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SYNTHESIS AND ANTIVIRAL ACTIVITY OF 1-{[2-(PHENOXY)ETHOXY]-METHYL}URACIL DERIVATIVES

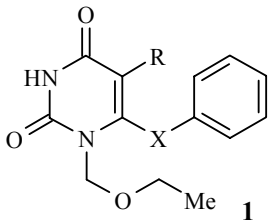
M. S. Novikov[1], A. A. Ozerov[1], Yu. A. Orlova[1], and R. W. Buckheit[2]

The synthesis of novel 1-{[2-(phenoxy)ethoxy]methyl}uracil derivatives with different substituents in positions and 6 of the pyrimidine ring has been carried out. It has been shown that the alkylation of trimethylsilyl derivatives of uracil with 2-(4-chlorophenoxy)- and 2-(4-methylphenoxy)ethoxymethyl chloride under Hilbert–Johnson reaction conditions gives $N_{(1)}$-substitution products. It was found that the 1-{[2-(phenoxy)ethoxy]methyl}uracil derivatives show viral inhibition properties relative to human immunodeficiency type 1 virus in vitro. The most active compounds are 5-bromo-6-methyluracil derivatives which suppress viral reproduction by 50% at 7.2 and 7.8 micromolar concentrations.

Keywords: 1-{[2-(phenoxy)ethoxy]methyl}uracil derivatives, synthesis, anti HIV-1 activity.

From the time of the discovery of the human immunodeficiency virus (HIV) as the etiological agent of acquired immunodeficiency syndrome (AIDS) [1, 2] up to the present time the HIV infection has been a serious clinical problem [3]. Preparations used in the clinic can be divided into two main classes *viz*., inhibitors of viral protease and of reverse transcriptase. Their use is accompanied by serious side effects and the creation of resistant strains of HIV [4]. Hence the search for novel HIV inhibitors is an extremely urgent problem.

The majority of non-nucleoside inhibitors of reverse transcriptase, as used in combination HIV infection and AIDS therapy, have a butterfly like shape according to X-ray data (Figure 1). In "wing 1" they have nitrogen atoms as part of a heterocycle in direct proximity to the catalytic site of the viral enzyme. In "wing 2" an aromatic fragment is located in a hydrophobic pocket of the enzyme and the lower "body" of the butterfly fits a lipophilic area of the reverse transcriptase [5-8]. Hence the derivatives of HEPT (1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine **1**) and their 6-benzyl analogs **2** show high anti-HIV-1 activity and the aromatic fragment forming "wing 2" is bonded to the uracil position 6 either *via* a sulphur atom or *via* a methylene group [9].



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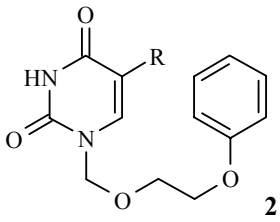

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1 X = S, CH_2; **1, 2** R = alkyl C_1-C_3

[1] Research Institute of Pharmacology, Volgograd State Medicinal University, Volgograd 400131, Russia; e-mail: ozerov@vlink.ru. [2] TherImmune Research Corporation, Maryland USA. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 5, pp. 726-731, May, 2005. Original article submitted September 24, 2003.

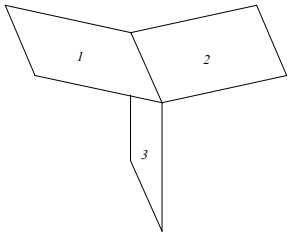
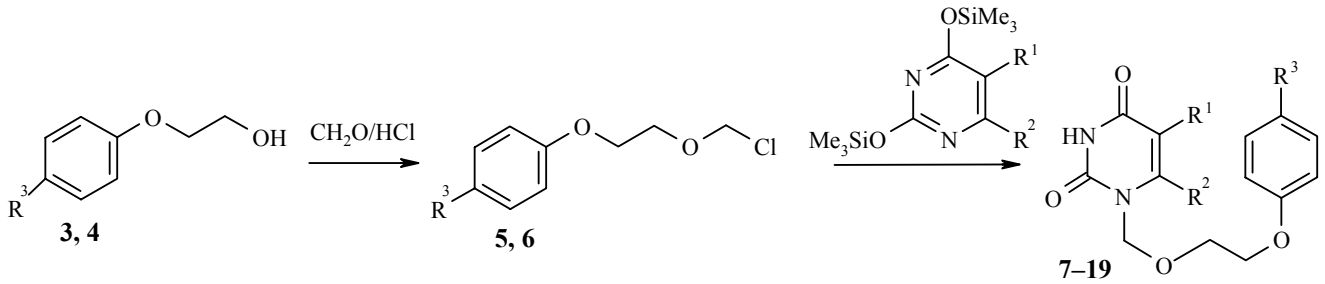


Fig. 1. Butterfly form of the non-nucleoside inhibitors of HIV-1 reverse transcriptase:
1: "wing 1" (pyrimidine base);
2: "wing 2" (aromatic nucleus);
3) "body" of the butterfly (substituent at position $N_{(1)}$)

We have proposed that the aromatic fragment might be bonded with the uracil residue through the $N_{(1)}$ atom rather than at position 6 and the overall butterfly structure of the HIV-1 reverse transcriptase inhibitor retained. Further, the optimum length of connecting chain should be a five to six carbon and oxygen fragment. In this case, "geometrical" analogs of 6-(phenylthio)- and 6-benzyluracils might be formed.

The closest structures corresponding to this criterion are 1-{[2-(phenoxy)ethoxy]methyl} uracils, in the chains of which there occur three methylene groups and two oxygen atoms. Their synthesis was carried out as shown in the scheme below.



7, **10**, **13**, **16** R^1 = H; **8**, **11**, **14**, **17** R^1 = Br; **9**, **12**, **15**, **18** R^1 = Me; **19** R^1 = Et;
7-9, **13-15** R^2 = H; **10-12**, **16-19** R^2 = Me; **3**, **5**, **7-12** R^3 = Cl; **4**, **6**, **13-19** R^3 = Me

In the presence of paraformaldehyde and gaseous hydrogen chloride the starting 2-(4-chlorophenoxy)ethanol (**3**) or 2-(4-methylphenoxy)ethanol (**4**) were converted to the corresponding 2-(4-chlorophenoxy)- (**5**) or 2-(4-methylphenoxy)ethoxymethyl (**6**) chlorides in 92 and 90% yields respectively. The subsequent step in the synthesis is condensation of the α-chloro ethers **5** and **6** with 2,4-bis(trimethylsilyloxy)pyrimidines in aqueous methylene chloride solution at room temperature. The target 1-{[2-(phenoxy)ethoxy]methyl}uracils **7-19** were obtained in 56-71% yields after preparative chromatography. The basic strategy in the synthesis has been reported by us previously [10].

The purity of the compounds **7-19** obtained was monitored using TLC, their composition proved by elemental analysis, and their structure by ^{1}H NMR spectroscopy and mass spectrometry. The physicochemical properties of compounds **7-19** are given in Table 1.

The *in* vitro antiviral activities of the synthesized compounds towards HIV-1 were measured at the TherImmune Research Corporation (Maryland, USA) using the CEM-SS cell culture. The screening results showed that several of the uracil derivatives **7-19** show marked antiviral activity. The most active compounds of this series proved to be the 1-{[2-(4-chlorophenoxy)ethoxy]methyl}- (**11**) and 1-{[2-(4-methylphenoxy)-ethoxy]methyl}- (**17**) derivatives of 5-bromo-6-methyluracil which showed a 50% inhibition of the reproduction of HIV-1 at 7.2 and 7.8 micromolar concentrations, respectively. However, due to their relatively high toxicity, their selectivity indices were equal to 4.3 and 7.7. The uracil derivatives 1-{[2-(4-chlorophenoxy)ethoxy]-methyl- (**7**) and 1-{[2-(4-methylphenoxy)ethoxy]methyl} (**13**) uracils had EC_{50} values of 23.7 and 16.1 micromolar and were an order less active but had lower toxicity and their selectivity indices were comparable with the indices for compounds **11** and **17**. The remaining compounds proved to be either inactive towards HIV-1 or showed weak viral inhibiting activity (Table 2).

TABLE 1. Parameters for the Compounds Synthesized

Compound	Empirical formula	Found, % / Calculated, %			mp, °C	R_f	Yield, %
		C	H	N			
7	$C_{13}H_{13}ClN_2O_4$	52.45 / 52.62	4.57 / 4.42	9.53 / 9.44	126-127	0.16	60
8	$C_{13}H_{12}BrClN_2O_4$	41.88 / 41.57	3.61 / 3.22	7.70 / 7.46	155-156	0.38	56
9	$C_{14}H_{15}ClN_2O_4$	53.97 / 54.11	5.04 / 4.87	8.72 / 9.02	121-123	0.22	71
10	$C_{14}H_{15}ClN_2O_4$	54.03 / 54.11	5.06 / 4.87	9.24 / 9.02	146-148	0.20	64
11	$C_{14}H_{14}BrClN_2O_4$	43.34 / 43.16	3.88 / 3.62	7.05 / 7.19	157-159	0.39	63
12	$C_{15}H_{17}ClN_2O_4$	55.21 / 55.48	5.07 / 5.28	8.39 / 8.63	167-168	0.31	57
13	$C_{14}H_{16}N_2O_4$	60.95 / 60.86	5.63 / 5.84	9.99 / 10.14	139-140	0.28	63
14	$C_{14}H_{15}BrN_2O_4$	47.50 / 47.34	4.41 / 4.26	7.78 / 7.89	70-73	0.49	70
15	$C_{15}H_{18}N_2O_4$	61.91 / 62.06	6.12 / 6.25	9.44 / 9.65	143-144	0.34	64
16	$C_{15}H_{18}N_2O_4$	62.18 / 62.06	6.13 / 6.25	9.46 / 9.65	140-143	0.32	59
17	$C_{15}H_{17}BrN_2O_4$	48.97 / 48.80	4.79 / 4.64	7.40 / 7.59	149-150	0.51	58
18	$C_{16}H_{20}N_2O_4$	62.95 / 63.14	6.51 / 6.62	9.03 / 9.20	141-143	0.43	56
19	$C_{17}H_{22}N_2O_4$	62.96 / 63.13	6.82 / 6.97	9.23 / 8.80	120-121	0.48	71

TABLE 2. *In vitro* anti-HIV-1-Activity of the Synthesized Compounds

Compound	EC_{50}, micromolar*	TC_{50}, micromolar*[2]	Selectivity index, TC_{50} / EC_{50}
7	23.7	>100.0	> 4.2
8	>100.0	48.2	—
9	50.9	>100.0	> 2.0
10	>100.0	>100.0	—
11	7.2	31.1	4.3
12	>100.0	96.3	—
13	16.1	>100.0	> 6.2
14	69.1	>100.0	> 1.5
15	>100.0	>100.0	—
16	>100.0	>100.0	—
17	7.8	60.0	7.7
18	76.8	>100.0	> 1.3
19	>100.0	19.8	—

* EC_{50}. The effective concentration giving a 50% protection of the cell from the cytopathic effect of the virus

*[2] TC_{50}. The cytotoxic concentration decreasing the survival of uninfected cells by 50%.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Bruker DRX-500 instrument (500 MHz) using CCl_4 (compounds **5** and **6**) or DMSO-d_6 (compounds **7-19**) and with TMS as internal standard. The interpretation of the spectra was carried out using the ACD/HNMR Predictor Pro 3.0 licensed program from Advanced Chemistry of Canada. Mass spectra were recorded on a Varian MAT-11 spectrometer (direct introduction, EI ionization method, 70 eV). TLC was performed on Silufol UV-254 plates in the system ethyl acetate-chloroform (1:1) and revealed using iodine vapour. Melting points were measured in glass capillaries on a Mel-Temp 3.0 instrument (Laboratory Devices Inc., USA).

2-(4-Chlorophenoxy)ethoxymethyl Chloride (5). A stream of dry hydrogen chloride was passed through a stirred mixture of 2-(4-chlorophenoxy)ethanol (**3**) (19.1 g, 0.111 mol) and paraformaldehyde (3.5 g, 0.117 mol) in anhydrous methylene chloride (100 ml) at 0-5°C for 2 h. The organic layer was separated, dried over $CaCl_2$, filtered, and the filtrate was evaporated in a vacuum to give compound **5** (22.6 g, 92%) as a viscous liquid which was used subsequently without further purification. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.66 (2H, t, *J* = 7, CH_2O); 3.96 (2H, t, *J* = 7, CH_2OAr); 5.41 (2H, s, CH_2Cl); 6.96-7.31 (4H, m, arom. H).

2-(4-Methylphenoxy)ethoxymethyl chloride (6) was prepared similarly in 90% yield. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.11 (3H, s, 4-CH_3); 3.72 (2H, t, *J* = 7, CH_2O); 4.10 (2H, t, *J* = 7, CH_2OAr); 5.51 (2H, s, CH_2Cl); 6.66-6.98 (4H, m, arom. H).

1-{[2-(4-Chlorophenoxy)ethoxy]methyl}uracil (7). A solution of the chloroether **5** (2.4 g, 10.86 mmol) in methylene chloride (20 ml) was added to a solution of 2,4-bis(trimethylsilyloxy)pyrimidine (2.75 g, 10.72 mmol) in anhydrous methylene chloride (30 ml) and stirred for 1 day at about 20°C. Ethanol (95%, 10 ml) was added and the product was stirred for 30 min, filtered, and the filtrated was evaporated to dryness in vacuo. The solid residue obtained was chromatographed on a silica gel column (2 × 35 cm) and eluted with a mixture of chloroform and methanol (10: 1). The fractions containing the target compound were combined, evaporated in vacuo, and the residue was recrystallized from ethyl acetate to give compound **7** (1.9 g, 60%) as a white, finely crystalline material with mp 126-127°C and R_f 0.16 (ethyl acetate–chloroform, 1: 1). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.81 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.08 (2H, t, *J* = 6, CH_2OAr); 5.14 (2H, s, NCH_2); 5.60 (1H, d, *J* = 8, H-5); 6.92-7.32 (4H, m, arom. H); 7.66 (1H, d, *J* = 8, H-6); 11.06 (1H, br. s, NH). Mass spectrum, *m/z*: 296 $[M]^+$.

Compounds 8-19 were prepared similarly

5-Bromo-1-{[2-(4-chlorophenoxy)ethoxy]methyl}uracil (8). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.82 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$; 4.09 (2H, t, *J* = 6, CH_2OAr); 5.14 (2H, s, NCH_2); 6.91-7.32 (4H, m, arom. H); 8.20 (1H, s, H-6); 11.64 (1H, br. s, NH). Mass spectrum, *m/z*: 375 [M].

1-{[2-(4-Chlorophenoxy)ethoxy]methyl}thiamine (9). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.78 (3H, s, CH_3); 3.81 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.08 (2H, t, *J* = 6, CH_2OAr); 5.12 (2H, s, NCH_2); 6.90-7.31 (4H, m, arom. H); 7.56 (1H, s, H-6); 11.26 (1H, br. s, NH). Mass spectrum, *m/z*: 310 $[M]^+$.

1-{[2-(4-Chlorophenoxy)ethoxy]methyl}-6-methyluracil (10). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.27 (3H, s, 6-CH_3); 3.81 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.09 (2H, t, *J* = 6, CH_2OAr); 5.30 (2H, s, NCH_2); 5.3 (1H, s, H-5); 6.91-7.33 (4H, m, C_6H_4);11.18 (1H, br. s, 3-NH). Mass spectrum, *m/z*: 310 $[M]^+$.

5-Bromo-1-{[2-(4-chlorophenoxy)ethoxy]methyl}-6-methyluracil (11). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.36 (3H, s, CH_3); 3.82 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.10 (2H, t, *J* = 6, CH_2OAr); 5.31 (2H, s, NCH_2); 6.90-7.32 (4H, m, arom. H); 11.49 (1H, br. s, NH). Mass spectrum, *m/z*: 389 $[M]^+$.

1-{[2-(4-Chlorophenoxy)ethoxy]methyl}-5,6-dimethyluracil (12). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.81 (3H, s, 5-CH_3); 2.26 (3H, s, 6-CH_3); 3.81 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.09 (2H, t, *J* = 6, CH_2OAr); 5.32 (2H, s, NCH_2); 6.90-7.32 (4H, m, C_6H_4); 11.21 (1H, br. s, 3-NH). Mass spectrum, *m/z*: 324 $[M]^+$.

1-{[2-(4-Methylphenoxy)ethoxy]methyl}uracil (13). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.22 (3H, s, CH_3); 3.81 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.04 (2H, t, *J* = 6, CH_2OAr); 5.12 (2H, s, NCH_2); 5.58 (1H, d, *J* = 8, H-5); 6.77-7.05 (4H, m, arom. H); 7.68 (1H, s, H-6); 11.14 (1H, br. s, NH). Mass spectrum, *m/z*: 276 $[M]^+$.

5-Bromo-1-{[2-(4-methylphenoxy)ethoxy]methyl}uracil (14). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.21 (3H, s, 4-CH_3); 3.84 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.04 (2H, t, *J* = 6, CH_2OAr); 5.15 (2H, s, NCH_2); 6.78-7.09 (4H, m, C_6H_4); 8.25 (1H, s, H-6); 11.76 (1H, br. s, 3-NH). Mass spectrum, *m/z*: 354 $[M]^+$.

1-{[2-(4-Methylphenoxy)ethoxy]methyl}thiamine (15). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.77 (3H, s, 5-CH_3); 2.22 (3H, s, 4-CH_3); 3.81 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.04 (2H, t, *J* = 6, CH_2OAr); 511 (2H, s, NCH_2); 6.78-7.07 (4H, m, arom. H); 7.54 (1H, s, H-6); 11.22 (1H, br. s, 3-NH). Mass spectrum, *m/z*: 290 $[M]^+$.

6-Methyl-1-{[2-(4-methylphenoxy)ethoxy]methyl}uracil (16). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.22 (3H, s, 4-CH_3); 2.28 (3H, s, 6-CH_3); 3.80 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.05 (2H, t, *J* = 6, CH_2OAr); 5.30 (2H, s, NCH_2); 5.52 (1H, s, H-5); 6.80-7.06 (4H, m, arom. H); 11.15 (1H, br. s, NH). Mass spectrum, *m/z*: 290 $[M]^+$.

5-Bromo-6-methyl-1-{[2-(4-methylphenoxy)ethoxy]methyl}uracil (17). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.21 (3H, s, CH_3); 2.35 (3H, s, 6-CH_3); 3.81 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.07 (2H, t, *J* = 6, CH_2OAr); 5.30 (2H, s, NCH_2); 6.81-7.07 (4H, m, arom. H); 11.29 (1H, br. s, NH). Mass spectrum, *m/z*: 369 $[M]^+$.

5,6-Dimethyl-1-{[2-(4-methylphenoxy)ethoxy]methyl}uracil (18). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.82 (3H, s, 5-CH_3); 2.22 (3H, s, 4-CH_3); 2.28 (3H, s, 6-CH_3); 3.79 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 4.03 (2H, t, *J* = 6, CH_2OAr); 5.32 (2H, s, NCH_2); 6.75-7.06 (4H, m, arom. H); 11.21 (1H, br. s, 3-NH). Mass spectrum, *m/z*: 304 $[M]^+$.

6-Methyl-1-{[2-(4-methylphenoxy)ethoxy]methyl}uracil (19). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.09 (3H, t, *J* = 7, CH_3); 2.34 (3H, s, CH_3); 2.50 (3H, s, 6-CH_3); 2.58 (2H, q, *J* = 7, CH_2); 3.81 (2H, t, *J* = 6, $\underline{CH_2}CH_2OAr$); 3.94 (2H, t, *J* = 6, CH_2OAr); 5.31 (2H, s, NCH_2); 6.80-7.05 (4H, m, arom. H); 11.27 (1H, br. s, NH). Mass spectrum, *m/z*: 318 $[M]^+$.

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