye, *Neurology* **2004**, *63*, S2.
[5] S. R. Ali, Y. Ma, R. R. Parajuli, Y. Balogun, W. Y. C. Lai, H. He, *Anal. Chem.* **2007**, *79*, 2583.
[6] K. Hayashi, Y. Iwasaki, R. Kurita, K. Sunagawa, O. Niwa, *Electrochem. Commun.* **2003**, *5*, 1037.
[7] C. Hu, X. Chen, S. Hu, *J. Electroanal. Chem.* **2006**, *586*, 77.
[8] Y. J. Jang, J. H. Jun, K. M. K. Swamy, K. Nakamura, H. S. Koh, Y. J. Yoon, J. Yoon, *Bull. Korean Chem. Soc.* **2005**, *26*, 2041.
[9] X. Lin, Y. Zhang, W. Chen, P. Wu, *Sens. Actuators B Chem.* **2007**, *122*, 309.
[10] M. Maue, T. Schrader, *Angew. Chem.* **2005**, *117*, 2305; *Angew. Chem. Int. Ed.* **2005**, *44*, 2265.
[11] K. E. Secor, T. E. Glass, *Org. Lett.* **2004**, *6*, 3727.
[12] J. Y. Yoon, A. W. Czamik, *Bioorg. Med. Chem.* **1993**, *1*, 267.
[13] V. Zucolotto, M. Ferreira, M. R. Cordeiro, C. J. L. Constantino, W. C. Moreira, O. N. Oliveira, *Sens. Actuators B Chem.* **2006**, *113*, 809.
[14] A. Coskun, E. U. Akkaya, *Org. Lett.* **2004**, *6*, 3107.
[15] G. Cohen, *J. Neural Transm. Suppl.* **1983**, *19*, 89.
[16] S. E. Frayne, I. J. Mitchell, P. T. Sharpe, M. A. Sambrook, A. R. Crossman, *Mol. Neuropharmacol.* **1991**, *1*, 53.
[17] J. A. Gingrich, M. G. Caron, *Annu. Rev. Neurosci.* **1993**, *16*, 299.
[18] U. K. Rinne, V. Sonninen, *Annales Medicinae Internae Fenniae* **1968**, *57*, 105.
[19] M. Bourin, D. J. David, P. Jolliet, A. Gardier, *Therapie* **2002**, *57*, 385.
[20] F. P. Bymaster, C. C. Felder, *Mol. Psychiatry* **2002**, *7*, S57.
[21] A. Erfurth, N. Michael, C. Stadtland, V. Arolt, *Neuropsychobiology* **2002**, *45 Suppl 1*, 33.
[22] N. E. Goeders, *J. Pharmacol. Exp. Ther.* **2002**, *301*, 785.
[23] N. E. Goeders, *Psychoneuroendocrinology* **2002**, *27*, 13.
[24] M. Hummel, E. M. Unterwald, *J. Cell. Physiol.* **2002**, *191*, 17.
[25] T. Maurice, R. Martin-Fardon, P. Romieu, R. R. Matsumoto, *Neurosci. Biobehav. Rev.* **2002**, *26*, 499.
[26] S. A. Asher, V. L. Alexeev, A. V. Goponenko, A. C. Sharma, I. K. Lednev, C. S. Wilcox, D. N. Finegold, *J. Am. Chem. Soc.* **2003**, *125*, 3322.
[27] R. Badugu, J. R. Lakowicz, C. D. Geddes, *Dyes Pigm.* **2004**, *61*, 227.
[28] H. S. Cao, D. l. Diaz, D. DiCesare, J. R. Lakowicz, M. D. Heagy, *Org. Lett.* **2002**, *4*, 1503.
[29] S. Gamsey, A. Miller, M. M. Olmstead, C. M. Beavers, L. C. Hirayama, S. Pradhan, R. A. Wessling, B. Singaram, *J. Am. Chem. Soc.* **2007**, *129*, 1278.
[30] J. P. Lorand, J. O. Edwards, *J. Org. Chem.* **1959**, *24*, 769.
[31] G. Springsteen, B. Wang, *Chem. Commun.* **2001**, 1608.
[32] G. Springsteen, B. Wang, *Tetrahedron* **2002**, *58*, 5291.
[33] W. Wang, X. Gao, B. Wang, *Curr. Org. Chem.* **2002**, *6*, 1285.
[34] C. J. Ward, P. Patel, P. R. Ashton, T. D. James, *Chem. Commun.* **2000**, 229.
[35] J. Yan, H. Fang, B. Wang, *Med. Res. Rev.* **2005**, *25*, 490.
[36] J. Yan, G. Springsteen, S. Deeter, B. Wang, *Tetrahedron* **2004**, *60*, 11 205.
[37] S. L. Wiskur, J. L. Lavigne, H. Ait-Haddou, V. Lynch, Y. H. Chiu, J. W. Canary, E. V. Anslyn, *Org. Lett.* **2001**, *3*, 1311.
[38] P. T. Lewis, C. J. Davis, F. R. Fronczek, R. M. Strongin, *Org. Lett.* **2001**, *3*, 2443.
[39] T. D. James, K. R. A. S. Sandanayake, R. Iguchi, S. Shinkai, *J. Am. Chem. Soc.* **1995**, *117*, 8982.
[40] M. Dowlut, D. G. Hall, *J. Am. Chem. Soc.* **2006**, *128*, 4226.
[41] W. Yang, H. He, D. G. Drueckhammer, *Angew. Chem.* **2001**, *113*, 1764; *Angew. Chem. Int. Ed.* **2001**, *40*, 1714.
[42] Gaussian 03, Revision C.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian, Inc., Wallingford CT, **2004**.
[43] A. Dornhofer, M. Biely, G. Wolber, T. Langer, *QSAR Mol. Modell. Ration. Des. Bioact. Mol. Proc. Eur. Symp. Struct.-Act. Relat. (QSAR) Mol. Modell. 15th* **2006**, 421.
[44] T. Langer, G. Wolber, *Pure Appl. Chem.* **2004**, *76*, 991.
[45] J. Gasteiger, C. Rudolph, J. Sadowski, *Tetrahedron Comput. Methodol.* **1990**, *3*, 537.
[46] A. McKillop, D. Kemp, *Tetrahedron* **1989**, *45*, 3299.
[47] K. A. Connors, *Binding Constants*, John Wiley and Sons, Toronto, **1987**.
[48] J. Wang, S. Jin, S. Akay, B. Wang, *Eur. J. Org. Chem.* **2007**, 2091.
[49] E. J. Land, C. A. Ramsden, P. A. Riley, *ARKIVOC* **2007**, *23*.

Received: November 5, 2007
Published online on May 21, 2008

