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SYNTHESIS AND ANTI-HIV-1 ACTIVITY OF 2-[2-(3,5-DIMETHYLPHENOXY)-ETHYLTHIO]PYRIMIDIN-4(3H)-ONES

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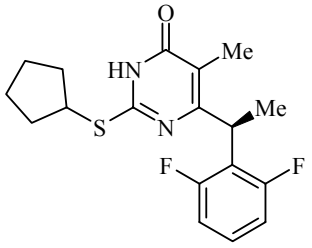
New 2-[2-(3,5-dimethylphenoxy)ethyl]thio derivatives of pyrimidin-4(3H)-one containing various substituents at positions 5 and 6 of the pyrimidine ring were synthesized. It was shown that alkylation of 2-thiouracils with 1-bromo-2-(3,5-dimethylphenoxy)ethane in DMF takes place exclusively at the sulfur atom. The obtained 6-benzyl and 6-(2,6-difluorobenzyl) derivatives have clearly defined virus-inhibiting properties with respect to type 1 human immunodeficiency virus in vitro and suppress its reproduction by 50% at concentrations of 1.3 and 11.2 μM respectively.

Keywords: 2-[2-(3,5-dimethylphenoxy)ethylthio]pyrimidin-4(3H)-ones, S-alkylation, anti-HIV-1 activity.

The search for new effective inhibitors of the reproduction of the human immunodeficiency virus (HIV) is the most important problem of modern medicine. A few years ago more that 15 000 new cases of HIV infection were established daily, and at present a progressive growth of the disease is being observed [1]. One of the most studied targets for chemotherapeutic action against HIV is reverse transcriptase (RT), which transforms the viral RNA into the proviral DNA. Blocking of its activity destroys the replicative cycle of HIV [2]. However, the inhibitors of reverse transcriptase employed for the treatment of HIV infection have a series of serious side effects, which restrict their clinical application. Moreover, prolonged therapy leads to the establishment of resistance in HIV to nucleosidic and nonnucleosidic inhibitors of reverse transcriptase. This gives rise to the need to search for new more selective and safer drugs [3].



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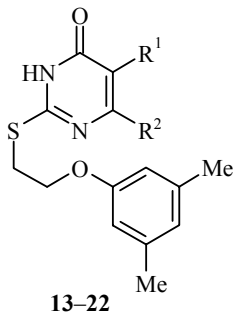

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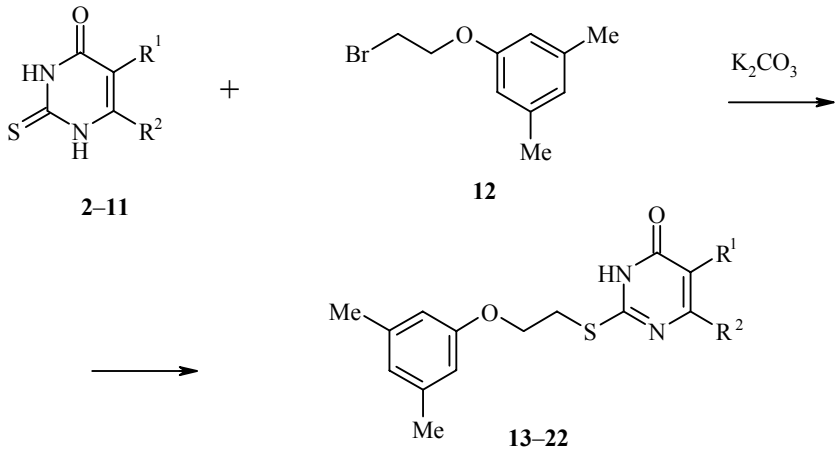
Earlier it was established that certain 2-(alkylthio)- and 2-(cycloalkylthio)pyrimidin-4(3H)-ones exhibit considerable antiviral activity against HIV-1 [4-8]. For example, the (*R*)-enantiomer of 5-methyl-6-(α-methyl-2,6-difluorobenzyl)-2-(cyclopentylthio)pyrimidin-4(3H)-one (**1**) inhibits by 50% the reproduction of HIV-1 *in vitro* at a concentration of only 0.002 μM [9]. However, compounds of this series containing an aromatic fragment as S-alkyl substituent have not been described before. In this connection we undertook the synthesis of new derivatives of pyrimidin-4(3H)-one containing at position 2 an aryloxyethylthio substituent capable of conformationally imitating the benzyl fragment of the highly active compound **1** and its analogs, and we studied their anti-HIV activity *in vitro*.

It is known that several methods have been used for the S-alkylation of 2-thiouracils: a) Treatment of an alkaline aqueous solution of 2-thiouracil with methyl iodide leads to the target 2-(methylthio)pyrimidin-4(3H)-one with a yield of 88% [10]; b) Treatment of an alkaline water–alcohol solution of 2-thiouracil with methyl iodide leads to the preferential formation of 2-(methylthio)pyrimidin-4(3H)-one with a yield of 38% and the side formation of 3-methyl-2-(methylthio)pyrimidin-4(3H)-one with a yield of 8% [11]; c) Alkylation of 2-thiouracils with alkyl halides in absolute methanol in the presence of sodium methoxide gives the corresponding 2-(alkylthio) derivatives with yields in the range of 50-78% [7, 12]; d) Alkylation with alkyl halides in the polar solvent DMF [12] or DMSO [13] in the presence of potassium carbonate frequently leads to the formation of a complex mixture of S-mono-, S,$N_{(1)}$-, and $N_{(3)}$,S-dialkyl compounds.



2, **5**-**11**, **13**, **16**–**22** R^1 = H; **3**, **14** R^1 = Me; **4**, **15** R^1 = CH_2Ph; **2**-**4**, **13**-**15** R^2 = Me;
5, **16** R^2 = Ph; **6**, **17** R^2 = CH_2Ph; **7**, **18** R^2 = $CH_2(1\text{-}C_{10}H_7)$; **8**, **19** R^2 = $CH_2(2,6\text{-}F_2C_6H_3)$;
9, **20** R^2 = $CH_2O(4\text{-}MeC_6H_4)$; **10**, **21** R^2 = $CH_2O(4\text{-}ClC_6H_4)$; **11**, **22** R^2 = $CH_2O(2\text{-}C_{10}H_7)$

We found that the treatment of the initial 6-methyl (**2**), 5,6-dimethyl (**3**), 5-benzyl-6-methyl (**4**), 6-phenyl (**5**), 6-benzyl (**6**), 6-(1-naphthylmethyl) (**7**), 6-(2,6-difluorobenzyl) (**8**), 6-[(4-methylphenoxy)methyl] (**9**), 6-[(4-chlorophenoxy)methyl] (**10**), and 6-[(2-naphthyloxy)methyl] (**11**) derivatives of 2-thiouracil, which were synthesized by the previously described methods [14, 15], in DMF solution with an equimolar amount of 1-bromo-2-(3,5-dimethylphenoxy)ethane (**12**) in the presence of potassium carbonate at 70-80°C leads to the exclusive formation of S-alkylated 2-[2-(3,5-dimethylphenoxy)ethylthio] derivatives of pyrimidin-4(3H)-one **13**-**22**. According to TLC, the formation of S,N-dialkylation side products was not detected. The bulky 2-(3,5-dimethylphenoxy)ethyl substituent at the sulfur atom probably screens the nitrogen atom of the pyrimidine ring and prevents further alkylation under the given conditions.

The physicochemical characteristics of compounds **13**-**22** are given in Table 1.

TABLE 1. The Characteristics of the Synthesized Compounds

Compound	Empirical formula	Found, % / Calculated, %			mp, °C	R_f	Yield, %
		C	H	N			
13	$C_{15}H_{18}N_2O_2S$	62.19 / 62.04	6.39 / 6.25	9.36 / 9.65	167-169	0.37	61
14	$C_{16}H_{20}N_2O_2S$	63.38 / 63.13	6.44 / 6.62	9.01 / 9.20	180-181	0.22	72
15	$C_{22}H_{24}N_2O_2S$	69.75 / 69.44	6.10 / 6.36	7.07 / 7.36	168-169	0.72	63
16	$C_{20}H_{20}N_2O_2S$	68.28 / 68.16	5.89 / 5.72	7.77 / 7.95	174-176	0.46	58
17	$C_{21}H_{22}N_2O_2S$	68.67 / 68.83	5.83 / 6.05	7.90 / 7.64	153-155	0.48	75
18	$C_{25}H_{24}N_2O_2S$	71.82 / 72.09	5.59 / 5.81	6.94 / 6.73	174-175	0.40	61
19	$C_{21}H_{20}F_2N_2O_2S$	62.95 / 62.67	5.11 / 5.01	7.21 / 6.96	168-169	0.52	71
20	$C_{22}H_{24}N_2O_3S$	66.83 / 66.64	6.33 / 6.10	6.87 / 7.06	192-194	0.32	61
21	$C_{21}H_{21}ClN_2O_3S$	60.27 / 60.50	5.30 / 5.08	6.50 / 6.72	163-164	0.24	52
22	$C_{25}H_{24}N_2O_3S$	69.56 / 69.42	5.80 / 5.59	6.77 / 6.48	190-192	0.18	33

The antiviral properties of the synthesized compounds *in vitro* against HIV-1 were studied at the TherImmune Research Corporation (Maryland, USA) according to the previously described method [16]. The investigations showed that some of the new compounds **13**-**22** exhibited appreciable virus-inhibiting activity. The highest activity was exhibited by 2-[2-(3,5-dimethylphenoxy)ethylthio] derivatives of 6-benzylpyrimidin-4-(3H)-one (**17**) and 6-(2,6-difluorobenzyl)pyrimidin-4(3H)-one (**19**), which inhibited the reproduction of HIV-1 by 50% at concentrations of 1.3 and 11.2 μM and had a selectivity index of 58.0 and 8.1 respectively. The compounds containing a naphthyl fragment at position 6 proved weakly active. At concentrations of 40.8 and 123.0 μM the derivatives of 6-(1-naphthylmethyl)pyrimidin-4(3H)-one (**18**) and 6-(naphthyloxymethyl)-pyrimidin-4(3H)-one (**22**) blocked the reproduction of the virus by only 25% (Table 2).

TABLE 2. The Anti-HIV-1 Activity of the Synthesized Compounds in Culture of CEM-SS Cells *in vitro**

Compound	Antiviral activity EC_{50} (EC_{25}), μM	Cytotoxicity, TC_{50}, μM	Therapeutic index, TC_{50} / EC_{50}
13	>200.0	39.4	—
14	>200.0	46.2	—
15	>100.0	>100.0	—
16	>100.0	>100.0	—
17	1.3	72.9	58.0
18	(40.8)	61.7	—
19	11.2	90.0	8.1
20	>100.0	92.7	—
21	>100.0	>100.0	—
22	(123.0)	>200.0	—

* EC_{50} is the effective concentration securing protection of the cells against the cytopathic effect of the virus by 50%; TC_{50} is the cytotoxic concentration reducing the viability of the uninfected cells by 50%.

Investigation of the structure–activity relation showed that the compounds containing methyl (**13**-**15**) or phenyl (**16**) group as substituent at position 6 of the pyrimidine ring do not possess virus-inhibiting characteristics. At the same time increase in the dimensions and conformational mobility of the substituent to benzyl (**17**) leads to substantial increase of anti-HIV activity. Further increase in the size of the substituent to 1-naphthylmethyl (**18**) leads to decrease in the activity of the compounds. With the symmetrical introduction of fluorine atoms at the *o*-position of the benzyl substituent in **19** the virus-inhibiting characteristics are reduced by an order of magnitude. The 6-(aryloxymethyl) derivatives **20**-**22**, which have an additional oxygen atom in the substituent, are significantly less active than their 6-arylmethyl analogs. Transfer of the benzyl fragment from position 6 to position 5 in compound **15** leads to complete loss of anti-HIV-1 activity.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Tesla BS-567A spectrometer (100 MHz) in a 1:1 mixture of DMSO-d_6 and acetone-d_6 with HMDS as internal standard. The spectra were interpreted by means of the licensed ACD/HNMR Predictor Pro 3.0 software of Advanced Chemistry Development (Canada). The mass spectra were recorded on a Varian MAT-111 spectrometer (direct injection, electron impact ionization, 70 eV). Thin-layer chromatography was conducted on Silufol UV-254 plates in the 1:1 ethyl acetate–hexane system with development in iodine vapor. The melting points were determined in glass capillaries on a Mel-Temp 3.0 instrument (Laboratory Devices Inc. USA).

2-[2-(3,5-Dimethylphenoxy)ethylthio]-6-methylpyrimidin-4(3H)-one (13). Mixture of 6-methyl-2-thiouracil (2.3 g, 16.18 mmol) and potassium carbonate (2.3 g, 16.64 mmol) in DMF (40 ml) was stirred at 70-80°C for 1 h. Solution of 1-bromo-2-(3,5-dimethylphenoxy)ethane **11** (3.7 g, 16.15 mmol) in DMF (20 ml) was added, and the mixture was stirred at the same temperature for a further 4 h. It was cooled to room temperature and filtered. The filtrate was evaporated under vacuum, and the residue was washed with cold water (100 ml). The insoluble solid residue was filtered off, dried in air, and recrystallized from ethanol (80 ml). We obtained 2.6 g (61%) of a white crystalline substance; mp 167-169°C. 1H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.15 (3H, s, CH_3); 2.20 (6H, s, CH_3); 3.45 (2H, t, *J* = 7, SCH_2); 4.16 (2H, t, *J* = 7, OCH_2); 5.95 (1H, s, 5-H); 6.54 (3H, s, H arom.). Mass spectrum (*m/z*): 290 $[M]^+$.

Compounds **14**-**22** were prepared similarly.

2-[2-(3,5-Dimethylphenoxy)ethylthio]-5,6-dimethylpyrimidin-4(3H)-one (14). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.16 (6H, s, 3-, 5-CH_3 arom.); 2.18 (3H, s, 5-CH_3); 2.32 (3H, s, 5-CH_3); 2.34 (3H, s, 6-CH_3); 3.46 (2H, t, *J* = 7, SCH_2); 4.17 (2H, t, *J* = 7, OCH_2); 6.58 (1H, s, H arom.). Mass spectrum (*m/z*): 304 $[M]^+$.

5-Benzyl-2-[2-(3,5-dimethylphenoxy)ethylthio]-6-methylpyrimidin-4(3H)-one (15). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.14 (6H, s, CH_3 arom.); 2.32 (3H, s, CH_3); 3.44 (2H, t, *J* = 7, SCH_2); 3.71 (2H, s, CH_2Ph); 4.15 (2H, t, *J* = 7, OCH_2); 6.60 (3H, s, H arom.); 7.01-7.56 (5H, m, C_6H_5). Mass spectrum (*m/z*): 380 $[M]^+$.

2-[2-(3,5-Dimethylphenoxy)ethylthio]-6-phenylpyrimidin-4(3H)-one (16). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.09 (6H, s, CH_3 arom.); 3.44 (2H, t, *J* = 7, SCH_2); 4.17 (2H, t, *J* = 7, OCH_2); 6.12 (1H, s, 5-H); 6.56 (3H, s, H arom.); 7.14-7.69 (5H, m, C_6H_5). Mass spectrum (*m/z*): 352 $[M]^+$.

6-Benzyl-2-[2-(3,5-dimethylphenoxy)ethylthio]pyrimidin-4(3H)-one (17). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.10 (6H, s, CH_3 arom.); 3.48 (2H, t, *J* = 7, SCH_2); 4.08 (2H, s, CH_2Ph); 4.18 (2H, t, *J* = 7, OCH_2); 5.93 (1H, s, 5-H); 6.54 (3H, s, H arom.); 6.96-7.54 (5H, m, C_6H_5). Mass spectrum (*m/z*): 366 $[M]^+$.

2-[2-(3,5-Dimethylphenoxy)ethylthio]-6-(1-naphthylmethyl)pyrimidin-4(3H)-one (18). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.10 (6H, s, CH_3 arom.); 3.46 (2H, t, *J* = 7, SCH_2); 4.11 (2H, t, *J* = 7, OCH_2); 4.34 (2H, s, $ArCH_2$); 5.98 (1H, s, 5-H); 6.60 (3H, s, H arom.); 6.78-7.82 (5H, m, C_6H_5). Mass spectrum (*m/z*): 416 $[M]^+$.

2-[2-(3,5-Dimethylphenoxy)ethylthio]-6-(2,6-difluorobenzyl)pyrimidin-4(3H)-one (19). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.11 (6H, s, CH_3 arom.); 3.45 (2H, t, *J* = 7, SCH_2); 4.12 (2H, s, $ArCH_2$); 4.20 (2H, t, *J* = 7, OCH_2); 5.98 (1H, s, 5-H); 6.54-7.12 (5H, m, H arom.). Mass spectrum (*m/z*): 402 $[M]^+$.

2-[2-(3,5-Dimethylphenoxy)ethylthio]-6-(4-methylphenoxymethyl)pyrimidin-4(3H)-one (20). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.10 (6H, s, CH_3 arom.); 2.06 (3H, s, CH_3); 3.44 (2H, t, *J* = 7, SCH_2); 4.18 (2H, t, *J* = 7, OCH_2); 5.12 (2H, s, CH_2OAr); 5.84 (1H, s, 5-H); 6.50-6.87 (7H, m, H arom.). Mass spectrum (*m/z*): 396 $[M]^+$.

6-(4-Chlorophenoxymethyl)-2-[2-(3,5-dimethylphenoxy)ethylthio]pyrimidin-4(3H)-one (21). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.12 (6H, s, CH_3 arom.); 3.46 (2H, t, *J* = 7, SCH_2); 4.19 (2H, t, *J* = 7, OCH_2); 5.10 (2H, s, CH_2OAr); 5.82 (1H, s, 5-H); 6.55 (3H, s, H arom.); 6.61-7.21 (4H, m, H arom.). Mass spectrum (*m/z*): 416 $[M]^+$.

2-[2-(3,5-Dimethylphenoxy)ethylthio]-6-(2-naphthyloxymethyl)pyrimidin-4(3H)-one (22). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm, *J* (Hz): 2.11 (6H, s, CH_3 arom.); 3.46 (2H, t, *J* = 7, SCH_2); 4.18 (2H, t, *J* = 7, OCH_2); 5.08 (2H, s, CH_2OAr); 5.81 (1H, s, 5-H); 6.54 (3H, s, H arom.); 6.90-7.72 (7H, m, H arom.). Mass spectrum (*m/z*): 432 $[M]^+$.

Investigation of the Anti-HIV-1 Activity. CEM-SS cells were suspended in a culture medium at the rate of 10^5 cells/ml and infected with HIV-1 (HTLV-III_B strain) with infection multiplication of 0.2. Immediately after infection solutions containing various concentrations of the investigated substances in DMSO were added, and incubation was carried out for 4 days at 37°C. The number of live cells was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and the concentration of the substance that protected the CEM-SS cells by 50% from the cytopathic effect of HIV-1 (EC_{50}) was established.

The cytotoxicity of the tested compounds was determined in parallel, and here the concentration of the substance that reduced the number of live CEM-SS cells by 50% (TC_{50}) was established. The therapeutic index, equal to the ratio of the cytotoxic concentration to the inhibitor concentration (TC_{50}/EC_{50}), was obtained by calculation.

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