ХИМИЯ ГЕТЕРОЦИКЛИЧЕСКИХ СОЕДИНЕНИЙ. — 2013. — № 4. — С. 607—614

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SYNTHESIS OF 3-ALKOXYCARBONYL-6-ALKYLSULFANYL-4-[2-(DIFLUOROMETHOXY)PHENYL]-1,4-DIHYDROPYRIDINES AND RELATED DERIVATIVES AS ANALOGUES OF COGNITION ENHANCER CEREBROCRAST

4-[2-(Difluoromethoxy)phenyl]-substituted 3-alkoxycarbonyl-6-alkylsulfanyl-3-cyano-2-methyl-1,4-dihydropyridines and related pyridine and 4,7-dihydrothieno[2,3-*b*]pyridine derivatives have been prepared and their memory-improving activity by using the passive avoidance responses in acquisition test and calcium overload-preventing activity in SH-SY5Y neuroblastoma cell line in the presence of agonist carbachol were examined. 1,4-Dihydropyridine derivatives bearing 2-propoxyethoxycarbonyl group in position 3 and possessing weak influence on calcium overload in neuronal cells, showed high activity comparable with that of cerebrocrast.

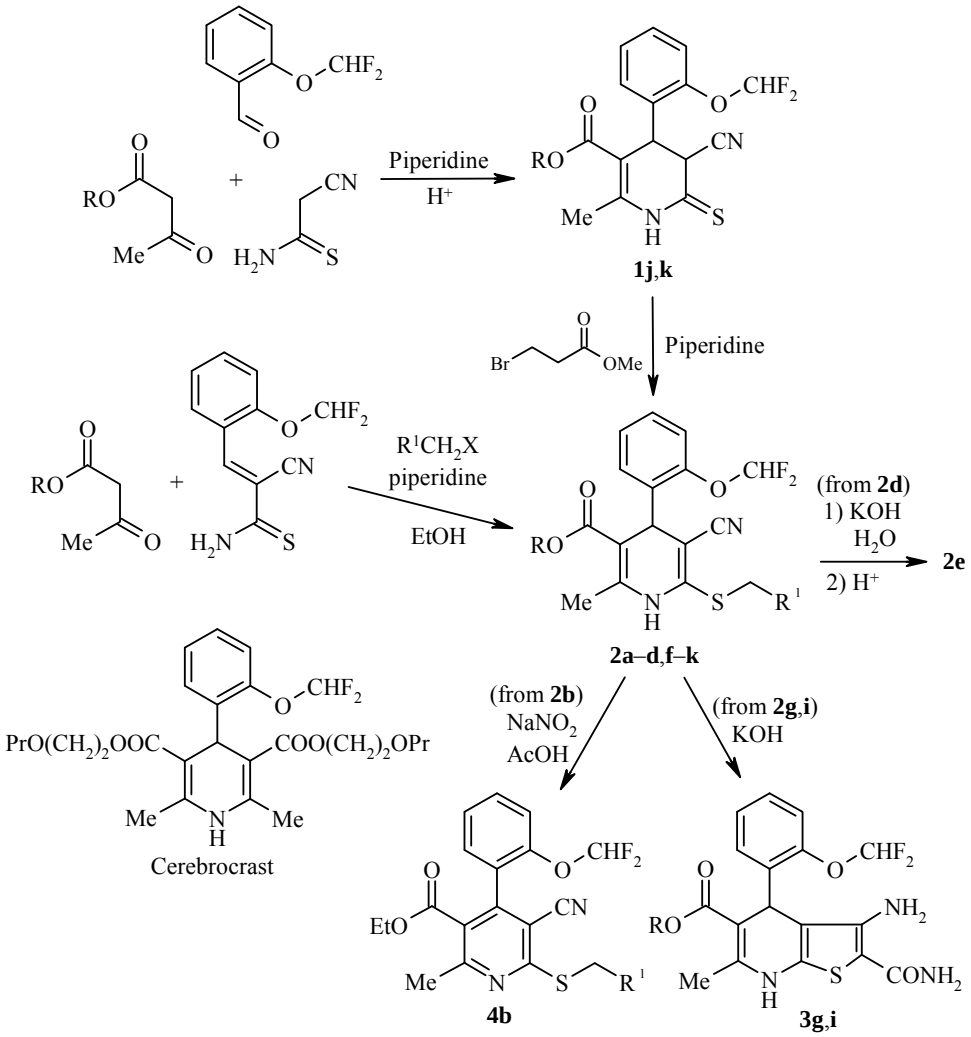
Keywords: 2-alkylsulfanyl-1,4-dihydropyridines, thieno[2,3-*b*]pyridines, calcium overload preventing activity, passive avoidance responses in acquisition test.

1,4-Dihydropyridine (DHP) structure, when appropriately substituted, can exert potent and selective actions at a diverse set of membrane structures, including ion channels, G-protein coupled receptors and enzymes [1]. Many DHPs possess pleiotropic properties [2–4]. Thus, depending on the chemical structure peculiarities, 1,4-DHPs have many regulatory activities: neuro- and radioprotection, antimutagenic, antidiabetic, anti-inflammatory, anti-ischaemic/antianginal, and antihypertensive, as well as growth-stimulating, life span-prolonging, and gene transfection properties [5]. Therefore, studies of specific properties of a group of DHP derivatives comprising minor changes of substituent structure could be useful for elucidation of biochemical interactions, which play a major role in the understanding of drug's structure–activity relationship, drug's development, and therapeutic success [6].

Several studies have shown that some 1,4-DHPs exert direct protective activity in experimental models of stroke [7] and neurodegenerative diseases [8]. As calcium overload has been linked to the apoptosis and death of the cell, it was suggested that the neuroprotective properties of 1,4-DHPs could be connected with their ability to prevent calcium overload in neurons [9]. 1,4-DHPs have been developed that simultaneously inhibit calcium influx in muscular cells, as well as prevent calcium overload in neuronal cells [10].

Decoration of 1,4-DHP ring with cyano and alkylsulfanyl substituents have resulted in compounds with cardiovascular [11, 12], hepatoprotective [13], antioxidant [14], and antiradical [15] properties. However, calcium channel blocking activities of such type of compounds are less pronounced.

This study was performed to elaborate synthesis of novel asymmetric 6-alkylsulfanyl-1,4-DHPs containing structural fragments of cognition enhancer cerebrocrast [16–19], examine their memory improving activity by making use of the passive avoidance responses in acquisition test and calcium overload preventing activity in SH-SY5Y neuroblastoma cell line.



a R = Me, R^1 = H; **b** R = Et, R^1 = H; **c** R = *i*-Pr, R^1 = H; **d** R = $(CH_2)_2CN$, R^1 = H;
e R = R^1 = H; **f** R = $(CH_2)_2OPr$, R^1 = H; **g** R = Et, R^1 = $CONH_2$; **h** R = Et, R^1 = COOEt;
i R = $(CH_2)_2OPr$, R^1 = $CONH_2$; **j** R = $(CH_2)_2OPr$, R^1 = CH_2COOMe;
k R = Me, R^1 = CH_2COOMe; X = Br, I

6-Alkylsulfanyl-substituted 3-alkoxycarbonyl-5-cyano-4-[2-(difluoromethoxy)-phenyl]-2-methyl-1,4-DHPs **2a**–**d**,**f** were prepared in 62–82% yields by Michael reaction of alkyl acetoacetates with 2-cyano-3-[2-(difluoromethoxy)phenyl]thioacryl-amide in the presence of stoichiometric amount of piperidine as catalyst in ethanol with subsequent treatment with twofold excess of iodomethane.

By treatment of compound **2d** with KOH water solution hydrolysis took place and acid **2e** in 97% yield was formed.

To enhance solubility and lipophilicity of 1,4-DHPs, an ester function (COOEt, CH_2COOMe groups) was introduced in 6-methylsulfanyl substituent, but ester group in position 3 (substituent R) was derivatized with 2-propoxyethyl group. 1,4-DHPs **2g**,**i** containing amide function in 6-methylsulfanyl substituent and lipophilic $COO(CH_2)_2OPr$ group in position 3 were synthesized as well. 1,4-DHPs **2g**–**i** were prepared in 70–82% yields similarly to compounds **2a**–**f**, only by making use of 1.05–1.10-fold excess of ethyl bromoacetate or iodoacetamide instead of iodo-

methane. 1,4-DHPs **2j**,**k** were prepared in 70–87% yields by alkylation of the corresponding thiones **1j**,**k** with methyl 3-bromopropionate which in turn were obtained by one-pot four-component condensation of acetoacetate, aromatic aldehyde, 2-cyanoethanethioacetamide and stoichiometric amount of piperidine in 57–79% yields. Although the summary yields were moderate (40–68%) the last pathway (obtaining of intermediates **1**) has advantage because the target compounds **2j**,**k** crystallize as pure substances from reaction mixture, but in the case of 5-component one-pot method, separation with flash chromatography was necessary.

Treating 2-(carbamoylmethyl)sulfanyl-3-cyano-1,4-DHPs **2g**,**i** with KOH in water–ethanol solution Thorpe's cyclization took place and 4,7-dihydrothieno-[2,3-*b*]pyridines **3g**,**i** in 59–73% yield were obtained.

Pyridines are the most possible metabolites of 1,4-DHPs *in vivo*. Compound **4b** was prepared in 29% yield by oxidation of 1,4-DHP **2b** with sodium nitrite in acetic acid.

The structures of synthesized compounds were established by spectroscopic and elemental analysis data. In the IR spectra of 1,4-DHPs **2a**–**k**, the absorption band of cyano group was observed at 2188–2207 cm^{-1} (characteristic for β-aminovinyl-carbonitriles) which disappeared after Thorpe's cyclization (in case of 4,7-dihydrothieno[2,3-*b*]pyridines **3g**,**i**). In the ^1H NMR spectra, characteristic singlet of 4-CH proton at 4.82–5.10 ppm was observed for 1,4-DHPs **2a**–**k** and at 5.24–5.32 for 4,7-dihydrothieno[2,3-*b*]pyridines **3g**,**i** partially confirming their structure.

Influence of compounds 2a–k, 3g,i, 4b on Ca^{2+} accumulation in cell line SH-SY5Y in the presence of agonist carbachol and on the passive avoidance responses (PAR) in acquisition test in male ICR mice (18–24 g, t 21°C, *n* = 6)

Compound	log *P**	IC_{50}, μM	PAR test	
			Dose, mg/kg	Latency, Δ*t*, s
Control saline	–	–	–	77.0±22.3
Cerebrocrast	5.08	>100	0.05	158.3±1.9**
2a	3.41	n. e.***	–	n. d.
2b	3.78	n. e.	5.00	112.0±9.4
2c	4.15	n. d.***	–	n. d.
2d	3.84	n.d.	–	n. d.
2e	2.79	n.d.	–	n. d.
2f	4.08	>100	0.05	152.3±3.6**
2g	2.51	n. d.	–	n. d.
2h	4.02	n. e.	5.00	153.0±9.5**
2i	2.81	n. e.	0.05	162.2±4.3**
2j	4.32	100	–	n. d.
2k	3.23	100	–	n. d.
3g	3.15	n. d.	–	n. d.
3i	3.45	n. d.	5.00	101.3±48.8
4b	4.20	n. d.	–	n. d.

* Calculated with program Molinspiration.

** $P < 0.05$ *vs* control.

*** n. d. – not determined, n. e. – no effect.

Cognitive enhancing effects of the studied substances **2a**–**k**, **3g**,**i** and **4b** are shown in Table. 1,4-DHPs **2f**,**i** at the dose 0.05 mg/kg in memory test (PAR, acquisition) in mice showed activity which is comparable with that of cerebrocrast. 1,4-DHP **2h** lacking 2-propoxyethyl ester group was active at the dose 5.0 mg/kg. Thus, prolongation of the side chains by replacing COOEt group with $COO(CH_2)_2OPr$ group in position 3 or SMe group with SCH_2COOEt group (compounds become more lipophilic), caused an increase of activity. Thorpe's cyclization of active 2-carbamoylmethylsulfanyl-1,4-DHP **2i** to the corresponding 4,7-thieno[2,3-*b*]pyridine **3i** led to the significant lowering of activity.

As are seen from Table, 1,4-DHPs **2** that have cyano and alkylsulfanyl substituents similarly to symmetric 1,4-DHP – cerebrocrast and asymmetric 1,4-DHP-3,5-dicarboxylates [10] have weak influence on prevention of calcium overload in neuronal cells. 1,4-DHP **2i** bearing 2-propoxyethoxycarbonyl group in position 3 was the most potent cognition enhancer.

In conclusion, these results together with the known data [16–19] allow to characterize 3-$PrO(CH_2)_2OCO$-1,4-DHP-moiety as pharmacophore determining the memory improvement.

EXPERIMENTAL

IR spectra were recorded on a Shimadzu IRPrestige-21 spectrometer in nujol. ^{1}H NMR spectra were recorded on a Varian Mercury-400 spectrometer (400 MHz) in DMSO-d_6 (compounds **2e**,**g**,**i**, **3g**,**i**) and $CDCl_3$ (remaining compounds), HMDS (δ 0.05 ppm) is used as standard. Elemental analyses were performed on a Carlo Erba Instrument Analyzer EA 1106. Melting points were determined on OptiMelt MPA100 apparatus and are uncorrected. The course of the reactions and the purity of substances were monitored by TLC on Kieselgel 60 F Merck plates with CH_2Cl_2–hexane–MeOH, 5:5:1, as eluent. All reagents were purchased from Aldrich or Acros and used without further purification. Synthesis of thione **1k** is published in [20].

2-Propoxyethyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-2-methyl-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate (1j). A mixture of 2-(difluoromethoxy)benzaldehyde (0.69 g, 4.0 mmol), 2-propoxyethyl acetoacetate (0.75 g, 4.0 mmol), and piperidine (0.04 ml, 0.4 mmol) in EtOH (20 ml) was stirred for 5 min at room temperature. Then 2-cyanoethanethioacetamide (0.4 g, 4.0 mmol) and piperidine (0.4 ml, 4.0 mmol) were added and the reaction mixture was stirred for 30 min. The resulting reaction mixture was acidified with 3M ethanolic solution of HCl (2.4 ml). The precipitate was separated by filtration, washed with cold MeOH (5 ml) and H_2O (20 ml). Yield 0.97 g (57%), yellow powder, mp 90–91°C. IR spectrum, ν, cm^{-1}: 3309 (N–H), 2198 (C≡N), 1714 (C=O). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.78–0.89 (3H, m, $OCH_2CH_2C\underline{H}_3$); 1.35–1.55 (2H, m, $OCH_2C\underline{H}_2CH_3$); 2.51 (1.5H, s) and 2.59 (1.5H, s, *cis*- and *trans*-2-CH_3); 3.21–3.33 (2H, m, $COOC\underline{H}_2CH_2O$); 3.47–3.54 (2H, m, $OC\underline{H}_2CH_2CH_3$); 4.15–4.24 (2H, m, $COOCH_2C\underline{H}_2O$); 4.17 (0.5H, d, *J* = 1.8) and 4.86 (0.5H, d, *J* = 1.8, *trans*-4,5-CH); 4.25 (0.5H, d, *J* = 7.5) and 5.08 (0.5H, d, *J* = 7.5, *cis*-4,5-CH); 6.25 (0.5H, q, *J* = 74.0) and 6.61 (0.5H, q, *J* = 74.0 *cis*- and *trans*-$OCHF_2$); 6.90–7.35 (4H, m, H Ar); 8.82 (1H, br. s, NH). Found, %: C 54.40; H 4.11; N 7.87; S 7.32. $C_{20}H_{22}F_2N_2O_4S$. Calculated, %: C 54.54; H 4.00; N 7.95; S 7.55.

Methyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (2a). A mixture of methyl acetoacetate (0.58 g, 5.0 mmol), 2-cyano-3-(2-difluoromethoxyphenyl)thioacrylamide (1.27 g, 5.0 mmol), and piperidine (0.55 ml, 5.5 mmol) in EtOH (10 ml) was heated to 40–50°C, then stirred for 1 h at the ambient temperature. Then MeI (1.24 ml, 20.0 mmol) was added, the reaction mixture was refluxed for 15 min, and cooled to 0°C. The precipitated crude product was recrystallized from EtOH. Yield 1.30 g (71%), slightly yellow powder, mp 161–163°C. IR spectrum, ν, cm^{-1}: 1672 (C=O), 2198 (C≡N), 3315 (N–H). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.36 (3H, s,

2-CH_3); 2.47 (3H, s, SCH_3); 3.56 (3H, s, $COOCH_3$); 5.06 (1H, s, 4-CH); 6.00 (1H, br. s, NH); 6.54 (1H, q, J = 73.2, $OCHF_2$); 7.00–7.40 (4H, m, H Ar). Found, %: C 55.61; H 4.45; N 7.72; S 8.81. $C_{17}H_{16}F_2N_2O_3S$. Calculated, %: C 55.73; H 4.40; N 7.65; S 8.75.

Ethyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (2b). Compound **2b** was prepared in the same manner as compound **2a** using ethyl acetoacetate instead of methyl acetoacetate. Yield 1.56 g (82%), slightly yellow powder, mp 173–175°C. IR spectrum, ν, cm^{-1}: 1693 (C=O), 2190 (C≡N), 3342 (N–H). ^{1}H NMR spectrum, δ, ppm (J, Hz): 1.08 (3H, t, J = 7.0, $OCH_2C\underline{H}_3$); 2.34 (3H, s, 2-CH_3); 2.44 (3H, s, SCH_3); 4.00 (2H, q, J = 7.0, $OC\underline{H}_2CH_3$); 5.07 (1H, s, 4-CH); 6.30 (1H, s, NH); 6.56 (1H, q, J = 73.2, $OCHF_2$); 7.00–7.30 (4H, m, H Ar). Found, %: C 56.81; H 4.57; N 7.52; S 8.39. $C_{18}H_{18}F_2N_2O_3S$. Calculated, %: C 56.83; H 4.77; N 7.36; S 8.43.

Isopropyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (2c). Compound **2c** was prepared in the same manner as compound **2a** using isopropyl acetoacetate instead of methyl acetoacetate. Yield 1.22 g (62%), slightly yellow powder, mp 114–116°C. IR spectrum, ν, cm^{-1}: 1694 (C=O), 2194 (C≡N), 3364 (N–H). ^{1}H NMR spectrum, δ, ppm (J, Hz): 0.90 (3H, d, J = 6.2) and 1.17 (3H, d, J = 6.2, $OCH(C\underline{H}_3)_2$); 2.38 (3H, s, 2-CH_3); 2.47 (3H, s, SCH_3); 4.88 (1H, quint, J = 6.2, $OCH(CH_3)_2$); 5.08 (1H, s, 4-CH); 6.08 (1H, s, NH); 6.57 (1H, q, J = 73.2, $OCHF_2$); 7.00–7.30 (4H, m, H Ar). Found, %: C 57.81; H 5.17; N 7.20; S 8.19. $C_{19}H_{20}F_2N_2O_3S$. Calculated, %: C 57.86; H 5.11; N 7.10; S 8.13.

Cyanoethyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (2d). A mixture of cyanoethyl acetoacetate (0.80 g, 5.0 mmol), 2-cyano-3-[2-(difluoromethoxy)phenyl]thioacrylamide (1.27 g, 5.0 mmol) and piperidine (0.55 ml, 5.5 mmol) in EtOH (10 ml) was heated to 40–50°C, stirred for 1 h at the ambient temperature. Then MeI (1.24 ml, 20 ml) was added, the reaction mixture was refluxed for 15 min, chilled to 0°C and poured into cold water (50 ml). The precipitated crude product was recrystallized from EtOH. Yield 1.31 g (65%), slightly yellow powder, mp 134–135°C. IR spectrum, ν, cm^{-1}: 1712 (C=O), 2207, 2266 (C≡N), 3288 (N–H). ^{1}H NMR spectrum, δ, ppm (J, Hz): 2.37 (3H, s, 2-CH_3); 2.46 (3H, s, SCH_3); 2.53 (2H, t, J = 7.0, $OCH_2C\underline{H}_2CN$); 4.17 (4H, t, J = 7.0, $OC\underline{H}_2CH_2CN$); 5.04 (1H, s, 4-CH); 6.18 (1H, br. s, NH); 6.58 (1H, q, J = 73.2, $OCHF_2$); 7.00–7.30 (4H, m, H Ar). Found, %: C 56.19; H 4.17; N 10.31; S 7.85. $C_{19}H_{17}F_2N_3O_3S$. Calculated, %: C 56.29; H 4.23; N 10.36; S 7.91.

5-Cyano-4-[2-(difluoromethoxy)phenyl]-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylic acid (2e). A mixture of 1,4-DHP **2d** (1.22 g, 3 mmol), 4M aqueous solution of KOH (1 ml) and EtOH (4 ml) was stirred at 30°C for 3 h. Then 3M ethanolic solution of HCl (1.3 ml) was added and the mixture stirred at the ambient temperature for 1 h. The precipitate was separated by filtration and washed with EtOH (2 ml) and water (10 ml). Yield 1.03 g (97%), colorless powder, mp 190–191°C. IR spectrum, ν, cm^{-1}: 1680 (C=O), 2204 (C≡N), 3272, 3334 (N–H, O–H). ^{1}H NMR spectrum, δ, ppm (J, Hz): 2.27 (3H, s, 2-CH_3); 2.40 (3H, s, SCH_3); 4.82 (1H, s, 4-CH); 7.08 (1H, q, J = 73.2, $OCHF_2$); 7.10–7.30 (4H, m, H Ar); 9.29 (1H, s, NH). Found, %: C 54.29; H 4.05; N 8.07; S 8.92. $C_{16}H_{14}F_2N_2O_3S$. Calculated, %: C 54.54; H 4.00; N 7.95; S 9.10.

2-Propoxyethyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (2f). Compound **2f** was prepared in the same manner as compound **2d** using 2-propoxyethyl acetoacetate instead of cyanoethyl acetoacetate. Yield 1.67 g (76%), colorless powder, mp 118–120°C. IR spectrum, ν, cm^{-1}: 1683 (C=O), 2206 (C≡N), 3296 (N–H). ^{1}H NMR spectrum, δ, ppm (J, Hz): 0.82 (3H, t, J = 7.0, $OCH_2CH_2C\underline{H}_3$); 1.42–1.52 (2H, m, $OCH_2C\underline{H}_2CH_3$); 2.30 (3H, s, 2-$CH_3$); 2.39 (3H, s, SCH_3); 3.35 (2H, t, J = 7.0, $OC\underline{H}_2CH_2CH_3$); 3.37–3.47 (2H, m, $COOCH_2C\underline{H}_2$); 3.98–4.08 (2H, m, $COOC\underline{H}_2CH_2$); 5.01 (1H, s, 4-CH); 6.03 (1H, s, NH); 6.50 (1H, q, J = 73.2, $OCHF_2$); 7.00–7.20 (4H, m, H Ar). Found, %: C 57.45; H 5.49; N 6.35; S 7.36. $C_{21}H_{24}F_2N_2O_4S$. Calculated, %: C 57.52; H 5.52; N 6.39; S 7.31.

Ethyl 6-(carbamoylmethyl)sulfanyl-5-cyano-4-[2-(difluoromethoxy)phenyl]-2-methyl-1,4-dihydropyridine-3-carboxylate (2g). Compound **2g** was prepared in the same manner

as compound **2d** using ethyl acetoacetate instead of cyanoethyl acetoacetate and iodoacetamide (10% excess) instead of MeI. Yield 1.48 g (70%), colorless powder, mp 199–201°C. IR spectrum, ν, cm^{-1}: 1672, 1695 (C=O), 2188 (C≡N), 3200, 3354 (N–H). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.02 (3H, t, *J* = 7.0, $OCH_2C\underline{H}_3$); 2.34 (3H, s, 2-CH_3); 3.60 (1H, d, *J* = 15.0) and 3.72 (1H, d, *J* = 15.0, SCH_2); 3.90 (2H, q, *J* = 7.0, $OC\underline{H}_2CH_3$); 4.97 (1H, s, 4-CH); 7.10–7.40 (4H, m, H Ar); 7.18 (1H, q, *J* = 73.2, $OCHF_2$); 7.62 (1H, br. s) and 7.92 (1H, br. s, $CONH_2$); 10.43 (1H, s, NH). Found, %: C 53.94; H 4.37; N 9.95; S 7.53. $C_{19}H_{19}F_2N_3O_4S$. Calculated, %: C 53.89; H 4.52; N 9.92; S 7.57.

Ethyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-6-(ethoxycarbonylmethyl)sulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (2h). Compound **2h** was prepared in the same manner as compound **2d** using ethyl acetoacetate instead of cyanoethyl acetoacetate and ethyl bromoacetate (5% excess) instead of MeI. Yield 1.58 g (70%), colorless powder, mp 120–122°C. IR spectrum, ν, cm^{-1}: 1676, 1706 (C=O), 2198 (C≡N), 3184, 3230 (N–H). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.08 (3H, t, *J* = 7.0, 3-$COOCH_2C\underline{H}_3$); 1.30 (3H, t, *J* = 7.0, $SCH_2COOCH_2C\underline{H}_3$); 2.38 (3H, s, 2-$CH_3$); 3.54 (2H, s, SCH_2); 3.98 (2H, q, *J* = 7.0, 3-$COOC\underline{H}_2CH_3$); 4.28 (2H, q, *J* = 7.0, $SCH_2COOC\underline{H}_2CH_3$); 5.10 (1H, s, 4-CH); 6.56 (1H, q, *J* = 73.2, $OCHF_2$); 7.00–7.30 (4H, m, H Ar); 8.48 (1H, s, NH). Found, %: C 55.67; H 4.83; N 6.25; S 7.17. $C_{21}H_{22}F_2N_2O_5S$. Calculated, %: C 55.74; H 4.90; N 6.19; S 7.09.

2-Propoxyethyl 6-(carbamoylmethyl)sulfanyl-5-cyano-4-[2-(difluoromethoxy)-phenyl]-2-methyl-1,4-dihydropyridine-3-carboxylate (2i). Compound **2i** was prepared in the same manner as compound **2d** using 2-propoxyethyl acetoacetate instead of cyanoethyl acetoacetate and iodoacetamide (10% excess) instead of MeI. Yield 1.97 g (82%), slightly yellow powder, mp 178–180°C. IR spectrum, ν, cm^{-1}: 1662, 1700 (C=O), 2194 (C≡N), 3184, 3356, 3466 (N–H). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.79 (3H, t, *J* = 7.0, $OCH_2CH_2C\underline{H}_3$); 1.37-1.47 (2H, m, $OCH_2C\underline{H}_2CH_3$); 2.36 (3H, s, 2-$CH_3$); 3.26 (2H, t, *J* = 7.0, $OC\underline{H}_2CH_2CH_3$); 3.37–3.47 (2H, m, $COOCH_2C\underline{H}_2O$); 3.60 (1H, d, *J* = 15.0) and 3.76 (1H, d, *J* = 15.0, SCH_2); 3.96–4.04 (2H, m, $COOC\underline{H}_2CH_2O$); 4.94 (1H, s, 4-CH); 7.10–7.30 (4H, m, H Ar); 7.12 (1H, q, *J* = 73.2, $OCHF_2$); 7.62 (1H, br. s) and 7.90 (1H, br. s, $CONH_2$); 10.47 (1H, s, NH). Found, %: C 54.80; H 5.18; N 8.68; S 6.82. $C_{22}H_{25}F_2N_3O_5S$. Calculated, %: C 54.88; H 5.23; N 8.73; S 6.66.

2-Propoxyethyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-6-[2-(methoxycarbonyl)-ethylsulfanyl]-2-methyl-1,4-dihydropyridine-3-carboxylate (2j). A mixture of 2-propoxyethyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**1j**) (0.85 g, 2.0 mmol), piperidine (0.23 ml, 2.3 mmol) and methyl 3-bromopropionate (0.25 ml, 2.3 mmol) in MeOH (20 ml) was heated for 1 h. The precipitated crystals were filtered off, washed with cold MeOH (2 ml), H_2O (5 ml) and MeOH (1 ml). Yield 0.71 g (70%), colorless crystals, mp 74–75°C. IR spectrum, ν, cm^{-1}: 1705, 1719 (C=O), 2198 (C≡N), 3259 (NH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.86 (3H, t, *J* = 7.0, $OCH_2CH_2C\underline{H}_3$); 1.49-1.62 (2H, m, $OCH_2C\underline{H}_2CH_3$); 2.43 (3H, s, 2-$CH_3$); 2.61–2.74 (2H, m, $SCH_2C\underline{H}_2$); 2.99–3.21 (2H, m, $SC\underline{H}_2CH_2$); 3.29 (2H, t, *J* = 7.0, $OC\underline{H}_2CH_2CH_3$); 3.46 (2H, t, *J* = 7.0, $COOCH_2C\underline{H}_2O$); 3.78 (3H, s, $COOCH_3$); 4.08 (2H, t, *J* = 7.0, $COOC\underline{H}_2CH_2O$); 5.08 (1H, s, 4-CH); 6.18–6.93 (1H, q, *J* = 73.2, 2-$OCHF_2$); 7.06–7.29 (4H, m, H Ar); 8.00 (1H, s, NH). Found, %: C 56.27; H 5.39; N 5.38; S 6.10. $C_{24}H_{28}F_2N_2O_6S$. Calculated, %: C 56.46; H 5.53; N 5.49; S 6.28.

Methyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-6-[2-(methoxycarbonyl)ethylsulfanyl]-2-methyl-1,4-dihydropyridine-3-carboxylate (2k). Methyl 5-cyano-4-(2-difluoromethoxyphenyl)-2-methyl-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**1k**) (0.71 g, 2.0 mmol) [20], piperidine (0.23 ml, 2.3 mmol) and methyl 3-bromopropionate (0.23 ml, 2.3 mmol) in MeOH (20 ml) were heated at 65°C for 2 h. The precipitated crystals were filtered off, washed with cold MeOH (2 ml), H_2O (5 ml) and MeOH (1 ml). Yield 0.76 g (87%), colorless crystals, mp 134–135°C. IR spectrum, ν, cm^{-1}: 1710 (C=O), 2201 (C≡N), 3183 (N–H). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.42 (3H, s, 2-CH_3); 2.67–2.74 (2H, m, $SCH_2C\underline{H}_2$); 2.96–3.24 (2H, m, $SC\underline{H}_2CH_2$); 3.55 (3H, s, 3-$COOCH_3$); 3.78 (3H, s, $S(CH_2)_2COOC\underline{H}_3$); 5.06 (1H, s, 4-CH); 6.54 (1H, q, *J* = 73.2, $OCHF_2$); 6.96–7.27 (4H, m,

H Ar); 8.03 (1H, s, NH). Found, %: C 54.66; H 4.55; N 6.36; S 7.13. $C_{20}H_{20}F_2N_2O_5S$. Calculated, %: C 54.79; H 4.60; N 6.39; S 7.31.

Ethyl 3-amino-2-carbamoyl-4-[2-(difluoromethoxy)phenyl]-6-methyl-4,7-dihydrothieno[2,3-*b*]pyridine-5-carboxylate (3g). Sample of DHP **2g** (0.42 g, 1 mmol) in EtOH (10 ml) was treated with 4M aqueous solution of KOH (0.3 ml), refluxed for 15 min, stirred for 1 h at ambient temperature. Then 3M ethanolic solution of HCl (0.5 ml) was added, stirred for 15 min and cooled to 0°C. The precipitate was separated by filtration, washed with cold EtOH (5 ml), H_2O (20 ml) and EtOH (2 ml). Yield 0.25 g (59%), yellow powder, mp 208–210°C. IR spectrum, ν, cm^{-1}: 1624, 1636 sh, 1656 sh, 1670 sh (C=O), 3166, 3260, 3342, 3430 (NH, NH_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.88 (3H, t, *J* = 7.0, $OCH_2C\underline{H}_3$); 2.37 (3H, s, 2-CH_3); 3.88 (2H, q, *J* = 7.0, $OC\underline{H}_2CH_3$); 5.24 (1H, s, 4-CH); 7.00–7.50 (8H, m, H Ar, NH_2, $CONH_2$); 7.18 (1H, q, *J* = 73.2, $OCHF_2$); 9.88 (1H, s, NH). Found, %: C 53.94; H 4.43; N 9.62; S 7.29. $C_{19}H_{19}F_2N_3O_4S$. Calculated, %: C 53.89; H 4.52; N 9.92; S 7.57.

2-Propoxyethyl 3-amino-2-carbamoyl-4-[2-(difluoromethoxy)phenyl]-6-methyl-4,7-dihydrothieno[2,3-*b*]pyridine-5-carboxylate (3i). Compound **3i** was prepared in the same manner as compound **3g** using compound **2i**. Yield 0.35 g (73%), yellow powder, mp 103–105°C. IR spectrum, ν, cm^{-1}: 1624, 1687 (C=O), 3218, 3316 sh, 3364, 3484 (NH, NH_2). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.83 (2H, t, *J* = 7.0, $OCH_2CH_2C\underline{H}_3$); 1.46–1.54 (2H, m, $OCH_2C\underline{H}_2CH_3$); 2.45 (3H, s, 6-$CH_3$); 3.28 (2H, t, *J* = 7.0, $OC\underline{H}_2CH_2CH_3$); 3.44–3.56 (2H, m, $COOCH_2C\underline{H}_2O$); 4.08–4.20 (2H, m, $COOC\underline{H}_2CH_2O$); 5.00 (2H, br. s, NH_2); 5.32 (1H, s, 4-CH); 6.80 (1H, q, *J* = 73.2, $OCHF_2$); 7.10–7.40 (6H, m, H Ar, $CONH_2$). Found, %: C 54.57; H 5.37; N 8.59; S 6.31. $C_{22}H_{25}F_2N_3O_5S$. Calculated, %: C 54.88; H 5.23; N 8.73; S 6.66.

Ethyl 5-cyano-4-[2-(difluoromethoxy)phenyl]-2-methyl-6-methylsulfanylpyridine-3-carboxylate (4b). Sample of 1,4-DHP **2b** (0.76 g, 2 mmol) in AcOH (7 ml) was treated with $NaNO_2$ (0.35 g, 5 mmol), heated for 15 min and stirred at ambient temperature for 1 h. Then 50% aqueous EtOH (10 ml) was added and the mixture cooled to 5°C. The precipitate was separated by filtration and washed with 50% aqueous EtOH (5 ml). Yield 0.22 g (29%), slightly yellow powder, mp 66–68°C. IR spectrum, ν, cm^{-1}: 1732 (C=O), 2220 (C≡N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.88 (3H, t, *J* = 7.0, $OCH_2C\underline{H}_3$); 2.66 (3H, s, 2-CH_3); 2.70 (3H, s, SCH_3); 4.00 (2H, q, *J* = 7.0, $OC\underline{H}_2CH_3$); 6.50 (1H, q, *J* = 73.2, $OCHF_2$); 7.00–7.60 (4H, m, H Ar). Found, %: C 56.88; H 4.14; N 7.32; S 8.50. $C_{18}H_{16}F_2N_2O_3S$. Calculated, %: C 57.14; H 4.26; N 7.40; S 8.47.

Biological evaluation of compounds 2 and 3. PAR test was performed according to the procedures given in [17, 21]. Cerebrocrast was used as references drug. The results obtained in the experiments were expressed as the mean values ± SEM and analyzed by Student's *t*-test. The criterion of statistical significances was $P < 0.05$.

Influence of compounds **2a**–**k** on Ca^{2+} concentration in cell line SH-SY5Y in presence of agonist carbachol was determined according to the procedures given in [10].

This work was supported by European Regional Development Fund (ERDF) project 2010/0227/2DP/2.1.1.1.0/10/APIA/VIAA/072.

REFERENCES

1. D. J. Triggle, *Mini-Rev. Med. Chem.*, **3**, 215 (2003).
2. R. P. Mason, P. Marche, T. H. Hintze, *Arterioscler. Thromb. Vasc. Biol.*, **23**, 2155 (2003).
3. R. Vitolina, A. Krauze, G. Duburs, A.Velena, *Int. J. Pharm. Sci. Res.*, **3**, 942 (2012).
4. S. Yamagishi, K. Nakamura, K. Takenaka, T. Matsui, H. Inoue, *Curr. Pharm. Des.*, **12**, 1543 (2006).
5. G. Duburs, B. Vigante, A. Plotniece, A. Krauze, A. Sobolevs, J. Briede, V. Klusa, A. Velena, *Chim. Oggi*, **28**, 68 (2008).

6. M. A. S. Fernandes, M. S. Santos, A. J. M. Moreno, L. Chernova, A. Krauze, G. Duburs, J. A. F. Vicente, *Toxicol. In Vitro*, **23**, 1333 (2009).
7. V. Lukic-Panin, T. Kamiya, H. Zhang, T. Hayashi, A. Tsuchiya, Y. Sehara, K. Deguchi, T. Yamashita, K. Abe, *Brain Res.*, **1176**, 143 (2007).
8. E. Ilijic, J. N. Guzman, D. J. Surmeier, *Neurobiol. Dis.*, **43**, 364 (2011).
9. R. Leon, R. C. de Los, J. Marco-Contelles, M. G. Lopez, A. G. Garcia, M. Villarroya, *Eur. J. Med. Chem.*, **43**, 668 (2008).
10. R. Vilskersts, B. Vigante, Z. Neidere, A. Krauze, I. Domracheva, L. Bekere, I. Shestakova, G. Duburs, M. Dambrova, *Lett. Drug Des. Discovery*, **9**, 322 (2012).
11. A. A. Krauze, R. O. Vitolina, M. R. Romanova, G. Ya. Dubur, *Khim.-Farm. Zh.*, **22**, 548 (1988). [*Pharm. Chem. J.*, **22**, 366 (1991).]
12. A. Krauze, L. Baumane, L. Sile, L. Cherniva, M. Vilums, R. Vitolona, G. Duburs, J. Stradins, *Khim. Geterotsikl. Soedin.*, 1022 (2004). [*Chem. Heterocycl. Compd.*, **40**, 876 (2004).]
13. A. A. Krauze, A. G. Odinecs, A. A. Verreva, S. K. Germane, A. N. Kozhukov, G. J. Dubur, *Khim.-Farm. Zh.*, **25**, 40 (1991). [*Pharm. Chem. J.*, **25**, **477** (1991).]
14. I. E. Kirule, A. A. Krauze, A. H. Velena, D. J. Antipova, G. J. Arnicane, I. A. Vucina, *Khim.-Farm. Zh.*, **26**, 59 (1992). [*Pharm. Chem. J.*, **26**, 411 (1992).]
15. D. Tirzite, A. Krauze, A. Zubareva, G. Tirzits, G. Duburs, *Khim. Geterotsikl. Soedin.*, 902 (2002). [*Chem. Heterocycl. Compd.*, **38**, 795 (2002).]
16. G. J. Dubur, M. M. Veveris, G. Weinheimer, E. A. Bisenieks, N. R. Makarova, A. A. Kimenis, J. R. Uldrikis, E. J. Lukevics, D. Dooley, H. Osswald, *Arzneim. Forsch.*, **39**, 1185 (1989).
17. G. Dubur, E. Bisenieks, S. Germane, V. Klusa, E. Bleidelis, I. Misane, Eur. Pat. Appl. EP 499983.
18. V. Klusa, *Drug Future*, **20**, 135 (1995).
19. M. Drigelova, B. Tarabova, G. Duburs, L. Lacinova, *Can. J. Physiol. Pharmacol.*, **87**, 923 (2009).
20. Z. Andzans, A. Krauze, I. Adlere, L. Krasnova, G. Duburs, *Khim. Geterotsikl. Soedin.*, 454 (2013).
21. S. K. Germane, O. E. Eberlinsh, A. N. Kozhukov, in *Scientific and Procedural Aspects of Biological Research on New Medicinal Drugs*, Zinatne, Riga, 1987, p. 87 (in Russian).

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Received 2.11.2012

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