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The derivatives of 2,6-dimethyl-1,4-dihydroisonicotinic acid and their antiplatelet properties

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Introduction

The search for antiplatelet agents is an important area of cardiological pharmacology. There are some agents for this purpose, but they exhibit various side effects. Some 4-phenyl-1,4-dihydropyridine derivatives that are calcium antagonists possess antiplatelet properties [1–3]. The related 1,4-dihydroisonicotinic acid (1,4-DIA) derivatives, which have not been reported to have calcium antagonist properties, are susceptible to free radical processes [4] and are suitable products for condensation reactions with amino acids, such as β-aminoethane sulfonic acid (taurine). On the other hand, taurine plays an important role in cell physiology, especially in blood platelets (thrombocytes) [5, 6]. The main disadvantage of taurine is its high rate of metabolism which complicates its clinical use. Thus, the aim of this investigation was the coupling of the 1,4-dihydropyridine and taurine moieties to increase the metabolic stability of taurine and to add dihydropyridine moiety properties to the new compounds [7].

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Abbreviations: ADP: adenosine 5'-diphosphate; ATP: adenosine 5'-triphosphate; ATPase: adenosine 5'-triphosphatase; DCC: *N,N'*-dicyclohexylcarbodiimide; EGTA: ethylene glycol-bis(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid; NADPH: nicotinamide adenine dinucleotide phosphate, reduced form; TBA: thiobarbituric acid; TRIS: tris(hydroxymethyl)aminomethane.

Results and discussion

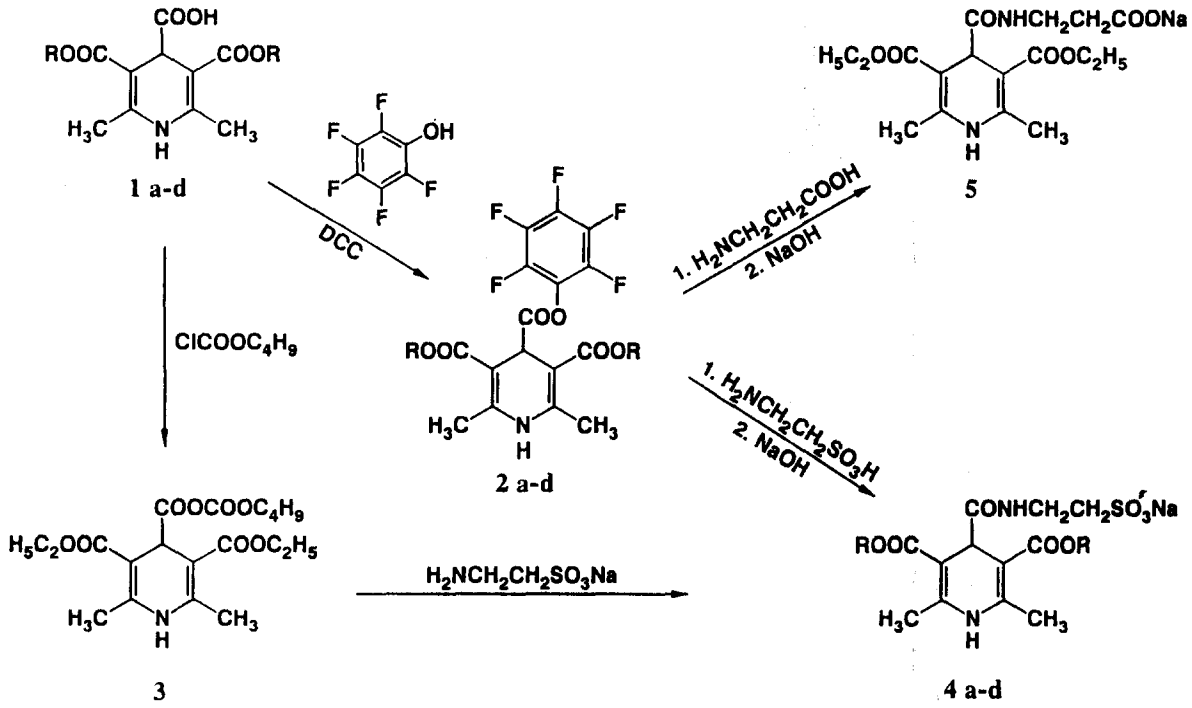
Chemistry

The taurine derivatives of substituted 1,4-DIA **4** were synthesized by using both activated ester and mixed anhydrides methods. Pentafluorophenyl esters of 2,6-dimethyl-3,5-dialkoxycarbonyl-1,4-DIA **2** and the mixed anhydride of 2,6-dimethyl-3,5-diethoxycarbonyl-1,4 DIA and the mono-*n*-butyl ester of carbonic acid **3** were used (scheme 1).

The pentafluorophenyl esters **2** were obtained from the corresponding compound **1** [8] and pentafluorophenol in the presence of dicyclohexylcarbodiimide. The mixed anhydride **3** was prepared from the corresponding derivative of **1** and butyl chloroformate. The taurine derivatives **4** were mainly obtained by condensation of corresponding pentafluorophenyl esters **2** and taurine with subsequent neutralization of prepared acid by alkali. For the large-scale preparation of **4b**, the mixed anhydride **3** and sodium salt of taurine were used. For comparison purposes, the structural analogue **5** of the taurine derivative **4b**, in which the taurine moiety was substituted by that of β-alanine, was synthesized.

Antiplatelet properties

The antiaggregating (antiplatelet) activity of compound **4** was examined in human blood using the nephelometric method. The results obtained, as well as the data for taurine and some structurally related 1,4-DIA derivatives, are presented in table I. The effect that the title compounds have on Ca^{2+}/Mg^{2+}-



Scheme 1.

Table I. The antiaggregation (antiplatelet) activity of compounds **4–5** and their effect on Ca^{2+}/Mg^{2+}-ATPase from human blood platelets.

Compound	*Conc (mM)*	*Primary aggregation (% of control)*	*Activity of Ca^{2+}/Mg^{2+}-ATPase (% of control)*
4a	1.0	100	100
4b	0.5	48.8 ± 11.9	147 ± 10
4c	1.5	50.0 ± 8.2	–
4d	1.0	100	100
5	2.0	71.5 ± 9.2	100
Taurine	15.0	60.1 ± 6.3	133 ± 8

ATPase activity of platelets (thrombocytes) is also described.

The data obtained show that **4b** is a more powerful antiaggregant than taurine since it remarkably decreased the level of platelet aggregation at a 30-fold lower concentration. Related analogues with different alkyl radicals in the ester groups of the dihydropyridine moiety were inactive (**4a**, **4d**), or less active (**4c**). Compound **5**, in which the taurine moiety is replaced by β-alanine, was less active than **4b**. In contrast, β-alanine had no appreciable effect upon platelet aggregation. The most characteristic property of **4b** was its action on the first, reversible stage of aggregation. Thus, the level of the second aggregation stage upon the action of **4b** was 80% of the control, compared with 60% for taurine. A microscopic investigation of the aggregation process indicated that the number of platelets in aggregates decreased in the presence of **4b**. On the other hand, activation of Ca^{2+}/Mg^{2+}-ATPase was one of the pronounced effects exhibited by taurine [9]. The obtained results for compounds **4–5** show that the increase in ATPase activity exhibited by **4b** is higher than that for taurine.

Recently, it was reported that taurine protects biomembranes against oxidative damage [10]. Our data for the action of **4b** on lipid peroxidation (induction of peroxidation by NADPH, ascorbic acid/Fe^{2+}, detection of peroxidation by TBA-reaction) of human blood plasma indicate that **4b** shows antioxidative properties. At the same time **4b** increases the level of superoxide dismutase activity in plasma by 17.5%, relative to 2.0% for taurine.

Thus, compound **4b** represents a new type of dihydropyridine derivative possessing a taurine moiety that exhibits antiplatelet activity mainly on the first, reversible stage of platelet aggregation in blood plasma.

Experimental protocols

Chemistry

Melting points were determined on a HMK microscope apparatus. Elemental analyses (C, H, N) were within ± 0.4% of the theoretical values. IR spectra were recorded on a Perkin–Elmer 580B spectrophotometer (in Nujol) and peak positions ν_{max} are expressed in cm^{-1}. UV spectra were recorded on a Hitachi 557 spectrophotometer; peak positions λ_{max} are expressed in nm; log ε values are presented in parentheses. ^{1}H-NMR spectra were recorded on a Bruker WH–90 spectrometer and chemical shifts are reported as δ values (ppm) relative to tetramethylsilane. The NMR data is presented in table II.

Pentafluorophenyl 2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydroisonicotinate ***2a***
A mixture of **1a** (13.5 g; 0.05 mol) and pentafluorophenol (9.9 g; 0.054 mol) was dissolved in 100 ml *N,N*-dimethylformamide and DCC (11.1 g; 0.054 mol) was added. The reaction mixture was filtered after 24 h storage and the filtrate was evaporated under reduced pressure. The residue was recrystallized from ethylacetate. Mp 153–155°C, yield: 18.7 g (86%). IR: 3360, 1768, 1715. UV (C_2H_5OH): 230 (4.37), 344 (3.75).

Pentafluorophenyl 2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydroisonicotinate ***2b***
Compound **2b** was prepared in the same manner as **2a**. Mp 138–140°C, yield: 89%. IR: 3360, 1757, 1710, 1688. UV (C_2H_5OH): 230 (4.39), 348 (3.78).

Pentafluorophenyl 2,6-dimethyl-3,5-di-n-propoxycarbonyl-1,4-dihydroisonicotinate ***2c***
Compound **2c** was prepared according to the method described for **2a**. Mp 98–100°C, yield: 71%. IR: 3395, 1760, 1700. UV (C_2H_5OH): 228 (4.36), 341 (3.77).

Pentafluorophenyl 2,6-dimethyl-3,5-di-(1-adamantyloxycarbonyl)-1,4-dihydroisonicotinate ***2d***
Compound **2d** was prepared similarly to **2a**. Mp 171–173°C (ethyl acetate), yield: 71%. IR: 3380, 1760, 1695. UV (C_2H_5OH): 230 (4.38), 343 (3.81).

n-Butyl 2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydroisonicotinoyl carbonate ***3***
n-Butyl chloroformate (7.2 g; 0.053 mol) was added to a solution of **1b** (14.9 g; 0.05 mol) and triethylamine (5.3 g; 0.053 mol) in 150 ml dichloromethane under stirring at 20°C.

Table II. ^{1}H-NMR spectral data for compounds **2–5**.

Compound (solvent)	*Chemical shifts (δ) and coupling constants*
2a ($CDCl_3$)	2.36 (6H, s, 2,6-CH_3); 3.75 (6H, s, 3,5-CH_3); 5.16 (1H, s, 4-H); 5.86 (1H, s, NH)
2b ($CDCl_3$)	1.30 (6H, t, J = 7 Hz, 3,5-CH_3); 2.33 (6H, s, 2,6-CH_3); 4.19 (4H, q, J = 7 Hz, 3,5-$COOCH_2$); 5.15 (1H, s, 4-H); 6.27 (1H, s, NH)
2c ($CDCl_3$)	0.97 (6H, t, J = 7.5 Hz, 3,5-CH_3); 1.51–1.89 (4H, m, 3,5-CH_2); 2.38 (6H, s, 2,6-CH_3); 4.12 (4H, t, J = 6.5 Hz, 3,5-$COOCH_2$); 5.23 (1H, s, 4-H); 6.03 (1H, s, NH)
2d ($CDCl_3$)	1.67 (12H, s, δ-H of adamantyl); 2.17 (18H, s, β + γ-H of adamantyl); 2.32 (6H, s, 2,6-CH_3); 5.14 (1H, s, 4-H); 5.67 (1H, s, NH)
3 ($CDCl_3$)	0.93 (3H, t, J = 7 Hz, δ-CH_3); 1.30 (6H, t, J = 7 Hz, 3,5-CH_3); 1.25–1.86 (4H, m, β + γ-CH_2); 2.33 (6H, s, 2,6-CH_3); 4.18 and 4.21 (2 x 2H, 2q, J = 7 Hz, 3- and 5-$COOCH_2$); 4.20 (2H, t, J = 6.5 Hz, 4-$COOCH_2$); 4.94 (1H, s, 4-H); 5.93 (1H, s, NH)
4a (DMSO-d_6)	2.22 (6H, s, 2,6-CH_3); 2.55–2.80 (2H, m, CH_2); 3.09–3.35 (2H, m, CH_2); 3.60 (6H, s, 3,5-CH_3); 4.32 (1H, s, 4-H); 7.36 (1H, t, J = 5 Hz, CONH); 8.83 (1H, s, NH)
4b (DMSO-d_6)	1.20 (6H, t, J = 7 Hz, 3,5-CH_3); 2.19 (6H, s, 2,6-CH_3); 2.30–2.55 (m, CH_2 + DMSO); 3.03–3.37 (2H, m, CH_2); 4.04 (4H, q, J = 7 Hz, 3,5-$COOCH_2$); 4.29 (1H, s, 4-H); 7.28 (1H, t, J = 5.5 Hz, CONH); 8.22 (1H, s, NH)
4c (DMSO-d_6)	0.90 (6H, t, J = 7 Hz, 3,5-CH_3); 1.40–1.84 (4H, m, 3,5-CH_2); 2.30–2.55 (m, CH_2 + DMSO); 3.05–3.38 (m, CH_2 + H_2O); 3.98 (4H, t, J = 6.5 Hz, 3,5-$COOCH_2$); 4.36 (1H, s, 4-H); 7.26 (1H, t, J = 5 Hz, CONH); 8.78 (1H, s, NH)
4d (DMSO-d_6)	1.67 (12H, s, δ-H of adamantyl); 2.15 (18H, s, β + γ-H of adamantyl); 2.18 (6H, s, 2,6-CH_3); 2.30–2.58 (2H, m, CH_2); 3.08–3.40 (2H, m, CH_2); 4.44 (1H, s, 4-H); 7.04 (1H, t, J = 5.8 Hz, CONH); 7.55 (1H, s, NH)
5 (DMSO-d_6)	1.17 (6H, t, J = 7 Hz, 3,5-CH_3); 1.89 (2H, t, J = 6.5 Hz, CH_2COO); 2.17 (6H, s, 2,6-CH_3); 3.03 (2H, distorted q, J = 5.4 Hz, NCH_2); 4.02 (4H, q, J = 7 Hz, 3,5-$COOCH_2$); 4.24 (1H, s, 4-H); 7.34 (1H, t, J = 5 Hz, CONH); 8.82 (1H, s, NH)

After 15 min the reaction mixture was washed with 100 ml water, dried (sodium sulphate), and the solvent was evaporated under reduced pressure to 1/3 of its initial volume. To the residue, hexane (10 ml) was added and crystallization was allowed to occur in a refrigerator. Mp 70–72°C, yield: 16.1 g (81%). IR: 3315, 3245, 1814, 1760, 1708. UV (C_2H_5OH): 228 (4.58), 338 (4.04).

Sodium 2-(2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydroisonicotinoyl)aminoethane sulfonate ***4a***
The reaction mixture consisting of **2a** (4.3 g; 0.01 mol), taurine (1.9 g; 0.015 mol), and triethylamine (2.5 g; 0.025 mol) in 80 ml *N,N*-dimethylformamide was stirred for 48 h at 20°C. The mixture was filtered and the filtrate was evaporated under reduced pressure. The precipitate was dissolved in ethanol, neutralized with NaOH to pH 7 and the product was separated by addition of acetone. Mp 178–180°C, yield: 2.2 g (54%). IR: 3560, 3490, 3350, 3295, 3205, 3080, 1710, 1690, 1680, 1650, 1625. UV (H_2O): 233 (4.27), 358 (3.82).

Sodium 2-(2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydroisonicotinoyl)aminoethane sulfonate ***4b***

Method A. Same as **4a**. Yield: 68%.
Method B. A solution of the sodium salt of taurine (13 g; 0.2 mol) in 100 ml water was added dropwise to a solution of **3** (40 g; 0.2 mol) in 400 ml ethanol under intensive stirring. The solvent was evaporated under reduced pressure and 50 ml ethanol was added to the precipitate. After standing at room temperature, the product crystallizes. Mp 174–177°C yield: 55 g (65%). IR: 3530, 3450, 3380, 3350, 3290, 3220, 3200, 3070, 1710, 1685, 1660, 1650, 1630. UV (H_2O): 234 (4.26), 359 (3.82).

Sodium 2-(2,6-dimethyl-3,5-di-n-propoxycarbonyl-1,4-dihydroisonicotinoyl)aminoethane sulfonate ***4c***
The synthesis was performed as described for the preparation of **4a**. Mp 148–150°C, yield: 71%. IR: 3520, 3360, 3305, 3230, 3200, 3080, 1706, 1690, 1675, 1655, 1620. UV (H_2O): 234 (4.37), 358 (3.92).

Sodium 2-(2,6-dimethyl-3,5-di-(1-adamantyloxycarbonyl)-1,4-dihydroisonicotinoyl)aminoethane sulfonate ***4d***
The synthesis was performed by analogy to **4a**. Mp 208–210°C, yield: 45%. IR: 3285, 3215, 3095, 1690, 1680, 1645, 1635, 1623. UV (H_2O): 235 (4.30), 357 (3.89).

Sodium 2-(2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydroisonicotinoyl)aminopropionate ***5***
Compound **5** was prepared by analogy to **4a** from **2b**, β-alanine and triethylamine. Mp 233–234°C, yield: 61%. IR: 3550, 3500, 3430, 3355, 3280, 3200, 3080, 1700, 1685, 1670, 1650, 1638. UV (H_2O): 233 (4.26), 360 (3.81).

Biological studies

Antiplatelet activity
Blood was obtained from healthy donors aged 25–40 yr, who had not received any pharmaceutical preparations during the 10 d before the trial. The blood was mixed with a 3.8% solution of sodium citrate and centrifuged at 140 *g* to obtain thrombocyte-enriched plasma. The latter was centrifuged at 1100 *g* for 15 min to give plasma with a reduced content of thrombocytes, to which the test compound was introduced. All operations were carried out in siliconized vessels. The number of thrombocytes was counted using a phase-contrast microscope. Aggregation was studied by the nephelometric method. Plasma with a low content of thrombocytes served as a control. The test volume of plasma was 1.3 ml. The induction of aggregation was effected by introducing 50–150 µl ADP in a concentration of 2–3.5 µM into the plasma. The change in light transmission of the plasma under the influence of the inductor in the absence of the test compound was taken as 100%.

Ca^{2+}/Mg^{2+}-ATPase activity
The activity of Ca^{2+}/Mg^{2+}-ATPase was determined as the increase in the amount of inorganic phosphorus cleaved from ATP under optimal conditions. The incubation medium for determination of the Ca^{2+}/M^{2+}-ATPase activity contains (in µM): ATP: 2.5; $MgCl_2$: 5; ouabain: 0.15; EGTA: 1; TRIS-HCl: 23; pH 7.5. The incubation was conducted for 20 min at 37°C.

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