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# SYNTHESIS AND NEUROPROTECTIVE PROPERTIES OF ISOSTERIC ANALOGS OF NICOTINE*

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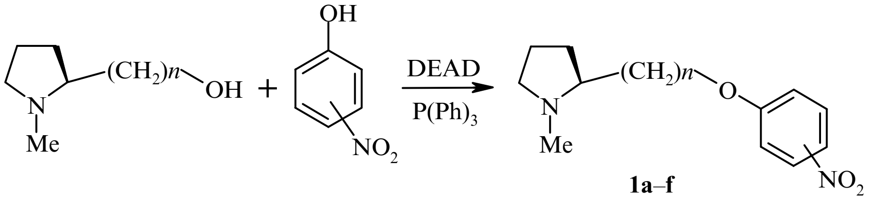
*A method has been developed for the synthesis of isosteric analogs of nicotine involving ethers of S(-)-1-2-pyrrolidinemethanol and S(-)-1-2-pyrrolidineethanol based on the Mitsunobu reaction. The results of testing the synthesized compounds as calcium channel blockers are presented.*

**Keywords:** prolinol and homoprolinol ethers, neuroprotectors, calcium channel blockers, Mitsunobu reaction

Pyridine series ethers, including pyrrolidine derivatives as the second fragment, show agonist activity towards acetylcholine receptors and, in some cases, exceed the activity of *S*(-)-nicotine [1]. In this work we have undertaken a focused search for compounds in the series of nicotine bioisosteric analogs ethers able not only to show inherent agonist activity (affinity towards the nicotine receptor) but also to possess marked neuroprotective properties. The neuroprotective properties of the substances appear as a specific block of neurotoxic glutamate induced input of calcium ions.

Nitrophenols can be considered as bioisosteric analogs of hydroxypyridines. They have been used for the synthesis of prolinol ethers *via* the Mitsunobu reaction [1-3].



**1a–f**

The reaction of *o*-, *m*-, and *p*-nitrophenols with N-methylprolinol and N-methylhomoprolinol in the presence of triphenylphosphine and diethylazodicarboxylate (DEAD) occurs in THF at room temperature in the course of 12 h (monitored by TLC). The yields of ethers, as calculated from the amount of unreacted nitrophenol, are given in Table 1.

The data for the yields of the corresponding ethers can be correlated with the increase in p$K_a$ of the acid component, the highest yield of the reaction products **1a-f** being achieved for *o*- and *p*-nitrophenols. Since the mechanism of the reaction assumes an acid-base type interaction of betaine diethylazodicarboxylate with the acid component (nitrophenol), the value of p$K_a$ 7 [4, 5] for this acid component plays a significant role in the successful course of the reaction.

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* Dedicated to A. N. Kost on the occasion of his 85th birthday.

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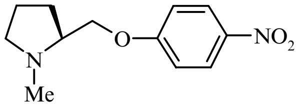
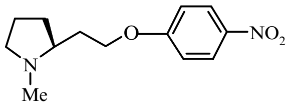
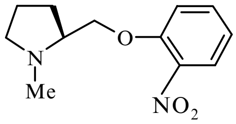
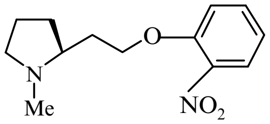
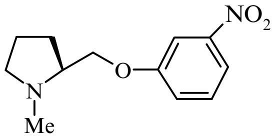
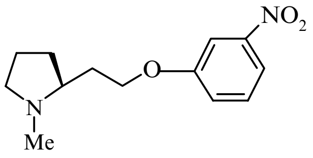
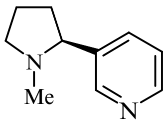
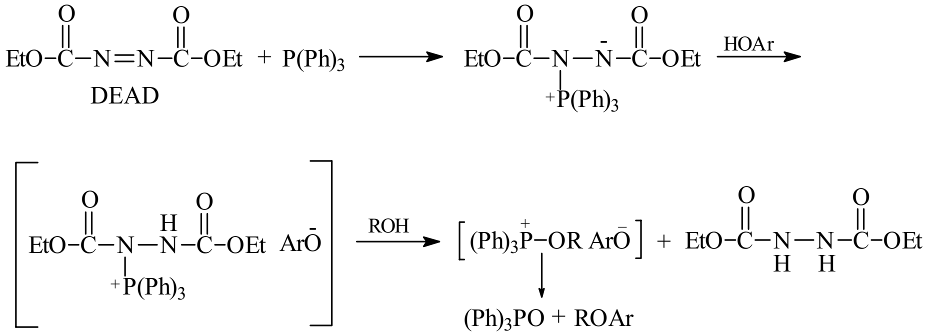
TABLE 1. Yields, $^1$H NMR Spectra and Activity of Compounds **2**

| Compound | Yield of ether, % | Activity of hydrochlorides, % | $^1$H NMR spectra of hydrochlorides, δ, ppm |
|---|---|---|---|
| **1a**<br> | 89 | **2a**<br>65 | 7.96 (2H, d, $\mathrm{H_{arom}}$);<br>6.86 (2H, d, $\mathrm{H_{arom}}$);<br>4.28 (1H, dd, CHO);<br>4.11 (1H, dd, CHO);<br>3.74-3.58 (1H, m, $\mathrm{CH(CH_2O)}$);<br>3.58-3.42 (1H, m, $\mathrm{CH(NCH_3)}$);<br>3.08-2.90 (1H, m $\mathrm{CH(NCH_3)}$);<br>2.78 (3H, s $\mathrm{NCH_3}$);<br>2.34-1.71 (4H, m, $\mathrm{CH_2CH_2}$)) |
| **1b**<br> | 90 | **2b**<br>73 | 8.15 (2H, d, $\mathrm{H_{arom}}$);<br>7.03 (2H, d, $\mathrm{H_{arom}}$);<br>4.24 (2H, dd, $\mathrm{CH_2O}$);<br>3.79-3.48 (2H, $\mathrm{CH_2}$, m, $\mathrm{(NCH_3)}$);<br>3.32-3.02 (1H, m, $\mathrm{CH(NCH_3)}$);<br>2.89 (3H, s, $\mathrm{NCH_3}$);<br>2.77-2.29 (2H, m, $\mathrm{CH_2(CH_2O)}$);<br>2.25-1.69 (4H, m, $\mathrm{CH_2CH_2}$)) |
| **1c**<br> | 98 | **2c**<br>74 | 7.89 (1H, dd, $\mathrm{H_{arom}}$);<br>7.65-7.43 (1H, m, $\mathrm{H_{arom}}$);<br>7.22 (1H, d, $\mathrm{H_{arom}}$):<br>7.08 (1H, t, $\mathrm{H_{arom}}$)<br>4.52 (1H, dd, CHO);<br>4.24 (1H, dd, CHO);<br>3.87-3.73 (1H, m, $\mathrm{CH(CH_2O)}$);<br>3.70-3.56 (1H, m, $\mathrm{CH(NCH_3)}$);<br>3.22-3.03 (1H, m, $\mathrm{CH(NCH_3)}$);<br>2.97 (3H, s, $\mathrm{NCH_3}$);<br>2.40-1.88 (4H, m, $\mathrm{CH_2CH_2}$) |
| **1d**<br> | 95 | **2d**<br>77 | 7.86 (1H dd, $\mathrm{H_{arom}}$);<br>7.66-7.44 (1H, m, $\mathrm{H_{arom}}$);<br>7.17 (1H, d, $\mathrm{H_{arom}}$);<br>7.04 (1H, t, $\mathrm{H_{arom}}$);<br>4.18 (2H, dd, $\mathrm{CH_2O}$);<br>3.78-3.42 (2H, m, $\mathrm{CH_2(NCH_3)}$);<br>3.24-3.06 (1H, m, $\mathrm{CH(NCH_3)}$);<br>2.92 (3H, s. $\mathrm{NCH_3}$);<br>2.50-2.22 (2H, m, $\mathrm{CH_2(CH_2O)}$);<br>2.20-1.71 (4H, m, $\mathrm{CH_2CH_2}$) |
| **1e**<br> | 61 | **2e**<br>80 | 7.83-7.60 (m, $\mathrm{H_{arom}}$);<br>7.44-7.12 (m, $\mathrm{H_{arom}}$);<br>4.42 (1H, dd, CHO);<br>4.24 (1H, dd, CHO);<br>3.98-3.77 (1H, m, $\mathrm{CH(CH_2O)}$);<br>3.77-3.56 (1H, m, $\mathrm{CH(NCH_3)}$);<br>3.31-3.12 (1H, m, $\mathrm{CH(NCH_3)}$);<br>3.02 (3H, s, $\mathrm{NCH_3}$);<br>2.5-1.90 (4H, m, $\mathrm{CH_2CH_2}$) |
| **1f**<br> | 53 | **2f**<br>79 | 7.81-7.62 (m, $\mathrm{H_{arom}}$);<br>7.44-7.10 (m, $\mathrm{H_{arom}}$);<br>4.44 (2H, m, $\mathrm{CH_2O}$);<br>3.95-3.67 (2H, m, $\mathrm{CH_2(NCH_3)}$);<br>3.52-3.24 (1H, m, $\mathrm{CH(NCH_3)}$);<br>3.00 (3H, s, $\mathrm{NCH_3}$);<br>2.94-2.50 (2H, m, $\mathrm{CH_2(CH_2O)}$);<br>2.48-1.90 (4H, m, $\mathrm{CH_2CH_2}$) |
|  | — | 77 | — |



This is in agreement with literature data for the synthesis of ethers of 3-hydroxypyridine (p$K_a$ 8.72 [4]), the yield of which does not exceed 15% in the Mitsunobu reaction [1, 2].

The increase in the length of the hydrocarbon chain by one methylene group on going from prolinol to homoprolinol does not influence the reaction course and yields of the corresponding ethers in a significant way.

For carrying out biological testing ethers **1a-f** were converted to the water soluble hydrochloride form. The structure and composition of hydrochlorides **2a-f** obtained were confirmed by $^1$H NMR spectroscopic data and by elemental analysis.

Hydrochlorides prepared were tested for physiological activity as glutamate receptor blockers. The results are presented in Table 1. It should be noted that, besides the series of ethers studied by us, we have also tested (*S*)-nicotine for physiological activity as glutamate receptor blocker for the first time. As is evident from the table, the most successful compound proved to be **2a**, since it has the highest physiological activity in the given series and exceeds the activity of (*S*)-nicotine.

Hence it was shown that, within the scope of the series of compounds investigated, bioisosteric analogs of nicotine which contain a pyrrolidine fragment have higher neuroprotective properties than the lead compound (*S*)-nicotine itself.

## EXPERIMENTAL

$^1$H NMR spectra were recorded on a Varian instrument working at 200 MHz using $D_2O$ solvent. TLC on Silufol plates was used to follow the course of the reactions. Chromatographic separations were carried out on Merck 60 (70-230 mesh, ASTM) silica gel columns. Testing of specific blocking of the neurotoxic glutamate induced entry of $Ca^{2+}$ ions was carried out at the Institute of Physiologically Active Substances, Russian Academy of Sciences.

THF was successively distilled over base, sodium, and lithium aluminium hydride and had bp 65°C. Diethyl ether was also successively distilled over base, sodium, and lithium aluminium hydride and had bp 34°C. Chloroform was extracted with concentrated sulfuric acid, washed with water, dried over $CaCl_2$, and distilled, bp 61°C. Triphenylphosphine was twice recrystallized from isopropanol and dried in vacuo, mp 79-80°C. Ethyl chloroformate was obtained from the Aldrich company; bp 93°C, $n_D^{20} = 1.3950$.

(*S*)-(-)-Methyl-2-pyrrolidinemethanol, Aldrich company; bp 67-69°C / 12 mm Hg, $n_D^{20} = 1.4690$, $[\alpha]^{19}$ -49.5° (c = 5, MeOH).

(*S*)-(-)-Methyl-2-pyrrolidineethanol, Aldrich company; bp 110-112°C / 14 mm Hg, $n_D^{20} = 1.4713$. 3-Hydroxypyridine, Aldrich company; mp 126-129°C, bp 151-153°C.

**Method for Estimation the Calcium Blocking Properties of the Compounds.** Reaction of the compounds with the system glutamate dependent $Ca^{2+}$ uptake was investigated on the synaptosome P2 fraction separated from neonate rat brains (8-11 days) by the method reported in [6]. Synaptosomes were placed in an incubating buffer A (132 mmol NaCl, 51 mmol KCl, 5 mmol HEPES) and held at a temperature of 0°C for the course of the whole experiment. Aliquots of the synaptosomes (50 μl) were placed in medium A containing the investigated compound and the $^{45}Ca^{2+}$ preparation. Uptake of $Ca^{2+}$ was stimulated by introduction of 20 μl glutamate into 10 ml of the solution. After incubating for 5 min at 30°C, the process was stopped by filtration through a GF/B filter and the preparation was washed three times with cold acetic acid. The radiolabel was then detected on an SL-4000 scintillation counter. The specific uptake of $Ca^{2+}$ was measured by the equation:

$$K_{(42/21)} = [(Ca_4 - Ca_2)/(Ca_2 - Ca_1)]\ 100\%,$$

where $Ca_1$ is the uptake in the control experiment (without glutamate and the test compound); $Ca_2$ is the uptake in the presence of glutamate (only glutamate induced uptake); $Ca_3$ is the uptake in the presence of the test compound (without glutamate); $Ca_4$ is the uptake in the presence of glutamate and the test compound. In separate examples the specific uptake of calcium is also calculated from the equation:

$$K_{(43/21)} = [(Ca_4 - Ca_3)/(Ca_2 - Ca_1)]\ 100\%$$
$$\text{or } K_{(3/1)} = [(Ca_3)/(Ca_1)]\ 100\%.$$

**Diethylhydrazodicarboxylate.** Ethyl chloroformate (30.7 g, 0.283 mol) was added dropwise with stirring to a solution of hydrazine hydrate (6.8 g, 0.136 mol) in alcohol (96%, 60 ml) cooled to 10°C at such a rate that the temperature did not go above 20°C. After the addition of half of ethyl chloroformate, a solution of $Na_2CO_3$ (12.2 g) in water (60 ml) was added dropwise simultaneously with the second half of ethyl chloroformate at a temperature below 20°C. Following this salt from walls was washed with water (25 ml) and stirred for a further 30 min. The precipitate was then filtered, washed with water (100 ml), and dried at 80°C to give 13.4 g (56%) of diethylhydrazodicarboxylate; mp 131-132°C.

**Diethylazodicarboxylate.** Chlorine was passed slowly with stirring through a mixture of diethylhydrazodicarboxylate (13.4 g, 76.16 mmol), water (60 ml), and benzene (60 ml) at a temperature not exceeding 15°C until the added weight amounted to 7 g. After this, the passage of chlorine was stopped. The mixture was stirred until a clear orange benzene layer was formed after interruption of stirring. The layer was separated, washed with water and $Na_2CO_3$ solution to a neutral reaction, and dried over $Na_2SO_4$. Solvent was evaporated and the residue was distilled in vacuo. Diethylazodicarboxylate (10.5 g, 81%) was obtained; bp 107-109°C / 15 mm Hg and $n_D^{20} = 1.4201$.

**General Method for Preparing Hydrochlorides of Nitrophenol Ethers 2a-f Using the Mitsunobu Reaction.** Diethylazodicarboxylate (1.02 ml, 6.5 mmol) was added dropwise with stirring at -3°C to a solution of triphenylphosphine (1.71 g, 6.5 mmol) in THF. Nitrophenol (0.625 g, 4.5 mmol) was added in one portion to the clear solution obtained. The product was stirred for 10 min. After this N-methylprolinol (4.34 mmol) or homoprolinol were added. The mixture was stirred in an argon stream for 24 h, solvent was evaporated, and the residue was chromatographed on a silica gel column eluting with chloroform. After separation of triphenylphosphine oxide and diethylhydrazodicarboxylate, elution was continued using a mixture of chloroform and methanol (10:1). The solvent was evaporated. The oil obtained was dissolved in a minimum volume of methanol, cooled, and diethyl ether saturated with HCl was added dropwise. The precipitated white solid was filtered off and washed with ether.

**4-[(1-Methyl-2(*S*)-pyrrolidinyl)methoxy]nitrobenzene hydrochloride (2a).** Mp 133°C. Found, %: C 49.38; H 6.25; N 9.99. $C_{12}H_{17}ClN_2O_3$. Calculated, %: C 52.83; H 6.29; N 10.28.

**4-[(1-Methyl-2(*S*)-pyrrolidinyl)ethoxy]nitrobenzene hydrochloride (2b).** Mp 93-94°C. Found, %: C 52.57, H 6.29; N 9.25. $C_{13}H_{19}ClN_2O_3$. Calculated, %: C 54.45; H 6.68; N 9.77.

**2-[(1-Methyl-2(*S*)-pyrrolidinyl)ethoxy]nitrobenzene hydrochloride (2d).** Found, %: C 53.05; H 6.54; N 9.32. $C_{13}H_{19}ClN_2O_3$. Calculated, %: C 54.45; H 6.68; N 9.77.

**2-[(1-Methyl-2(*S*)-pyrrolidinyl)methoxy]nitrobenzene hydrochloride (2c).** Found, %: C 50.08; H 6.43; N 9.95. $C_{12}H_{17}ClN_2O_3$. Calculated, %: C 52.83; H 6.29; N 10.28.

**3-[(1-Methyl-2(*S*)-pyrrolidinyl)ethoxy]nitrobenzene hydrochloride (2f).** Found, %: C 51.45; H 6.18; N 9.50. $C_{13}H_{19}ClN_2O_3$. C 54.45; H 6.68; N 9.77.

**3-[(1-Methyl-2(*S*)-pyrrolidinyl)methoxy]nitrobenzene hydrochloride (2e).** Found, %: C 48.99; H 6.30; N 10.47. $C_{12}H_{17}ClN_2O_3$. Calculated, %: C 52.83; H 6.29; N 10.28.

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