Synthesis and biological evaluation of novel amino-substituted derivatives of pyrido[2,3-*d*]pyrimidine as inhibitors of protein kinase CK2

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Aim. A search for human protein kinase CK2 inhibitors in a series of new amino-substituted pyrido[2,3-*d*]pyrimidine derivatives. **Methods.** Organic synthesis, analytical and spectral methods, molecular docking, *in vitro* biochemical testing. **Results.** Synthesis of new pyrido[2,3-d]pyrimidine derivatives with various aminogroups in position[s] 4 and 6 of the heterocycle was developed. Two compounds inhibiting kinase CK2 in micromolar concentrations were found among these derivatives. **Conclusion.** New pyrido[2,3-d]pyrimidin-7-ones containing aminogroups in position[s] 4 and 6 of heterocyclic system and new 4-amino-substituted pyrido[2,3-d]pyrimidin-7-amine derivatives have been synthesized. The inhibition activity of new pyrido[2,3-d]pyrimidines has been examined and the optimization modes have been suggested. Methyl-2-[(7-aminopyrido[2,3-d]pyrimidine-6-yl)amino]benzoate and N-(4-anilino-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidine-6-yl)-3,4-dimethoxy- benzamide were found to inhibit protein kinase CK2 at IC50 of 6 and 19.5 µM, respectively.

K e y w o r d s: pyrido[2,3-*d*]pyrimidine derivatives, synthesis, protein kinase CK2, the inhibition activity

Introduction

In recent years the development of protein kinase inhibitors excites an increasing attention in medical chemistry. Protein kinase CK2 (*Casein kinase* 2), one of significant molecular targets, is the polysubstrate serine/threonine kinase present in all eukaryotic cells. This enzyme is an important link of numerous signal ways of a cell and is involved in various pathological processes [4, 5]. This protein kinase is known to have an increased activity in inflammatory tissues and many tumors. Therefore, protein kinase CK2 inhibitors are of great therapeutical importance as anti-inflammatory and antitumor drugs [6–10]. This

state is supported by the fact that "Silmitasertib" (CX-4945) is verified on the second stage of clinical testing and might be a perspective antitumor drug [4, 11]. Hence, the research for new protein kinase inhibitors is fairly relevant.

CK2 inhibitors were found among derivatives benzotriazole and benzimidazole [12, 13], quinoline [14, 15], indolo[1,2-*a*]quinazoline [16], thieno[2,3-*d*]pyrimidine [17, 18], pyrazine [19], pyrazolo[1,5-*a*]pyrimidine [20] and other compounds.

The inhibition activity of pyrido[2,3-*d*]pyrimidine derivatives toward CK2 kinase has not been studied. Noteworthy, the substituted pyrido[2,3-*d*]pyrimidines are the inhibitors of EGFR [21-24], Cyclin-Dependent kinases [25, 26], Src-tyrosine kinase [27, 28] and c-Jun N-Terminal kinase (JNK) [29,30]. It was found that among 2-substituted pyrido[2,3-*d*]pyrimidin-7-one derivatives there are powerful selective inhibitors of CDK4/6 kinase (Palbociclib) [25], Abl kinase (PD173955) [31, 32] and p38 MAP kinase (Pamapimod) [33] which are effective in the treatment of autoimmune and cancer diseases.

In this work the search for CK2 inhibitors amongst amino-substituted pyrido[2,3-*d*]pyrimidine derivatives was carried out.

Materials and Methods

Chemistry

^{1}H and ^{13}C NMR spectra were acquired on Varian Mercury 400 (400 and 100 MHz for ^{1}H and ^{13}C nuclei, respectively) and Bruker Avance DRX-500 (500 and 125 MHz for ^{1}H and ^{13}C nuclei, respectively) instruments, with TMS as internal standard. ^{13}C NMR signals were assigned by using APT method. LCMS spectra were performed on Agilent 1100 Series HPLC equipped with diode array and Agilent LC/MSD SL mass selective detector, ionization method − chemical ionization at atmospheric pressure, m/z scan range from 80 to 1000. Elemental analysis was performed at the Analytical laboratory of the Institute of Bioorganic Chemistry and Petrochemistry of the NAS of Ukraine. Melting points were determined on Boetius hot stage apparatus. The reaction progress and purity of the obtained compounds were controlled by TLC on Silufol UV-254 plates using a 19:1 mixture of $CHCl_3$–MeOH as eluent.

***Methyl (6-amino-4-chloro-7-oxopyrido [2,3-d]pyrimidin-8(7H)-yl)acetate* (2)**. Triethylamine (4.20 ml, 30 mmol) was added to a suspension of aldehyde **1** (1.77 g, 10 mmol) and glycine methyl ester hydrochloride (2.51 g, 20 mmol) in methanol (20 ml). Reaction mixture was stirred for 5 h at 20–25 °C. The resulting precipitate was filtered and recrystallized from acetonitrile. Yield 0.96 g (36 %). M.p. = 192–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.65 s (3H, OCH_3), 5.15 s (2H, CH_2), 6.46 br.s (2H, NH_2), 6.80 s (1H, H-5), 8.54 s (1H, H-2); ^{13}C NMR (100 MHz, DMSO-d_6), δ: 43.2 (CH_2N), 52.7 (CH_3O), 97.4 (CH), 114.7 (C), 140.6 (C), 148.7 (C), 150.7 (CH), 153.3 (C), 158.5 (C=O), 176.5 (C=O); MS: m/z 269 MH[+]. Calcd. for $C_{10}H_9ClN_4O_3$: C 44.71; H 3.38; Cl 13.20; N 20.85. Found: C 44.78; H 3.35; Cl 13.16; N 20.81.

***6-Amino-4-chloropyrido[2,3-d]pyrimidin-7(8H)-one* (5)**. Triethylamine (3.37 ml, 22 mmol) was added to an aldehyde **4** (1.58 g, 10 mmol) and glycine methyl ester hydrochloride (1.38 g, 11 mmol) in methanol (20 ml) and reaction mixture was refluxed within 7 h.

The formed precipitate was filtered and recrystallized from DMF. Yield 0.77 g (39%). M.p. = 318–320 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 6.27 br.s (2H, NH$_2$), 6.73 s (1H, H-5), 8.46 s (1H, H-2), 12.80 br.s (1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6), δ: 97.9 (C), 114.1 (CH), 141.6 (C), 149.5 (C), 151.6 (CH), 152.5(C), 159.6(C=O); MS: m/z 197 MH$^+$. Calcd. for C$_7$H$_5$ClN$_4$O: C 42.77; H 2.56; Cl 18.03; N 28.50. Found: C 42.81; H 2.54; Cl 17.96; N 28.45.

General method of obtaining substituted pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones 3a-f, 6a-g. One of compounds **2**, **5** (1 mmol), respective amine (1 mmol) and triethylamine (1 mmol) was refluxed for 6–8 h. Completion of chemical reaction was controlled by TLC method. Triethylamine (1 mmol) and corresponding acid chloride (1 mmol) were added to reaction mixture and obtained suspension was refluxed within 2–3 h. The resulting precipitate was filtered and recrystallized from propan-2-ol.

Methyl [6-(benzoylamino)-4-(methylamino)-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl] acetate (3a). Yield 0.21 g (57 %). M.p. = 247–249 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 2.97 s (3H, CH$_3$), 3.67 s (3H, OCH$_3$), 5.16 s (2H, CH$_2$), 7.54–7.63 m (3H, CH$_{arom}$), 7.96–7.98 m (2H, *J* 7.2 Hz, CH$_{arom}$), 8.30 br.s (1H, NH), 8.37s (1H, H-5), 8.84 s (1H, H-2), 9.56 br.s (1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6), δ: 28.1 (CH$_3$), 43.0 (CH$_2$), 52.7 (OCH$_3$), 97.9 (C), 112.8 (C), 127.0 (2CH), 128.6 (CH), 128.8 (2CH), 132.2 (CH), 132.3 (C), 149.2 (C), 156.5 (CH), 158.7 (C), 159.3 (C), 166.0 (C), 168.4 (C); MS: m/z 368 MH$^+$. Calcd. for C$_{18}$H$_{17}$N$_5$O$_4$: C 50.85; H 4.66; N 19.06. Found: C 50.87; H 4.71; N 19.02.

Methyl [4-(ethylamino)-6-[(2-fluorobenzoyl)amino]-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl]acetate (3b). Yield 0.21 g (53 %). M.p. = 219–221 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 1.19 t (3H, *J* 7.2 Hz, CH$_3$), 3.50–3.54 m (2H, CH$_2$), 3.67 s (3H, OCH$_3$), 5.16 s (2H, CH$_2$), 7.41 q (2H, *J* 6.8 Hz, CH$_{arom}$), 7.67 d (1H, *J* 6.8 Hz, CH$_{arom}$), 7.97 d (1H, *J* 7.2 Hz, CH$_{arom}$), 8.34 s (1H, H-5), 8.37 br.s. (1H, NH), 8.97 s (1H, H-2), 9.80 br.s (1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6), δ: 14.9 (CH$_3$), 36.2 (CH$_2$), 43.2 (CH$_2$), 52.8 (OCH$_3$), 97.3 (C), 116.5 (CH), 117.2 (C), 121.7 (C), 125.7 (CH), 126.3 (CH), 131.5 (CH), 134.9 (CH), 149.9 (C), 157.0 (CH), 158.3 (C), 159.0 (C), 161.2 (C), 161.8 (C), 168.8 (C); MS: m/z 400 MH$^+$. Calcd. for C$_{19}$H$_{18}$FN$_5$O$_4$: C 57.14; H 4.54; F 4.76; N 17.54. Found: C 57.18; H 4.57; F 4.72; N 17.50.

Methyl [6-[(2-ethylbutanoyl)amino]-4-[(2-methoxyethyl)amino]-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl]acetate (3c). Yield 0.22 g (54 %). M.p. = 138–140 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 0.84–0.90 m (6H, 2CH$_3$), 1.43–1.50 m (2H, CH$_2$), 1.53–1.60 m (2H, CH$_2$), 2.58–2.65 m (1H, CH), 3.53 t (2H, *J* 7.6 Hz, CH$_2$), 3.68 s (6H, 2OCH$_3$), 5.14 s (2H, CH$_2$), 8.30-8.35 m (2H, H-5, NH), 8.85 s (1H, H-2), 9.44 br.s (1H, NH), the protons of the CH$_2$ group are overlapped with water; ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.0 (2CH$_3$), 25.7 (2CH$_2$), 43.1 (CH$_2$), 49.0 (CH), 49.2 (CH$_2$), 52.4 (OCH$_3$), 58.2 (OCH$_3$), 70.6 (CH$_2$), 97.4 (C), 116.3 (C), 126.7 (CH), 149.8 (C), 156.3 (CH), 158.4 (C), 159.1 (C), 168.9 (C), 175.5 (C); MS: m/z 406 MH$^+$. Calcd. for C$_{19}$H$_{27}$N$_5$O$_5$: C 56.29; H 6.71; N 17.27. Found: C 56.32; H 6.74; N 17.23.

Methyl [6-(acetylamino)-4-[(4-methoxyphenyl)amino]-7-oxopyrido[2,3-d]pyrimidin-

8(7H)-yl]acetate (3d). Yield 0.23 g (58 %). M.p. = 216–218 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 2.19 s (3H, CH$_3$), 3.68 s (3H, OCH$_3$), 3.76 s (3H, OCH$_3$), 5.17 s (2H, CH$_2$), 6.94 d (2H, *J* 8.8 Hz, CH$_{arom}$), 7.48 d (2H, *J* 8.8 Hz, CH$_{arom}$), 8.33 s (1H, H-5), 8.97 s (1H, H-2), 9.63 br.s (1H, NH), 9.73 br.s (1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6), δ: 24.1 (CH$_3$), 43.1 (CH$_2$), 53.3 (OCH$_3$), 55.4 (OCH$_3$), 98.2 (C), 114.3 (2CH), 116.3 (C), 125.5 (2CH), 127.3 (CH), 132.2 (C), 150.3 (C), 156.3 (C), 156.5 (CH), 157.7 (C), 158.4 (C), 168.9 (C), 169.9 (C); MS: m/z 398 MH$^+$. Calcd. for C$_{19}$H$_{19}$N$_5$O$_5$: C 57.43; H 4.82; N 17.62. Found: C 57.48; H 4.85; N 17.56.

Methyl [6-(benzoylamino)-4-[(4-methoxyphenyl)amino]-7-oxopyrido[2,3-d]pyrimidin-8(7H)-yl]acetate (3e). Yield 0.27 g (59 %). M.p. = 222–224 ºC. ^{1}H NMR (400 MHz, CDCl$_3$), δ: 3.79 s (3H, OCH$_3$), 3.82 s (3H, OCH$_3$), 5.31 s (2H, CH$_2$), 6.92 d (2H, *J* 8.8 Hz, CH$_{arom}$), 7.44 d (2H, *J* 8.8 Hz, CH$_{arom}$), 7.43–7.60 m (4H, CH$_{arom}$, NH), 7.90–7.92 m (2H, CH$_{arom}$), 8.43 s (1H, H-5), 9.04 s (1H, H-2), 9.29 br.s (1H, NH); MS: m/z 460 MH$^+$. Calcd. for C$_{24}$H$_{21}$N$_5$O$_5$: C 62.74; H 4.61; N 15.24. Found: C 62.78; H 4.63; N 15.17.

Methyl [4-[(4-methoxyphenyl)amino]-7-oxo-6-(pentanoylamino)pyrido[2,3-d]pyrimidin-8(7H)-yl]acetate (3f). Yield 0.23 g (52 %). M.p. = 127–129 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 0.90 t (3H, *J* 6.8 Hz, CH$_3$), 1.30–1.39 m (2H, CH$_2$), 1.55–1.63 m (2H, CH$_2$), 3.68 s (3H, OCH$_3$), 3.76 s (3H, OCH$_3$), 5.17 s (2H, CH$_2$), 6.94 d (2H, *J* 8.8 Hz, CH$_{arom}$), 7.48 d (2H, *J* 8.8 Hz, CH$_{arom}$), 8.33 s (1H, H-5), 9.01 s (1H, H-2), 9.52 br.s (1H, NH), 9.75 br.s (1H, NH), the protons of the CH$_2$ group are overlapped with water; ^{13}C NMR

(125 MHz, DMSO-d_6), δ: 13.8 (CH$_3$), 22.3 (CH$_2$), 27.7 (CH$_2$), 36.2 (CH$_2$), 43.1 (CH$_2$), 53.0 (OCH$_3$), 55.8 (OCH$_3$), 98.3 (C), 114.4 (2CH), 116.0 (C), 125.2 (2CH), 127.3 (CH), 132.2 (C), 150.3 (C), 155.7 (C), 156.5 (CH), 157.7 (C), 158.4 (C), 168.9 (C), 172.8 (C); MS: m/z 440 MH$^+$. Calcd. for C$_{22}$H$_{25}$N$_5$O$_5$: C 60.13; H 5.73; N 15.94. Found: C 60.18; H 5.77; N 15.88.

3,4-Dimethoxy-N-[7-oxo-4-(propylamino)-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl]benzamide (6a). Yield 0.20 g (54 %). M.p. = 261–263 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 0.91 t (3H, *J* 7.2 Hz, CH$_3$), 1.57–1.64 m (2H, CH$_2$), 3.43 q (2H, *J* 7.2 Hz, CH$_2$), 3.84 s (6H, 2OCH$_3$), 7.11 d (1H, *J* 8.4 Hz, CH$_{arom}$), 7.52 s (1H, CH$_{arom}$), 7.59 d (1H, *J* 8.4 Hz, CH$_{arom}$), 8.29 s (1H, H-5), 8.36 t (1H, *J* 6.4 Hz, NH), 8.78 s (1H, H-2), 9.39 br.s (1H, NH), 12.67 br.s (1H, NH); MS: m/z 384 MH$^+$. Calcd. for C$_{19}$H$_{21}$N$_5$O$_4$: C 59.52; H 5.52; N 18.27. Found: C 59.56; H 5.49; N 18.23.

N-[4-(Isobutylamino)-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl]-3,4-dimethoxybenzamide (6b). Yield 0.21 g (53 %). M.p. = 236–238 ºC. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 0.91 d (6H, *J* 6.5 Hz, 2CH$_3$), 1.95-2.02 m (1H, CH), 3.85 s (6H, 2OCH$_3$), 7.11 d (1H, *J* 8.5 Hz, CH$_{arom}$), 7.53 s (1H, CH$_{arom}$), 7.59 d (1H, *J* 8.5 Hz, CH$_{arom}$), 8.16 t (1H, *J* 6.0 Hz, NH), 8.25 s (1H, H-5), 8.79 s (1H, H-2), 9.38 br.s (1H, NH), 12.54 br.s (1H, NH); MS: m/z 398 MH$^+$. Calcd. for C$_{20}$H$_{23}$N$_5$O$_4$: C 60.44; H 5.83; N 17.62. Found: C 60.48; H 5.81; N 17.56.

N-(4-Anilino-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl)-4-methoxybenzamide (6c). Yield 0.22 g (56 %). M.p. = 283–285 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.85 s (3H,

OCH$_3$), 7.09–7.11 m (3H, CH$_{arom}$), 7.33–7.38 m (2H, CH$_{arom}$), 7.67 d (2H, *J* 10.0 Hz, CH$_{arom}$), 7.96 d (2H, *J* 10.0 Hz, CH$_{arom}$), 8.35 s (1H, H-5), 9.04 s (1H, H-2), 9.41 br.s (1H, NH), 9.77 br.s (1H, NH), 12.77 br.s (1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6), δ: 56.0 (OCH$_3$), 91.9 (C), 114.6 (C), 117.3 (2CH), 123.1 (CH), 124.1 (C), 126.2 (2CH), 127.8 (CH), 128.0 (C), 129.4 (2CH), 129.6 (2CH), 151.3 (C), 156.6 (C), 157.1 (CH), 159.6 (C), 162.9 (C), 164.7 (C); MS: m/z 388 MH$^+$. Calcd. for C$_{21}$H$_{17}$N$_5$O$_3$: C 65.11; H 4.42; N 18.08. Found: C 65.14; H 4.46; N 18.03.

***N-(4-Anilino-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl)-3,4-dimethoxybenzamide* (6d).** Yield 0.25 g (60 %). M.p. = 225–227 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.85 s (6H, 2OCH$_3$), 7.11–7.13 m (2H, CH$_{arom}$), 7.34–7.38 m (3H, CH$_{arom}$), 7.55 s (1H, CH$_{arom}$), 7.68 d (2H, *J* 8.0 Hz, CH$_{arom}$), 8.36 s (1H, H-5), 9.02 s (1H, H-2), 9.46 br.s (1H, NH), 9.76 br.s (1H, NH), 12.76 br.s (1H, NH); MS: m/z 418 MH$^+$. Calcd. for C$_{22}$H$_{19}$N$_5$O$_4$: C 63.30; H 4.59; N 16.78. Found: C 63.35; H 4.61; N 16.64.

***3,4-Dimethoxy-N-{4-[(4-methoxyphenyl)amino]-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl}benzamide* (6e).** Yield 0.24 g (54 %). M.p. = 278–280 ºC. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 3.76 s (3H, OCH$_3$), 3.86 s (6H, 2OCH$_3$), 6.89 d (2H, *J* 10.0 Hz, CH$_{arom}$), 7.06 d (1H, *J* 10.0 Hz, CH$_{arom}$), 7.52 d (3H, *J* 10.0 Hz, CH$_{arom}$), 7.57 d (1H, *J* 10.0 Hz, CH$_{arom}$), 8.25 s (1H, H-5), 9.04 s (1H, H-2), 9.33 br.s (1H, NH), 9.37 br.s (1H, NH), 12.68 br.s (1H, NH); MS: m/z 448 MH$^+$. Calcd. for C$_{23}$H$_{21}$N$_5$O$_5$: C 61.74; H 4.73; N 15.65. Found: C 61.78; H 4.75; N 15.62.

3,4-Dimethoxy-N-(7-oxo-4-piperidin-1-yl-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl)ben-

***zamide* (6f).** Yield 0.23 g (56 %). M.p. = 273–275 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 1.63–1.73 m (6H, 3CH$_2$), 3.56–3.66 m (4H, 2CH$_2$), 3.85 s (6H, 2OCH$_3$), 7.11 d (1H, *J* 8.4 Hz, CH$_{arom}$), 7.50 s (1H, CH$_{arom}$), 7.56 d (1H, *J* 8.4 Hz, CH$_{arom}$), 8.38 s (1H, H-5), 8.73 s (1H, H-2), 9.33 br.s (1H, NH), 12.74 br.s (1H, NH); MS: m/z 410 MH$^+$. Calcd. for C$_{21}$H$_{23}$N$_5$O$_4$: C 61.60; H 5.66; N 17.10. Found: C 61.64; H 5.70; N 17.06.

***N-(4-Azepan-1-yl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl)-3,4-dimethoxybenzamide* (6g).** Yield 0.24 g (57 %). M.p. = 228–230 ºC. ^{1}H NMR (500 MHz, DMSO-d_6), δ: 1.54–1.61 m (4H, 2CH$_2$), 1.86–1.93 m (4H, 2CH$_2$), 3.77–3.87 m (10H, 2OCH$_3$, 2CH$_2$), 7.10 d (1H, *J* 9.0 Hz, CH$_{arom}$), 7.48 s (1H, CH$_{arom}$), 7.54 d (1H, *J* 9.0 Hz, CH$_{arom}$), 8.27 s (1H, H-5), 8.99 s (1H, H-2), 9.27 br.s (1H, NH), 12.61 br.s (1H, NH); MS: m/z 424 MH$^+$. Calcd. for C$_{22}$H$_{25}$N$_5$O$_4$: C 62.40; H 5.95; N 16.54. Found: C 62.43; H 5.97; N 16.50.

***4-Chloropyrido[2,3-d]pyrimidin-7-amine* (7)** was synthesized according to method [34].

***Methyl 2-[(7-aminopyrido[2,3-d]pyrimidin-4-yl)amino]benzoate* (8).** The mixture of pyrido[2,3-*d*]pyrimidine **7** (0.18 g, 1 mmol), methyl 2-aminobenzoate (0.14 ml, 1.1 mmol) and triethylamine (0.15 ml, 1.1 mmol) in acetonitrile (2 ml) was refluxed for 7 h. The formed precipitate was filtered and recrystallized from ethanol– DMF (2:1). Yield 0.15 g (53 %). M.p. = 174–176 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.78 s (3H, OCH$_3$), 6.93 d (1H, *J* 9.2 Hz, H-6), 7.31 t (1H, *J* 8.0 Hz, CH$_{arom}$), 7.66-7.79 m (3H, NH$_2$, CH$_{arom}$), 7.97 d (1H, *J* 8.0 Hz, CH$_{arom}$), 8.23 d (1H, *J* 9.2 Hz, H-5), 8.39 d (1H, *J* 8.0 Hz, CH$_{arom}$), 8.57 s (1H, H-2), 11.24 br.s (1H, NH); ^{13}C

NMR (125 MHz, DMSO-d_6), δ: 52.8 (OCH$_3$), 101.2 (C), 113.8 (CH), 114.0 (C), 125.3 (CH), 131.0 (CH), 133.4 (CH), 133.9 (CH), 139.0 (CH), 152.7 (C), 154.5 (C), 158.5 (CH), 159.6 (C), 162.9 (C), 167.6 (C); MS: m/z 296 MH$^+$. Calcd. for C$_{15}$H$_{13}$N$_5$O$_2$: C 61.06; H 4.47; N 23.65. Found: C 61.01; H 4.44; N 23.72.

Biochemical testing

Compounds were tested using *in vitro* kinase assay [35]. Each test was done in triplicate in a reaction volume of 30μL, containing 6 µg of peptide substrate RRRDDDSDDD (New England Biolabs); 10 units of recombinant human CK2 holoenzyme (New England Biolabs); 50 µM ATP and γ-labeled 32P ATP, diluted to specific activity 100 µCi/µM; CK2 buffer (20 mM Tris-HCl, pH 7.5; 50 mM KCl; 10 mM MgCl$_2$) and inhibitor in varying concentrations. Incubation time was 20 min at 30ºC. The reaction was stopped by adding an equal volume of 10 % o-phosphoric acid and the reaction mixture was loaded onto 20-mm discs of phosphocellulose paper (Whatman). Disks were washed three times with 1 % o-phosphoric acid solution, air-dried at room temperature, and counted by the Cherenkov method in a beta-counter (LKB). As a negative control an equal volume of DMSO was added to the reaction mixture. Percent inhibition was calculated as ratio of substrate-incorporated radioactivity in the presence of inhibitor to the radioactivity incorporated in control reactions, i.e. in the absence of inhibitor. Serial dilutions of inhibitor stock solution were used to determine its IC$_{50}$ concentration.

Molecular docking

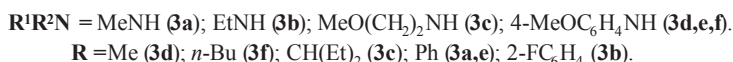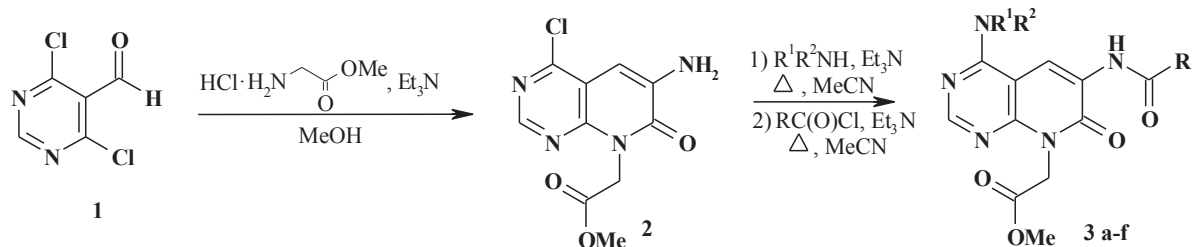*Preparation of ligand and receptor molecules.* Ligands were prepared by MGL Tools 1.5.6 [36] and Vega ZZ (command line) [37]. The catalytic subunit of protein kinase CK2 complex with a stable analogue of ATP (PDB code 3NSZ) was used as a target for docking [38]. Water molecules, ions, and ligands were removed from the PDB file. The receptor was prepared using MGL Tools and AutoGrid. Hydrogen atoms were removed from nonpolar atoms. The incoming formats of receptor and ligands data were converted into PDBQT-format with Vega ZZ in AUTODOCK force field.

Flexible docking. Autodock 4.2.6 programs package was used for the receptor-based flexible docking [36]. Parameters for docking calculation were set as described previously [39]. Autodock results were scored with Autodock scoring function and visual analysis of the best-scored complexes was performed using Discovery Studio Visualizer 4.0 (http://accelrys.com/).

Results and discussion

To identify new inhibitors of protein kinase CK2 among pyrido[2,3-*d*]pyrimidines, the receptor-based virtual screening of the virtual library (3408 pyridopyrimidine derivatives) was carried out. The molecular docking of ligands was performed with AutoDock 4.2.6 program targeting ATP-binding site of CK2. The most promising 14 compounds which had the best estimated binding energies were selected for further synthesis and biochemical evaluation.
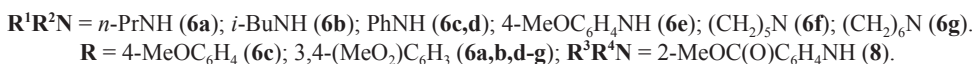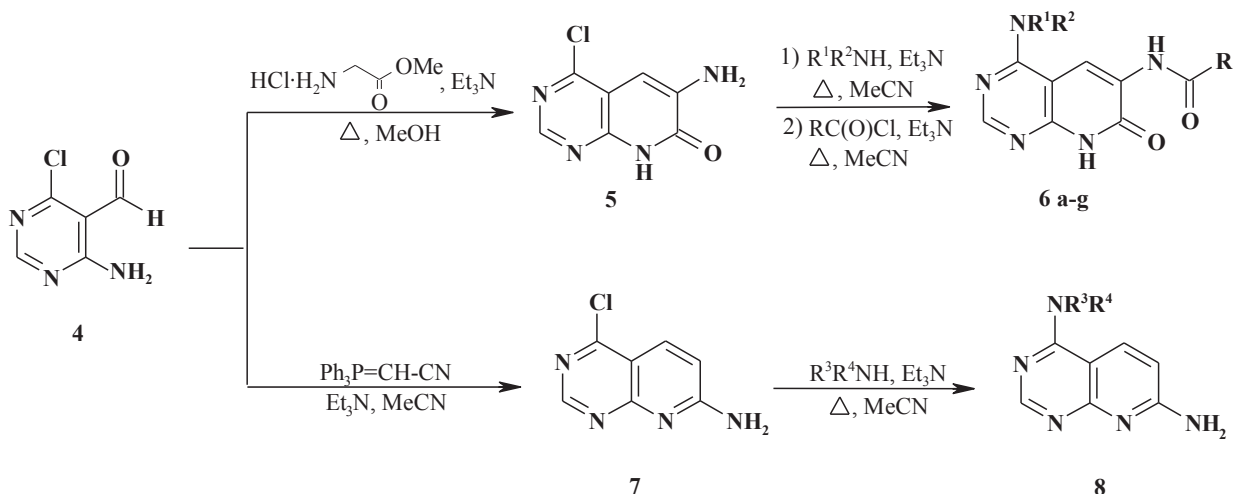
4,6-Dichloropyrimidin-5-carbaldehyde **1** was chosen as the initial compound for synthesis of pyrido[2,3-*d*]pyrimidine derivatives containing aminogroups in positions 4 and 6 of core heterocycle (**Scheme 1**). Owing to
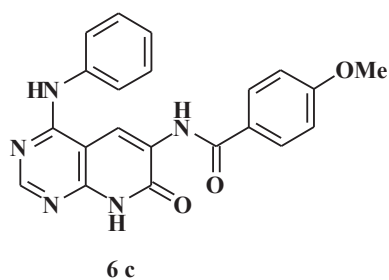
R^{1}R^{2}N = MeNH (**3a**); EtNH (**3b**); MeO(CH$_2$)$_2$NH (**3c**); 4-MeOC$_6$H$_4$NH (**3d,e,f**).
R = Me (**3d**); n-Bu (**3f**); CH(Et)$_2$ (**3c**); Ph (**3a,e**); 2-FC$_6$H$_4$ (**3b**).

Scheme 1. Synthesis of amino-substituted pyrido[2,3-d]pyrimidin-7-ones derivatives **3**.

interaction of aldehyde **1** with glycine methyl ester in methanol in the presence of triethyl-amine, intromolecular cyclization with following formation of 6-amino-4-chloro-7-oxopyrido[2,3-d]pyrimidine **2** takes place. Therefore, replacement of the chlorine atom with the aliphatic amines fragments in the compound **2** and the reaction of acylation lead to the formation of the target products **3**.
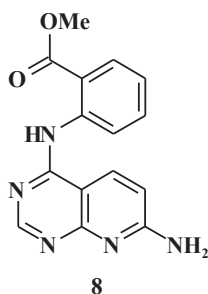
In order to synthesize pyrido[2,3-d]pyrimi-dine derivatives **6, 8** as an initial reagent it was utilized 4-amino-6-chloropyrimidin-5-carbal-dehyde (**Scheme 2**). 6-Amino-4-chloro-7-oxopyrido[2,3-d]pyrimidine **5** was obtained by reaction of aldehyde **4** with glycine methyl ester in the presence of triethylamine. 7-Aminopyrido[2,3-d]pyrimidine **7** was synthe-sized via Wittig reaction. Products of cyclization

R^{1}R^{2}N = n-PrNH (**6a**); i-BuNH (**6b**); PhNH (**6c,d**); 4-MeOC$_6$H$_4$NH (**6e**); (CH$_2$)$_5$N (**6f**); (CH$_2$)$_6$N (**6g**).
R = 4-MeOC$_6$H$_4$ (**6c**); 3,4-(MeO$_2$)C$_6$H$_3$ (**6a,b,d-g**); R^{3}R^{4}N = 2-MeOC(O)C$_6$H$_4$NH (**8**).

Scheme 2. Synthesis of pyrido[2,3-d]pyrimidine derivatives **6, 8**.

6 c

IC$_{50}$ = 19,5μM

8

IC$_{50}$ = 6 μM

Fig. 1. Structures and IC$_{50}$ values of pyrido[2,3-*d*]pyrimidine derivatives.

5 and **7** were shown to be convenient synthons for the preparation of new 4-amino-substituted pyrido[2,3-*d*]pyrimidine derivatives **6**, **8**.

The structures of synthesized compounds were proven by elemental analysis, ¹H and ¹³C NMR data and LCMS methods.

14 pyrido[2,3-*d*]pyrimidine derivatives were synthesized and their effect on the enzyme activity was investigated. In vitro experiments revealed that 2 compounds inhibit the activity of protein kinase CK2 (Fig. 1).

The complex of compound **6c** with the ATP-acceptor site of protein kinase CK2 obtained with molecular docking shows that the pyrido[2,3-*d*]pyrimidine heterocycle of compound **6c** is located in the adenine pocket of the ATP-binding site (Fig. 2). The hydrogen bond with the hinge region is formed between the NH-group of heterocycle and the carbonyl group of the amino acid residue Val116. The

substituent at the position 6 of heterocycle is directed into deep pocket of the ATP-binding site and forms stacking with Phe113. The substituent at the position 4 of heterocycle resides in the ribose pocket of the ATP-binding site and forms hydrophobic contact with Leu45.

In case of compound **8**, pyrido[2,3-*d*]pyrimidine heterocycle is located mirrored and deeper in the ATP acceptor site in comparison with compound **6c** (Fig. 3). The hydrogen bond interactions with the hinge region of CK2 occur between the backbone NH and carboxyl oxygen of Val116 and N-atom at position 6 of heterocycle and the amino group at position 7 of pyrido[2,3-*d*]pyrimidine. The substituent at position 4 of heterocycle is directed into deep pocket of the ATP binding site and forms stacking with Phe113 and additional hydrogen bond with Lys68. A higher activity of compound **8** (IC$_{50}$ = 6 μM) compared to compound **6c** (IC$_{50}$ = 19,5 μM) can be explained by its tighter interaction with CK2.

There are some ways of chemical optimization of pyrido[2,3-*d*]pyrimidines to increase their activity toward protein kinase CK2. Taking into account the similarity of binding mode of compound **8** with binding modes of flavones and thienopyrimidines obtained earlier [17,40,41] the introduction of hydrophobic groups, in particular, methyl, ethyl, phenyl or halogens (Br or Cl), into the position 5 of pyrido[2,3-*d*]pyrimidine can significantly increase the inhibitory activity of its derivatives. Also, improvement can be achieved by substituting 2-aminobenzoic acid methyl ester to 2-aminobenzoic acid or especially 3-aminobenzoic acid at position 4 of heterocycle. Such replacement will allow pyridopyrimidine derivatives to form ion bonds with Lys68 that
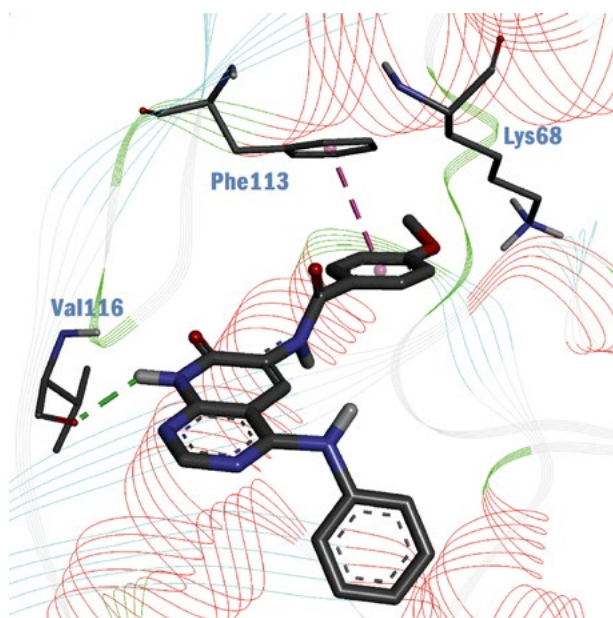
Fig. 2. The complex of compound **6c** with ATP-binding site of human protein kinase CK2. Hydrogen bonds are indicated by green dotted lines, hydrophobic bonds – by purple dotted lines.

Fig. 3. The complex of compound **8** with ATP-binding site of human protein kinase CK2. Hydrogen bonds are indicated by green dotted lines, hydrophobic bonds – by purple dotted lines.
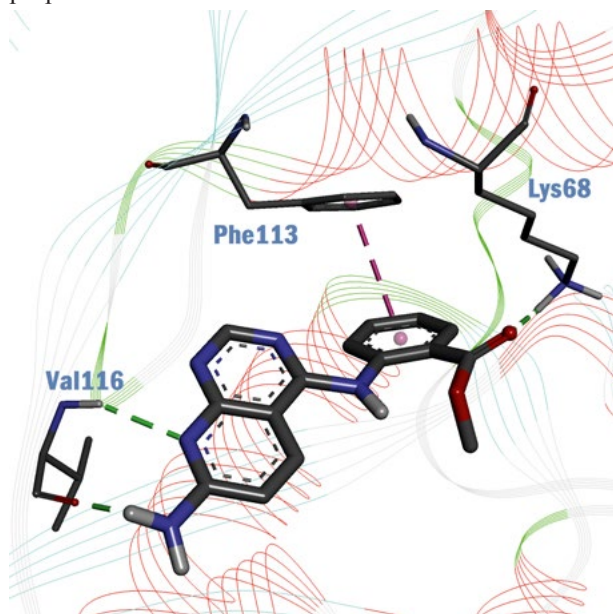
will significantly enhance their interaction with the ATP acceptor site of protein kinase CK2.

Conclusion

A convenient approach to the synthesis of the new pyrido[2,3-*d*]pyrimidine derivatives containing amino groups in positions 4, 6 and 7 of hetero-cyclic system has been developed. The results of biochemical research have shown the perspective of the search for protein kinase CK2 inhibitors among pyrido[2,3-*d*]pyrimidines derivatives. Methyl 2-[(7-aminopyrido[2,3-*d*]pyrimidin-4-yl)amino]benzoate and *N*-(4-anilino-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-6-yl)-3,4-dime-thoxybenzamide were determined to inhibit ki-nase CK2 in IC_{50} 6 and 19,5 μM respectively.

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375

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Синтез та біологічна оцінка нових амінозаміщених похідних піридо[2,3-*d*]піримідину як інгібіторів протеїнкінази СК2

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Мета. Пошук нових інгібіторів протеїнкінази СК2 людини в ряду нових амінозаміщених похідних піридо[2,3-*d*]піримідину. **Методи:** органічний синтез, аналітичні та спектральні методи, молекулярний докінг, біохімічне тестування *in vitro*. **Результати.** Розроблено методи синтезу нових похідних піридо[2,3-*d*]піримідину з різноманітними аміногрупами в положеннях 4, 6, 7 гетероциклу. Серед синтезованих похідних піридо[2,3-*d*]піримідину виявлено дві сполуки, що інгібують кіназу СК2 в мікромолярних концентраціях. **Висновки.** Синтезовано нові піридо[2,3-*d*]піримідин-7-они, котрі містять аміногрупи в положенні 4, 6 гетероциклічної системи, а також 4-амінозаміщені похідні піридо[2,3-*d*]піримідин-7-аміну. Досліджено інгібувальну активність похідних піридо[2,3-*d*]піримідину та запропоновано напрями хімічної оптимізації. Встановлено, що метил 2-[(7-амінопіридо[2,3-*d*]піримідин-4-іл)аміно]бензоат та *N*-(4-аніліно-7-оксо-7,8-дигідропіридо [2,3-*d*]піримідин-6-іл)-3,4-диметоксибензамід інгібують протеїнкіназу СК2 з IC$_{50}$ 6 та 19,5 µМ відповідно.

К л ю ч о в і   с л о в а: похідні піридо[2,3-*d*]піримідину, синтез, протеїнкіназа СК2, інгібувальна активність.

Синтез и биологическая оценка новых аминозамещенных производных пиридо[2,3-*d*] пиримидина в качестве ингибиторов протеинкиназы СК2

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Цель. Поиск новых ингибиторов протеинкиназы СК2 человека в ряде новых аминозамещенных производных пиридо[2,3-*d*]пиримидина. **Методы:** органический синтез, аналитические и спектральные методы, молекулярный докинг, биохимическое тестирование *in vitro*. **Результаты.** Разработаны методы синтеза новых производных пиридо[2,3-*d*]пиримидина с различными аминогруппами в положениях 4, 6, 7 гетероцикла. Среди синтезированных производных пиридо[2,3-*d*] пиримидина обнаружено два соединения, ингибирующие киназы СК2 в микромолярных концентрациях. **Выводы.** Синтезированы новые пиридо[2,3-*d*]пиримидин-7-оны, содержащие аминогруппы в положениях 4, 6 гетероциклической системы, а также 4-аминозамещенные производные пиридо[2,3-*d*]пиримидин-7-амина. Исследована ингибирующая активность производных пиридо[2,3-*d*]пиримидина и предложены направления химической оптимизации. Установлено, что метил-2-[(7-аминопиридо[2,3-*d*]пиримидин-4-ил) амин]бензоат и *N*-(4-анилин-7-оксо-7,8-дигидропиридо[2,3-*d*]пиримидин-6-ил)-3,4-диметоксибензамид ингибируют протеинкиназу СК2 с IC$_{50}$ 6 и 19,5 µМ соответственно.

К л ю ч е в ы е   с л о в а: производные пиридо[2,3-*d*] пиримидина, синтез, протеинкиназа СК2, ингибирующая активность.