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A combinatorial library of substituted 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones and their anticancer activities

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Aim. Synthesis of a combinatorial library of substituted 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones and evaluation of their *in vitro* anticancer activity against a panel of 60 different human tumor cell lines derived from nine cancer types. **Methods.** Organic synthesis; biological tests; spectral methods; statistic methods. **Results.** An efficient protocol for the synthesis of new 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones has been developed. *In vitro* screening of the anticancer activity showed that the obtaining compounds can effectively inhibit the growth of certain cancer cell lines. **Conclusions.** The synthesis of a library of 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones is described. The set of compounds (25 examples) inhibits the growth of certain renal cancer lines.

Keywords: combinatorial library, heterocyclization, 1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-d]pyrimidin-5-ones, pyrido[1,2-*a*:2',3'-*d*]pyrimidinones, *in vitro* screening, anticancer activity.

Introduction

Nitrogen-containing heterocycles are essential structural motifs found in many naturally occurring alkaloids, bioactive molecules, and materials [1, 2]. Among them, 1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones are the privileged structural scaffolds that have shown broad spectrum of potent biological activity. As the recent research evidenced , they find wide applications in medicinal chemistry as human and insect pest chitinase inhibitors [3], speckle-type POZ protein (SPOP) inhibitors against kidney cancer [4], antitubercular drug candidates [5], hepatitis C virus inhibitors [6], anti-HIV-1 agents [7] (Figure 1).

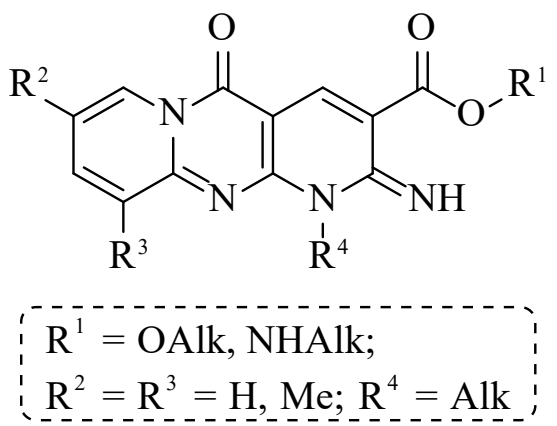


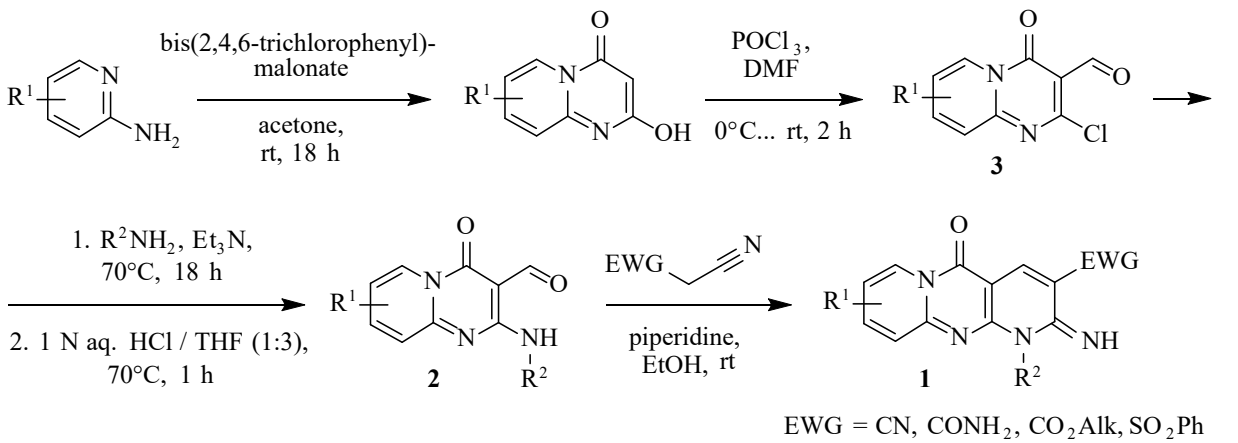
Fig. 1. Substituted 1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones as privileged structural motif with a broad spectrum of biological activity.

The same compounds were also found as a part of the *Cantharellus cibarius* fungi extracts, which had the anti-angiogenic activity [8].

Interestingly, the requirements of 1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-one construction result in an electron withdrawing group in position 3. Thus, the compounds of the general formula **1** were synthesized by the condensation of corresponding aminoaldehydes **2** with methylene active nitriles, as shown in Scheme 1 [6].

Noteworthy, the obtained compounds **1** contained in their structure an ester, amide, or nitrile group at the 3rd position, and only one derivative contained a sulfonyl group. Additionally, all compounds demonstrated good anti-HCV activity without cytotoxicity, which encourages expanding the compound range for further research. On the other hand, sulfonyl group-containing heterocyclic compounds constitute an important class of drug candidates in medicinal chemistry [9, 10]. A few examples of 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones with antiviral activity are also known [11, 12].

Therefore, the purpose of the presented study was to develop a convenient method for the synthesis of substituted 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*] pyrimidin-5-ones and survey their anticancer activity.



Scheme 1.

Materials and Methods

Chemistry

Our proposed approach to the synthesis of target compounds was based primarily on a known strategy (see Scheme 1); however, one significant modification was made. As specified in the Scheme 1, heterocyclization resulted in the formation of 2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones from aminoaldehydes **2**. Synthesis of products **2** was carried out from the corresponding chloro-aldehydes **3** in 2 steps by replacing chlorine with an amine residue to give Schiff's base, followed by subsequent hydrolysis.

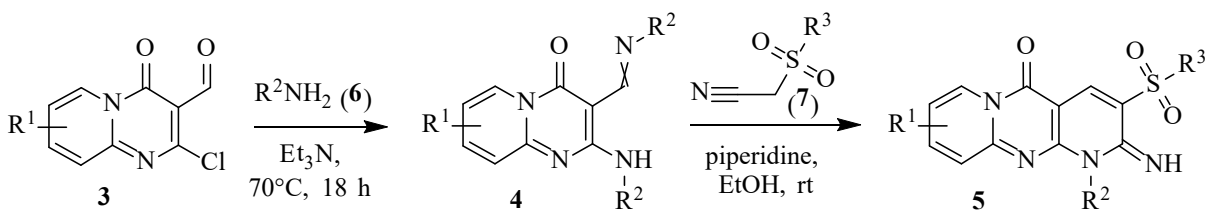
We found that the interaction of sulfonylacetonytrils with the corresponding Schiff's bases **4** in the mild reaction conditions resulted in 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones **5** (Scheme 2). Thus, this modification of the technique made it possible to reduce the number of synthetic stages and simplify the procedure operationally. Thus, products **4** were easily separated from the reaction mixture and, according to HPLC, had a purity of at least 90 %, allowing for their use in the next stage without additional purification. The output and purity of target products also improved.

This method's utility and scope were tested on a wide range of commercially available primary amines **6***{1-32}* and synthetically available chloro-aldehydes **3***{1-3}* and sulfonyl acetonitriles **7***{1-5}*, resulting in the creation of a combinatorial library of 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*] pyrimidin-5-ones **5** with varying substituents in position 1, sulfonyl group, and pyridine cycle. The representative substrates are listed in Figure 2.

For the designation of obtained products we use the numbering scheme accepted in combinatorial chemistry: for example, for a product **5***{2-16-5}*, the first number in parentheses corresponds to the index of the corresponding chloro-aldehyde **3***{1-3}*, the second number — to the index of the amine **6***{1-32}*, the third number — to the index of sulfonyl acetonitrile **7***{1-5}* (Figure 2).

As the result, 251 out of 294 experiments allowed us to obtain the target 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones (85.4 % success rate) with good purity (>95 % according to HPLC).

The solvents were purified according to the standard procedures. All materials were purchased from commercial sources and used without further purification. The success rate was calculated as the number of successful



Scheme 2.

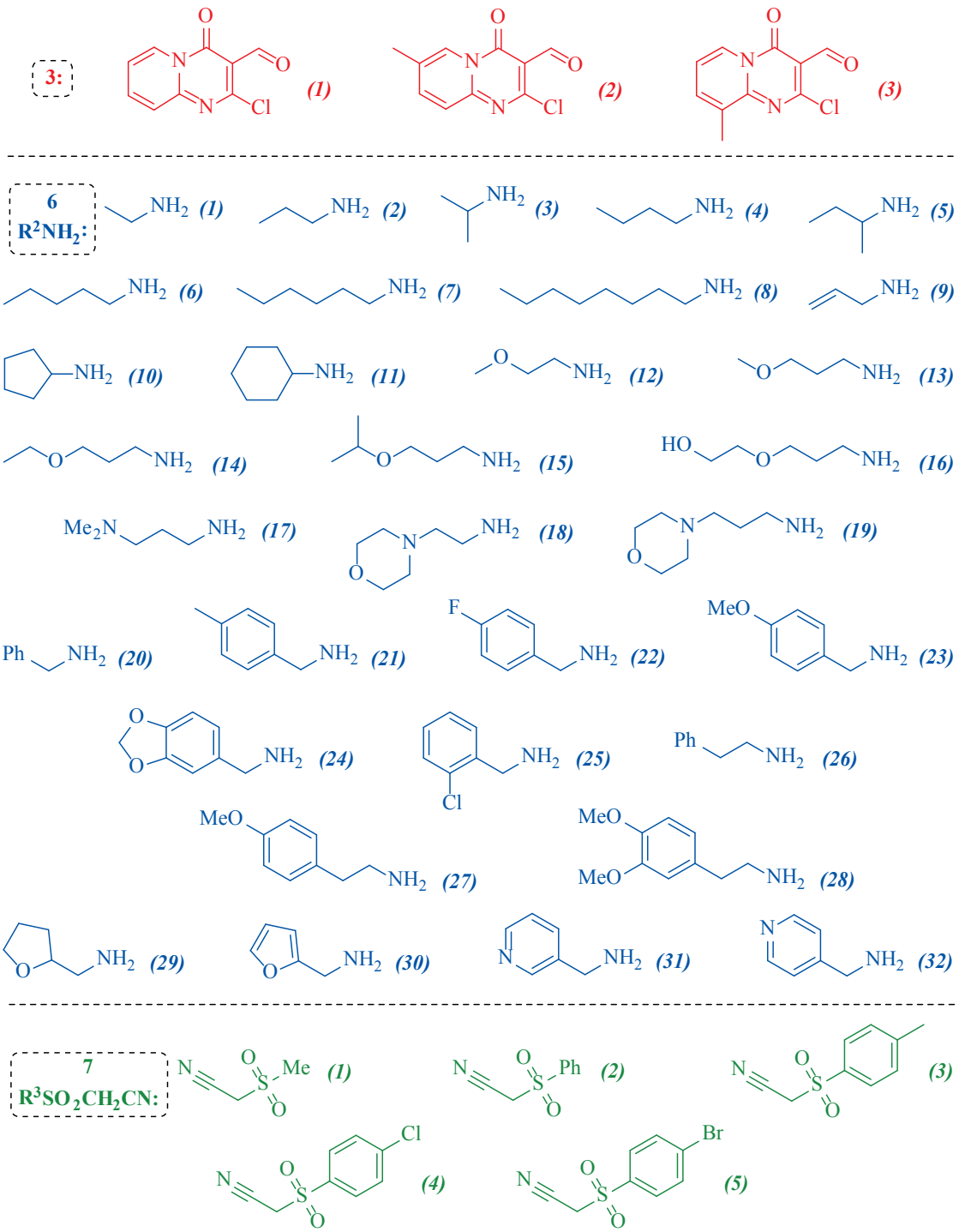


Fig. 2. The representative starting substrates

experiments divided by the total number of experiments. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra of obtained products were recorded at Varian Unityplus 400 spectrometer in DMSO-d_6 solution. Chemical shifts are reported in ppm downfield from TMS as internal standards. Mass spectra were recorded on an LC-MS instrument with chemical ionization (CI). The LC-MS data were acquired on an Agilent 1100 Series high performance liquid chromatograph equipped with a diode matrix with an Agilent LC/MS mass selective detector allowing fast switching of the positive/negative ionization modes (chemical ionization). The melting points were measured on the MPA100 OptiMelt automated melting point system. Elemental analyses were performed at the Analytical Laboratory of the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine, their results were found to be in good agreement (±0.4 %) with the calculated values. We have described 25 compounds that were selected for anticancer screening; this number of compounds also corresponds to the generally accepted practice of describing compound libraries in combinatorial chemistry.

A representative procedure for the synthesis of 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones 5.

To a suspension of **4** (5 mmol) in ethanol (50 mL) compound **7** (5 mmol), dry acetic acid (1 mL) and piperidine (0.3 mL) were added. The reaction mixture was boiled for 1 h, after 12 h the precipitate was filtered off and washed with ethanol.

1-Ethyl-2-imino-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-1-2}*). Yield: 1.6 g, 84 %, mp 221–223 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (1H, d, J = 7.0 Hz, H-7), 8.47 (1H, s, H-4), 8.10 (1H, br. s, NH), 8.04 (1H, t, J = 7.0 Hz, H-9), 7.97 (2H, d, J = 7.6 Hz, H_{SPh}-2,6), 7.73 (1H, t, J = 7.6 Hz, H_{SPh}-4), 7.66 (2H, t, J = 7.6 Hz, H_{SPh}-3,5), 7.57 (1H, d, J = 8.9 Hz, H-10), 7.32 (1H, t, J = 7.0 Hz, H-8), 4.39 (2H, q, J = 7.0 Hz, NCH_2), 1.16 (3H, t, J = 7.0 Hz, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.5, 154.6, 151.9, 151.6, 141.0, 139.5, 136.8, 134.1, 129.8, 128.3, 127.1, 125.3, 122.6, 116.0, 94.2, 36.9, 11.8. HPLC (CI) m/z 381.1 $[M+1]^+$.

2-Imino-3-(phenylsulfonyl)-1-propyl-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-2-2}*). Yield: 1.41 g, 71 %, mp 190–192 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (1H, d, J = 7.0 Hz, H-7), 8.48 (1H, s, H-4), 8.09–7.94 (4H, m, NH, H-9, H_{SPh}-2,6), 7.74 (1H, t, J = 7.6 Hz, H_{SPh}-4), 7.65 (2H, t, J = 7.6 Hz, H_{SPh}-3,5), 7.58 (1H, d, J = 8.7 Hz, H-10), 7.33 (1H, t, J = 6.8 Hz, H-8), 4.24 (2H, t, J = 6.5 Hz, NCH_2), 1.61–1.49 (2H, m, $CH_2CH_2CH_3$), 0.85 (3H, t, J = 6.5 Hz, $CH_2CH_2CH_3$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.6, 154.8, 152.2, 151.5, 141.0, 139.4, 136.8, 134.1, 133.3, 129.8, 128.3, 127.2, 125.3, 122.6, 116.0, 94.2, 43.1, 19.5, 11.1. HPLC (CI) m/z 395.1 $[M+1]^+$.

*2-Imino-1-octyl-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-8-2}).* Yield: 1.47 g, 63 %, mp 156–158 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (1H, d, J = 7.0 Hz, H-7), 8.46 (1H, s, H-4), 8.07 (1H, s, NH), 8.03 (1H, t, J = 8.1 Hz, H-9), 7.97 (2H, d, J = 7.7 Hz, H_{SPh}-2,6), 7.73 (1H, t, J = 7.7 Hz, H_{SPh}-4), 7.64 (2H, t, J = 7.7 Hz, H_{SPh}-3,5), 7.51 (1H, d, J = 8.9 Hz, H-10), 7.31 (1H, t, J = 7.0 Hz, H-8), 4.29 (2H,

t, J = 7.5 Hz, NCH_2), 1.60–1.52 (2H, m, NCH_2CH_2), 1.29–1.13 (10H, m, $(CH_2)_5$), 0.82 (3H, t, J = 6.7 Hz, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.5, 154.7, 152.1, 151.4, 140.9, 139.4, 136.7, 134.1, 129.7, 128.3, 127.1, 125.2, 122.6, 116.0, 94.1, 41.5, 31.2, 28.5, 26.2, 25.9, 22.0, 13.9. HPLC (CI) m/z 464.2 $[M+1]^+$.

1-Allyl-2-imino-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-9-2}*). Yield: 1.22 g, 62 %, mp 191–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (1H, d, J = 7.0 Hz, H-7), 8.49 (1H, s, H-4), 8.09–8.02 (2H, m, H-9, NH), 7.98 (2H, d, J = 7.6 Hz, H_{SPh}-2,6), 7.74 (1H, t, J = 7.6 Hz, H_{SPh}-4), 7.65 (2H, t, J = 7.6 Hz, H_{SPh}-3,5), 7.58 (1H, d, J = 8.8 Hz, H-10), 7.34 (1H, t, J = 7.0 Hz, H-8), 5.84 (1H, ddt, J = 15.9, 10.3, 5.2 Hz, $CH{=}CH_2$), 5.09–5.00 (2H, m, $CH{=}CH_2$), 4.93 (2H, d, J = 5.2 Hz, NCH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.6, 154.7, 151.9, 151.5, 141.1, 139.4, 137.0, 134.1, 132.1, 129.8, 128.4, 127.2, 125.3, 122.6, 116.9, 116.2, 94.2, 43.4. HPLC (CI) m/z 393.1 $[M+1]^+$.

1-Cyclopentyl-2-imino-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-10-2}*). Yield: 1.24 g, 59 %, mp 195–197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (1H, d, J = 7.0 Hz, H-7), 8.45 (1H, s, H-4), 8.21 (1H, s, NH), 8.02 (1H, t, J = 7.9 Hz, H-9), 7.98 (2H, d, J = 7.8 Hz, H_{SPh}-2,6), 7.74 (1H, t, J = 7.8 Hz, H_{SPh}-4), 7.65 (2H, t, J = 7.8 Hz, H_{SPh}-3,5), 7.54 (1H, d, J = 8.9 Hz, H-10), 7.31 (1H, t, J = 6.9 Hz, H-8), 5.98 (1H, p, J = 8.8 Hz, NCH), 2.32–2.18 (2H, m, H_{cPent}), 2.05–1.96 (2H, m, H_{cPent}), 1.73–1.60 (2H, m, H_{cPent}), 1.58–1.47 (2H, m, H_{cPent}). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.7, 155.1, 153.0, 150.6, 140.8, 139.5, 136.7, 134.1, 129.8, 128.2, 127.1, 125.2, 122.8, 116.0, 94.8, 54.0, 27.5, 25.7. HPLC (CI) m/z 421.2 $[M+1]^+$.

1-Cyclohexyl-2-imino-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-11-2}*). Yield: 1.48 g, 68 %, mp 258–260 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (1H, d, J = 7.2 Hz, H-7), 8.46 (1H, s, H-4), 8.19 (1H, br. s, NH), 8.04 (1H, t, J = 7.0 Hz, H-9), 7.97 (2H, d, J = 7.4 Hz, H_{SPh}-2,6), 7.74 (1H, t, J = 7.4 Hz, H_{SPh}-4), 7.65 (2H, t, J = 7.4 Hz, H_{SPh}-3,5), 7.59 (1H, d, J = 8.8 Hz, H-10), 7.31 (1H, t, J = 7.0 Hz, H-8), 5.49–5.33 (1H, m, NCH), 2.84–2.69 (2H, m, H_{cHex}), 1.79–1.69 (2H, m, H_{cHex}), 1.67–1.57 (1H, m, H_{cHex}), 1.52–1.40 (2H, m, H_{cHex}), 1.35–1.13 (3H, m, H_{cHex}). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.7, 155.5, 153.1, 150.6, 140.8, 139.5, 136.8, 134.1, 129.8, 128.2, 127.2, 125.3, 122.5, 116.0, 94.5, 55.2, 28.1, 26.2, 25.1. HPLC (CI) m/z 435.1 $[M+1]^+$.

2-Imino-1-(2-methoxyethyl)-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-12-2}*). Yield: 1.76 g, 86 %, mp 197–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (1H, d, J = 7.0 Hz, H-7), 8.44 (1H, s, H-4), 8.09 (1H, br. s, NH), 8.03 (1H, t, J = 7.8 Hz, H-9), 7.98 (2H, d, J = 7.6 Hz, H_{SPh}-2,6), 7.73 (1H, t, J = 7.6 Hz, H_{SPh}-4), 7.64 (2H, t, J = 7.6 Hz, H_{SPh}-3,5), 7.55 (1H, d, J = 8.8 Hz, H-10), 7.31 (1H, t, J = 7.0 Hz, H-8), 4.49 (2H, t, J = 6.6 Hz, NCH_2CH_2), 3.51 (2H, t, J = 6.6 Hz, NCH_2CH_2), 3.21 (3H, s, OCH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.4, 154.9, 152.2, 151.4, 140.4, 139.4, 136.4, 134.1, 129.7, 128.3, 127.2, 125.2, 122.6, 116.1, 94.1, 67.3, 57.9, 40.1. HPLC (CI) m/z 411.2 $[M+1]^+$.

2-Imino-1-(2-morpholinoethyl)-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-

a:2',3'-d]pyrimidin-5-one (**5***{1-18-2}*). Yield: 1.39 g, 60 %, mp 235–237 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (1H, d, J = 7.0 Hz, H-7), 8.47 (1H, s, H-4), 8.13 (1H, br. s, NH), 8.03 (1H, t, J = 7.8 Hz, H-9), 7.96 (2H, d, J = 7.6 Hz, H_{SPh}-2,6), 7.72 (1H, t, J = 7.6 Hz, H_{SPh}-4), 7.64 (2H, t, J = 7.6 Hz, H_{SPh}-3,5), 7.53 (1H, d, J = 8.8 Hz, H-10), 7.32 (1H, t, J = 7.1 Hz, H-8), 4.45 (2H, br. t, J = 7.0 Hz, NCH_2CH_2–morph), 3.43 (4H, br. t, J = 4.6 Hz, CH_2OCH_2), 2.54 (2H, br. t, J = 7.0 Hz, CH_2–morph), 2.41 (4H, br. t, J = 4.6 Hz, CH_2NCH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.4, 154.8, 152.2, 151.3, 141.0, 139.4, 136.7, 134.0, 129.7, 128.3, 127.2, 125.1, 122.6, 116.0, 94.1, 66.1, 53.9, 53.4, 38.6. HPLC (CI) m/z 466.2 $[M+1]^+$.

1-(4-Fluorobenzyl)-2-imino-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-22-2}*). Yield: 2 g, 87 %, mp 232–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (1H, d, J = 7.0 Hz, H-7), 8.49 (1H, s, H-4), 8.10 (1H, s, NH), 8.03 (1H, t, J = 8.7 Hz, H-9), 7.98 (2H, d, J = 7.6 Hz, H_{SPh}-2,6), 7.73 (1H, t, J = 7.6 Hz, H_{SPh}-4), 7.63 (2H, t, J = 7.6 Hz, H_{SPh}-3,5), 7.57 (1H, d, J = 8.7 Hz, H-10), 7.33 (3H, m, H-8, H_{Ar}-2,6), 7.00 (2H, t, J = 8.8 Hz, H_{Ar}-3,5), 5.47 (2H, s, NCH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.1 (d, J = 243.0 Hz), 155.6, 154.8, 152.3, 151.4, 141.2, 139.4, 137.0, 134.1, 133.4, 129.9 (d, J = 8.1 Hz), 129.8, 128.4, 127.2, 125.3, 122.7, 116.3, 114.7 (d, J = 21.2 Hz), 94.3, 43.6. HPLC (CI) m/z 461.1 $[M+1]^+$.

2-Imino-1-phenethyl-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-26-2}*). Yield: 1.43 g, 63 %, mp 249–251 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (1H, d, J = 7.1 Hz, H-7), 8.50 (1H, s, H-4), 8.12 (1H, br. s, NH), 8.09 (1H, t, J = 7.8 Hz, H-9), 8.01 (2H, d, J = 7.8 Hz, H_{SPh}-2,6), 7.75 (1H, t, J = 7.8 Hz, H_{SPh}-4), 7.71–7.62 (3H, m, H-10, H_{SPh}-3,5), 7.40–7.32 (2H, m, H_{Ph}-2,6), 7.29–7.19 (3H, m, H_{Ph}-3–5), 7.14 (1H, t, J = 7.0 Hz, H-8), 4.51 (2H, t, J = 7.8 Hz, NCH_2CH_2), 2.85 (2H, t, J = 7.8 Hz, NCH_2CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.6, 154.6, 152.1, 151.5, 141.0, 139.6, 138.8, 137.1, 134.1, 129.8, 128.6, 128.3, 127.2, 126.5, 125.4, 122.7, 116.1, 95.2, 43.9, 33.0. HPLC (CI) m/z 457.2 $[M+1]^+$.

2-Imino-1-(4-methoxyphenethyl)-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-27-2}*). Yield: 1.37 g, 56 %, mp 226–228 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (1H, d, J = 7.0 Hz, H-7), 8.49 (1H, s, H-4), 8.16–8.04 (2H, m, H-9, NH), 8.01 (2H, d, J = 7.6 Hz, H_{SPh}-2,6), 7.75 (1H, t, J = 7.7 Hz, H_{SPh}-4), 7.71–7.60 (3H, m, H-10, H_{SPh}-3,5), 7.35 (1H, t, J = 6.4 Hz, H-8), 7.14 (2H, d, J = 8.2 Hz, H_{Ar}-2,6), 6.80 (2H, d, J = 8.2 Hz, H_{Ar}-3,5), 4.44 (2H, br. t, J = 7.2 Hz, NCH_2), 3.68 (3H, s, OCH_3), 2.77 (2H, t, J = 7.9 Hz, NCH_2CH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.2, 156.8, 156.1, 155.2, 152.5, 151.8, 141.4, 139.8, 137.2, 134.4, 130.3, 130.1, 128.8, 127.7, 125.9, 122.5, 116.6, 114.2, 94.7, 55.4, 43.9, 31.6. HPLC (CI) m/z 487.0 $[M+1]^+$.

2-Imino-3-(phenylsulfonyl)-1-((tetrahydrofuran-2-yl)methyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-29-2}*). Yield: 1.2 g, 55 %, mp 196–198 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (1H, d, J = 7.0 Hz, H-7), 8.46 (1H, s, H-4), 8.11–7.95 (4H, m, NH, H-9, H_{SPh}-2,6), 7.73 (1H, t, J = 7.6 Hz, H_{SPh}-4), 7.64 (2H, t, J = 7.6 Hz, H_{SPh}-3,5), 7.53 (1H, d, J = 8.9 Hz,

H-10), 7.30 (1H, t, J = 6.9 Hz, H-8), 4.55–4.42 (1H, m, OCH), 4.35–4.24 (1H, m, OCH), 4.20 (1H, dd, J = 12.5, 5.4 Hz, OCH), 3.72 (1H, q, J = 7.7 Hz, NCH), 3.52 (1H, q, J = 7.1 Hz, NCH), 1.97–1.84 (1H, m, H_{Fur}), 1.79–1.66 (2H, m, H_{Fur}), 1.67–1.55 (1H, m, H_{Fur}). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.5, 155.2, 152.5, 151.2, 141.0, 139.4, 136.9, 134.1, 129.7, 128.3, 127.2, 125.2, 122.6, 116.1, 94.1, 74.1, 66.8, 44.3, 28.5, 24.7. HPLC (CI) m/z 437.1 $[M+1]^+$.

1-(Furan-2-ylmethyl)-2-imino-3-(phenylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-30-2}*). Yield: 1.6 g, 74 %, mp 199–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (1H, d, J = 7.0 Hz, H-7), 8.49 (1H, s, H-4), 8.13 (1H, br. s, NH), 8.06 (1H, t, J = 7.0 Hz, H-9), 7.98 (2H, d, J = 7.4 Hz, H_{SPh}-2,6), 7.73 (1H, t, J = 7.5 Hz, H_{SPh}-4), 7.69–7.58 (3H, m, H-10, H_{SPh}-3,5), 7.45 (1H, br. s, H_{Fur}-5), 7.35 (1H, t, J = 7.0 Hz, H-8), 6.29 (1H, br. s, H_{Fur}-3), 6.19 (1H, br. s, H_{Fur}-4), 5.51 (2H, s, NCH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.5, 154.7, 151.7, 151.4, 150.3, 141.7, 141.2, 139.4, 137.0, 134.2, 129.8, 128.5, 127.2, 125.3, 122.6, 116.3, 110.4, 108.1, 94.2, 37.9. HPLC (CI) m/z 433.2 $[M+1]^+$.

2-Imino-3-(phenylsulfonyl)-1-(pyridin-3-ylmethyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{1-31-2}*). Yield: 1.6 g, 72 %, mp 227–229 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (1H, d, J = 7.0 Hz, H-7), 8.57 (1H, d, J = 2.3 Hz, H_{Py}-2), 8.51 (1H, s, H-4), 8.37 (1H, dd, J = 4.8, 1.6 Hz, H_{Py}-6), 8.10 (1H, s, NH), 8.06 (1H, t, J = 7.0 Hz, H-9), 7.99 (2H, d, J = 7.7 Hz, H_{SPh}-2,6), 7.73 (1H, t, J = 7.5 Hz, H_{SPh}-4), 7.69 (1H, d, J = 8.7 Hz, H_{Py}-4), 7.66–7.58 (3H, m, H-10, H_{SPh}-3,5), 7.35 (1H, t, J = 7.0 Hz, H-8), 7.22 (1H, dd, J = 8.7, 4.8 Hz, H_{Py}-5), 5.52 (2H, s, NCH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.6, 154.9, 152.3, 151.5, 149.5, 148.0, 141.3, 139.3, 137.1, 135.6, 134.2, 132.9, 129.8, 128.5, 127.2, 125.3, 123.2, 122.6, 116.3, 94.4, 42.3. HPLC (CI) m/z 444.1 $[M+1]^+$.

1-Butyl-2-imino-8-methyl-3-(p-tolylsulfonyl)-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{2-4-3}*). Yield: 1.9 g, 87 %, mp 234–236 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (1H, s, H-7), 8.43 (1H, s, H-4), 8.06 (1H, br. s, NH), 7.89 (1H, dd, J = 9.0, 2.2 Hz, H-9), 7.83 (2H, d, J = 8.0 Hz, H_{STol}-2,6), 7.48–7.40 (3H, m, H-10, H_{STol}-2,6), 4.29 (2H, br. t, J = 7.4 Hz, NCH_2), 2.38 (3H, s, CH_3–Ar), 2.39 (3H, s, CH_3–Ar), 1.55 (2H, q, J = 7.5 Hz, $CH_2CH_2CH_2CH_3$), 1.29 (2H, h, J = 7.5 Hz, $(CH_2)_2CH_2CH_3$), 0.88 (3H, t, J = 7.5 Hz, $(CH_2)_3CH_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.3, 154.4, 151.8, 150.1, 145.1, 143.3, 136.0, 130.1, 127.3, 125.8, 125.4, 124.7, 122.6, 116.4, 93.6, 41.2, 28.1, 21.0, 19.6, 17.4, 13.6. HPLC (CI) m/z 437.3 $[M+1]^+$.

3-((4-Chlorophenyl)sulfonyl)-1-(3-ethoxypropyl)-2-imino-8-methyl-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{2-14-4}*). Yield: 1.25 g, 51 %, mp 199–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (1H, s, H-7), 8.45 (1H, s, H-4), 8.03 (1H, br. s, NH), 7.98 (2H, d, J = 8.2 Hz, H_{SAr}-2,6), 7.91 (1H, d, J = 9.1 Hz, H-9), 7.67 (2H, d, J = 8.2 Hz, H_{SAr}-3,5), 7.44 (1H, d, J = 9.1 Hz, H-10), 4.35 (2H, br. s, NCH_2), 3.40–3.33 (2H, m, $N(CH_2)_2CH_2O$), 3.30 (2H q, J = 7.0 Hz, OCH_2CH_3), 2.38 (3H, s,), 1.86–1.75 (2H, m, $NCH_2CH_2CH_2O$), 1.03 (3H, t, J = 7.0 Hz, OCH_2CH_3). ^{13}C NMR (151 MHz,

DMSO-d_6) δ 155.2, 154.5, 152.1, 150.2, 143.5, 139.2, 138.3, 137.2, 129.8, 129.2, 126.0, 125.5, 124.7, 121.9, 94.1, 67.7, 65.0, 40.0 (in solvent signal), 26.5, 17.4, 15.0. HPLC (CI) m/z 487.2 $[M+1]^+$.

1-Benzyl-3-((4-chlorophenyl)sulfonyl)-2-imino-10-methyl-1,2-dihydro-5H-dipyrido[1,2-a:2',3'-d]pyrimidin-5-one (**5***{3-20-4}*). Yield: 1.51 g, 62 %, mp 228–230 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (1H, d, J = 7.0 Hz, H-7), 8.52 (1H, s, H-4), 8.17 (1H, br. s, NH), 7.99 (2H, d, J = 8.6 Hz, H_{SAr}-2,6), 7.91 (1H, d, J = 7.1 Hz, H-9), 7.67 (2H, d, J = 8.2 Hz, H_{Ph}-2,6), 7.29–7.11 (6H, m, H-8, H_{SAr}-3,5, H_{Ph}-3–5), 5.58 (2H, s, NCH_2), 2.38 (3H, s, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 155.8, 154.0, 152.5, 150.7, 139.7, 139.2, 138.2, 137.5, 137.2, 133.3, 129.9, 129.2, 128.0, 127.4, 126.7, 126.3, 122.2, 115.6, 94.1, 44.6, 17.4. HPLC (CI) m/z 491.1 $[M+1]^+$.

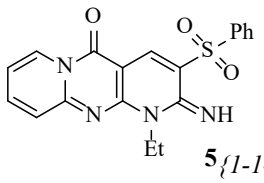
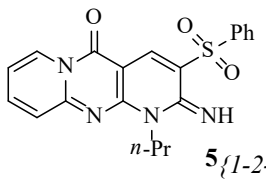
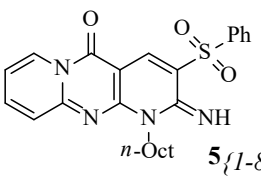
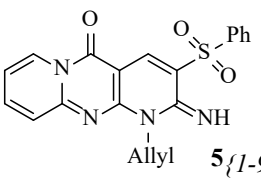
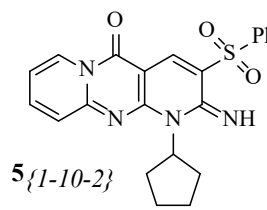
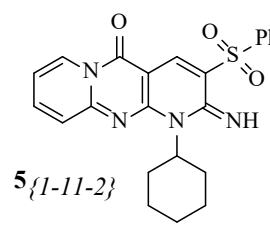
From the resulting library of compounds, 25 derivatives were selected for primary screening on 60 different human tumor cell lines derived from nine cancer types.

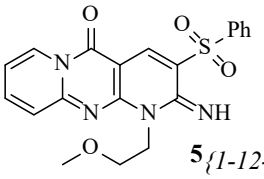
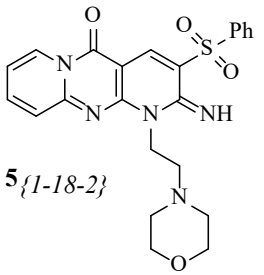
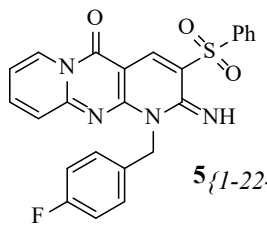
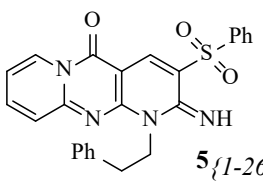
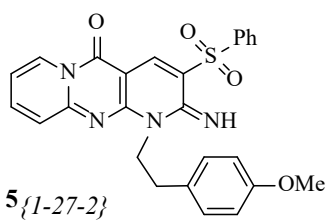
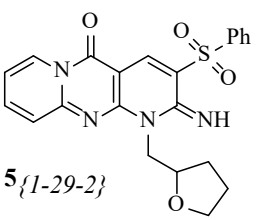
One Doses Full NCI 60 Cell Panel Assay. The newly synthesized compounds were submitted to National Cancer Institute NCI, Bethesda, Maryland, U.S.A., under the Developmental Therapeutic Program DTP [23]. The cell line panel engaged a total of 60 different human tumor cell lines derived from nine cancer types. The selected compounds **5** were assigned NCI codes (see *Table 1*). Correspondingly, a primary *in vitro* one dose anticancer screening was initiated, in which complete NCI 60 panel lines were inoculated onto a series of standard 96-well microtiter plates at day 0 at 5000–40,00 cells/well in RPMI 1640 medium containing 5 % fetal bovine serum and 2 mM L-glutamine, and then preincubated in the absence of a drug at 37 °C, and 5 % CO_2 for 24 h. The test compounds were then added in the same concentration of 10^{-5} M to all 60 cell lines (see [24] for the drug solution preparation) and incubated for further 48 h in the same incubation conditions. Following this, the media was removed, the cells were fixed *in situ*, washed, and dried. The sulforhodamine B assay was used for the cell density determination based on the measurement of cellular protein content. After an incubation period, cell monolayers were fixed with 10 % (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye was removed by repeatedly washing with 1 % (vol/vol) acetic acid. The bound stain was resolubilized in 10 mM Tris base solution and measured spectrophotometrically on automated microplate readers for OD determination at 510 nm.

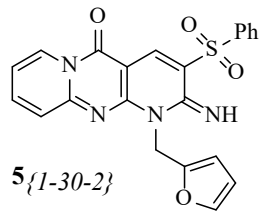
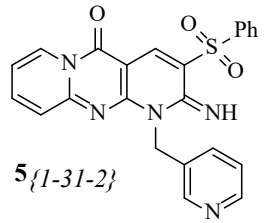
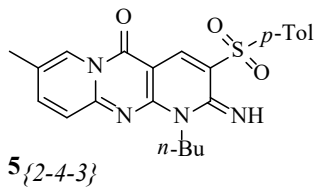
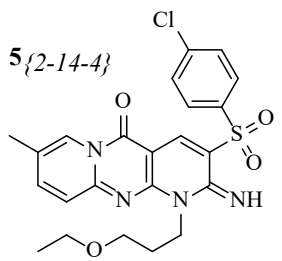
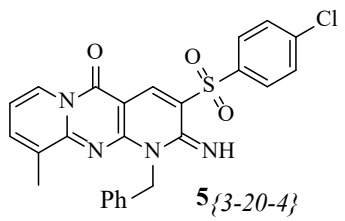
The findings suggest that the studied derivatives have, on average, a low inhibitory effect on the growth of malignant cells. However, certain derivatives clearly manifest selective suppression of certain Renal Cancer lines (Figure 3). This fact is aligned with the data of work [4], which studied the effect of substituents on the dipyridopyrimidin-5-one activity.

The table shows the quantitative indicators of Renal Cancer lines inhibition (limited to those lines for which the GP was less than 90 %), as well as several indicators of the most efficient inhibition of other cancer types. Noteworthy, with the only exception of product **5***{1-27-2}*, the highest percentage of inhibition was observed specifically for indi-

Table 1. **The effect of compounds 5 on cancer cells growth according to One Doses Full NCI 60 Cell Panel Assay (C = 10^{-5} mol/l)**

Compound /	NCI code	Growth Percent, %		
		Mean / range *	Renal Cancer lines	Another lines
 **5***{1-1-2}*	**814042**	98.8 / 40.5	76.6 (A498) 77.9 (UO-31) 84.4 (RXF 393) 85.2 (TK-10) 86.8 (CAKI-1)	84.2 (Non-Small Cell Lung Cancer NCI-H322M)
 **5***{1-2-2}*	**814047**	94.0 / 72.9	39.8 (A498) 61.4 (UO-31) 82.5 (CAKI-1) 86.0 (TK-10)	76.8 (Breast Cancer MDA-MB-231/ATCC) 77.3 (Breast Cancer T-47D) 78.4 (Melanoma LOX IMVI 2)
 **5***{1-8-2}*	**814053**	85.2 / 92.9	23.3 (ACHN) 33.0 (786-0) 46.1 (A498) 52.9 (UO-31) 58.6 (CAKI-1) 78.0 (TK-10) 83.7 (SN12C)	46.3 (Ovarian Cancer OVCAR-4) 49.3 (Non-Small Cell Lung Cancer NCI-H460) 50.9 (CNS Cancer U251)
 **5***{1-9-2}*	**811860**	100.4 / 79.9	46.4 (A498) 70.6 (UO-31) 80.8 (CAKI-1)	79.6 (CNS Cancer SNB-75)
 **5***{1-10-2}*	**814052**	87.7 / 118.6	-1.4 (A498) 50.9 (UO-31) 64.6 (CAKI-1) 80.7 (786-0) 82.5 (TK-10) 86.0 (ACHN) 86.0 (RXF 393) 87.2 (SN12C)	39.4 (Non-Small Cell Lung Cancer HOP-92)
 **5***{1-11-2}*	**814045**	99.2 / 64.4	62.8 (UO-31) 79.4 (A498) 81.0 (SN12C) 85.2 (CAKI-1)	63.3 (Non-Small Cell Lung Cancer HOP-92) 69.3 (CNS Cancer SNB-75)

Compound /	NCI code	Growth Percent, %		
		Mean / range *	Renal Cancer lines	Another lines
 **5***{1-12-2}*	**814048**	104.1 / 31.9	86.2 (UO-31)	91.1 (Melanoma LOX IMVI)
 **5***{1-18-2}*	**814049**	99.5 / 60.8	59.9 (A498) 89.1 (UO-31)	86.5 (Non-Small Cell Lung Cancer HOP-92) 87.2 (Breast Cancer T-47D)
 **5***{1-22-2}*	**814055**	95.3 / 68.0	43.2 (A498) 63.1 (UO-31)	80.5 (Melanoma UACC-62)
 **5***{1-26-2}*	**811863**	97.8 / 103.5	21.4 (A498) 75.4 (UO-31) 85.6 (CAKI-1) 88.5 (SN12C)	82.0 (Non-Small Cell Lung Cancer NCI-H522)
 **5***{1-27-2}*	**814056**	107.0 / 31.8	for all lines > 100	94.1 (Melanoma SK-MEL-2)
 **5***{1-29-2}*	**811862**	103.1 / 64.7	56.4 (UO-31) 84.1 (A498) 85.2 (CAKI-1)	73.9 (CNS Cancer SNB-75)

Compound /	NCI code	Growth Percent, %		
		Mean / range *	Renal Cancer lines	Another lines
 **5**{1-30-2}	**814043**	100.9 / 57.8	62.9 (A498) 75.4 (UO-31)	87.2 (Non-Small Cell Lung Cancer HOP-92) 89.3 (Leukemia SR)
 **5**{1-31-2}	**811861**	101.4 / 69.5	51.9 (A498) 70.2 (UO-31) 88.1 (CAKI-1)	83.3 (CNS Cancer SNB-75)
 **5**{2-4-3}	**765474**	103.5 / 75.4	82.2 (UO-31) 81.8 (TK-10)	88.9 (Non-Small Cell Lung Cancer NCI-H226) 89.2 (Colon Cancer KM12)
 **5**{2-14-4}	**765478**	98.7 / 98.4	63.1 (UO-31) 82.56 (786-0) 87.5 (RXF 393)	77.6 (Leukemia MOLT-4) 79.1 (Non-Small Cell Lung Cancer NCI-H226)
 **5**{3-20-4}	**765475**	93.8 / 57.6	70.6 (UO-31) 81.8 (TK-10) 87.0 (ACHN) 88.4 (CAKI-1)	78.3 (Melanoma UACC-62) 79.2 (Leukemia CCRF-CEM) 79.8 (Leukemia MOLT-4)

* for 60 tumor cell lines panel

vidual Renal Cancer lines. Derivative **5***{1-27-2}* with a *para*-methoxyphenylethyl substituent was atypical - it did not manifest the ability to slow down the growth of Renal Cancer lines at all. Interestingly, the phenethylamine derivative **5***{1-26-2}*, which differs from **5***{1-27-2}* only by the absence of a methoxy group, inhibits, though weakly, some cancer lines, primarily the A498 line Renal Cancer (see also Figure 3 for differences in these substances' activity). Still, the difference in the anticancer activity of derivatives **5***{1-10-2}*

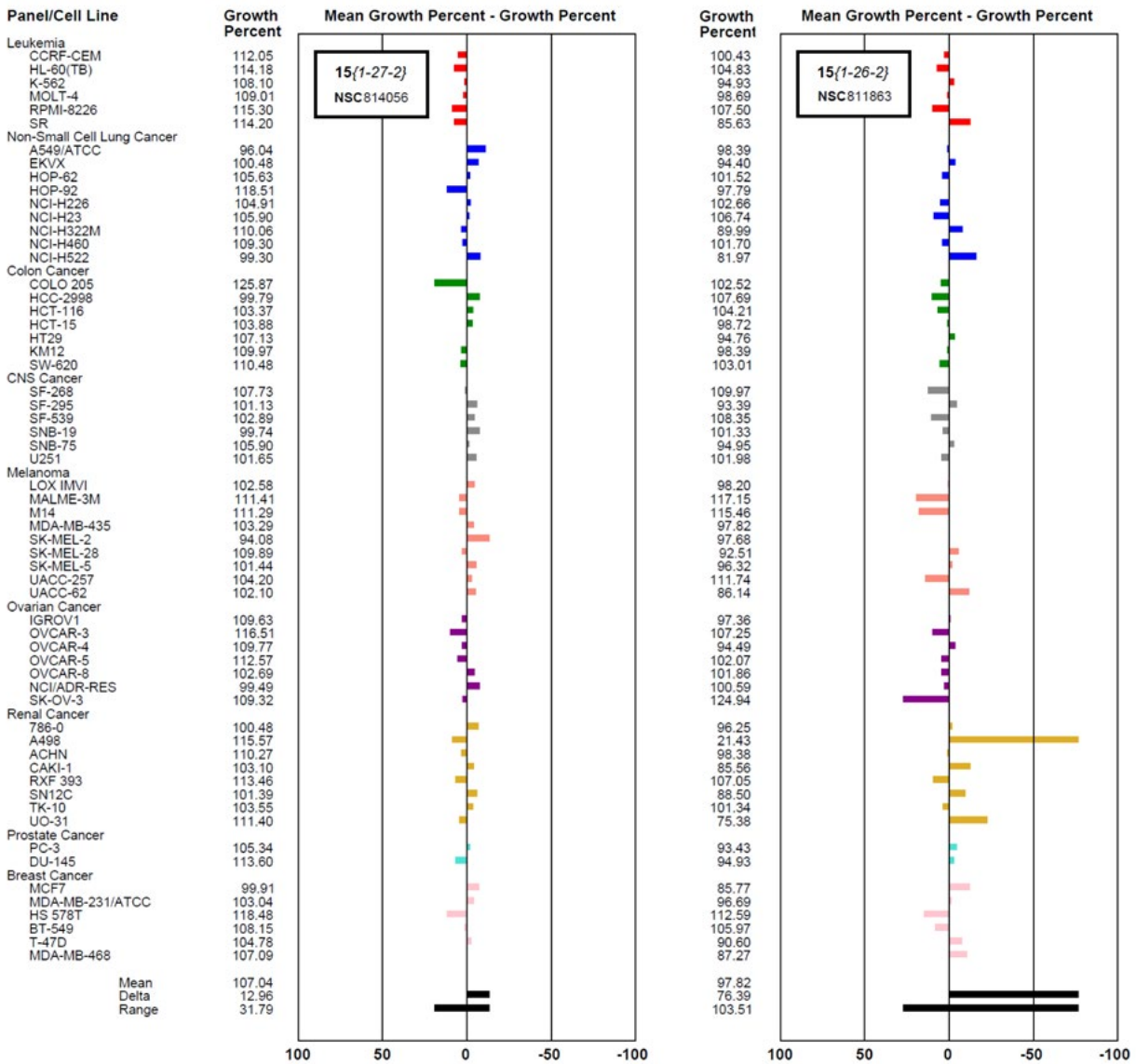


Fig. 3. Differences in these substances' activity

and **5***{1-11-2}* draws the most attention: cyclopentyl derivative **5***{1-10-2}* showed significantly higher inhibition rates than cyclohexyl derivative **5***{1-11-2}*. The significant impact of a slight variation in the structure on the activity level leaves hope of discovering derivatives of dipyridopyrimidin-5-one with a higher anticancer effect in the future.

Compounds' **5** record inhibition values of Renal Cancer line growth (highlighted) are presented in the table together with promising indicators of other cancer cell type suppression.

Conclusions

We have developed an efficient protocol for the synthesis of the library of 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*] pyrimidin-5-ones in mild conditions. The reaction is operationally simple, lasts short time, and provides 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones in moderate to high yields. As the result, 251 out of 294 experiments allowed us to obtain target 3-sulfonyl-2-imino-1,2-dihydro-5*H*-dipyrido[1,2-*a*:2',3'-*d*]pyrimidin-5-ones (85.4 % success rate). A series of compounds (25 examples) with a sulfonyl group in position 3 can effectively inhibit the growth of certain renal cancer lines. Future work will focus on improving their biophysical properties to yield drug-like pre-clinical candidates for *in vivo* animal studies.

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Disclaimer

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Комбінаторна бібліотека заміщених 3-сульфоніл-2-іміно-1,2-дигідро-5*H*-дипіридо[1,2-*a*:2',3'-*d*]піримідин-5-онів та їх протиракова активність

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Мета. Синтез комбінаторної бібліотеки заміщених 3-сульфоніл-2-іміно-1,2-дигідро-5*H*-дипіридо[1,2-*a*:2',3'-*d*]піримідин-5-онів та проведення оцінки їх протиракової дії *in vitro* на 60 різних лініях людських пухлинних клітин, отриманих з дев'яти типів раку. **Методи.** Органічний синтез; біологічні тести; спектральні методи; статистичні методи. **Результати.** Розроблено ефективний підхід до синтезу нових 3-сульфоніл-2-іміно-1,2-дигідро-5*H*-дипіридо[1,2-*a*:2',3'-*d*]піримідин-5-онів. *In vitro* скринінг протиракової активності показав, що отримані сполуки можуть ефективно пригнічувати ріст певних ліній ракових клітин. **Висновки.** Описано синтез бібліотеки 3-сульфоніл-2-іміно-1,2-дигідро-5*H*-дипіридо[1,2-*a*:2',3'-*d*]піримідин-5-онів. Бібліотека сполук (25 прикладів) пригнічує ріст деяких ліній раку нирки.

Ключові слова: комбінаторна бібліотека, гетероциклізація, 1,2-дигідро-5*H*-дипіридо[1,2-*a*:2',3'-*d*] піримідин-5-они, піридо[1,2-*a*:2',3' -*d*]піримідинони, скринінг *in vitro*, протиракова активність.

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