Supramolecular Inhibition of Neurodegeneration by a Synthetic Receptor

Shengke Li,^{†,||} Huanxian Chen,^{†,||} Xue Yang,[†] David Bardelang,^{*,‡} Ian W. Wyman,[§] Jianbo Wan,[†] Simon M. Y. Lee,[†] and Ruibing Wang^{*,†}[†]State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau, China[‡]Aix-Marseille Université, CNRS, Institut de Chimie Radicale, UMR 7273, 13013 Marseille, France[§]Department of Chemistry, Queen's University, Kingston, ON K7L 3N6, Canada

S Supporting Information

ABSTRACT: Cucurbit[7]uril (CB[7]) was found *in vitro* to sequester the neurotoxins MPTP (*N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and MPP⁺ (*N*-methyl-4-phenylpyridine). The CB[7]/neurotoxin host–guest complexes were studied in detail with ¹H NMR, electrospray ionization mass spectrometry, UV–visible spectroscopic titration, and molecular modeling by density functional theory. The results supported the macrocyclic encapsulation of MPTP and MPP⁺, respectively, by CB[7] in aqueous solutions with relatively strong affinities and 1:1 host–guest binding stoichiometries in both cases. More importantly, the progression of MPTP/MPP⁺ induced neurodegeneration (often referred to as a Parkinson's disease model) was observed to be strongly inhibited *in vivo* by the synthetic CB[7] receptor, as shown in zebrafish models. These results show that a supramolecular approach could lead to a new preventive and/or therapeutic strategy for counteracting the deleterious effects of some neurotoxins leading to neurodegeneration.

KEYWORDS: Neurodegeneration, neurotoxin, supramolecular complexation, cucurbituril, inhibition

Parkinson's disease (PD) is one of the most common neurodegenerative diseases, with more than four million people suffering from it globally.¹ Although the etiology of PD remains largely unknown, accumulated evidence suggests that environmental factors, such as exposure to neurotoxins, play an important role in inducing PD via the generation of ROS (reactive oxygen species) and RNS (reactive nitrogen species).² For instance, it has been demonstrated that MPTP (*N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Scheme 1), a well-known neurotoxin, can lead to selective degeneration of the dopaminergic neurons in the substantia nigra and cause a PD

syndrome in humans.¹ Therefore, MPTP has been frequently used to induce PD in various vertebrates ranging from zebrafish, to mice and to primates.³ During the neurodegeneration process, MPTP is converted into its active metabolite MPP⁺ (*N*-methyl-4-phenylpyridine, Scheme 1) by monoamine oxidase B (MAO-B) in the inner mitochondrial membrane. The toxin species MPP⁺ is then taken up via the dopamine transporter (DAT) into the dopamine neurons where it leads to the increased generation of reactive oxygen species.⁴ Current therapies for PD mainly provide symptomatic improvement by replacing neurotransmitters or controlling their metabolism to maintain their activities.³ As there is no cure of PD, restraint of neurodegeneration from exposure to external neurotoxins becomes one of the strategies for PD prevention.⁵ However, there are no neuroprotective agents available to effectively impede the activities of neurotoxins.⁵ As a result, there is a real need to develop a neuroprotective agent that may effectively counteract the actions of clinically relevant neurotoxins.

Cucurbit[*n*]uril (CB[*n*], *n* = 5–8, 10, 14) are pumpkin-shaped macrocycles that consist of *n* glycoluril units and 2*n* methylene groups, forming a hydrophobic cavity with two

Scheme 1. Molecular Structures of CB[7], MPTP-H⁺, and MPP⁺

Received: September 23, 2015

Accepted: October 16, 2015

Published: October 16, 2015

hydrophilic carbonyl-laced portals.^{6,7} Within the CB[n] family, CB[7] (Scheme 1) has stimulated the greatest attention in the fields of biomedical sciences and drug delivery due to its superior water-solubility and size that can accommodate many biologically important guest molecules.^{8–10} For over a decade, we have been actively investigating the host–guest chemistry^{11–17} and biocompatibility¹⁸ of CB[7]. Recently, CB[n] (especially CB[7]) and derivatives have been demonstrated to reverse or inhibit the biological activities of a group of guest molecules due to strong host–guest complexations. For instance, acyclic CB[n] derivatives have demonstrated significant reversal affects to NMBA (neuromuscular blocking agents) *in vivo*.¹⁹ Very recently, our group reported the ability of CB[7] to reverse the effects of general anesthesia induced by tricaine *in vivo*.¹¹ It has also been shown that the myotoxicity and cardiotoxicity of cisplatin was significantly reduced after this drug had been encapsulated by CB[7].²⁰ CB[7] was also found to inhibit amyloid fibrillation, thus potentially finding therapeutic applications to prevent or treat amyloidosis.²¹ Additionally, the olfactory responses of tilapia fish to odorants was suppressed by CB[7] encapsulation.²² Similarly, another synthetic host was demonstrated to be able to sequester spermine and reverse its biological effects *in vitro*.²³ Based on these studies, we report herein the supramolecular host–guest complexations of CB[7] toward MPTP and MPP⁺, and an unprecedented discovery of CB[7]’s ability to inhibit MPTP/MPP⁺-induced neurodegeneration using *in vivo* zebrafish models.

¹H NMR spectra of MPTP in the presence of CB[7] showed significant upfield shift resonances (up to ~1.0 ppm, Figure 1a)

Figure 1. ¹H NMR (400 MHz) spectra of MPTP in the presence of 1.2 equiv of CB[7] (a), with 0.5 equiv of CB[7] (b), and without CB[7] (c) in D₂O. The CB[7] and HOD protons are labeled as (●) and (○), respectively.

with respect to the spectrum of free MPTP (Figure 1c). Upon the addition of 0.5 equiv of CB[7], the peaks became very broad (with some resonances disappearing), which indicates that the exchange rate between the free and CB[7]-bound MPTP was intermediate on the ¹H NMR time scale.

After the addition of 1.2 equiv of CB[7], the resonances corresponding to the aromatic protons (H₆, H₇, H₈), the ethylene proton (H₅) in the tetrahydropyridine ring, and the methylene protons (H₃) showed significant upfield shifts,

indicating that they were encapsulated in the CB[7] cavity. Meanwhile, the methylene protons adjacent to the nitrogen atom (H₂ and one of the H₄ protons) exhibited slight upfield shifts, suggesting that they were situated within the cavity but near the carbonyl gates. The downfield shift of the methyl protons (H₁) suggests that the methyl group was positioned outside of the cavity but close to the electron-rich carbonyl-lined portal. Notably, one of the methylene protons (H₄) did not shift in the presence of CB[7], which may indicate that this proton was situated between the shielding and deshielding zones where the shielding and deshielding effects cancel each other. Concerning MPTP’s neurotoxin metabolite MPP⁺ (SI Figure S1), all of the guest protons except those of the methyl showed an upfield shift upon the addition of 1.2 equiv of CB[7] suggesting that they were encapsulated within the CB[7] cavity. As for MPTP, the downfield shift of the methyl protons is consistent with their positioning near the portal, outside of the CB[7] cavity. No splitting of proton resonances was observed in line with a fast exchange rate between the free and included MPP⁺ on the NMR time scale. The Job’s plots agreed with the formation of 1:1 complexes (UV–visible continuous variation method, Figures S2–S7), which was further confirmed by electrospray ionization mass spectrometry (ESI-MS). Binding constants were determined by UV–vis titrations in a phosphate buffered saline (PBS) solution (0.01 M) at pH = 7.4 to mimic a physiological environment. When increasing amounts of CB[7] were added into an MPTP solution, the absorbance peak of the guest at 242 nm decreased, along with a subtle red shift (Figure 2).

Figure 2. UV–vis titration of MPTP with increasing amounts of CB[7] in PBS solution (0.01 M). The inset shows the best fit between experimental points and a 1:1 binding model affording a binding constant $K_a \approx 4.8 \times 10^4 \text{ M}^{-1}$.

This behavior was consistent with that expected for the encapsulation of MPTP in the hydrophobic cavity of CB[7]. Plots of the absorbance of MPTP at 242 nm against the CB[7] concentration in PBS solution were fitted according to a nonlinear least-squares method,²⁴ consistent with the previously determined 1:1 stoichiometry and affording a binding constant K_a of $4.8 \pm 0.2 \times 10^4 \text{ M}^{-1}$ (Figure 2). The binding constant of MPP⁺ toward CB[7] was similarly determined and was found to be $1.05 \pm 0.05 \times 10^5 \text{ M}^{-1}$, which is twice the binding constant for the MPTP–CB[7] complex (Figure S8). These binding constants are comparable to those of viologen and derivatives whose structures are quite close to that of MPP⁺^{25–28} and are considered reasonably strong due to the presence of competitive salts (PBS buffer).^{27,29}

To gain further insights regarding inclusion complexation, DFT calculations were performed and the most likely structures are shown in Figure 3. Positively charged guests were

Figure 3. Side views of DFT calculated supramolecular inclusion complexes of MPTP-H⁺-CB[7] (left) and MPP⁺-CB[7] (right).

considered due to the well-known propensity of CB[7] to stabilize cationic charges via ion–dipole interactions.³⁰ The two inclusion complexes were calculated by DFT (B3LYP/6-31G(d)) gradually deepening the guest (starting conformations) prior to minimization (details in SI). For the MPTP-H⁺@CB[7] complex, the two six-membered rings of MPTP were included in the cavity of CB[7] (Figure 3a), leaving the methyl group outside but still close to one of the host carbonyl rims. This calculated structure agreed well with the complexation geometry deduced from ¹H NMR spectra. For the MPP⁺@CB[7] complex, the ¹H NMR data are only consistent with one of the two lowest energy calculated structures ($\Delta E = 1.6 \text{ kcal}\cdot\text{mol}^{-1}$), the one with the aromatic rings included (Figure 3b). The other conformer showed the guest methyl group inside the cavity leaving the aromatic rings bulk exposed and is thus much less likely.

After assignment of the inclusion behavior and binding strength of MPTP and MPP⁺ toward CB[7] in physiologically relevant media, the *in vivo* inhibition of MPTP/MPP⁺ induced PD by CB[7] was investigated in detail in zebrafish models. Zebrafish have been widely accepted as a suitable *in vivo* model for the investigation of neurodegeneration as well as novel therapeutic and preventative agents for PD. For instance, zebrafish have a nervous system that is similar to those of mammals and humans, highly conserved and endogenous genes, and neuronal populations that are directly related to human neurodegenerative diseases.³¹ The effect of MPTP on dopaminergic neurons in zebrafish larvae is mediated by the same pathways that have been observed among mammalian species, and clinical neuro-protective agents have been demonstrated to have equivalent protective effects on zebrafish as on humans.³² To assess the preventive potential of CB[7] against MPTP/MPP⁺-induced neurodegeneration in zebrafish, we visualized the morphology of dopaminergic neurons in the larval brains by immunostaining of tyrosine hydroxylase (TH) with mouse monoclonal anti-TH antibody. TH, the rate limiting enzyme in dopamine synthesis in the brain, is the gold standard marker in the identification of dopaminergic neurons.³³ Ventral diencephalic TH populations are highly sensitive to MPTP exposure, and a decreased TH population leads to a pronounced reduction in the number of dopaminergic cells in the diencephalon.³⁴ Of note, MPP⁺ alone cannot effectively induce neurodegeneration with animal models as it cannot cross the blood–brain barrier.³⁵ For the

present study (experiment detail in SI), zebrafish larvae exposed to MPTP (250 μM , the lowest effective concentration capable of inducing PD effects monitored by immunostaining) exhibited significant TH population recession of approximately 50%, as compared to the control group (Figure 4).

Figure 4. CB[7] protection against MPTP-induced TH deficiency in zebrafish larvae. (A) Representative images of whole-mount immunostained zebrafish brains from different treatment groups. (B) Statistical analysis of TH positive region (quantified by the fluorescent area) in each treatment group. Data are expressed as mean $\pm$ SEM ($n = 20$ –22 in each group). * $P < 0.05$, *** $P < 0.001$.

Significantly, the groups of larvae exposed to the same amount of MPTP in the presence of CB[7] dramatically attenuated MPTP-induced neurodegeneration, with the TH population reduced by less than 20% in comparison with the control group. Additionally, no toxic phenotype was observed in the major organs of this group of zebrafish, in contrast to the MPTP treated group. Moreover, exposure to 100 μM of CB[7] alone did not yield statistically significant differences in the TH region of the treated fish, when compared to control group, thus suggesting that CB[7] alone at this concentration has no neurotoxicity in this case.

Meanwhile, MPTP is known to remarkably alter the swimming behavior of zebrafish as a consequence of injury to dopaminergic neurons and induction of PD.³⁶ To further confirm the impeditive effect of CB[7] against MPTP-induced neurodegeneration, we evaluated the locomotion activity with wild-type zebrafish (experimental detail in SI). Zebrafish embryos (1 day postfertilization) were coincubated with 50 μM (the lowest effective concentration capable of inducing PD effects monitored by locomotion behaviors of zebrafish) of the neurotoxin MPTP in the absence and in the presence of 100 μM of CB[7] for 5 days. As shown in Figure 5, the administration of MPTP to zebrafish larvae resulted in an approximately 65% reduction in the locomotion activity.

Notably, CB[7] served to counteract the MPTP-induced locomotion deficiency among the zebrafish larvae, and the fish that were exposed to MPTP in the presence of CB[7] swam a similar distance as the control group (Figure S9). In addition, exposure to 100 μM of CB[7] alone had little influence on the locomotion behaviors of the treated fish, when compared to control group, thus confirming the biocompatibility of CB[7]. These results reaffirm that CB[7] significantly alleviated the neurotoxicity of MPTP.

Figure 5. CB[7] attenuated MPTP-induced locomotion deficiency in zebrafish larvae. Zebrafish embryos at 1 dpf were incubated with MPTP (50 μ M) in the absence and in the presence of CB[7] (100 μ M) for 5 days. (A) Representative figures showing the swimming traces of zebrafish larvae for 45 min in various treatment groups. Different colors of lines indicated the speed of the movement. Red line, Active with velocity >6 mm/s; green line, low activity with velocity between 3 and 6 mm/s; black line, inactive with velocity <3 mm/s. (B) The total swimming distance of each zebrafish observed in 45 min is reported on the graph. Data are expressed as mean $\pm$ SEM ($n = 20$ –22 in each group). * $P < 0.05$, ** $P < 0.01$.

It is well-known that MPTP is catalyzed by the enzyme MAO-B in the brain and converted to MPP⁺.⁴ In addition, its toxicity is mainly attributed to the inhibition of mitochondrial complex I of the electron transport chain, which causes the loss of ATP and the generation of ROS, resulting in cell apoptosis.² We speculate that the alleviation of MPTP/MPP⁺ induction of neurodegeneration by CB[7] likely proceeds through one or more of the following three pathways: (1) MPTP is well-known for its ability to penetrate the blood–brain barrier (BBB).³⁷ CB[7]’s complexation might limit its ability to penetrate the BBB, thus inhibiting its neurotoxicity; (2) if the complex may transport through the BBB, CB[7] might protect MPTP from undergoing oxidation by MAO-B; (3) even if MPP⁺ is generated by MAO-B, CB[7] may serve as a synthetic receptor of MPP⁺ to limit its transportation by DAT to mitochondrial complex I. Once again, the strong encapsulation of MPTP and MPP⁺ may limit access of these guest molecules to their corresponding biological targets (such as MAO-B and DAT), as the binding constants between MPTP/MPP⁺ and CB[7] are comparable to the binding affinities between MPTP/MPP⁺ and their biological receptors.^{38,39} These preliminary results are promising, and we are currently working to clarify the mechanism behind the CB[7]’s inhibition of neurotoxin-induced neurodegeneration.

In summary, we have demonstrated the supramolecular host–guest complexation between macrocyclic CB[7] and a well-known neurotoxin, MPTP, as well its metabolite MPP⁺. For the first time we report the effectiveness of CB[7] as an inhibitory agent against MPTP/MPP⁺-induced neurodegeneration using zebrafish models. This preliminary finding may lead to a new approach for the prevention of PD caused by exposure to a variety of neurotoxins and likely further expand the scope of CB[7] for biological and biomedical applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.5b00372.

Experimental methods and procedures, additional spectra, original data of zebrafish experiments, and atomic coordinates of the CB[7] complexes (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: david.bardelang@univ-amu.fr.

*E-mail: rwang@umac.mo.

Author Contributions

^{||}These authors contributed equally to this work.

Funding

The Start-up Research Grant (SRG2014-00025) from University of Macau is gratefully acknowledged for providing the financial support to this project.

Notes

The authors declare no competing financial interest.

Biographies

Dr. Ruibing Wang received his Ph.D. in Organic Chemistry from Queen’s University, Kingston, Canada. After working at the National Research Council (NRC) of Canada, he joined the industrial sector as a Senior Scientist for research and development of Radiopharmaceuticals and Interventional Medicine. He has recently been appointed as an Assistant Professor of Biomedical Sciences at University of Macau. His current projects address the use of synthetic receptors, such as cucurbiturils, in pharmaceutical and biomedical sciences. His research interests span broad fields of supramolecular medicine, molecular imaging, and Chinese medicine.

Dr. David Bardelang studied free radical stabilization in cyclodextrins during his Ph.D. thesis at Université de Provence, Marseille, France. After postdoctoral training at University of Birmingham, U.K., and National Research Council, Canada, where he worked on various facets of supramolecular chemistry (DNA, helicates, gels, and solid–solid, solid–liquid, and solid–gas recognition), he was recruited by CNRS in 2009 to develop supramolecular perspectives of free radicals for which EPR spectroscopy is the technique of choice. His research interests include crystalline structures of free radicals with void spaces amenable for guest recognition, cyclodextrins, and a growing research effort on cucurbiturils.

■ REFERENCES

- (1) Langston, J. W.; Ballard, P.; Tetrud, J. W.; Irwin, I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **1983**, *219*, 979–980.
- (2) Lin, M. T.; Beal, M. F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* **2006**, *443*, 787–795.
- (3) Duty, S.; Jenner, P. Animal models of Parkinson’s disease: a source of novel treatments and clues to the cause of the disease. *Br. J. Pharmacol.* **2011**, *164*, 1357–1391.
- (4) Hare, D. J.; Adlard, P. A.; Doble, P. A.; Finkelstein, D. I. Metallobiology of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. *Metallomics* **2013**, *5*, 91–109.
- (5) Del-Bel, E.; Tumas, V. Parkinson’s disease: Some current therapies and their limitations. *Frontiers in Parkinson’s Disease Research* **2012**, *93*.
- (6) Lee, J. W.; Samal, S.; Selvapalam, N.; Kim, H.-J.; Kim, K. Cucurbituril Homologues and Derivatives: New Opportunities in Supramolecular Chemistry. *Acc. Chem. Res.* **2003**, *36*, 621–630.

- (7) Lagona, J.; Mukhopadhyay, P.; Chakrabarti, S.; Isaacs, L. The Cucurbit[n]uril Family. *Angew. Chem., Int. Ed.* **2005**, *44*, 4844–4870.
- (8) Macartney, D. H. Encapsulation of Drug Molecules by Cucurbiturils: Effects on their Chemical Properties in Aqueous Solution. *Isr. J. Chem.* **2011**, *51*, 600–615.
- (9) Walker, S.; Oun, R.; McInnes, F. J.; Wheate, N. J. The Potential of Cucurbit[n]urils in Drug Delivery. *Isr. J. Chem.* **2011**, *51*, 616–624.
- (10) Ghosh, I.; Nau, W. M. The strategic use of supramolecular pKa shifts to enhance the bioavailability of drugs. *Adv. Drug Delivery Rev.* **2012**, *64*, 764–783.
- (11) Chen, H.; Chan, J. Y. W.; Li, S.; Liu, J. J.; Wyman, I. W.; Lee, S. M. Y.; Macartney, D. H.; Wang, R. In vivo reversal of general anesthesia by cucurbit[7]uril with zebrafish models. *RSC Adv.* **2015**, *5*, 63745–63752.
- (12) Li, S.; Miao, X.; Wyman, I. W.; Li, Y.; Zheng, Y.; Wang, Y.; Macartney, D. H.; Wang, R. High-affinity host–guest complex of cucurbit[7]uril with a bis(thiazolium) salt. *RSC Adv.* **2015**, *5*, 56110–56115.
- (13) Miao, X.; Li, Y.; Wyman, I.; Lee, S. M. Y.; Macartney, D. H.; Zheng, Y.; Wang, R. Enhanced in vitro and in vivo uptake of a hydrophobic model drug coumarin-6 in the presence of cucurbit[7]uril. *MedChemComm* **2015**, *6*, 1370–1374.
- (14) Bardelang, D.; Casano, G.; Poulhes, F.; Karoui, H.; Filippini, J.; Rockenbauer, A.; Rosas, R.; Monnier, V.; Siri, D.; Gaudel-Siri, A.; Ouari, O.; Tordo, P. Spin exchange monitoring of the strong positive homotropic allosteric binding of a tetradical by a synthetic receptor in water. *J. Am. Chem. Soc.* **2014**, *136*, 17570–17577.
- (15) Wang, R.; Bardelang, D.; Waite, M.; Udachin, K. A.; Leek, D. M.; Yu, K.; Ratcliffe, C. I.; Ripmeester, J. A. Inclusion complexes of coumarin in cucurbiturils. *Org. Biomol. Chem.* **2009**, *7*, 2435–2439.
- (16) Wang, R.; Yuan, L.; Macartney, D. H. Inhibition of C(2)-H/D exchange of a bis(imidazolium) dication upon complexation with cucurbit[7]uril. *Chem. Commun.* **2006**, 2908–2910.
- (17) Wang, R.; Yuan, L.; Macartney, D. H. A green to blue fluorescence switch of protonated 2-aminoanthracene upon inclusion in cucurbit[7]uril. *Chem. Commun.* **2005**, 5867–5869.
- (18) Chen, H.; Chan, J. Y. W.; Yang, X.; Wyman, I. W.; Bardelang, D.; Macartney, D. H.; Lee, S. M. Y.; Wang, R. Developmental and organ-specific toxicity of cucurbit[7]uril: in vivo study on zebrafish models. *RSC Adv.* **2015**, *5*, 30067–30074.
- (19) Ma, D.; Zhang, B.; Hoffmann, U.; Sundrup, M. G.; Eikermann, M.; Isaacs, L. Acyclic cucurbit[n]uril-type molecular containers bind neuromuscular blocking agents in vitro and reverse neuromuscular block in vivo. *Angew. Chem., Int. Ed.* **2012**, *51*, 11358–11362.
- (20) Oun, R.; Floriano, R. S.; Isaacs, L.; Rowan, E. G.; Wheate, N. J. The neurotoxic, myotoxic and cardiotoxic activity of cucurbituril-based macrocyclic drug delivery vehicles. *Toxicol. Res.* **2014**, *3*, 447–455.
- (21) Lee, H. H.; Choi, T. S.; Lee, S. J.; Lee, J. W.; Park, J.; Ko, Y. H.; Kim, W. J.; Kim, K.; Kim, H. I. Supramolecular inhibition of amyloid fibrillation by cucurbit[7]uril. *Angew. Chem., Int. Ed.* **2014**, *53*, 7461–7465.
- (22) Da Silva, J. P.; Choudhury, R.; Porel, M.; Pischel, U.; Jockusch, S.; Hubbard, P. C.; Ramamurthy, V.; Canario, A. V. Synthetic versus natural receptors: supramolecular control of chemical sensing in fish. *ACS Chem. Biol.* **2014**, *9*, 1432–1436.
- (23) Vial, L.; Ludlow, R. F.; Leclaire, J.; Pérez-Fernández, R.; Otto, S. Controlling the Biological Effects of Spermine Using a Synthetic Receptor. *J. Am. Chem. Soc.* **2006**, *128*, 10253–10257.
- (24) Hirose, K. A practical guide for the determination of binding constants. *J. Inclusion Phenom. Mol. Recognit. Chem.* **2001**, *39*, 193–209.
- (25) Moon, K.; Kaifer, A. E. Modes of binding interaction between viologen guests and the cucurbit[7]uril host. *Org. Lett.* **2004**, *6*, 185–188.
- (26) Ong, W.; Gómez-Kaifer, M.; Kaifer, A. E. Cucurbit[7]uril: A Very Effective Host for Viologens and Their Cation Radicals. *Org. Lett.* **2002**, *4*, 1791–1794.
- (27) Ong, W.; Kaifer, A. E. Salt effects on the apparent stability of the cucurbit[7]uril-methyl viologen inclusion complex. *J. Org. Chem.* **2004**, *69*, 1383–1385.
- (28) Kim, H. J.; Jeon, W. S.; Ko, Y. H.; Kim, K. Inclusion of methylviologen in cucurbit[7]uril. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 5007–5011.
- (29) Houk, K. N.; Leach, A. G.; Kim, S. P.; Zhang, X. Binding affinities of host-guest, protein-ligand, and protein-transition-state complexes. *Angew. Chem., Int. Ed.* **2003**, *42*, 4872–4897.
- (30) Saleh, N.; Koner, A. L.; Nau, W. M. Activation and stabilization of drugs by supramolecular pKa shifts: drug-delivery applications tailored for cucurbiturils. *Angew. Chem., Int. Ed.* **2008**, *47*, 5398–5401.
- (31) Babin, P. J.; Goizet, C.; Raldua, D. Zebrafish models of human motor neuron diseases: advantages and limitations. *Prog. Neurobiol.* **2014**, *118*, 36–58.
- (32) Pienaar, I. S.; Gotz, J.; Feany, M. B. Parkinson's disease: insights from non-traditional model organisms. *Prog. Neurobiol.* **2010**, *92*, 558–571.
- (33) Zhu, Y.; Zhang, J.; Zeng, Y. Overview of tyrosine hydroxylase in Parkinson's disease. *CNS Neurol. Disord.: Drug Targets* **2012**, *11*, 350–358.
- (34) Lam, C. S.; Korzh, V.; Strahle, U. Zebrafish embryos are susceptible to the dopaminergic neurotoxin MPTP. *Eur. J. Neurosci.* **2005**, *21*, 1758–1762.
- (35) Sallinen, V.; Torkko, V.; Sundvik, M.; Reenila, I.; Khrustalyov, D.; Kaslin, J.; Panula, P. MPTP and MPP+ target specific aminergic cell populations in larval zebrafish. *J. Neurochem.* **2009**, *108*, 719–731.
- (36) Cui, W.; Zhang, Z.; Li, W.; Hu, S.; Mak, S.; Zhang, H.; Han, R.; Yuan, S.; Li, S.; Sa, F.; Xu, D.; Lin, Z.; Zuo, Z.; Rong, J.; Ma, E. D.; Choi, T. C.; Lee, S. M.; Han, Y. The anti-cancer agent SU4312 unexpectedly protects against MPP(+)-induced neurotoxicity via selective and direct inhibition of neuronal NOS. *Br. J. Pharmacol.* **2013**, *168*, 1201–1214.
- (37) Smeyne, R. J.; Jackson-Lewis, V. The MPTP model of Parkinson's disease. *Mol. Brain Res.* **2005**, *134*, 57–66.
- (38) Giros, B.; el Mestikawy, S.; Godinot, N.; Zheng, K.; Han, H.; Yang-Feng, T.; Caron, M. G. Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol. Pharmacol.* **1992**, *42*, 383–390.
- (39) Kinemuchi, H.; Arai, Y.; Toyoshima, Y. Participation of brain monoamine oxidase B form in the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Relationship between the enzyme inhibition and the neurotoxicity. *Neurosci. Lett.* **1985**, *58*, 195–200.