SEARCH FOR NEW DRUGS

SYNTHESIS AND NEUROTROPIC ACTIVITY OF (HETERYLPHENYLMETHYL)-AMINES AND -UREAS

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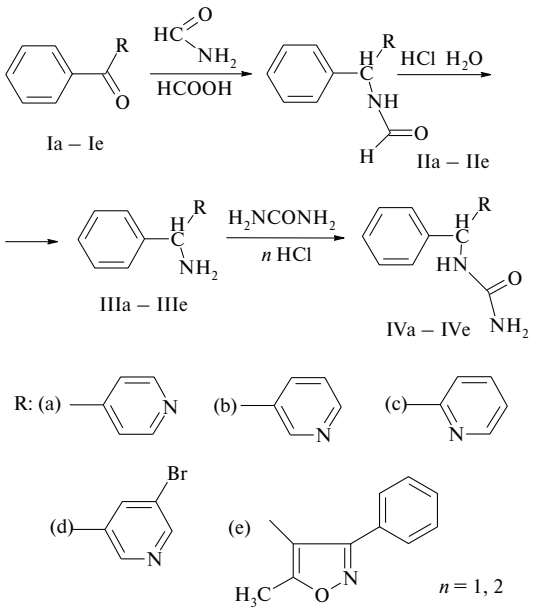
Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 35, No. 7, pp. 3 – 6, July, 2001.

Original article submitted November 21, 2000.

The antiepileptic drugs widely used in clinical practice, such as diphenin, phenobarbital, and benzonal, belong to the cyclic derivatives of urea [1]. Opening of the hydantoin ring between carbon in position 5 and nitrogen leads to a noncyclic urea derivative (α-diphenylacetylurea, known as the drug fenuron) still retaining the anticonvulsant properties [2].

In the search for new substances possessing neurotropic activity in the series of benzohydrylamines and benzohydrylureas, it was of interest to substitute a heterocyclic residue for one of the phenyl fragments. We have studied the pharmacological activity of a series of (heterylphenylmethyl)amines (IIIa – IIIe) and (heterylphenylmethyl)ureas (IVa – IVe) synthesized by the following scheme:



The initial compounds (Ia – Ie) were prepared by acylating benzene with chloroanhydrides of the corresponding heterocyclic acids by a conventional method [3] under Friedel – Crafts reaction conditions. The synthesis of new compounds proceeded by interaction of ketones I with ammonium formate under modified Leuckart reaction conditions [4], followed by acid hydrolysis of the intermediate formamides II to amines III. Finally, compounds IV were obtained using the condensation of compounds III with urea by the known method described in [5, 6].

In addition, we synthesized a series of amine IIIa analogs, including N-methyl, piperidine, and acetyl derivatives (compounds V – VII, respectively), and N-[(4-pyridyl)phenylmethyl]-N′-methylurea (VIII) representing an analog of compound IVa. [(4-Pyridyl)phenylmethyl]aminomethane dihydrochloride (compound V) was obtained using the interaction of ketone Ia with methylamine and formic acid under Leuckart reaction conditions [4], followed by hydrolysis of the reaction mass with hydrochloric acid without isolation of the intermediate formyl derivative. Compound VI was synthesized by reducing ketone Ia with aluminum isopropylate to the corresponding alcohol [7], followed by chlorine substitution for the oxy group with the aid of thionyl chloride, and condensation with piperidine by analogy with the method described in [8]. The acylation of amine IIIa with acetic anhydride using a procedure analogous to that described in [6] led to N-[(4-pyridyl)phenylmethyl]acetamide (compound VII), while interaction of the same amine IIIa with methyl isocyanate by analogy with the reaction described in [9] yielded compound VIII.

The yields and some physicochemical characteristics of the synthesized compounds are presented in Table 1.

EXPERIMENTAL CHEMICAL PART

The IR absorption spectra were measured on an UR-10 spectrophotometer (Germany) using samples prepared as nujol mulls. The mass spectra were obtained using an

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MKh-1303 spectrometer (Russia) equipped with a system of direct sample injection into the ion source operated at an electron impact energy of 30 eV. The chemical purity of the synthesized compounds was checked by TLC using Silufol UV-254 plates (Czech Republic) eluted in the chloroform – ethanol – NH_4OH (20 : 2 : 1) solvent system with R_f ~ 0.45 (for compound I), 0.6 (II), 0.5 (III), 0.05 (IV), 0.55 (V), 0.7 (VI), 0.3 (VII), and 0.2 (VIII).

The molecular masses of all compounds determined by mess spectrometry coincide with the calculated values, and the character of fragmentation is consistent with the proposed structures. The melting points (uncorrected) were determined using a Koefler heating stage. The data of elemental analyses correspond to the results of calculations using the empirical formulas.

N-(Heterylphenylmethyl)formamides (IIa – IIe). General method. To 422 mmole of a 25% aqueous ammonia is slowly added with stirring 422 mmole of formic acid 99,5%. The mixture is gradually (over 1.5 – 2 h) heated up to 160°C (while water and formic acid are simultaneously distilled off) and cooled to 90°C. Then 81.9 mmole of ketone I is added and the mixture is stirred for 15 min. After that, the pH of the medium, checked by a universal indicator, must not exceed 6.5. If the reaction mass shows an alkaline or neutral reaction, formic acid (~16 ml) is added to pH 4 – 4.5 and the reaction mixture is again heated to 160°C and kept at this temperature for ~6 h (until water is completely distilled off and gaseous carbon dioxide ceases to evolve). Then the reaction mass is cooled to 140°C and poured at this temperature into 90 ml of water, after which the mixture is cooled to 25°C and kept for 1 h at 0 – 5°C. The oil and aqueous layers are separated and the the latter is treated with 4 × 30 ml of chloroform. The extract is evaporated and the residue combined with the main oil fraction. Then 100 ml of benzene is added and the mixture is boiled in the presence of 1 g of activated charcoal. The charcoal is separated by filtration and washed with 20 ml of benzene. The total filtrate is mixed with 25 ml of hexane, cooled to 10°C, and filtered. The residue is washed with benzene (2 × 5 ml) and dried to obtain the target compound IIa – IIe (Table 1).

(Heterylphenylmethyl)amine hydrochlorides (IIIa – IIIe). General method. A mixture of 36 mmole of compound II, 20 ml of anhydrous isopropyl alcohol, and 7.8 ml of concentrated hydrochloric acid is boiled for ~3 h until the initial formamide II is fully consumed (TLC monitoring). Upon cooling down to room temperature, the mixture is neutralized with a 40% aqueous sodium hydroxide solution to pH 9 – 10 and amine III is extracted with ether (2 × 25 ml). The ether extracts are combined, dried over magnesium sulfate, and filtered. To this ether solution is added a solution of hydrogen chloride in isopropyl alcohol to pH 1 – 2, after which the resulting suspension is poured into 200 ml of acetone. The precipitate is filtered and dried to obtain dihydrochlorides of compounds IIIa and IIIb or hydrochlorides of compounds IIIc – IIIe (Table 1).

TABLE 1. Yields and Some Physicochemical Characteristics of (Heterylphenylmethyl)-formamides (IIa – IIe), -amines (IIIa – IIIe, V – VII), and -ureas (IVa – IVe)

Compound	Yield, %	M.p., °C	Empirical formula	IR spectrum: ν, cm^{-1}	Mass spectrum (M + H)
IIa	66	102.5 – 103.5	$C_{13}H_{12}N_2O$	3160 (NH), 1670(C=O)	213
IIb	81	104.0 – 105.5	$C_{13}H_{12}N_2O$	3170(NH), 1670(C=O)	213
IIc	70	107.0 – 109.0	$C_{13}H_{12}N_2O$	3165(NH), 1675(C=O)	213
IId	86	134.5 – 136.0	$C_{13}H_{11}BrN_2O$	3160 (NH), 1670(C=O)	291, 292
IIe	85	123.5 – 125.0	$C_{18}H_{16}N_2O$	3150(NH), 1690(C=N)	292
IIIa	92	230*	$C_{12}H_{12}N_2 \cdot 2HCl$	3455 (NH_3^+), 2580 (NH^+)	185
IIIb	51	250 – 260*	$C_{12}H_{12}N_2 \cdot 2HCl$	3450 (NH_3^+), 2600 (NH^+)	185
IIIc	60	224.5*	$C_{12}H_{12}IN_2 \cdot HCl$	3450 (NH_3^+)	185
IIId	59	243.5 – 244.5*	$C_{12}H_{11}BrN_2 \cdot HCl$	3445 (NH_3^+)	264, 265
IIIe	58	136 – 138*	$C_{17}H_{16}IN_2 \cdot HCl$	3400 (NH_3^+), 1690 (C = N)	264
IVa	78	226.5 – 227	$C_{13}H_{13}N_3O$	3420, 3285, 3150 (NH, NH_2), 1650 (C=O)	227
IVb	68	165.5 – 167	$C_{13}H_{13}N_3O \cdot H_2O$	3420, 3300, 3160 (NH, NH_2), 1650 (C=O)	227
IVc	61	197.5 (decomp.)	$C_{13}H_{13}N_3O$	3470.3310 (NH, NH_2), 1640 (C=O)	227
IVd	68	223.5 – 225	$C_{13}H_{11}N_2BrO$	3420, 3255 (NH_2), 3200 (NH), 1660 (C=O)	306
IVe	50	181 – 182	$C_{18}H_{17}N_3O_2$	3490, 3440, 3350 (NH, NH_2), 1650 (C=O)	307
V	39**	217 – 219*	$C_{13}H_{14}IN_2 \cdot 2HCl$	3420 (NH_2+), 2650 (NH+)	198
VI	38**	170 – 172*	$C_{17}H_{20}N_2 \cdot 2HCl$	2600 (NH+)	252
VII	82	131 – 132.5*	$C_{14}H_{14}N_2O$	3300 (NH), 1635 (C=O)	226
VIII	80	192 (decomp.)	$C_{14}H_{15}IN_3O \cdot HCl$	2325, 3270 (NH^+), 1670 (C=O)	242

* Amine salts melt with decomposition.
** Yields of compounds V and VI are calculated with respect to the initial ketone and carbinol, respectively.

(Heterylphenylmethyl)ureas (IVa – IVe). General method. To a thoroughly stirred mixture of 126 mmole of urea and 8.5 ml of water are added 21 mmole of amine III and 2.25 ml of concentrated hydrochloric acid. The reaction mass is slowly (over 1 h) heated from 20°C up to 104 – 105°C, boiled at this temperature for 3 h, and cooled down to ~20°C. The precipitate is filtered, washed with water until neutral reaction, dried, and recrystallized from methyl, ethyl, or isopropyl alcohol to obtain compounds IVa – IVe (Table 1).

N-[(4-Pyridyl)phenylmethyl]aminomethane dihydrochloride (V). To a solution of 7.2 g (104 mmole) of methylamine hydrochloride in 10 ml of water was added 4.3 g (104 mmole) of solid sodium hydroxide and the mixture was stirred for 10 min. To the resulting solution was gradually added 5.5 ml (140 mmole) of a 99% formic acid solution and the mixture was kept for 5 min (after which the pH must not exceed 3.5 – 4.0), gradually (over 2 h) heated to 172°C (until aqueous formic acid is completely distilled off), and cooled to 60°C. Then 3.66 g (20 mmole) of 4-benzoylpyridine (Ia) and 4.7 ml of a 99% formic acid are added and the mixture is stirred for 10 min, checked for pH (not to exceed 3.5 – 4.0), boiled at 148 – 180°C for ~11 h until the initial compound Ia is completely consumed (TLC monitoring) and gaseous carbon dioxide ceases to evolve, and cooled to ~ 20°C. Then 50 ml of water is added and the mixture is adjusted at pH 9 by adding solid sodium hydroxide, after which the oil and aqueous layers are separated and the the latter is treated with 3 × 25 ml of chloroform. The extracts are combined with the main oil fraction and the solvent is distilled off in vacuum. The residue is mixed with 4.3 ml of concentrated hydrochloric acid and the mixture is boiled for 3 h, charged with 6 ml of water and 1.0 g of activated charcoal, and treated at 70 – 75°C for 20 min, after which the charcoal is separated by filtration. The filtrate is discolored by adding sodium hydrosulfite, cooled to ~20°C, and adjusted to pH 9 by adding solid sodium hydroxide. The oil and aqueous layers are separated and the the latter is treated with 2 × 5 ml of chloroform. The extract is combined with the main oil fraction, the solvent is distilled off in vacuum, and the residue is dissolved at 40°C in 3 ml of isopropyl alcohol. To this solution are gradually added a 20% solution of hydrogen chloride in isopropyl alcohol (to pH 2) and then 60 ml of acetone. The mixture is cooled to 5 – 10°C and kept at this temperature for 1 h. The precipitate is filtered, washed with acetone (2 × 3 ml), dried, and recrystallized from 40 ml of anhydrous isopropyl alcohol to obtain 2.12 g of compound V (Table 1).

N-[(4-Pyridyl)phenylmethyl]piperidine dihydrochloride (VI). To an ice-cold solution of 3.5 ml of freshly distilled thionyl chloride in 10 ml of chloroform is added dropwise a solution of 6 g (32.5 mmole) of [(4-pyridyl)phenyl]methylcarbinol synthesized by a conventional method [10]. The mixture is gradually (over 1 h) heated to boiling (~60°C), treated at this temperature until the evolution of gaseous hydrogen chloride and sulfur dioxide completely ceases (~7 h), and cooled to room temperature. The precipitate (~0.5 g of the initial carbinol by TLC data) is separated by filtration and the filtrate is evaporated in vacuum to dryness and the residue (7 g) is dissolved at 40°C in 30 ml of water. The solution is charged with 0.5 g of activated charcoal and treated at this temperature for 20 min. Then charcoal is separated by filtration and washed with 2 × 5 ml of warm water. The filtered solution at ~20°C is adjusted to pH 9 by adding an aqueous Na_2CO_3 solution. The oil and aqueous layers are separated and the the latter is treated with 3 × 100 ml of chloroform. The extract is combined with the main oil fraction and the solvent is distilled off in vacuum at 40 – 45°C. The oil residue (5.5 g) is mixed with 30 ml of freshly distilled DMF, 4 ml of distilled piperidine, and 5.0 g of calcined and triturated potassium carbonate. The mixture is stirred at 45 – 50°C for ~20 h until complete consumption of the initial chlorine-substituted compound (TLC monitoring). The precipitate is filtered and washed with 5 ml of DMF. The filtrate is eva-

TABLE 2. Acute Toxicity and Neurotropic Activity of (Heterylphenylmethyl)-amines and -ureas

Compound	LD_{50}, mg/kg	Dose, mg/kg	Hexenal test (60 mg/kg)	Corazole test (1%, i.v.)
			Lateral position, min	Threshold dose, mg/kg
IIIa	225.0 ± 26.5	Control	35.0 ± 7.3	37.6 ± 2.4
		23	127.0 ± 11.6*	48.4 ± 3.2*
IIIb	288.0 ± 24.8	Control	45.0 ± 1.2	40.0 ± 2.8
		30	91.8 ± 11.2*	49.8 ± 3.1*
IIIc	101.0 ± 4.8	Control	53.4 ± 3.1	35.4 ± 3.1
		10	54.9 ± 7.1	39.9 ± 3.7
IIId	470.8 ± 21.8	Control	47.8 ± 3.1	36.8 ± 3.1
		47	71.0 ± 6.4*	39.0 ± 2.4
IIIe	396.0 ± 31.6	Control	51.8 ± 3.6	35.5 ± 2.6
		40	84.6 ± 5.8*	47.6 ± 3.7*
IVa	> 2000	Control	48.8 ± 2.6	41.7 ± 2.6
		200	355.8 ± 21.6*	40.6 ± 2.8
IVb	838 ± 35.4	Control	45.5 ± 2.1	38.9 ± 3.1
		84	81.5 ± 10.3*	37.8 ± 2.8
IVc	> 1000	Control	57.0 ± 8.5	35.7 ± 2.1
		100	78.5 ± 6.4*	37.4 ± 2.9
IVd	> 1000	Control	50.7 ± 3.2	41.9 ± 3.2
		100	36.0 ± 4.1*	44.0 ± 4.1
IVe	> 1000	Control	42.0 ± 4.2	41.3 ± 3.1
		100	87.6 ± 8.5*	51.8 ± 2.4*
V	215.5 ± 19.7	Control	35.6 ± 4.8	40.9 ± 3.8
		20	69.5 ± 5.8*	38.4 ± 3.1
VI	280.0 ± 25.6	Control	37.0 ± 5.4	41.5 ± 2.8
		28	132.0 ± 10.8*	42.6 ± 3.1
VII	410.0 ± 21.5	Control	51.7 ± 5.6	39.7 ± 3.4
		40	175.0 ± 8.5*	41.8 ± 2.7
VIII	390.8 ± 27.8	Control	47.9 ± 4.3	40.9 ± 3.3
		40	197.5 ± 4.8*	41.5 ± 4.1

* $p < 0.05$ relative to control.

porated in vacuum to obtain 6.6 g of a dark-brown gummy residue. The residue is doubly dissolved in 30 ml of a 8% hydrochloric acid, treated with activated charcoal, and reprecipitated with solid sodium carbonate. The precipitate is dissolved in 15 ml of anhydrous isopropyl alcohol. To this solution is gradually added a 20% solution of hydrogen chloride in isopropyl alcohol until reaching pH ~ 3. Upon cooling, the precipitate is filtered, washed with acetone (2 × 5 ml), and dried in vacuum at 50°C to obtain 3.9 g of compound VI (Table 1).

N-[(4-Pyridyl)phenylmethyl]acetamide (VII). A mixture of 5.14 g (20 mmole) of compound IIIa is stirred for 15 min with a 12% sodium hydroxide solution prepared from 1.7 g (41.6 mmole) NaOH and 12 ml of water. To this mixture is added 4.4 ml (45.7 mmole) of acetic anhydride and the reaction mass is treated at 45 – 50°C. Then a 10% sodium hydroxide solution (~30 ml) is added to pH 11 and the mixture is cooled to 5°C and kept at this temperature for 1 h. The precipitate is filtered, washed with water to pH 7, and dried at 70°C. Then 60 ml of benzene is added and the mixture is boiled for 30 min with stirring, cooled to 5°C, filtered, and dried at 70°C to obtain 3.7 g of compound VII (Table 1).

N-[(4-Pyridyl)phenylmethyl]-N′-methylurea hydrochloride(VIII). To a mixture of 5.14 g (20 mmole) of compound IIIa and 50 ml of anhydrous dichloroethane at ~20°C is added 6 ml (42.8 mmole) of triethylamine and the reaction mass is stirred for 1 min. The precipitate of triethylamine hydrochloride is separated by filtration and washed with 5 ml of dichloroethane. To the filtrate, cooled down to 2°C with stirring on ice, is gradually (over 1 h) added dropwise a solution of 11 ml (22 mmole) of methyl isocyanate in 20 ml of dichloroethane, after which the reaction mass is allowed to stand at 2 – 7°C for 16 h. Then 10 ml of water is added and the mixture is stirred for 30 min and allowed to stand until phase separation. The organic layer is evaporated in vacuum to dryness and the brown residue is dissolved in 20 ml of anhydrous ethanol at 20°C. To this solution are added with stirring first ~6 ml (dropwise) of a 20% solution of hydrogen chloride in isopropyl alcohol until reaching pH 1, and then 140 ml of an acetone – benzene (2 : 1) mixture. The mixture is cooled to 3 – 5°C and allowed to stand at this temperature for 16 h. Then the precipitate is filtered, washed with 3 ml of acetone, and dried at 60°C to obtain 2.2 g of compound VIII (Table 1).

EXPERIMENTAL PHARMACOLOGICAL PART

The biological tests were performed on mongrel male mice weighing 22 – 25 g kept under standard vivarium conditions (12-h illumination; air temperature 21 – 22°C; free access to water and meals). The acute toxicity of the synthesized compounds was determined for a single intraperitoneal injection and characterized by LD_{50} values calculated by the Kerber method [10]. The spontaneous motor activity was characterized by the number of horizontal movements over a time period of 10 min determined for the test mice placed in individual compartments of a DFA-ER-20 type actometer. The rectal temperature was measured with an electronic thermometer of the TEMP type 10 min before and 30 min after introduction of the test compounds.

The neurotropic activity was studied by methods described in [11, 12]. The properties of the synthesized compounds were characterized by their effects on (i) hexenal (60 mg/kg) sleep duration determined by measuring the time of stay (min) in the lateral position, (ii) apomorphine (2 mg/kg) stereotypy, and (iii) arecoline (25 mg/kg) tremor. The anticonvulsant activity was studied on the models of convulsions induced by maximum electroshock (MES) or by intravenous injections of a 1% corazole solution [13, 14].

The test substances were introduced, 30 min before the model-inducing agents, by intraperitoneal injections in a dose of 0.1 LD_{50} in the form of suspensions with Tween-80. Each control and test group contained 10 animals. The experimental data were statistically processed in terms of the Student *t*-criterion; the results were considered as reliable for $p < 0.05$.

It was established that most of the synthesized compounds potentiate, to a more or less pronounced extent, the hypnotic effect of hexenal (Table 2). In the series of substituted (heterylphenylmethyl)ureas (IV), the corresponding amines (III), and their derivatives, the maximum increase in hexenal sleep was observed for compounds IVa, VII, VIII, and IIIa. Among the substances tested for the anticonvulsant activity, only compounds IIIa, IIIb, IIIe, and IVe produced reliable anticonvulsant effect with respect to corazole-induced convulsions (Table 2). All the compounds were ineffective in the MES test, did not change the motor activity and rectal temperature, and did not influence the apomorphine and arecoline effects.

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