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SYNTHESIS AND PROPERTIES OF METHYL 6-ALKYLSULFANYL-4-(2-CHLOROPHENYL)-1,4-DIHYDROPYRIDINE-3-CARBOXYLATES

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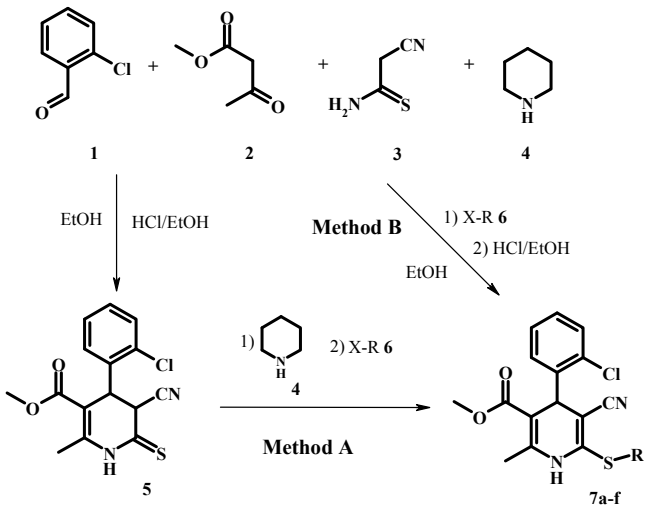
Methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7** with optimum lipophilicity have been obtained by treatment of methyl 4-(2-chlorophenyl)-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate **5** with alkyl halides **6**. Preparation of target compounds **7** was improved using one-pot five-component synthesis. Methyl 6-ethylsulfanyl-, 6-cyclopropylmethylsulfanyl- and 6-isobutylsulfanyl-1,4-dihydropyridine-3-carboxylates **7b**,**e**,**f** have shown calcium channel blocking activity in *in vitro* models – SH-SY5Y cell lines (human neuroblastoma).

Key words: *1,4-dihydropyridine-3-carboxylate, 6-thioxo-1,4,5,6-tetrahydropyridine, one-pot five-component synthesis, calcium channel blocking activity.*

INTRODUCTION

6-Alkylsulfanyl-1,4-dihydropyridines display antioxidant [1], hepatoprotective [2] and antiradical [3] activities. 6-Methylsulfanyl-1,4-dihydropyridine-3-carboxylates possess pronounced coronary circulation-stimulating and blood pressure-decreasing activities [4,5]. Moreover, the advantage of 1,4-dihydropyridines (DHP) containing sulfur atom is their low toxicity [5].

This study was performed in order to synthesize methyl 6-alkylsulfanyl-4-(2-chlorophenyl)-1,4-dihydropyridine-3-carboxylates and test their calcium channel blocking activity in *in vitro* models (SH-SY5Y cell lines – human neuroblastoma). To improve their cardiovascular activity, various alkyl groups were introduced in the position 6 in the 1,4-DHP molecule.



X = Br, I

a) R=Me, b) R=Et, c) R=Pr, d) R=*i*-Pr, e) R=$CH_2CH(CH_2)_2$, f) R=$CH_2CH(CH_3)_2$

EXPERIMENTAL

All reagents were purchased from *Aldrich, Acros, Fluka* or *Merck* and used without further purification. TLC was performed on 20×20 cm Silica gel TLC-PET F254 foils (*Fluka*). NMR spectra were recorded with a *Varian Mercury 200BB* spectrometer (200 MHz). Chemical shifts are reported in ppm relative to hexamethyldisiloxane (δ 0.055). Multiplicities are abbreviated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The coupling constants are expressed in Hz. Melting points were determined on an *OptiMelt* (*SRS Stanford Research Systems*). Elemental analyses were performed on an *EA 1106* (*Carlo Erba Instruments*). IR spectra have been recorded on a *Perkin-Elmer 580 B* spectrometer (in nujol) and peak positions ν_{max} were expressed in cm^{-1}. Compounds were recrystallized from methanol.

Synthesis of 5-cyano-4-(2-chlorophenyl)-2-methyl-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylic acid methyl ester (5)

To stirred mixture of aromatic aldehyde **1** (2.0 mmol), methyl acetoacetate **2** (2.0 mmol), 2-cyanothioacetamide **3** (2.0 mmol) in ethanol (20 ml), piperidine **4a** (2.0 mmol) was added. The mixture was stirred at room temperature for 1 h, and then 5 ml of 3 *N* hydrochloric acid in ethanol were added and stirred for 1 h at room temperature. The precipitate was filtered and washed with 10 ml of ethanol and 10 ml of water to give 0.53 g (84%), of compound **5** as yellow powder; m.p. 142–143 °C. IR spectrum, ν, cm^{-1}: 1690 (C=O); 2258 (C≡N); 3242 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 2.45 and 2.56 (3H, s and s, *cis*- and *trans*-2-Me); 3.59 and 3.61 (3H, s and s, *cis*- and *trans*-OMe); 4.18 and 4.88 (1H and 1H, d and d, J = 1.96 Hz, *trans*-4-H and 5-H); 4.19 and 5.15 (1H and 1H, d and d, J = 7.43 Hz, *cis*-4-H and 5-H); 6.82-7.38 (4H, m, C_6H_4Cl); 8.67 (1H, br.s, NH).

Elemental analysis data. Calculated for $C_{15}H_{13}ClN_2O_2S$, %: C 56.16; H 4.08; N 8.73. Found, %: C 56.14; H 3.86; N 8.70.

Synthesis of methyl 4-(2-chlorophenyl)-5-cyano-2-methyl-6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates (7). General method

Mixture of 6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate **5** (0.32 g, 1.0 mmol) and alkyl halide **6** (1.0 mmol) in 7 ml of ethanol was shortly heated until dissolution, stirred for 1 h at ambient temperature. The precipitate was filtered, washed with ethanol (5 ml) and water (5 ml).

Methyl 4-(2-chlorophenyl)-5-cyano-2-methyl-6-methylsulfanyl-1,4-dihydropyridine-3-carboxylate (7a)

Yield 0.31 g (93%), colourless powder; m.p. 210–212 °C. IR spectrum, ν, cm^{-1}: 1695 (C=O); 2200 (C≡N); 3200, 3260 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 2.38 (3H, s, 2-Me); 2.46 (3H, s, 6-S-Me); 3.54 (3H, s, OMe); 5.30 (1H, s, 4-H); 6.00 (1H, s, NH); 7.00–7.50 (4H, m, C_6H_4).

Found, %: C 57.34; H 4.26; N 8.59; S 9.55. Calculated for $C_{16}H_{15}ClN_2O_2S$, %: C 57.40; H 4.52; N 8.37; S 9.58.

Methyl 4-(2-chlorophenyl)-5-cyano-6-ethylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (7b)

Yield 0.28 g (82%), colourless powder; m.p. 157–159 °C. IR spectrum, ν, cm^{-1}: 1697 (C=O); 2211 (C≡N); 3070, 3247 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.27 and 2.93 (5H, t and q, J = 7.0 Hz, 6-S-Et); 2.38 (3H, s, 2-Me); 3.56 (3H, s, OMe); 5.31 (1H, s, 4-H); 6.07 (1H, s, NH); 7.10–7.36 (4H, m, C_6H_4).

Found, %: C 58.34; H 4.74; N 8.00; S 9.12. Calculated for $C_{17}H_{17}ClN_2O_2S$, %: C 58.53; H 4.91; N 8.03; S 9.19.

Methyl 4-(2-chlorophenyl)-5-cyano-2-methyl-6-propylsulfanyl-1,4-dihydropyridine-3-carboxylate (7c)

Yield 0.31 g (86%), colourless powder; m.p. 125–126 °C. IR spectrum, ν, cm^{-1}: 1660 (C=O); 2200 (C≡N); 3438 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.97, 1.52–1.71 and 2.71–2.99 (7H, t, m and m, J = 7.48 Hz, 6-S-Pr); 2.37 (3H, s, 2-Me); 3.55 (3H, s, OMe); 5.29 (1H, s, 4-H); 6.15 (1H, s, NH); 7.12–7.36 (4H, m, C_6H_4).

Found, %: C 58.84; H 5.34; N 7.64; S 9.03. Calculated for $C_{18}H_{19}ClN_2O_2S$, %: C 59.58; H 5.28; N 7.72; S 8.84.

Methyl 4-(2-chlorophenyl)-5-cyano-6-isopropylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (7d)

Yield 0.30 g (83%), colourless powder; m.p. 137–139 °C. IR spectrum, ν, cm^{-1}: 1660 (C=O); 2210 (C≡N); 3438 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.27 and 1.29 (6H, dd, J_{1-1} = 2.20 Hz, J_{1-2} = 4.40 Hz, 6-S-CH($\underline{CH_3}$)$_2$); 2.39 (3H, s, 2-Me); 3.45–3.58 (1H, m, 6-S-$\underline{CH}$(CH_3)$_2$); 3.56 (3H, s, OMe); 5.33 (1H, s, 4-H); 6.09 (1H, s, NH); 7.10–7.37 (4H, m, C_6H_4).

Found, %: C 59.24; H 5.28; N 7.71; S 8.72. Calculated for $C_{18}H_{19}ClN_2O_2S$, %: C 59.58; H 5.28; N 7.72; S 8.84.

Methyl 4-(2-chlorophenyl)-5-cyano-6-cyclopropylmethylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (7e)

Yield 0.34 g (92%), colourless powder; m.p. 132–133 °C. IR spectrum, ν, cm^{-1}: 1660 (C=O); 2200 (C≡N); 3438 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.22 and 0.57 (4H, m and m, 6-S-CH_2CH($\underline{CH_2}$)$_2$); 0.90–1.07 (1H, m, 6-S-$CH_2\underline{CH}$(CH_2)$_2$); 2.38 (3H, s, 2-Me); 2.68–2.94 (2H, m, 6-S-$\underline{CH_2}$CH(CH_2)$_2$); 3.55 (3H, s, OMe); 5.31 (1H, s, 4-H); 6.19 (1H, s, NH); 7.10–7.36 (4H, m, C_6H_4).

Found, %: C 60.41; H 5.17; N 7.43; S 8.74. Calculated for $C_{19}H_{19}ClN_2O_2S$, %: C 60.87; H 5.11; N 7.47; S 8.55.

Methyl 4-(2-chlorophenyl)-5-cyano-6-isobutylsulfanyl-2-methyl-1,4-dihydropyridine-3-carboxylate (7f)

Yield 0.30 g (79%), colourless powder; m.p. 142–144 °C. IR spectrum, ν, cm^{-1}: 1660 (C=O); 2200 (C≡N); 3438 (NH). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.97 (6H, t, J = 6.59 Hz, 6-S-CH_2CH($\underline{CH_3}$)$_2$); 1.70–1.87 (1H, m, 6-S-$CH_2\underline{CH}$(CH_3)$_2$); 2.39 (3H, s, 2-Me); 2.65–2.88 (2H, m, 6-S-$\underline{CH_2}$CH(CH_3)$_2$); 3.56 (3H, s, OMe); 5.29 (1H, s, 4-H); 6.06 (1H, s, NH); 7.13–7.36 (4H, m, C_6H_4).

Found, %: C 60.30; H 5.65; N 7.69; S 8.62. Calculated for $C_{19}H_{21}ClN_2O_2S$, %: C 60.55; H 5.62; N 7.43; S 8.51.

One-pot reaction for synthesis of compounds 7

To stirred mixture of 2-chlorobenzaldehyde **1** (0.5 mmol), methyl acetoacetate **2** (0.5 mmol), 2-cyanothioacetamide **3** (0.5 mmol) in ethanol (2 ml), piperidine **4** (0.5 mmol) was added. The mixture was stirred at room temperature for 1 h. Alkyl halide **6** (0.5 mmol) was added and reaction mixture was heated for 5 min. Reaction mixture was acidified with 0.12 ml of 3*N* hydrochloric acid in ethanol and stirred for 1 h at room temperature. The precipitate was filtered and washed with 10 ml of ethanol and 10 ml of water.

***In vitro* activity assay**

Measurement of intracellular Ca^{2+}

Monolayer tumor cells SH-SY5Y (human neuroblastoma, cells were obtained from the ATTC®) (American Type Culture Collection) and were grown in standard medium DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% of fetal bovine serum. 3×10^4 cells/well were placed in 96-well plates for 24 h. Changes in intracellular concentration $[Ca^{2+}]_i$ were assayed using Fluo-4 NW Calcium assay kit (Invitrogen) according to modificaton of the known method [6, 7]. The cells were preincubated with compounds (100 μM) for 15 min followed by stimulation with 20 nM carbachol. Fluorescence intensity was monitored using fluorescence spectrophotometer (Thermo Ascient, Finland) with excitation at 494 nm and emmision at 516 nm. The IC_{50} values were calculated using the program Graph Pad Prism® 3.0.

RESULTS AND DISCUSSION

The aim of this work is to elaborate the method for the synthesis of methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7** by modifying substituents at position 6 in DHP molecule. The introduction of prolonged and lipophilic 6-alkylsulfanyl group in DHP carboxylate could increase the bioavailability of the title compound in the human body.

Methyl 4-(2-chlorophenyl)-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate **5** was prepared in 84% yield according to the standard procedure [5]. Methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7a**–**f** have been obtained by alkylation of intermediate **5** with alkyl halides **6** (method A) and by one-pot five-component condensation (method B) as well (see Scheme). Using the conventional method A, compounds **7** were obtained in 79–93% yield. Summary yields of compounds **7** in two-step synthesis were 69–78% (Table 1.). According to the our previous studies [5, 8, 9] target compounds **7** were obtained in five-component one-pot synthesis (method B). It is worth to mention, that in the method B, the sequence of starting compounds **1**–**4** and **6** is important for reaching high yields of target products **7** [10]. Condensation of aromatic aldehyde **1**, methyl acetoacetate **2**, 2-cyanothioacetamide **3**, piperidine **4** and alkyl halides **6**, followed by acidification with HCl/EtOH solution gave methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7** in 77–93% yield (Table 1.). This approach enables the preparation of methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7** in a shorter time (1–2 hours), under mild conditions and in higher yields than in the two-step synthesis.

Table 1. The yields and lipophilicity values of methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates (**7**)

Compound	R	Method A, Yield, %, (Summary yield[1], %)	Method B, Yield[2], %	log *P*[4]
a	Me	93 (78)	93 (85[3])	3.47
b	Et	82 (69)	872	3.85
c	Pr	86 (72)	77	4.35
d	*i*-Pr	83 (70)	79	4.21
e	$CH_2CH(CH_2)_2$	92 (77)	82	4.34
f	$CH_2CH(CH_3)_2$	79 (66)	88	4.59

N o t e s : [1] calculated on 2-chlorobenzaldehyde; [2] determined by HPLC; [3] isolated yield; [4] theoretical calculations [14].

Theoretical calculations of lipophilicity of compounds under study show that introduction of longer alkyl chain in the position 6 of 1,4-DHP ring causes the increase of value of log *P* (partition coefficient in the system *octanol–water*). According to the Lipinski's Rule of Five [11, 12] the new synthesized compounds **7** possess the optimum lipophilicity – log *P* = 3.47–4.59 (Table 1.).

The structures of intermediate **5** and reaction products **7** were established by their spectral data (^{1}H NMR, IR) and elemental analysis data. In the IR spectra, characteristic absorption bands of 5-C≡N group for 6-thioxo-1,4,5,6-tetrahydropyridine **5** at 2258 cm^{-1} and for 1,4-dihydropyridines **7** at 2200–2210 cm^{-1} were observed. Absorption bands of $\nu_{C=O}$ of compounds are in agreement with the type of conjugation of C=O groups. In the ^{1}H NMR spectra (taken in $CDCl_3$ solution) signals characteristic of *cis-trans* isomers of 6-thioxo-1,4,5,6-tetrahydropyridines **5** were observed, the *cis* isomer being in superiority over *trans* isomer [13]. In the ^{1}H NMR spectrum the characteristic 4-H proton signals of 1,4-DHPs **7** appeared as singlets at δ 5.29–5.33 ppm.

Methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7** were tested on calcium channel blocking activity in *in vitro* models (SH-SY5Y cell lines) as effectors of agonist carbachol. Compounds **7a, 7c** and **7d** show no activity, while compounds **7b**,**e**,**f** have antagonistic activity in concentration $IC_{50} > 100$ μ*M*.

CONCLUSIONS

Methyl 6-alkylsulfanyl-1,4-dihydropyridine-3-carboxylates **7** with optimum lipophilicity have been obtained in alkylation reactions of methyl 4-(2-chlorophenyl)-6-thioxo-1,4,5,6-tetrahydropyridine-3-carboxylate **5** with alkyl halides **6**. Preparation of target compounds **7** was improved using one-pot five-component synthesis. Methyl 6-ethylsulfanyl-, 6-cyclopropylmethylsulfanyl- and 6-isobutylsulfanyl-1,4-dihydropyridine-3-carboxylates **7b**,**e**,**f** show calcium channel blocking activity in *in vitro* models – SH-SY5Y cell lines (human neuroblastoma).

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6-ALKILSULFANIL-4-(2-HLORFENIL)-1,4-DIHIDROPIRIDĪN-3-KARBONSKĀBES METILESTERU SINTĒZE UN ĪPAŠĪBAS

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K O P S A V I L K U M S

6-Alkilsulfanil-5-ciano-4-(2-hlorfenil)-2-metil-1,4-dihidropiridīn-3-karbonskābes metilesteri **7**, kas raksturojas ar optimālo lipofilitāti, iegūti 5-ciano-4-(2-hlorfenil)-2-metil-6-tiokso-1,4,5,6-tetrahidropiridīn-3-karbonskābes metilestera (**5**) alkilēšanas reakcijās ar halogēnalkāniem **6**. Uzlabots mērķprodukta **7** iegūšanas paņēmiens, izstrādājot vienreaktora sintēzes metodi. 6-Etilsulfanil-, 6-ciklopropilmetilsulfanil- un 6-izobutilsulfanil-1,4-dihidropiridīn-3-karbonskābes metilesteri **7b**,**e**,**f** uzrāda L-tipa kalcija kanālu bloķējošo aktivitāti *in vitro* modeļos – šūnu līnijās SH-SY5Y.