SYNTHESIS AND STUDY OF THE ANTIRADICAL AND ANTIOXIDANT ACTIVITY OF FORIDON ANALOGS

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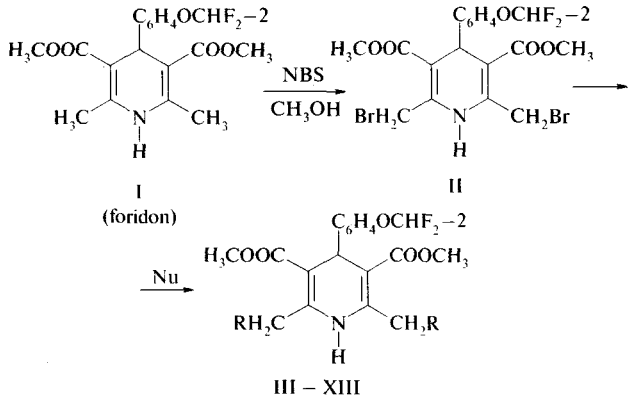
The antioxidant properties of calcium antagonists may affect their therapeutic action in the course of treatment of some cardiovascular disorders [1]. Among the 1,4-dihydropyridine (1,4-DHP) derivatives, antioxidant activity was initially found in some 4-unsubstituted compounds [2, 3] and then was also revealed in a group of 4-aryl-1,4-DHP derivatives including nifedipine, nisoldipine, felodipine, nicardipine, and lacidipine [4 – 6], but not in foridon (I) [3].

The purpose of this work was to study how various 2,6-substituents introduced into foridon analogs affect the antiradical (ARA) and antioxidant (AOA) activity of the initial compound.

Some of the foridon analogs studied in this work were synthesized as described previously [7]. Below we describe the synthesis of four new representatives (IV, V, VIII, IX) of this group. All the studied compounds (III – XIII) were obtained using a common pathway of synthesis via a dibromoderivative (II) of foridon. The nucleophilic displacement of bromine takes place within several hours under mild conditions (room temperature). The reaction products are stable crystalline substances (Table 1). The proposed structures of these compounds were confirmed by spectroscopic data (Table 2).



(NBS = N-bromosuccinimide; Nu = nucleophile; for R see Table 3).

The electronic spectra of the synthesized substances retain a longwave absorption band in the region of 366 – 371 nm characteristic of 3,5-dicarbonyl-1,4-DHP. The IR spectra exhibit an absorption band at 3240 – 3300 cm^{-1} corresponding to stretching vibrations ν_{NH} in the heterocycle. Compounds IV and V exhibit additional absorption peaks in the 3300 – 3400 cm^{-1} range related to vibrations of the N–H bonds in substituents R. The absorption bands of $\nu_{C=O}$ in the ester groups are shifted to 1690 – 1702 cm^{-1} due to conjugation with the DHP cycle. The ν_{CH} stretching vibrations in the N-methyl groups of compound VIII are manifested by a well distinguished band at 2788 cm^{-1}. In addition, the IR spectra of all foridon derivatives (III – XIII) contain two absorption bands (in the vicinity of 1645 and 1585 cm^{-1}) attributed to $\nu_{C=C}$, $\nu_{C(arom)-C(arom)}$, and δ_{NH}.

In the ^1H NMR spectra, the protons of methylene groups in positions 2 and 6 give signals forming a singlet (compounds IV and IX) or an AB-quartet with the geminal spin-spin coupling constant $J = 17$ Hz (compound VIII). The signals due to protons of the substituents R and other moieties correspond to the proposed structures and agree with the ^1H NMR spectrum of foridon [8].

An analysis of the results of our experiments (Table 3) showed that foridon possesses neither ARA nor AOA. At the same time, all the 2,6-disubstituted foridon analogs (except compound XIII) exhibit more or less pronounced AOA, the maximum antioxidant effect being observed for compounds

TABLE 1. Yields and Physicochemical Characteristics of Newly Synthesized Compounds

Compound	Empirical formula	Yield, %	M.p., °C	R_f
IV	$C_{26}H_{37}F_2N_3O_5$	31	122 – 124	0.17
V	$C_{37}H_{37}F_2N_3O_5$	23	139 – 141	0.57
VIII	$C_{22}H_{29}F_2N_3O_5$	62	124 – 125	0.40
IX	$C_{30}H_{45}F_2N_3O_5$	5	46 – 48	0.28

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III, IV, V, and VI (containing a secondary amino group in positions 2 and 6) and for compound VIII.

As is known, the secondary amines generally belong to antioxidants [9]. For this reason, we have compared the AOA of compound VI to the properties of cyclohexyl-N-methylamine. It was found that the latter substance possesses a lower AOA (1.48 ± 0.03) compared to that of VI (2.91 ± 0.05). This fact indicates that the DHP system is a factor significantly contributing to the AOA of compound VI.

The most pronounced ARA in the series of substances studied was observed for compound XII, which can be related to the presence of another radical-trapping center in this molecule (probably, with the ilide formation), differing from that found in compounds III – VI. It should be noted that compound XIII, which also exhibits a comparatively high ARA, is not only free of antioxidant properties but rather produces a prooxidant effect. This fact confirms that AOA is not always determined entirely by the ability of compounds to react with free radicals. A compound cannot be classified as antioxidant based only on the data on its antiradical activity. It is well known that some compounds, capable of trapping radicals and inhibiting transoxidation, may form rather active secondary radicals (the so-called inhibitor radicals) and initiate new transoxidation chains [10].

An important role for the optimum manifestation of some kinds of physiological activity of a given compound, including the antioxidant effect, belongs to localization of this compound in the lipid layer [11]. The presence of calcium antagonist in the lipid bilayer is now also recognized as an additional condition positively affecting the AOA. There are data [12] that the coefficients of distribution of the lipophilic 1,4-DHP derivatives between cell membranes and the ambient hydrophilic medium may fall within 5,000 – 150,000 to favor reaching local concentrations of these compounds on a millimolar level. We judged the affinity of a 1,4-DHP derivative to liposomal membranes by the degree of quenching of the fluorescence from anthracene probes interacting with the membranes at the boundary between the hydrophobic and polar phospholipid fragments. According to the published data [12], the 1,4-DHP derivatives are localized within the same region. Our experimental data on the affinity of foridon analogs to the model lipid membranes are presented in Table 3. As seen, compounds possessing more pronounced AOA are usually also capable (except for compound VIII) of tightly binding to the model lipid bilayer.

EXPERIMENTAL CHEMICAL PART

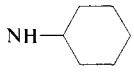
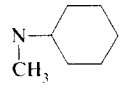
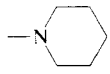
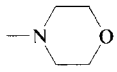
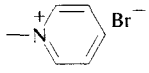
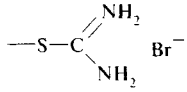
The course of reactions was monitored and the purity of the reaction products was checked by TLC on Silufol UV-254 plates (Kavalier) eluted in an acetone – methanol – water (8 : 2 : 1) system. The spots were visualized under UV illumination (254 or 366 nm). The melting temperatures were determined on a Boetius heating table (VEB Analytik Dresden, Germany). The ^{1}H NMR spectra were obtained with a Bruker WH-90/DS spectrometer operating at a working frequency of 90 MHz. The samples were dissolved in $CDCl_3$ and the chemical shifts were referenced to those of a TMS internal standard. The UV spectra were recorded on a Hitachi 557 UV-VIS spectrophotometer using 5×10^{-5} M ethanol solutions. The IR spectra were measured on a Perkin-Elmer 580B spectrophotometer using samples prepared as nujol mulls. The data of elemental analyses agree with the results of analytical calculations according to the empirical formulas.

2,6-Bis(*tert*-butylaminomethyl)-4-(2-difluoromethoxy phenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ether (IV). To a solution of 13.13 g (0.025 mole) of compound II [8] in 150 ml of chloroform were added 10.6 ml (0.1 mole) of *tert*-butylamine and the mixture was stirred for 1.5 h at room temperature and allowed to stand overnight at – 10°C. Then the mixture was triply washed with water (3×150 ml), dried over K_2CO_3, and evaporated in vacuum. The residue was treated with diethyl ether and recrystallized from a mixture of diethyl and petroleum ethers to obtain 3.89 g of compound IV in the form of colorless crystals.

TABLE 2. Spectroscopic Characteristics of Newly Synthesized Compounds

Compound	UV spectrum: λ_{max}, nm (log ε)	IR spectrum: ν_{max}, cm^{-1}	^{1}H NMR spectrum: δ, ppm
IV	238 (4.36); 371 (3.89)	3347 (NH); 3242 (NH ring); 1690 (C=O); 1674; 1632, 1575 (C=C)	10.23 (bs, 1H, NH), 6.80 – 7.35 (m, 4H, C_6H_4), 6.45 (t, 1H, $^2J_{HF}$ 75 Hz, $OCHF_2$), 5.30 (s, 1H, 4-H), 3.93 (s, 4H, 2,6-CH_2N), 3.54 (s, 6H, OCH_3), 1.10 (s, 2H, $NHC(CH_3)_3$)
V	240 (4.29); 369 (3.83)	3370, 3330 (NH); 3242 (NH ring); 1696 (C=O); 1640, 1580 (C=C)	9.73 (bs, 1H, NH), 6.8 – 7.6 (m, 14H, $C_6H_4 + 2C_6H_5$), 6.42 (t, 1H, $^2J_{HF}$ 74.5 Hz, $OCHF_2$), 5.25 (s, 1H, 4-H), 3.55 – 4.35 (m, 6H, CH_2, CH), 3.5 (s, 6H, OCH_3), 1.63 (bs, 2H, NH)
VIII	240 (4.34); 367 (3.92)	3298 (NH ring); 2788 (NCH_3); 1693 (C=O); 1648, 1598 (C=C)	9.40 (bs, 1H, NH), 6.80 – 7 (m, 4H, C_6H_4), 6.47 (t, 1H, $^2J_{HF}$ 75 Hz, $OCHF_2$), 5.35 (s, 1H, 4-H), 3.56, 3.70 (AB-q, 4H, $^2J_{HH}$ 17 Hz, 2CH_2N), 3.56 (s, 6H, OCH_3), 2.31 (s, 12H, $N(CH_3)_2$)
IX	238 (4.29); 366 (3.82)	3268 (NH ring); 1702 (C=O); 1688 sh; 1650, 1585 (C=C)	9.52 (bs, 1H, NH), 6.80 – 7 (m, 4H, C_6H_4), 6.44 (t, 1H, $^2J_{HF}$ 75 Hz, $OCHF_3$), 5.28 (s, 1H, 4-H), 3 (s, 4H, 2,6-CH_2N), 3.52 (s, 6H, OCH_3), 2.41 (t, 8H, ^3J 7.5 Hz, NCH_2CH_2), 1.10 – 1.73 (m, 8H, $CH_2CH_2CH_3$), 0.85 (t, 12H, ^3J 7 Hz, CH_2CH_3)

TABLE 3. Antioxidant (AOA) and Antiradical (ARA) Activity of Compounds I and III – XIII and Their Affinity to Model Phospholipid Membranes

Compound	R	AOA: τ/τ_0	ARA: k, liter/(mole · sec)	Affinity: ln F_0/F
I	H	1.00 ± 0.01	< 0.1	0.33 ± 0.02
III	$NH(CH_2)_3CH_3$	2.51 ± 0.05	1.11 ± 0.19	0.26 ± 0.02
IV	$NHC(CH_3)_3$	1.59 ± 0.03	0.12 ± 0.04	0.17 ± 0.01
V	$NHCH(C_6H_5)CH_3$	1.76 ± 0.04	0.21 ± 0.04	0.41 ± 0.02
VI		2.91 ± 0.05	0.48 ± 0.01	0.31 ± 0.02
VII		1.36 ± 0.03	< 0.1	0.31 ± 0.02
VIII	$N(CH_3)_2$	1.94 ± 0.04	0.17 ± 0.02	0.08 ± 0.01
IX	$N(C_3H_7)_2$	1.32 ± 0.03	0.08 ± 0.01	0.41 ± 0.03
X		1.23 ± 0.04	< 0.1	0.39 ± 0.02
XI		1.26 ± 0.02	< 0.1	0.27 ± 0.02
XII		1.13 ± 0.03	33.71 ± 2.84	0.11 ± 0.01
XIII		0.47 ± 0.01	2.62 ± 0.27	0.24 ± 0.01

2,6-Bis[(1-phenylethylamino)methyl]-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ether (V). Compound V was obtained by a procedure similar to that described above for the synthesis of compound IV, using a reaction of dibromoderivative II with racemic α-phenylethylamine. The final product was recrysallized from an acetone – hexane mixture (Tables 1 and 2).

2,6-Bis(dimethylaminomethyl)-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ether (VIII). To a solution of 2.63 g (0.005 mole) of compound II in 60 ml of methanol was added with stirring 9.0 ml (~0.07 mole) of a 40% aqueous dimethylamine solution, The mixture was stirred for 2 h at room temperature and allowed to stand overnight. Then the mixture was poured into water. The precipitated yellow substance was filtered and crystallized from aqueous methanol to obtain 1.40 g of compound VIII.

2,6-Bis(dipropylaminomethyl)-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ether (IX). Compound IX was obtained by a procedure similar to that described above for the synthesis of compound VIII, using a reaction of compound II with 3.28 ml (0.024 mole) of dipropylamine. The reaction mixture poured into water forms a dark oil that was triply extracted with chloroform. The extracts were combined and dried over $CaCl_2$. Finally, the solvent was distilled off in vacuum and the residue was chromatographed on a column filled with aluminum oxide (neutral Brockman II from Reanal, Budapest). The column was eluted with a hexane – acetone (9 : 1) mixture. Compound IX was obtained in the form of a dark-yellow oil, which crystallized upon treatment with a small amount of absolute ethanol followed by prolonged (1 month) storage at – 10 °C. Yield of compound IX, 1.11 g; solvent for crystallization, aqueous isopropyl alcohol.

EXPERIMENTAL BIOLOGICAL PART

Antiradical activity characterization. ARA of the synthesized compounds was evaluated by their ability to react with a stable radical of 1,1-diphenyl-2-picrylhydrazide (DPPH) [13]. A mixture of 2.5 ml of a 10^{-4} M DPPH solution in ethanol with 50 μl of a 5×10^{-3} M 1,4-DHP solution in ethanol (with a final 1,4-DHP concentration of 10^{-4} M) was incubated at 30 °C. The rate of DPPH reduction was estimated by measuring the decrease in the optical absorption at 517 nm (Hitachi 557 UV-VIS spectrophotometer). The ARA was characterized by a reaction rate constant determined by the formula

$$k = \Delta[\text{DPPH}]/([\text{DPPH}]_0 \cdot [\text{DPPH}]_t \cdot t)$$

and expressed in liter/(mole · sec).

Antioxidant activity characterization. AOA of the synthesized compounds was evaluated as described in [14]. A mixture of 1 ml of a β-carotene solution in chloroform (prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform), 0.02 ml of linolic acid methyl ether, and 0.1 g of Tween-40 in a round-bottom flask was thoroughly stirred and the chloroform was distilled off on a rotor evaporator at 40°C. To the residue was added by portions with vigorous shaking 50 ml of a 0.1 M phosphate buffer (pH 7.4) freshly saturated with oxygen immediately prior to reaction. The resulting aqueous emulsion of β-carotene and methyl linoleate was rapidly distributed by 5-ml portions into Bausch & Lomb cells containing 0.02 ml aliquots of 5×10^{-4} M ethanol solutions of the 1,4-DHP derivatives studied. The same volume (0.02 ml) of pure ethanol was added to the control cells. The cells were closed and incubated at 37°C, with periodic measurements of the optical absorption at 460 nm (Spectronic 70 spectrophotometer). The AOA was characterized by the ratio τ/τ_0, where τ and τ_0 are the induction periods (i.e., the times during which the β-carotene concentration decreases by one-third) with and without the compound studied in the test solution, respectively.

Characterization of the affinity of 1,4-DHP to model phospholipid membranes. The model phospholipid membranes were prepared by rapidly injecting an ethanol solution of egg phosphatidylcholine into a 13-fold excess of a 0.01 M Tris-HCl buffer (pH 7.4) intensively stirred with a magnetic stirrer. The final lipid concentration in liposomes was 0.5 mg/ml. To 1 ml of this liposome suspension were sequentially added 5 μl of a 1 mM ethanol solution of an anthracene fluorescent probe and 5 μl of a 1 mM ethanol solution of the 1,4-DHP derivative tested (5 μl of pure ethanol were added to the control samples). The fluorescence intensity was measured on a Hitachi 850 spectrophotometer. A 1-cm-thick flat optical cell was oriented at 45° to the incident beam. The fluorescence signal was excited at a wavelength of 340 nm and measured at 380 nm. The intensity of fluorescence from anthracene probes in the liposome suspension was measured before and after adding 1,4-DHP to obtain the F_0 and F values, respectively. The liposome affinity of the derivative studied was judged by the degree of quenching of the fluorescence from anthracene molecules bound to the membranes and evaluated as $\ln(F_0/F)$. Data presented in Table 3 were obtained by averaging over the results of 5 – 6 independent measurements.

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