Supplemental Material

Supramolecular therapeutics to treat the side effects induced by a depolarizing neuromuscular blocking agent

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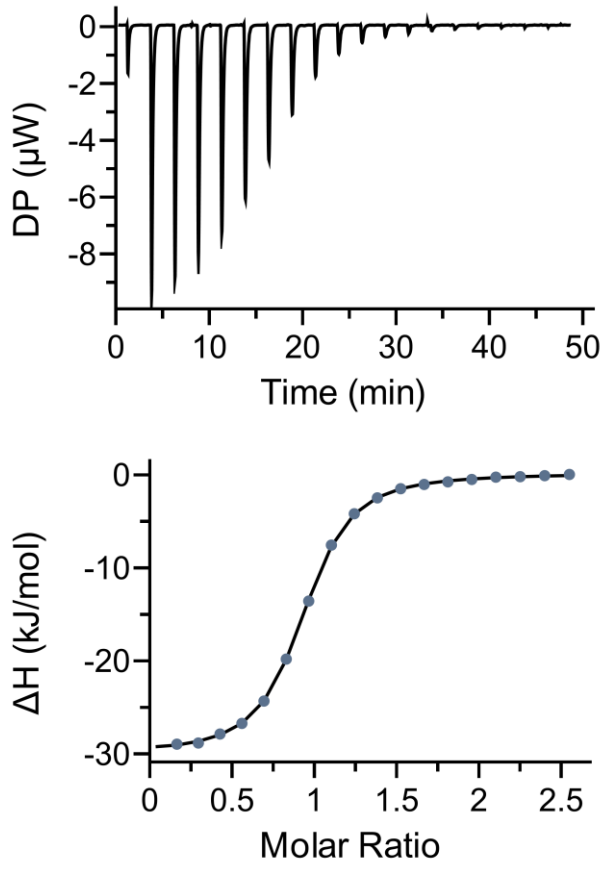

Figure S1. ITC titration of WP[6] and Sch in PBS at 25 °C. One binding site model was utilized to fit the data, affording an association constant *Ka* of $2.79 \times 10^5\ M^{-1}$ (ΔH: -30.2 kJ/mol, ΔG: -31.1 kJ/mol and -T ΔS: -0.894 kJ/mol).

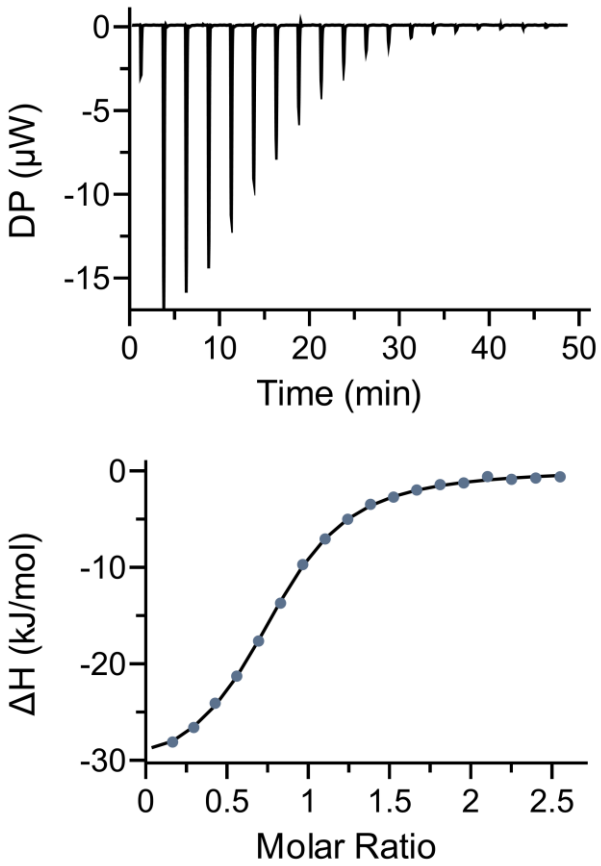

Figure S2. ITC titration of SC[4]A and Sch in PBS at 25 °C. One binding site model was utilized to fit the data, affording an association constant *Ka* of $7.52 \times 10^4\ M^{-1}$ (ΔH: -32.3 kJ/mol, ΔG: -27.8 kJ/mol and -T ΔS: 4.45 kJ/mol).

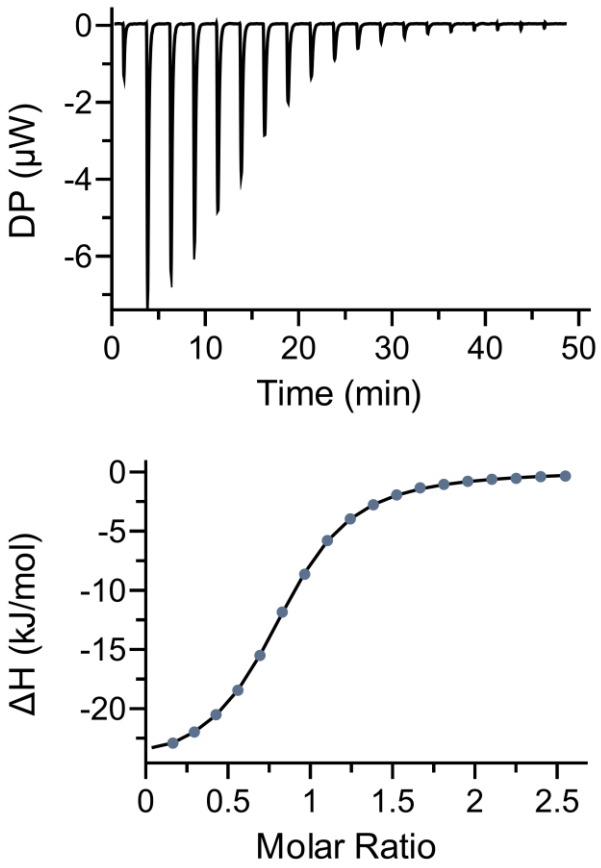

Figure S3. ITC titration of CB[7] and Sch in PBS at 25 °C. One binding site model was utilized to fit the data, affording an association constant *Ka* of 1.00×10^{5} M^{-1} (ΔH: -25.4 kJ/mol, ΔG: -28.6 kJ/mol and -T ΔS: -3.14 kJ/mol).

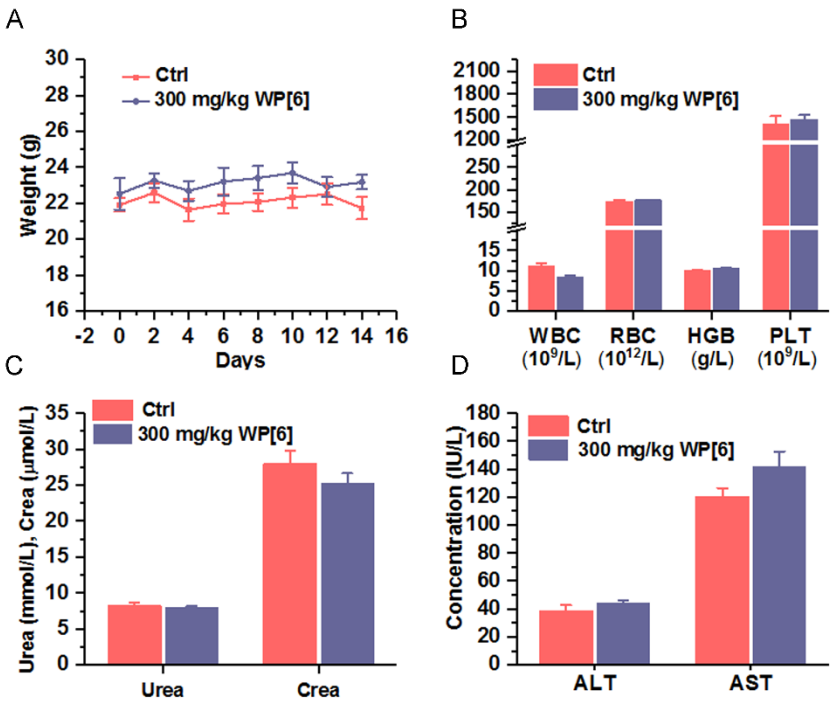

Figure S4. Acute toxicity evaluation of WP[6] in mice. (A) Weight changes of mice i.v. administered with WP[6]. Data are presented as mean ± SEM, *n* = 6 for each group. **(B)** Hematological parameteres of the blood samples collected from the mice at Day 14 after i.v. administration of WP[6]. Data are presented as mean ± SEM, *n* = 6 for each group. **(C to D)** Renal function (C) and hepatic function (D) biomarkers in the blood samples collected from the mice at Day 14 after i.v. administration of WP[6]. Data are presented as mean ± SEM, *n* = 6 for each group.

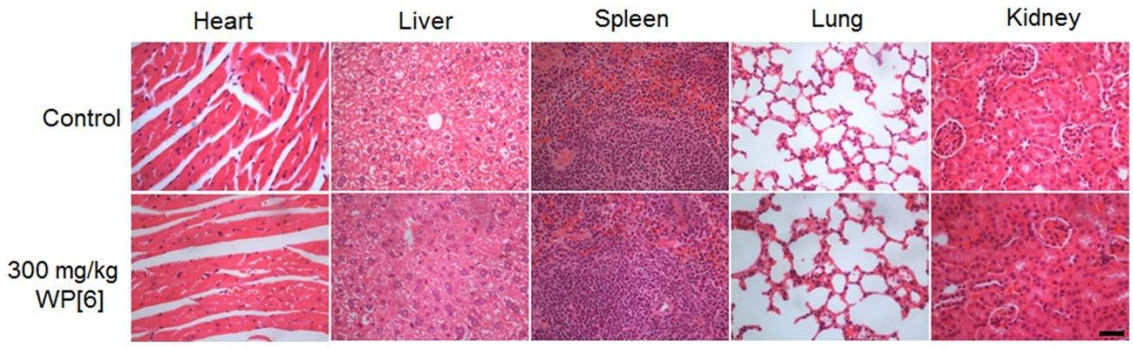

Figure S5. H&E sections of major organs from mice with *i.v.* administration of WP[6]. Mice were sacrificed on Day 14 post the *i.v.* administration of WP[6]. Scale bar = 20 μm.

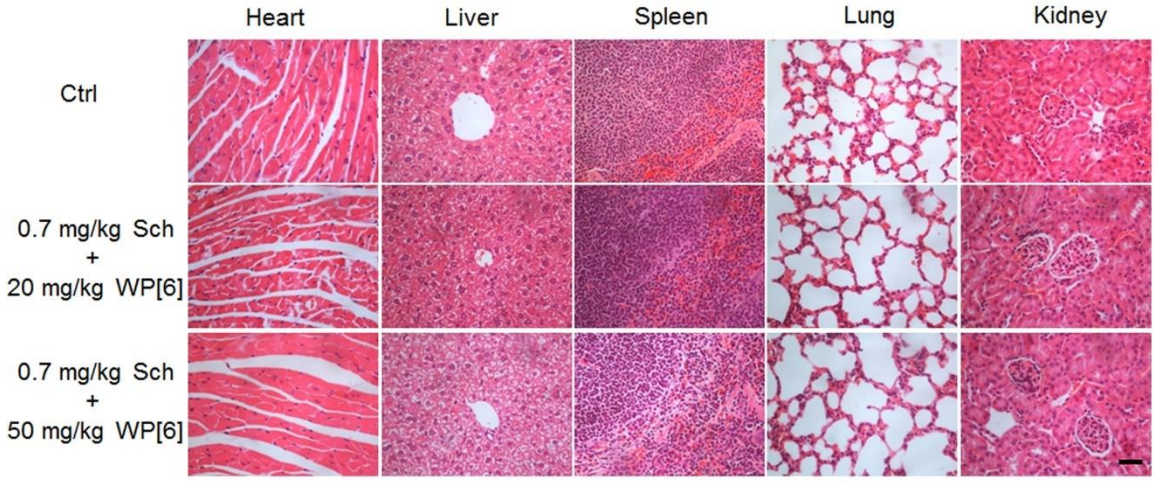

Figure S6. Histopathological analysis of major organs from survived mice. Mice were sacrificed on Day14 post treatment of WP[6] after the administration of Sch. Scale bar = 20 μm.

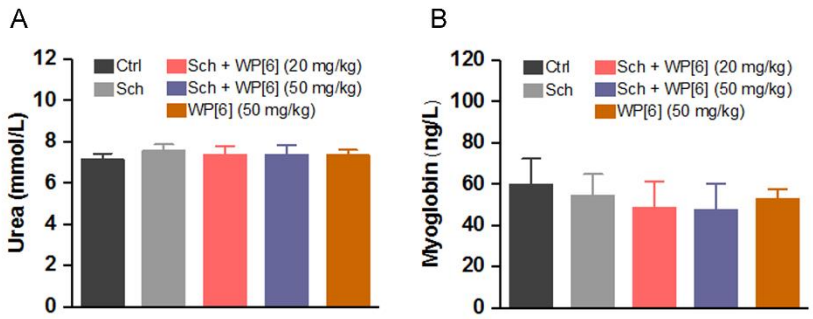

Figure S7. Serum levels of urea and myoglobin. Blood samples were collected from the rats that *i.m.* administered with WP[6] (20 mg/kg or 50 mg/kg) at 30 s after the *i.m.* administration of Sch (1 mg/kg).

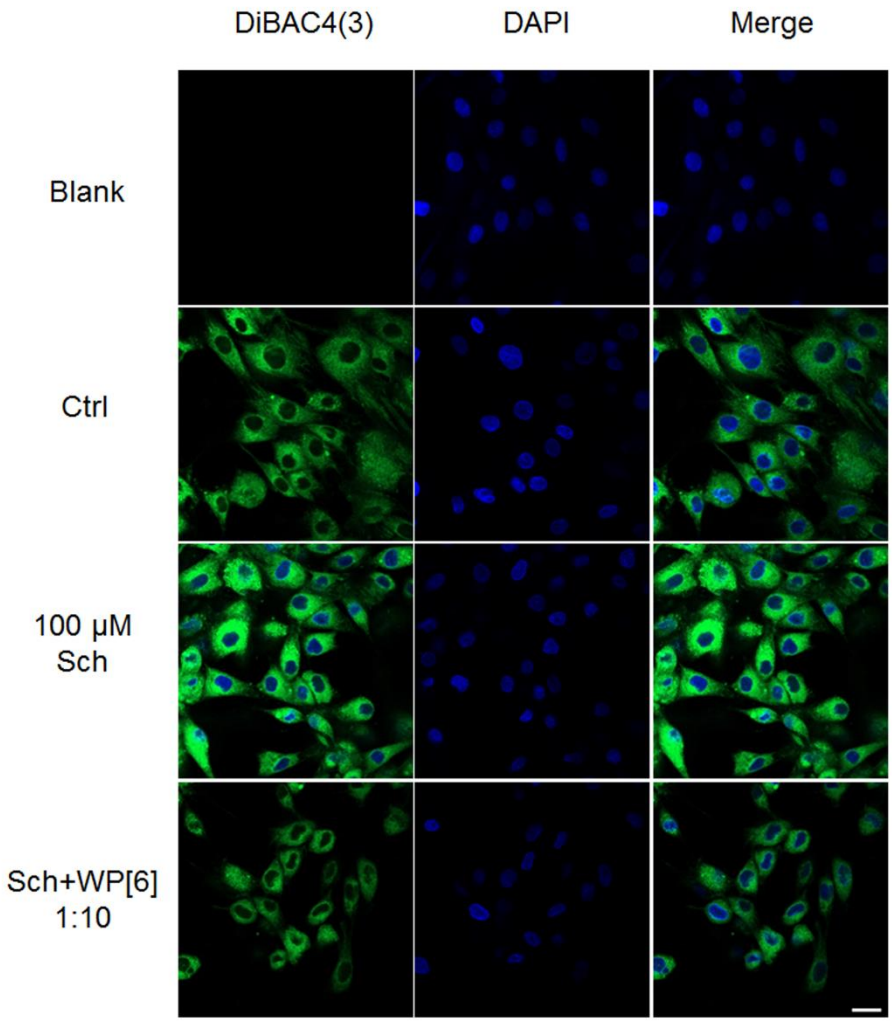

Figure S8. Confocal laser scanning microscopy images of L6 cells. 2×10^5 L6 cells were incubated with 100 μM Sch or Sch+WP[6] with various concentrations of WP[6] in HBSS for 10 min at 37℃, after the solution were removed, cells were then incubated with 200 nM DiBAC4(3) in HBSS for 20 min at 37 ˚C, cells were then fixed with 4% paraformaldehyde for 15 min and stained with DAPI for 2 min. Confocal laser scanning microscopy were then used for the fluorescence observation. Scale bar = 20 μm.

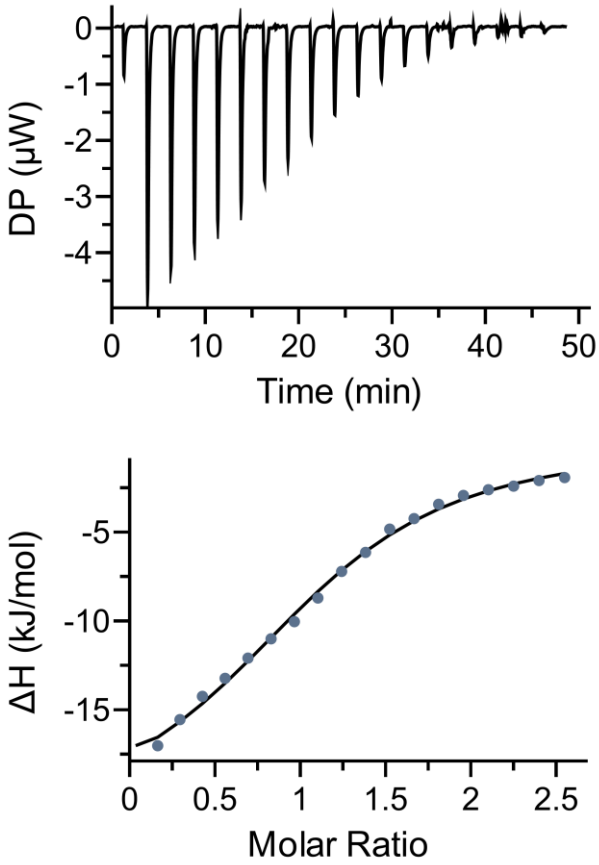

Figure S9. ITC titration of WP[5] and Sch in water at 25 °C. One binding site model was utilized to fit the data, affording an association constant *Ka* of $2.33\times10^{4}\ M^{-1}$ (ΔH: -21.7 kJ/mol, ΔG: -25.0 kJ/mol and -T ΔS: -3.30 kJ/mol).

Table S1. Binding parameters of Sch and cholinergic receptors. (*Ka*, M^{-1})

	M_2 mAchR	M_3 mAchR	Neuronal nAchR	Musclar nAChR
solutions	PBS buffer pH 7.4	PBS buffer pH 7.4	Tris-HCl buffer pH 7.4	HEPES pH7.4
Sch	5.75×10^3	8.13×10^3	5.55×10^4	9.26×10^4

Data taken or calculated with EC_{50} from supplemental references (1-3).

Table S2. Pharmacological properties of NMBAs

NMBAs	ED_{95} (mg/kg)	Onset of action	Duration of action	Approximate metabolism (%)	Main potential side effects	Reversal agents
Succinylcholine	0.260	~60 s	~10 min	>90	Myalgia, vagal stimulation, fasciculations, hyperkalemia, increased intraocular and intracranial pressure, malignant hyperthermia, cardiac arrhythmias and arrest, acute rhabdomyolysis	None
Benzylisoquinolines						
Tubocurarine	0.480	3-5 min	70-90 min	0	Histamine release, hypotension, compensatory tachycardia, ganglion block	Cholinesterase inhibitors
Atracurium	0.230	110 s	43 min	60-90	Histamine release, epileptogenic properties in high concentration	Cholinesterase inhibitors
Cisatracurium	0.050	2-3 min	30-40 min	80	Bronchospasm	Cholinesterase inhibitors
Mivacuronium	0.067	2.5 min	15-20 min	95-99	Histamine release, hypotension	Cholinesterase inhibitors
Gantacurium	0.190	1.5-3 min	15 min	>95	Histamine release, transient hypotension, tachycardia,	Cholinesterase inhibitors and Cysteine
CW002	0.077	90 s	28.8-36.1 min	*	Cardiopulmonary side effects	Cholinesterase inhibitors and Cysteine
Aminosteroids						
Pancuronium	0.067	2-3 min	60-90 min	10-20	Increase in heart rate, blood pressure and cardiac output	Cholinesterase inhibitors and Sugammadex
Rocuronium	0.310	1-3 min	30-70 min	<1	Vagolytic property, anaphylactoid reactions	Cholinesterase inhibitors and Sugammadex
Vecuronium	0.043	2-3 min	25-40 min	30-40	cardiac vagolytic effects, tachycardia	Cholinesterase inhibitors and Sugammadex

Data taken from the supplemental references (4-13). * No human results.

Table S3. Binding parameters for vecuronium, rocuronium and receptors. (*Ka*, M^{-1})

	Vecuronium	Rocuronium
M_1 mAchR	~1.38×10^6	~2.63×10^5
M_2 mAchR	~7.94×10^6	~2.51×10^5
M_3 mAchR	~1.48×10^6	~2.19×10^4
M_4 mAchR	~2.04×10^7	~1.05×10^5
M_5 mAchR	~1.58×10^6	~1.26×10^5
neuronal nAchR	~9.31×10^7	~7.27×10^7
muscular nAchR	~3.32×10^7	~1.10×10^7
Sugammadex	~5.72×10^6	~1.79×10^7
Calabadion 1	~5.80×10^6	~8.40×10^6
Calabadion 2	~1.60×10^9	~3.40×10^9

Data taken or calculated with EC_{50} from supplemental references (14-17).

Supplemental References

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