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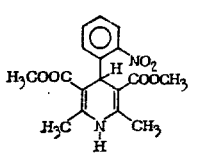
SYNTHESIS, BIOLOGICAL ACTIVITY, AND AFFINITY FOR MODEL BIOLOGICAL MEMBRANES OF ALKYL- AND CYCLOALKYL-1,4-DIHYDROPYRIDINE-3,5-DICARBOXYLATES

N. V. Makarova, G. V. Belevich,
É. A. Bisenieks, M. M. Veveris,
and G. Ya. Dubur

UDC 616.22:547.822.1/.012.1

Many 1,4-dihydropyridines [1,4-DHP) are known to possess high cardiovascular activity [7]. The most interesting 1,4-DHP derivatives are those which have been termed calcium channel blockers or calcium antagonists [8, 9]. These are effective hypotensive and coronary dilating drugs, some of which are being used currently with success for the treatment of cardiovascular diseases [8, 9]. A typical example of these drugs is nifenidine (adalate, corinfar, or fenigidine) (I) [8]. Since the molecular effects of calcium channel blockers are expressed at the cell membrane level, it would be expected that an increase in the affinity of these compounds for the membranes would increase their biological activity. We have therefore synthesized a number of novel compounds (IIc-k) which are structuraly close to the known calcium antagonist ryodipine (IIa) [11, 12] but are more lipophilic than the latter, with a view to identifying novel active compounds, and to establishing relationships between the chemical structures, certain physicochemical properties, and biological activity.



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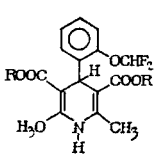

*II*a-k

R = CH_3(riodipine)[2] (IIa); C_2H_5 [2] (IIb); $C(CH_3)_3$ (IIc); menthyl (IId); C_9H_{19} (IIe); $C_{10}H_{21}$(IIf); (1-adamantyl[5] (IIg); $C_{11}H_{23}$(IIh); $C_{12}H_{25}$ (IIj); $C_{14}H_{29}$ (IIi); $C_{16}H_{33}$ (IIk).

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 22, No. 7, pp. 810-815, July, 1988. Original article submitted October 27, 1987.

 0091-150X/88/2207-0534$12.50

TABLE 1. Alkyl and Cycloalkyl 2,6-Dimethyl-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylates (IIc-k)

Compound	Yield, %	mp, °C	Empirical formula	UV spectrum, λ_{max}, nm (log ε)	IR spectrum, cm^{-1}	
					C=O	NH
IIa	44	128—30	$C_{24}H_{31}F_2NO_5$	206 (4,32) 236 (4,29) 360 (3,84)	1 693	3 340
IIb	42	177—8	$C_{36}H_{47}F_2NO_5$	208 (4,21) 238 (4,29) 363 (3,88)	1 677	3 320
IIe	25	59—60	$C_{34}H_{51}F_2NO_5$	207 (4,32) 239 (4,32) 362 (3,86)		
IIf	27	61—2	$C_{36}H_{55}F_2NO_5$	206 (4,31) 239 (4,30) 362 (3,84)	1 700	3 340
IIg	45	126—8	$C_{36}H_{43}F_2NO_5$	207 (4,16) 238 (4,24) 366 (3,82)	1 692	3 270
IIh	57	46—7	$C_{38}H_{59}F_2NO_5$	206 (4,31) 239 (4,28) 362 (3,82)	1 700	3 330
IIi	23	51—3	$C_{40}H_{63}F_2NO_5$	206 (4,27) 238 (4,24) 362 (3,79)	1 705	3 340
IIj	49	54—5	$C_{44}H_{71}F_2NO_5$	206 (4,28) 239 (4,27) 362 (3,82)	1 707	3 350
IIk	49	63—5	$C_{44}H_{79}F_2NO_5$	206 (4,27) 239 (4,29) 362 (3,80)	1 705	3 350

Compounds (IIc-k) were obtained from the appropriate alkyl and cycloalkyl acetoacetates, 2-difluoromethoxybenzaldehyde, and ammonia (the Hansch synthesis). The properties and yields of the new compounds are given in Tables 1 and 2. Data on the lipophilicity of the compounds is given in Table 3. It will be seen that on increasing the length of the carbon chain of the substituent R in the 3- and 5-positions, the lipophilicity of (IIa-k) increases.

If model phospholipid membranes (liposomes) containing anthracene as a fluorescent probe are treated with a 1,4-DHP derivative, the fluorescence is extinguished (Fig. 1). This extinction basically arises from nonradiant energy overbalance [1]. The magnitude of the extinction depends on the concentration of the extinguishing agent bonded to the membrane (Fig. 2), and therefore serves as a measure of binding. The results characterizing the affinity of 1,4-DHP derivatives for liposomal membranes are given in Table 3. It will be seen that starting with (IId), the affinity for the membranes decreases sharply despite the increasing lipophilicity of the compounds.

The hypotensive activity of (IIa-k) and nifedipine was measured in narcotized cats. All the test compounds, by the intraperitoneal route, caused a decrease in systemic arterial pressure (Table 3), without any significant effects on the frequency of cardiac contractions. The hypotensive activity of different compounds varied considerably, and was in general inversely proportional to increases in the number of carbon atoms in the substituent R, and directly proportional to the affinity for phospholipid membranes. Departures from this behavior were seen in (IId) and (IIg), which have methyl and 1-adamantyl substituents. In the case of (IId), it was not possible to measure the ED_{30}, since in the dose range studied (up to 3 mg/kg intravenously), arterial pressure was not reduced to the extent of 30%. It is interesting that (IIg) has high anti-angina activity [5].

Amongst these newly-synthesized 1,4-DHP derivatives, therefore, the only compound with high hypotensive activity is (IIc). All of these compounds are less active in reducing arterial pressure than the previously known compounds (I), (IIa), and (IIb). Increasing the length of the side chain substituents, although it increased lipophilicity, did not facilitate binding to the lipid bilayer. It is, however, worthy of note that compounds with the highest hypotensive activity bind well to liposomes, where as those with the lowest activity have poor affinity for lipid membranes. It is possible that binding to the lipid bilayer of biological membranes is a necessary and important factor in the mode of action of calcium channel blockers.

TABLE 2. PMR Spectral Parameters for (IIc-k) in $CDCl_3$

Compound	Chemical shift δ (ppm) and spin-spin coupling constant J(Hz)					
	C_6H_4 (m)	$OCHF_2$ (t, F=75)	NH br.s	4-H (s)	2,6-CH_3 (s)	3,5-R
II c	7,38—6,90	6,40	5,46	5,11	2,21	1,37 (18H, s., C $(CH_3)_3$
II d	7,38—6,89	6,43	5,63	5,18	2.25, 2,30	4,83—4,46 (2H, m., α-Hmenthyl, 2,0—0,5 (.menthyl prot.)
II e	7,39—6,90	6,44	5,87	5,22	2,25	3,97 (4H, t., J=6,5, 1′ — CH_2); 1,64—1,42 (4H, m., 2′=CH_2); 1,26 (24H, br., s 3—8=CH_2); 0,87 (6H, def. t., CH_3)
II f	7,39—6,90	6,45	5,77	5,22	2,27	3,97 (4H, t, J=6,5, 1′=CH_2); 1,65—1,45 (4H, m., 2=CH_2); 1,26 28H, br.s, 3=9′-CH_2; 0,88 (6H, def. t., CH_3)
II g	7,38—6,95	6,39	5,63	5,07	2,18	2,08—1,97 (18H, br. s, (β+γ) H adamantyl); 1,60 (12H, br.s., δ=H adamantyl)
II h	7,49—7,00	6,45	5,74	5,22	2,25	3,97 (4H, t, J=6,5,1′-CH_2); 1,64—1,48 (4H, m., 2′-CH_2); 1,23 (32H, t., 3 — 10=CH_2); 0,87 (6H, def.t., CH_3)
II i	7,42—6,92	6,46	5,63	5,22	2,28	3,99 (4H, t, J=6,5,1′-CH_2); 1,71—1,45 (4H, m., 2′-CH_2); 1,26 (36H, s., 3—11—CH_2); 0,88 (6H, def. t, CH_3)
II j	7,40—6,90	6,45	5,65	5,22	2,28	3,98 (4H, t., J=6,5,1′-CH_2); 1,72—1,46 (4H, m., 2′-CH_2); 1,25 (40H, s, 3-13-CH_2); 0,88 (6H, def.t, CH_3)
II k	7,40—6,90	6,45	5,63	5,22	2,28	3,98 (4H, t., J-6,5,1′-CH_2); 1,74—1,46 (4H, m., 2′-CH_2); 1,25 (44H, s., 3-15CH_3); 0,88 (6H, def.t., CH_3)

TABLE 3. Parameters of Lipophilicity, Membrane Binding, Hemodynamics, and Acute Toxicity of Compounds I and IIa-k

Compound	R_M	$\ln(F_0/F)$	$(\ln F_0/F)/\pi R_0^3 \times 10^{-2}$, nm^{-3}	ED_{30} mg/kg	Change in arterial pressure (at a dose of 1 mg/kg), %	LD_{50} (intraperitoneal), mg/kg
I	—0,6	0,27	6,88	0,022	—70	190
IIa	—0,5	0,32	8,94	0,023	—70	360
IIb	...	0,42	11,13	0,06	—70	...
IIc	—0,35	0,39	10,61	0,3	—62	...
IId	—0,35	0,05	1,31	—*	+5	...
IIe	—0,32	0,05	1,42	0,3	—45	...
IIf	—0,21	...	...	0,5	—40	...
IIg	—0,18	0,04	0,94	3,2	—7	8 400
IIh	—0,1	0,02	0,60	0,7	—36	3 000
IIi	—0,03	0,05	1,32	2,6	—18	2 000
IIj	0,19	0,03	0,74	2,8	—14	3 000
IIk	0,39	0,04	1,02	3,5	—8	5 000

*In doses up to 3 mg/kg, no reduction in pressure by 30% was observed.

EXPERIMENTAL (CHEMISTRY)

UV spectra were obtained on a Hitachi-557 in ethanol, IR spectra on a Perkin-Elmer-580 in Nujol, and PMR spectra on a Brucker WH 90/DS, internal standard tetramethylsilane in $CDCl_3$.

Synthesis of Alkyl and Cycloalkyl 2,6-Dimethyl-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylates. The appropriate acetoacetic ester (0.05 mole), 4.3 g (0.025 mole) of 2-difluoromethoxybenzaldehyde, and 3 ml (3.06 g, 0.046 mole) of 25% aqueous ammonia were dissolved in 30-50 ml of ethanol, and boiled for 6 h. After cooling, the bright yellow crystalline solid which separated was filtered off and recrystallized from ethanol. The elemental analyses were in agreement with the calculated values.

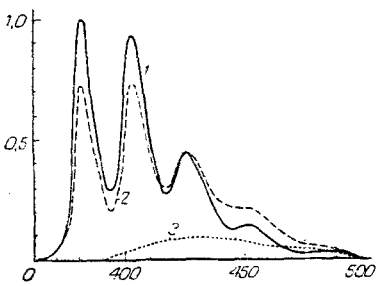

Fig. 1

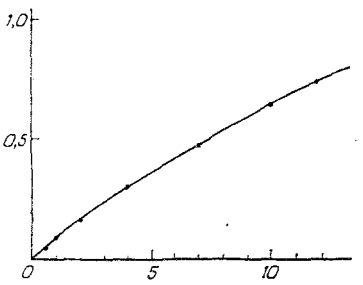

Fig. 2

Fig. 1. Extinction of anthracene fluorescence by ryodipine in liposomal membranes from chicken phosphatidylcholine (0.4 g/liter). Spectra 1 and 2 – fluorescence of 5 μM anthacene in the absence and presence of 5 μM of ryodipine respectively; 3 – fluorescence spectrum of 5 μM ryodipine in liposomes in the absence of anthracene. Fluorescence excited at 340 nm. Horizontal axis, wavelength of fluorescence (nm); vertical axis, intensity of fluorescence (arbitrary units).

Fig. 2. Dependence of the extinction of fluorescence of anthracene on concentration of ryodipine in membrane suspensions, F_0 and F are the intensities of fluorescence of anthracene in the absence and presence of ryodipine respectively. Liposome concentration 0.4 g/liter, anthracene 5 μM. Horizontal axis, concentration of ryodipine (μM); vertical axis in F_0/F.

Measurement of Lipophilicity of 1,4-DHP Derivatives. The lipophilicity of the compounds obtained was characterized by the quantity $R_M = \log(1/R_f - 1)$ [10]. The R_f values were measured on revered phase Whatman KC-18 DF plates in the mobile system ethanol–water [24:1].

EXPERIMENTAL (BIOLOGY)

Determination of Pharmacological Activity. In order to assess and compare the biological activity of the test compounds, their effects on systemic arterial pressure in narcotized cats were examined, and the following parameters found: 1) the equiactive ED_{30} doses, i.e., the dose of each compound in milligrams per kilogram body weight at which the arterial pressure was reduced by 30% below its initial value; 2) the hypotensive activity of the compounds when administered in the same dose (1 mg/kg intravenously), as the change in arterial pressure as a percentage of the initial value. In acute experiments in cats (weight 2.6-3.9 kg) of both sexes, narcotized with α-glucochloralose and urethane (80 and 200 mg/kg intraperitoneally), the arterial pressure in the common carotid artery was measured (RP-1500 electromanometer), together with the ECG in standard II leads in a Narko Bayo-Systems DM P-4B physiograph (USA). Solutions or suspensions of the test compounds were prepared in dimethlyacetamide, diluted with distilled water, and introduced via a cannula inserted into the femoral vein. The acute toxicities (LD_{50}) of the test compounds were also measured by intraperitoneal administration.

Determination of the Affinity of 1,4-DHP Derivatives for Model Phospholipid Membranes. Model phospholipid membranes were prepared by rapid spraying of an ethanolic solution of chicken phosphatidylcholine* into a vigorously stirred buffer solution (0.01 M Tris-HCl, pH 7.4) [4].

Fluorescence measurements were carried out with a Hitachi MPF-4 spectrofluorimeter with a flat cell, thickness of fluorescing layer 1 mm, established from the diagonal of the right angle formed by the optical axes of both monochromators. The width of the optical slits of the monochromators did not exceed 5 nm.

*Obtained from Kar'kov Biological Preparations Factory.

The binding of 1,4-DHP derivatives to the liposomal membranes was assessed by the extent of extinction of the fluorescence of anthracene, used as a fluorescence probe bonded to the membrane. For this purpose, the intensity of fluorescence of anthracene (5 μM, Sigma) in a suspension of liposomes (0.4 g/liter) was measured both before and after addition of 3 μM 1,4-DHP (F_0 and F respectively). Fluorescence in these experiments was excited at 340 nm, and measured at 380 nm. The results were presented in the form $(\ln F_0/F)\pi R_0^2$, where R_0 is the critical radius for energy transfer, calculated from the fluorescence spectrum of anthracene in liposomes and the UV spectra of the 1,4-DHP [6]. The quantum yield for the fluorescence of anthracene in liposomes was taken to be 0.2 [3], the absorption spectra of 1,4-DHP in ethanol being used.

To prepare mother liquors of the compounds, the anthracene was dissolved in ethanol (1 mM), and compounds (I) and (IIa-k) in dimethyl sulfoxide (1-2 mM) (Merck) (West Germany).

All measurements were carried out at room temperature.

The authors express their sincere thanks to Prof. G. E. Dobretsov for valuable discussions of the fluorescence data.

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