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Design, synthesis, and biological evaluation of tricyclic heterocycle-tetraamine conjugates as potent NMDA channel blockers

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Abstract—We have developed a new class of *N*-methyl-D-aspartate (NMDA) channel blockers having a conjugate structure that consists of a nitrogenous heterocyclic head and a tetraamine tail. Among them, dihydrodibenzazepine-homospermine conjugate (**8**) exhibited potent antagonistic activity at NR1/NR2A or NR1/NR2B NMDA subtype receptors compared with the lead compound, AQ343 (**1**), or memantine, as well as weak cytotoxicity. Its superior biological profiles compared with known compounds point to its potential use as therapeutic agents for neurological disorders.

N-Methyl-D-aspartate (NMDA) receptors, which are ligand-gated cation channels embedded in the cell membrane of neurons, have been implicated in learning and memory, and may also play a central role in various conditions leading to neuronal degeneration. Overexcitation of NMDA receptors leads to excessive Ca^{2+} influx through receptor-associated ion channels, resulting in neuronal cell injury or death. Therefore, NMDA receptor antagonists could be of therapeutic benefit to a number of neurological disorders, such as stroke, Alzheimer's disease, or Parkinson's disease. Memantine, which possesses an aminoadamantane structure, was recently approved by the European Union and US FAD for the treatment of dementia.[1]

In our continuing research on NMDA receptor channel blockers,[2] we have found that polycycle-polyamine conjugates, such as AQ343 (**1**) and AQ444 (**2**), are reversible and voltage-dependent NMDA blockers, particularly at NR1/NR2A and NR1/NR2B receptors,[3] and their antagonistic activities (IC_{50} values) are more potent than that of memantine. Further, we have revealed that the angle between the polycyclic head moiety and the linear polyamine tail, as well as the length of the polyamine itself, is important for interactions with the NMDA channel and for the activity of these compounds. Based on these findings, we further investigated new types of NMDA antagonists by utilizing AQ343 as the lead compound in order to develop more efficient NMDA channel blockers having potential clinical applications. Here we report the synthesis and biological evaluation of tricyclic heterocycle-tetraamine conjugates as NMDA channel blockers.

As the head moiety of new conjugates, we have adopted tricyclic nitrogenous heterocycles, i.e., 5*H*-dibenz[*b*,*f*]azepine (A), 10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine (B), phenothiazine (C), and carbazole (D), in the present study. Tricyclic heterocycle-tetraamine conjuncts (**3**–**14**) were synthesized by coupling carboxylic acid derivatives of tricyclic heterocycles with tetraamines, including spermine, N^1-(3-(4-aminobutylamino)propyl)butane-1,4-diamine, and homospermine, i.e., N^1-(4-(4-aminobutylamino)butyl)butane-1,4-diamine, as follows.

Starting from 1,4-diaminobutane (**15**), N^1-nitrosobenzenesulfonyl-N^4-Boc derivative (**17**)[4] was prepared

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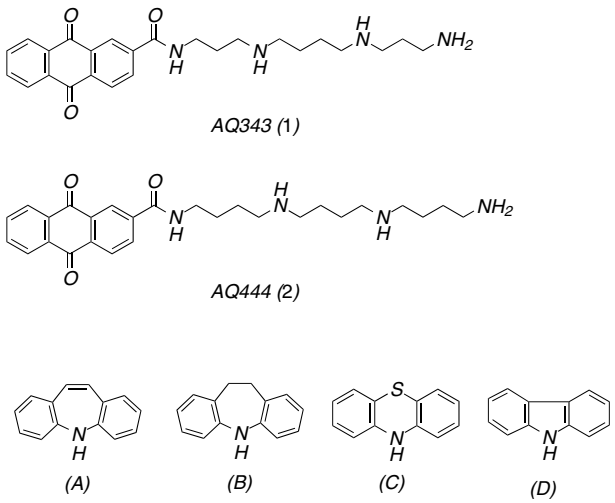
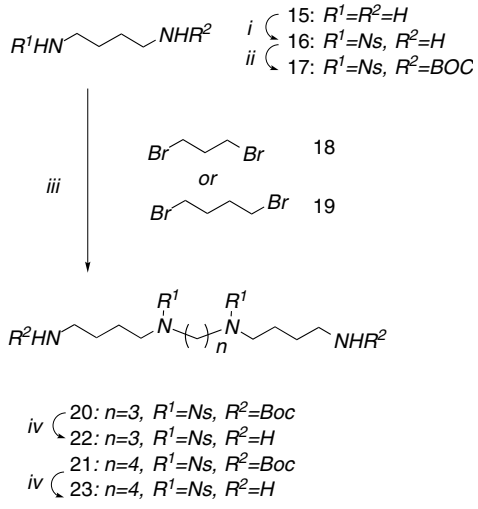


Figure 1. Structures of AQ343, AQ444, and tricyclic heterocycles.

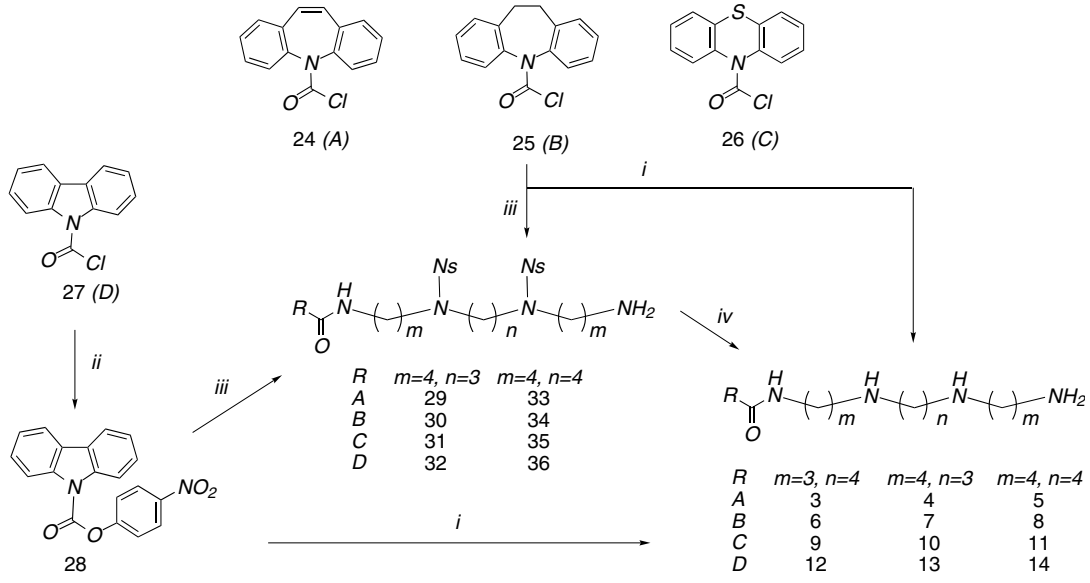


Scheme 1. Reagents and conditions: (i) NsCl (0.33 equiv), EtOH, 0 °C, 30 min, 62%; (ii) Boc_2O, CH_2Cl_2, rt, 2 h, quant.; (iii) **18** or **19**, DMF, 60 °C, 15 h, 96% for **20**, 95% for **21**; (iv) TFA, CH_2Cl_2, rt, 1.5 h, quant.

by successive treatment with 2-nitrobenzenesulfonyl chloride[5] (0.33 eq.) and Boc_2O (Scheme 1). Then, amination of 1,3-dibromopropane (**18**) or 1,4-dibromobutane (**19**) with **17** afforded linear polyamine derivative (**20**) or (**21**), respectively, which was then treated with trifluoroacetic acid to remove the Boc group, giving terminal amine-free tetraamine derivatives (**22** and **23**) in good yields (Fig. 1).

Next, coupling of the heterocyclic head moiety with the polyamine tail was carried out. Spermine-conjugated dibenzazepine, dihydrodibenzazepine, and phenothiazine compounds (**3**, **6**, **9**) were, respectively, prepared by condensation of their carbamoyl chlorides (**24**–**26**) and a large excess of spermine itself. In the case of compound **12**, due to the high reactivity of the carbamoyl chloride derivative of carbazole (D), chloride **27** was initially converted into *p*-nitrophenyl carbamate (**28**) and then condensed with a large excess of spermine. In turn, N^1-(3-(4-aminobutylamino)propyl)butane-1,4-diamine- or N^1-(4-(4-aminobutylamino)butyl)butane-1,4-diamine-conjugated dibenzazepine, dihydrodibenzazepine, and phenothiazine compounds were, respectively, prepared by coupling their carbamoyl chlorides (**24**–**26**) with protected tetraamines (**22** or **23**), followed by the removal of Ns groups using thiophenol in the presence of Cs_2CO_3 or K_2CO_3 in DMF.[5] As in the preparation of conjugate **12**, the *p*-nitrophenyl carbamate of carbazole (**28**) was used for the syntheses of tetraamines (**13** and **14**). The structures of all the synthetic compounds were confirmed by analysis of spectroscopic data (^1H, ^{13}C NMR, MS, HRMS, UV, IR).

The antagonistic effects of prepared compounds (**3**–**14**) were studied using recombinant NMDA receptors expressed in *Xenopus laevis* oocytes. Most NMDA receptors in the adult central nervous system contain combinations of NR1 and NR2, with NR2A and



Scheme 2. Reagents and conditions: (i) spermine (4.8–5 equiv), CH_2Cl_2, rt (for **3**, **6**) or 0 °C (for **9**, **12**); (ii) *p*-nitrophenol, Et_3N, CH_2Cl_2, rt; (iii) tetraamine **22** (2 equiv) or **23** (2 equiv), CH_2Cl_2, rt, or 40 °C (for **36**); (iv) PhSH, Cs_2CO_3 or K_2CO_3 (for **5**, **13**, **14**), DMF, rt.

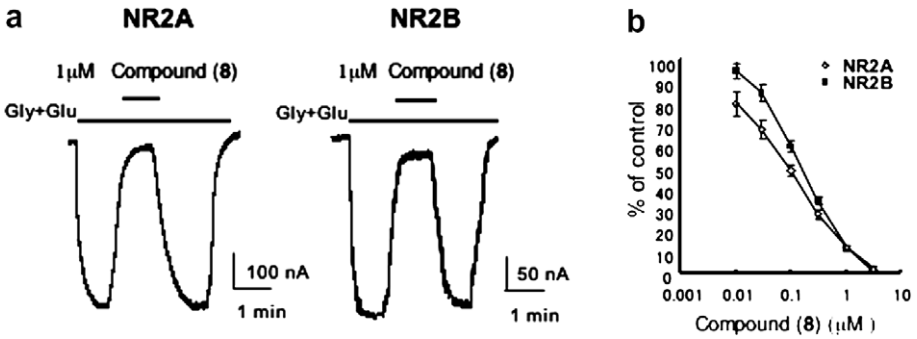
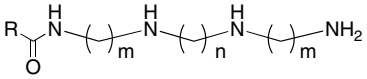


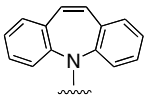
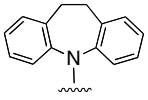
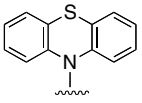
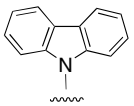
Figure 2. Effect of dihydrodibenzazepine-homospermine **8** on NR1/NR2A and NR1/NR2B receptors. Channel blocking activity of compound **8** was measured using an oocyte voltage clamped at −70 mV. Receptors were activated by superfusing glycine (Gly) and glutamate (Glu) (10 μM). Macroscopic currents were recorded with a two-electrode voltage clamp using a Gene Clamp 500 amplifier (molecular devices). (a) Representative traces are shown to illustrate the effect of 1 μM compound **8**. (b) Concentration–inhibition curves were determined at NR1/NR2A and NR1/NR2B receptors.

NR2B predominating in forebrain areas such as the cerebral cortex.[6] Thus, IC_{50} values were obtained from concentration–inhibition curves with five or six concentrations of each compound at NR1/NR2A or NR1/NR2B NMDA receptors.[7] Representative traces of the effect of 1 μM compound **8** on NR1/NR2A and NR1/NR2B receptors are shown in Figure 2a, and concentration and inhibition curves of compound **8** are shown in Figure 2b.

Compared to memantine and the anthraquinone derivative, AQ343 (**1**), almost all the conjugates (**3**–**14**) prepared in the present study were potent antagonists of recombinant NMDA receptors (Table 1). Particularly, dihydrodibenzazepine-tetraamine (**8**) (IC_{50} 0.072 μM at NR1/NR2A), phenothiazine-tetraamine (**10**) (IC_{50} 0.078 μM at NR1/NR2A), and carbazole-spermine (**12**) (IC_{50} 0.083 μM at NR1/NR2B) were the most effective compounds of this series, showing approximately

Table 1. Biological assay for memantine and compounds **1** and **3**–**14**



Compound	R	*m*	*n*	Channel block IC_{50}[a] (μM)		Cytotoxicity[b]	Selectivity index
				NR1/NR2A	NR1/NR2B	IC_{50} (μM)	Cytotoxicity/channel block[c]
Memantine	—	—	—	0.911 ± 0.043	1.02 ± 0.077	147 ± 10.3	152
1 (AQ343)		3	4	0.392 ± 0.040	0.491 ± 0.060	14.9 ± 0.38	33.7
3		3	4	0.203 ± 0.032	0.199 ± 0.021	97.6 ± 1.49	486
4		4	3	0.198 ± 0.013	0.335 ± 0.103	115 ± 2.89	432
5		4	4	0.240 ± 0.045	0.293 ± 0.063	132 ± 8.77	495
6		3	4	0.958 ± 0.064	0.442 ± 0.063	102 ± 4.21	146
7		4	3	0.117 ± 0.013	0.270 ± 0.015	169 ± 15.9	873
8		4	4	0.072 ± 0.017	0.142 ± 0.023	133 ± 8.15	1242
9		3	4	0.215 ± 0.009	0.348 ± 0.032	57.1 ± 4.60	202
10		4	3	0.078 ± 0.021	0.108 ± 0.009	32.5 ± 3.81	349
11		4	4	0.127 ± 0.019	0.108 ± 0.030	40.4 ± 2.69	344
12		3	4	0.145 ± 0.051	0.083 ± 0.019	31.3 ± 9.48	275
13		4	3	0.211 ± 0.013	0.189 ± 0.043	42.3 ± 4.21	212
14		4	4	0.111 ± 0.029	0.166 ± 0.033	84.2 ± 2.17	607

[a] Oocytes were injected with NR1 plus NR2 cRNAs in a ratio of 1:5 (0.1–4 ng of NR1 plus 0.5–20 ng of NR2). Microscopic currents were recorded at −70 mV. Values are means ± SEM of four oocytes.[7]

[b] FM3A cells (1×10^4) were cultured in ES medium in the presence or absence of various concentrations of each compound at 37 °C for 48 h. The number of cells was counted in the presence of 0.05% Trypan blue. IC_{50} values are means ± SD of triplicate determinations.[8]

[c] Selectivity index (cytotoxicity/channel block) was determined using the mean value of IC_{50} of NR1/N2A and NR1/NR2B NMDA receptors.[8]

12-fold stronger activity than memantine. Furthermore, it was found that the optimal length of the tetraamine part (343, 434, or 444) required to exhibit potent activity depended on the structure of the head moiety (Scheme 2).

The degree of inhibition of cell growth (IC_{50}) was then examined using mouse mammary carcinoma FM3A cells and five or six concentrations of each compound.[8] Compounds (**3**–**8**) having a dibenzazepine or dihydrodibenzazepine head showed weak cytotoxicity similar to memantine, which was nearly 10-fold weaker than our lead compound, AQ343 (**1**). Because of the decrease in cytotoxicity of the newly synthesized compounds, the ratio of channel blocking activity to cytotoxicity (see selectivity index in Table 1) of our compounds was superior to that of memantine. In particular, the selectivity index of dihydrodibenzazepine-homospermine (**8**) was 8-fold higher than that of memantine.

In summary, we have succeeded in developing a new class of NMDA channel blockers having a conjugate structure that consists of a nitrogenous heterocyclic head and a tetraamine tail. Some of the compounds have potent antagonistic activities compared with the lead compound, AQ343, or memantine. Further, several compounds have appropriate biological profiles, i.e., weak cytotoxicity, for the development of therapeutic agents for neurological disorders. Investigations, including in vivo experiments using these compounds, are under way.

Acknowledgments

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