Methyl *N*-(carbazolyl)acetyl-2-aminotetrahydrobenzothiophene-3-carboxylates as modulators of NMDA receptors

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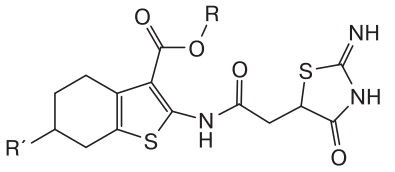
Methyl *N*-(carbazolyl)acetyl-2-aminotetrahydrothiophene-3-carboxylates were synthesized by alkylation of carbazoles with the corresponding chloroacetylamides of 2-aminotiophenes. The radioligand binding method showed the presence of the modulating effect of the synthesized compounds on the neuronal NMDA receptors.

Key words: methyl 2-aminothiophene-3-carboxylates, NMDA receptors, radioligand binding.

2-Aminotetrahydrobenzothiophene-3-carboxylic acid derivatives belong to a class of biologically active compounds promising in the search for drugs for the treatment of various neurodegenerative diseases. Thus, it was shown that methyl 2-amino-6-*tert*-butyl-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carboxylate (TBTC) is a selective antagonist of the retinoid X-α-receptor and improves the cognitive abilities of transgenic mice simulating Alzheimer's disease,[1] while *N*-acylamides represent a new class of regulators of calcium-activated chloride channel conductivity (the most active compound is CaCCnh-01).[2] Aminothiophene derivatives **1** containing the iminothioimidazolinone ring exhibit the properties of positive and negative allosteric modulators of NMDA receptors, and they were used as an example to demonstrate the possibility of both inhibition and potentiation of the receptor active sites.[3,4] In our earlier works, we showed that TBTC and thiourea **2** have both the blocking and the potentiating activity with respect to calcium-activated chloride channel current,[5] while 2-aminothiophenes **3** having a 4-trifluoromethylimidazolone ring at the amino group increase the binding of labeled ligands to the binding site of both MK-801 and ifenprodil, *i.e.*, act as positive allosteric modulators of NMDA receptors.[6]

One of the approaches of rational molecular design in the search for new therapeutically promising compounds[7,8] is the introduction of pharmacophoric substituents at the amino group of the 2-aminothiophene fragment. It was shown earlier in other[9,10] and our[11] works, that amino-substituted carbazole derivatives exhibit neuroprotective and proneurogenic activity. The purpose of the present work is the synthesis of methyl *N*-carbazolylacetyl-2-aminotetrahydrobenzothiophene-3-carboxylates and the



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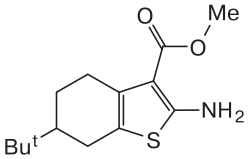
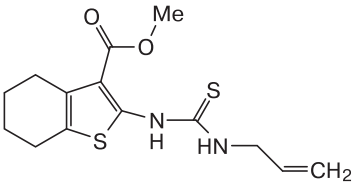


TBTC



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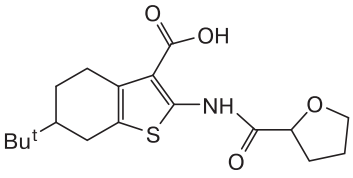
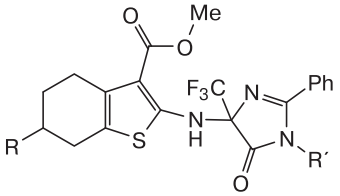


CaCCnh-01



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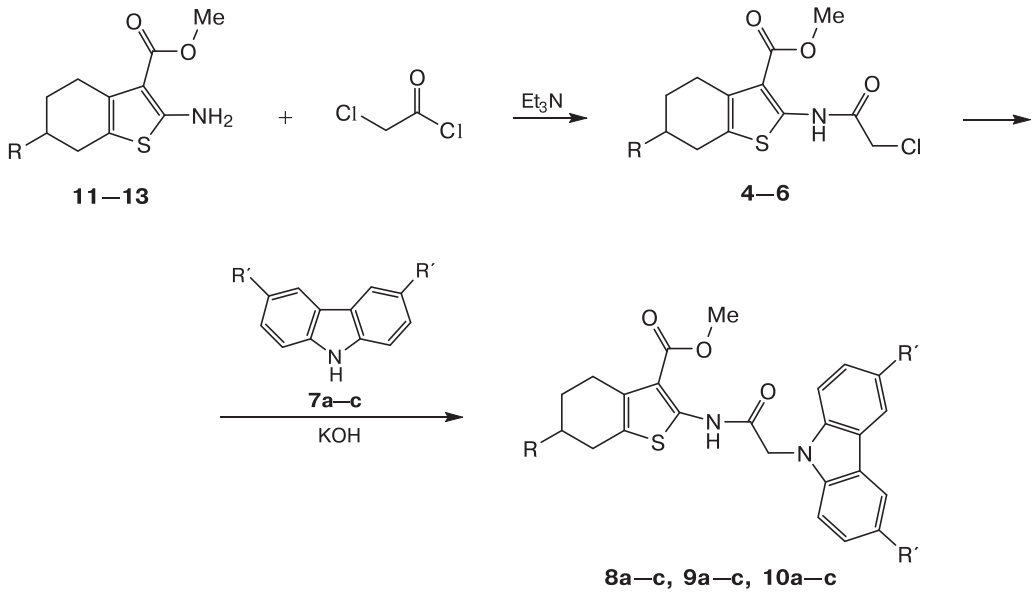
Scheme 1



R = H (**4**, **8**, **11**), Me (**5**, **9**, **12**), Bu^t (**6, 10**, **13**); R′ = H (**a**), Cl (**b**), Br (**c**)

Table 1. Yields, melting points, and elemental analysis data for compounds **4—6**, **8a—c**, **9a—c**, and **10a—c**

Compound	Yield (%)	M.p./°C	Molecular formula	Found (%) / Calculated		
				C	H	N
4	66	111—112	$C_{12}H_{14}ClNO_3S$	50.40	4.85	4.69
				50.09	4.90	4.87
5	59	116—118	$C_{13}H_{16}ClNO_3S$	51.63	5.19	4.50
				51.74	5.34	4.64
6	71	126—127	$C_{16}H_{22}ClNO_3S$	55.95	6.32	4.30
				55.89	6.45	4.07
8a	77	196—198	$C_{24}H_{22}N_2O_3S$	68.94	5.48	6.63
				68.88	5.30	6.69
8b	81	226—227	$C_{24}H_{20}Cl_2N_2O_3S$	59.25	4.27	5.52
				59.14	4.14	5.75
8c	86	232—234	$C_{24}H_{20}Br_2N_2O_3S$	50.25	3.65	4.55
				50.02	3.50	4.86
9a	78	220—221	$C_{25}H_{24}N_2O_3S$	61.15	5.16	7.21
				69.42	5.59	6.48
9b	79	215—217	$C_{25}H_{22}Cl_2N_2O_3S$	59.92	4.61	5.42
				59.88	4.42	5.59
9c	84	215—217	$C_{25}H_{22}Br_2N_2O_3S$	50.58	3.58	4.50
				50.86	3.76	4.75
10a	85	160—162	$C_{28}H_{30}N_2O_3S$	70.84	6.50	5.73
				70.86	6.37	5.90
10b	82	222—224	$C_{28}H_{28}Cl_2N_2O_3S$	61.65	5.41	5.28
				61.88	5.19	5.15
10c	81	263—265	$C_{28}H_{28}Br_2N_2O_3S$	53.44	4.21	4.62
				53.18	4.46	4.43

study of the biological activity of the synthesized compounds as modulators of NMDA receptors.

We showed that chloroacetylamidothiophenes **4—6** in solution in DMF in the presence of finely dispersed KOH

Table 2. ^{1}H NMR spectra of compounds **4—6**, **8a—c**, **9a—c**, and **10a—c** in $CDCl_3$

Compound	^{1}H NMR, δ (*J*/Hz)
4	1.67—1.94 (m, 4 H, CH_2); 2.58—2.89 (m, 4 H, CH_2); 3.91 (s, 3 H, CH_3O); 4.27 (s, 2 H, CH_2Cl); 12.15 (s, 1 H, NH)
5	1.07 (d, 3 H, $\underline{CH_3}CH$, $J_{H,H}$ = 6.5); 1.27—1.50 (m, 1 H, $CH_3\underline{CH}$); 1.78—2.00 (m, 2 H, CH_2); 2.16—2.36 (m, 1 H, CH_2); 2.54—2.90 (m, 2 H, CH_2); 2.86—3.03 (m, 1 H, CH_2); 3.90 (s, 3 H, CH_3O); 4.26 (s, 2 H, CH_2Cl); 2.09 (s, 1 H, NH)
6	10.93 (s, 9 H, $\underline{CH_3}CH$, $J_{H,H}$ = 6.5); 1.14—1.56 (m, 2 H, $CH_3\underline{CH}$ + CH_2); 1.92—2.08 (m, 1 H, CH_2); 2.29—2.78 (m, 3 H, CH_2); 2.93—3.11 (m, 1 H, CH_2); 3.88 (s, 3 H, CH_3O); 4.25 (s, 2 H, CH_2Cl); 12.09 (s, 1 H, NH)
8a	1.60—1.96 (m, 4 H, CH_2); 2.52—2.82 (m, 4 H, CH_2); 3.01 (s, 3 H, CH_3O); 5.17 (s, 2 H, CH_2N); 7.22—7.65 (m, 6 H, $C_{Ar}H$); 8.20 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 7.6); 10.70 (s, 1 H, NH)
8b	1.62—1.92 (m, 4 H, CH_2); 2.52—2.82 (m, 4 H, CH_2); 3.37 (s, 3 H, CH_3O); 5.12 (s, 2 H, CH_2N); 7.33 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.8); 7.49 (dd, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.8, 1.8); 8.07 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 1.8); 11.08 (s, 1 H, NH)
8c	1.64—1.91 (m, 4 H, CH_2); 2.56—2.75 (m, 4 H, CH_2); 3.38 (s, 3 H, CH_3O); 5.10 (s, 2 H, CH_2N); 7.29 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.7); 7.62 (dd, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.7, 1.9); 8.22 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 1.9); 11.09 (s, 1 H, NH)
9a	1.06 (d, 3 H, $\underline{CH_3}CH$, $J_{H,H}$ = 6.3); 1.22—1.46 (m, 1 H, $CH_3\underline{CH}$); 1.72—1.99 (m, 2 H, CH_2); 2.15—2.37 (m, 1 H, CH_2); 2.43—2.95 (m, 3 H, CH_2); 3.01 (s, 3 H, CH_3O); 5.16 (s, 2 H, CH_2N); 7.26—7.64 (m, 6 H, $C_{Ar}H$); 8.19 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 7.6); 10.69 (s, 1 H, NH)
9b	1.06 (d, 3 H, $\underline{CH_3}CH$, $J_{H,H}$ = 6.5); 1.21—1.46 (m, 1 H, $CH_3\underline{CH}$); 1.73—1.99 (m, 2 H, CH_2); 2.15—2.36 (m, 1 H, CH_2); 2.43—2.94 (m, 3 H, CH_2); 3.37 (s, 3 H, CH_3O); 5.11 (s, 2 H, CH_2N); 7.33 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.8); 7.49 (dd, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.8, 2.0); 8.07 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 2.0); 11.07 (s, 1 H, NH)
9c	1.07 (d, 3 H, $\underline{CH_3}CH$, $J_{H,H}$ = 6.5); 1.22—1.46 (m, 1 H, $CH_3\underline{CH}$); 1.72—1.98 (m, 2 H, CH_2); 2.14—2.36 (m, 1 H, CH_2); 2.44—2.94 (m, 3 H, CH_2); 3.38 (s, 3 H, CH_3O); 5.11 (s, 2 H, CH_2N); 7.29 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.7); 7.63 (dd, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.7, 1.9); 8.23 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 1.9); 11.08 (s, 1 H, NH)
10a	0.90 (s, 9 H, $\underline{CH_3}CH$, $J_{H,H}$ = 6.5); 1.09—1.54 (m, 2 H, $CH_3\underline{CH}$ + CH_2); 1.82—2.08 (m, 1 H, CH_2); 2.21—2.52 (m, 2 H, CH_2); 2.57—3.01 (m, 2 H, CH_2); 3.10 (s, 3 H, CH_3O); 5.17 (s, 2 H, CH_2N); 7.17—7.63 (m, 6 H, $C_{Ar}H$); 8.13 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 7.6); 10.74 (s, 1 H, NH)
10b	0.90 (s, 9 H, $\underline{CH_3}CH$, $J_{H,H}$ = 6.5); 1.06—1.52 (m, 2 H, $CH_3\underline{CH}$ + CH_2); 1.85—2.06 (m, 1 H, CH_2); 2.22—2.54 (m, 2 H, CH_2); 2.56—2.75 (m, 1 H, CH_2); 2.78—3.01 (m, 1 H, CH_2); 3.33 (s, 3 H, CH_3O); 5.08 (s, 2 H, CH_2N); 7.29 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.7); 7.45 (dd, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.7, 1.9); 8.04 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 1.9); 11.02 (s, 1 H, NH)
10c	0.90 (s, 9 H, $\underline{CH_3}CH$, $J_{H,H}$ = 6.5); 1.08—1.50 (m, 2 H, $CH_3\underline{CH}$ + CH_2); 1.84—2.06 (m, 1 H, CH_2); 2.21—2.53 (m, 2 H, CH_2); 2.55—2.74 (m, 1 H, CH_2); 2.77—3.00 (m, 1 H, CH_2); 3.33 (s, 3 H, CH_3O); 5.06 (s, 2 H, CH_2N); 7.24 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.8); 7.57 (dd, 2 H, $C_{Ar}H$, $J_{H,H}$ = 8.8, 2.0); 8.17 (d, 2 H, $C_{Ar}H$, $J_{H,H}$ = 2.0); 11.00 (s, 1 H, NH)

easily alkylate carbazoles **7a—c** at the nitrogen atom to form the target compounds **8—10** (Scheme 1). To complete the reaction, the mixture was stirred for 3 h at 20 °C. After work-up with water and extraction with chloroform, aminothiophenes **8—10** were isolated and purified by column chromatography. The composition and structure of compounds **8a—c**, **9a—c**, and **10a—c**, which are crystalline solids obtained in 86—93% yield, were confirmed by elemental analysis and NMR spectroscopy (Tables 1 and 2). Chloroacetylamidothiophenes **4**,[12,13] **5**, and **6** were obtained in 58—86% yield by the reaction of 2-aminothiophenes **11—13** with chloroacetyl chloride in the presence of Et_3N in solution in DMF with subsequent work-up with water, extraction with dichloromethane, and crystallization.

The biological activity of compounds **8a—c**, **9a—c**, and **10a—c** was studied by radioligand binding,[14,15] which allows one to quantify the effect of compounds on neuronal, in particular, NMDA receptors — one of the three main types of ionotropic glutamate receptors of the central nervous system of mammals playing a key role in the mechanisms of neuroprotection and neurotoxicity.

Table 3 presents the results on the effect of compounds **8a—c**, **9a—c**, and **10a—c** on the binding of labeled MK-801 and ifenprodil to NMDA receptor binding sites in the *in vitro* experiments at a concentration of 10^{-4} mol L^{-1}. The results show that almost all of the test compounds increase the binding of labeled ligands to both the MK-801 binding site and the ifenprodil binding site, *i.e.*, act as positive allosteric modulators of NMDA receptors, which recently are considered as potential therapeutic agents for the treatment of neuropsychiatric diseases.[16,17] The introduction of the carbazolylacetyl fragment into the aminothiophene molecules significantly increases the binding to the sites as compared to the previously described aminothiophenes containing five-membered trifluoromethyl-containing heterocycles at the nitrogen atom.[6] Compounds with the Bu^t-substituent in the cyclohexyl ring and dibromocarbazole derivatives showed the highest binding.

Table 3. Effects of compounds **8a—c**, **9a—c,** and **10a—c** on binding of[^{3}H]MK-801 and [^{3}H]ifenprodil with their sites on NMDA receptors

Compound	χ* (%)	
	[^{3}H]MK-801	[^{3}H]ifenprodil
3**	212	223
8a	170±9.3	228±18.7
8b	356±9.8	662±30.3
8c	220±10.5	437±28.5
9a	155±4.3	229±15.2
9b	200±8.7	300±5.5
9c	225±11.4	314±9.4
10a	290±11.6	648±24.7
10b	247±12.2	437±12.4
10c	560±18.6	1177±23.3

* χ is the change in binding compared to control. Binding with control is taken for 100%.
** Compound **3** with R = Bu^t and R′ = H is the most active modulator of compounds with structure **3** (see Ref. 6).

In conclusion, the modification of 2-aminotetrahydrobenzothiophenes at the amino group with carbazolylacetyl substituents was accomplished, it was found that the synthesized compounds can be considered as a new class of positive modulators of NMDA receptors.

Experimental

^{1}H NMR spectra were recorded on a Bruker DPX 200 spectrometer (200.13 MHz) relative to tetramethylsilane (internal standard). Melting points were determined in a glass capillary. The starting 2-aminothiophenes **11—13** were synthesized according to the procedure described earlier,[18] carbazoles **7a—c** (Aldrich) were used without prior purification.

Methyl 2-chloroacetylamino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (4), methyl 2-chloroacetylamino-6-methyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (5), and methyl 6-*tert*-butyl-2-chloroacetylamino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (6) (general procedure). Chloroacetyl chloride (1.1 g, 0.1 mol) was added to a solution of 2-aminothiophene **11—13** (0.01 mol) and Et_3N (1.0 g, 0.01 mol) in DMF (20 mL) at 20 °C with stirring. The reaction mass was stirred for 30 min, poured into H_2O (50 mL), extracted with chloroform (2×10 mL), dried over Na_2SO_4. Chloroform was evaporated, the residue was crystallized from a mixture hexane—chloroform, 10 : 1.

Methyl 6-alkyl-2-{[9*H*-carbazol-9-yl)acetyl]amino}-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylates (8—10) (general procedure). A mixture of carbazole **7a** (0.17 g, 1 mmol), chloroacetamide **4** (0.29 g, 1 mmol), KOH (0.06 g, 1 mmol) in DMF (1.5 mL) was stirred at 20 °C for 3 h. After addition of water (10 mL), the mixture was extracted with dichloromethane, which then was evaporated. The residue was chromatographed on silica gel (60 mesh, eluent methanol—chloroform, 1 : 10).

Yields, melting points, elemental analysis data, and spectral characteristics of compounds **4—6**, **8a—c**, **9a—c**, and **10a—c** are given in Tables 1 and 2.

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