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Original Article

In vitro proliferative activity of 6-substituted uracil derivatives

[Actividad proliferativa *in vitro* de derivados de uracilo 6-sustituidos]

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Abstract

Context: Previously, we investigated the relationship between the nature of the substituent at the 5-position of the uracil ring and the action of the corresponding uracil derivatives on immortalized lung cells. In the present study, we analyzed the impact of some 6-substituted uracil-derivatives on the regeneration potential of the lung cells (LC).

Aims: To evaluate uracil derivatives capable of stimulating lung cell proliferation to create drugs that accelerate lung regeneration.

Methods: The level of cell proliferation, maximum tolerated dose, and toxic effect of 6-substituted uracil derivatives (9 compounds) were studied on the immortalized lung epithelial cells and compared with the known drug 6-methyluracil.

Results: The maximum tolerated dose of compounds for the LC line depends on the chemical structure of the compounds. The highest level of cell proliferation and tolerated was demonstrated when was used 3-methyl-6-cyclopropyluracil and 1-butyl-6-methyluracil.

Conclusions: 3-methyl-6-cyclopropyluracil and 1-butyl-6-methyluracil exhibit a high proliferative activity *in vitro* so they could be recommended for additional studies of regenerative activity *in vivo*.

Keywords: cytotoxicity; lung epithelial cells; proliferation; 6-substituted uracil derivatives; synthesis.

Resumen

Contexto: Previamente, investigamos la relación entre la naturaleza del sustituyente en la posición 5 del anillo de uracilo y la acción de los derivados de uracilo correspondientes sobre las células pulmonares inmortalizadas. En el presente estudio, analizamos el impacto de algunos derivados de uracilo 6-sustituidos sobre el potencial de regeneración de las células pulmonares (CP).

Objetivos: Evaluar derivados del uracilo capaces de estimular la proliferación de células pulmonares para crear fármacos que aceleren la regeneración pulmonar.

Métodos: Se estudiaron el nivel de proliferación celular, la dosis máxima tolerada y el efecto tóxico de los derivados de uracilo 6-sustituidos (9 compuestos) en las células epiteliales pulmonares inmortalizadas y se compararon con el fármaco conocido 6-metiluracilo.

Resultados: La dosis máxima tolerada de compuestos para la línea CP depende de la estructura química de los compuestos. El nivel más alto de proliferación celular y tolerado se demostró cuando se utilizó 3-metil-6-ciclopropiluracilo y 1-butil-6-metiluracilo.

Conclusiones: 3-metil-6-ciclopropiluracilo y 1-butil-6-metiluracilo exhiben una alta actividad proliferativa *in vitro* por lo que pudieran recomendarse para estudios adicionales de actividad regenerativa *in vivo*.

Palabras Clave: células epiteliales pulmonares; citotoxicidad; derivados de uracilo 6-sustituidos; proliferación; síntesis.

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INTRODUCTION

Pyrimidines occupy an important position in the medicinal world as it has a number of diverse biological properties (Rosenfeldt et al., 1998; Lagoja et al., 2005; Brulikova and Hlavac, 2011; Gimadieva et al., 2014; Pałasz and Ciez, 2015; Shaker et al., 2016; Kumar et al., 2019). Pyrimidine derivatives are reported to have diverse pharmacological activities such as anticonvulsant, analgesic, sedative, anti-depressive, antipyretic, anti-inflammatory, antiviral, anti-HIV, antimicrobial and antitumor activities (Prekupec et al., 2005; Wang et al., 2010; Li et al., 2012; Yan and Ma, 2012; Novikov and Geisman, 2014; Sharma et al., 2014; Stevaert and Naesens, 2016; Bhat, 2017). Drugs of the pyrimidine series are characterized by a variety of mechanisms of action, relative safety, as well as a wide range of therapeutic effects. They are considered as the basis for the synthesis of new potential biologically active compounds.

Great interest in uracil derivatives exists since the beginning of the last century because they possess a very low toxicity and have a latitude therapeutic action - stimulate nucleic and protein metabolism, accelerate cell growth, exert anti-inflammatory action, increase immunity (Wang et al., 2010; Li et al., 2012; Gimadieva et al., 2014).

Injuries of the lung epithelium in various pathologies testify to the strong need for the development of drugs for a specific tissue environment that combine the possibility of inflammatory and tissue regeneration, which can be achieved by creating a drug based on pyrimidine.

Estimation of the potential cytotoxicity of pharmaceutical substances is a necessary stage of their studies at the preclinical stage (Riss and Moravec, 2004; Gordon et al., 2016).

Previously, we investigated the relationship between the nature of the substituent at the 5-position of the uracil ring and the action of the corresponding uracil derivatives on immortalized lung cells (Kabal`nova et al., 2017). In the present study we analyzed the impact of some 6-substituted uracil-derivatives on the regeneration potential of the immortalized fetal calf lung cells (LC). We found that the 3-methyl-6-cyclopropyl-uracil and 1-butyl-6-methyluraci have the best perspectives for further studies of their biological activity.

MATERIAL AND METHODS

Analytical studies

NMR spectra were recorded on a Bruker Avance-III 500 MHz instrument with PABBO X{^{1}H} direct detection probe in 5 mm NMR tubes, at 298 K. Gradient selected {^{1}H, ^{13}C} HSQC and {^{1}H, ^{13}C} HMBC spectra were recorded using the standard Bruker sequence. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

All melting points were taken on a Büchi apparatus and were uncorrected.

High-performance liquid chromatography (HPLC) analysis was carried out on a Waters® Breeze™ HPLC system with a spectrophotometric detector. A Zorbax RXC18 250 × 4.6 mm column was used; 5 µm (Agilent, USA). The eluent CH_3CN/H_2O was used as a mobile phase; the flow rate was 1 mL/min. Detection was carried out at a wavelength of 215 nm.

Materials and chemicals

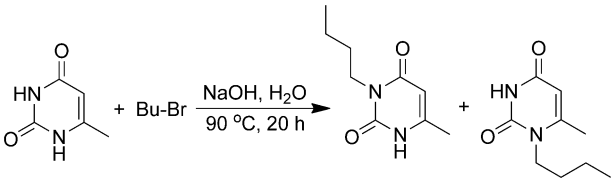
Commercially available 6-methyluracil (>99%) and 1-methyl-6-aminouracil (>98%) were recrystallized from water. The other compounds were synthesized as described below, some of their characteristics are listed in the Table 1.

N-Butyl derivatives of 6-methyluracil were synthesized by alkylating 10 g (79.3 mmol) of 6-methyluracil in 100 mL of 1 M sodium hydroxide solution with butyl bromide at 90°C for 20 h. The mixture of 1-butyl and 3-butyl derivatives (1:10) were separated by fractional crystallization from acetonitrile (first crystallized 3-butyl-6-methyl-uracil). The residue containing a mixture of 1- and 3- derivatives in a ratio of 2:3 was separated by column chromatography of the hexane/ethanol/

Table 1. Some characteristics of synthesized compounds.

Chemical name	Molecular formula	Mass (g/mol)	$T_{m.p.}$ (°C)	Yield (%)	Identification
6-*iso*-Propyl-2-thiouracil	$C_7H_{10}N_2OS$	170.2	179	72.5	Elem. anal.; ^{1}H, ^{13}C and ^{15}N NMR
6-tret-Butyl-2-thiouracil	$C_8H_{12}N_2OS$	184.3	173-172	60.0	Elem. anal.; ^{1}H, ^{13}C and ^{15}N NMR
3-Methyl-6-cyclopropyluracil	$C_8H_{10}N_2O_2$	166.2	178-180 (subl.)	16.7	Elem. anal.; ^{1}H, ^{13}C and ^{15}N NMR
1-Butyl-6-methyluracil	$C_9H_{14}N_2O_2$	182.2	132-133	7.6	Elem. anal.; ^{1}H and ^{13}C NMR
6-Ethyluracil	$C_6H_8N_2O_2$	140.1	309-310	20.0	Elem. anal.; ^{1}H, ^{13}C and ^{15}N NMR
3-Methyl-6-ethyluracil	$C_7H_{10}N_2O_2$	154.2	220-222	82.0	Elem. anal.; ^{1}H, ^{13}C and ^{15}N NMR
3-Butyl-6-methyluracil	$C_9H_{14}N_2O_2$	182.2	165-166	78.0	Elem. anal.; ^{1}H and ^{13}C NMR

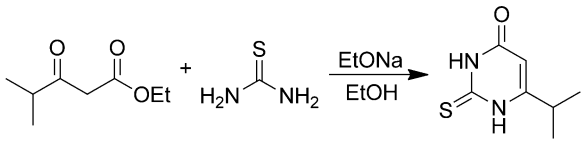
ethyl acetate eluent in a ratio of 3/1/15, respectively.



1-Butyl-6-methyluracil. The yield was 1.1 g. Colorless crystals, m. p. 132-133°C [lit. 132-133°C (Kato et al., 1981)]; ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS, δ, ppm): 0.918 (t, J=7.4 Hz, 3H, $CH_2C\underline{H}_3$), 1.344 (sex, J=7.5 Hz, 2H, $C\underline{H}_2CH_3$), 1.55-1.63 (m, 2H, N(1)$CH_2C\underline{H}_2$), 2.234 (s, 3H, CCH_3), 3.759 (t, J=7.9 Hz, 2H, N(1)CH_2), 5.550 (s, 1H, CH), 10.131 (br.s., 1H, N(3)H). ^{13}C-NMR (125 MHz) (DMSO-d_6/TMS, δ, ppm): 13.62 ($CH_2\underline{C}H_3$), 19.68 ($H_3CC(6)$ and $\underline{C}H_2CH_3$), 30.85 (N(1)$CH_2\underline{C}H_2$), 44.17 (N(1)CH_2), 101.88 (C5), 151.65 (C2), 153.95 (C6), 163.36 (C4). Anal. calc. for $C_9H_{14}N_2O_2$, %: C, 59.32; H, 7.74; N, 15.37. Found, %: C, 59.33; H, 7.75; N, 15.35.

3-Butyl-6-methyluracil. The yield was 11.3 g (78%). White crystals, m. p. 165-166°C; ^{1}H-NMR (500 MHz) ($CDCl_3$/TMS, δ, ppm): 0.921 (t, J=7.4 Hz, 3H, $CH_2C\underline{H}_3$), 1.347 (sex, J=7.4 Hz, 2H, $C\underline{H}_2CH_3$), 1.55-1.63 (m, 2H, N(3)$CH_2C\underline{H}_2$), 2.143 (s, 3H, CCH_3), 3.889 (t, J=7.5 Hz, 2H, N(3)CH_2), 5.580 (s, 1H, CH), 10.708 (s, 1H, N(1)H). ^{13}C-NMR (125 MHz) ($CDCl_3$/TMS, δ, ppm): 13.73 ($CH_2\underline{C}H_3$), 18.53 (C(6)$\underline{C}H_3$), 20.07 ($\underline{C}H_2CH_3$), 29.70 (N(3)$CH_2\underline{C}H_2$), 40.12 (N(3)CH_2), 100.26 (C5), 149.96 (C6), 153.46 (C2), 163.46 (C4). Anal. calc. for $C_9H_{14}N_2O_2$, %: C, 59.32; H, 7.74; N, 15.37. Found, %: C, 59.31; H, 7.76; N, 15.38.

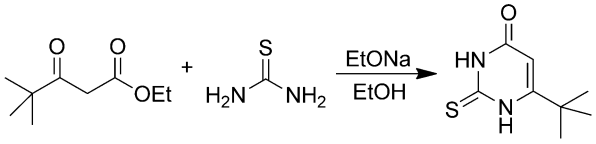
6-*iso*-Propyl-2-thiouracil. Synthesized from 4.8 g (63.2 mmol) of thiourea and 5.1 mL (31.6 mmol) of ethyl 4-methyl-3-oxopentanoate (Paknikar and Fondekar, 2001) in the presence of 40.0 mmol ethoxide of sodium in 30 mL of ethanol (Anderson et al., 1945). After recrystallization from water, the purity was 98% (by HPLC).



The yield was 3.9 g as white crystals with m. p. 179°C (lit. 179-180°C (Anderson et al., 1945)). ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS, δ, ppm): 1.139 (d, J=6.9 Hz, 6H, CH_3), 2.677 (sep, J=6.9 Hz, 1H, $C\underline{H}Me_2$), 5.679 (s, 1H, C(5)H), 12.214 (s, 1H, N(1)H), 12.353 (s, 1H, N(3)H). ^{13}C-NMR (125 MHz) (DMSO-d_6/TMS, δ, ppm): 20.31 (CH_3), 30.24 ($\underline{C}HMe_2$), 100.25 (C5), 161.27 (C=O), 162.14 (C6), 175.99 (C=S). ^{15}N-NMR (50 MHz) (DMSO-d_6, δ,

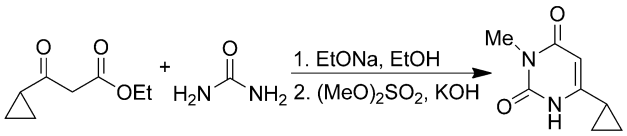
ppm): 158.3 (N1), 180.6 (N3). Anal. calc. for $C_7H_{10}N_2OS$, %: C, 49.39; H, 5.92; N, 16.46; S, 18.84. Found, %: C, 49.38; H, 5.94; N, 16.48; S, 18.81.

6-*tret*-Butyl-2-thiouracil. Synthesized from 4.4 g (58.0 mmol) of thiourea and 5 g (29.0 mmol) of ethyl 4,4-dimethyl-3-oxopentanoate (Vlassa and Barábas, 1980) in the presence of 40.0 mmol ethoxide of sodium in 30 mL of ethanol (Golubyatnikova et al., 2017). After recrystallization from water, the purity was 98% (by HPLC).



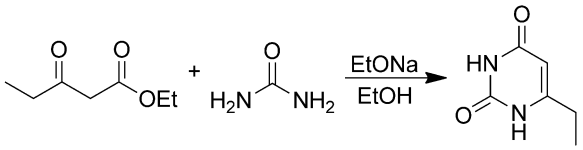
The yield was 3.2 g as white crystals with m. p. 173-172°C [lit. 178°C (Anderson et al., 1945)]; ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS, δ, ppm): 1.196 (s, 3H, CH_3), 5.618 (s, 1H, CH), 11.772 (s, 1H, N(1)H), 12.344 (s, 1H, N(3)H). ^{13}C-NMR (125 MHz) (DMSO-d_6/TMS, δ, ppm): 27.77 (CH_3), 34.94 ($\underline{C}Me_3$), 100.98 (C5), 161.63 (C=O), 163.80 (C6), 176.95 (C=S). ^{15}N-NMR (50 MHz) (DMSO-d_6, δ, ppm): 154.4 (N1), 180.4 (N3). Anal. calc. for $C_8H_{12}N_2OS$, %: C, 52.15; H, 6.56; N, 15.20; S, 17.40. Found, %: C, 52.14; H, 6.58; N, 15.18; S, 17.39.

3-Methyl-6-cyclopropyluracil. 6-Cyclopropyluracil was synthesized from 3.6 g (60.5 mmol) of urea and 5 mL (30.3 mmol) of ethyl 3-cyclopropyl-3-oxopropionate (Ragavan et al., 2013) in the presence of 40.0 mmol of sodium ethoxide in 30 mL of ethanol according to typical method (Basnak and Farkas, 1979). It was recrystallized from water. The yield was 0.97 g (21%). White crystals, m. p. 214-223°C (dec.) [lit. 215-224°C (Basnak and Farkas, 1976)]; ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS, δ, ppm): 0.83-0.89 and 0.95-1.02 (m, 2H и 2H, cycloprop. CH_2), 1.62-1.70 (m, 1H, cycloprop. $\underline{C}H(CH_2)_2$), 5.142 (s, 1H, C(5)H), 10.869 (s, 1H, N(3)H), 10.898 (s, 1H, N(1)H). ^{13}C-NMR (125 MHz) (DMSO-d_6/TMS, δ, ppm): 8.80 (CH_2), 12.25 ($\underline{C}H(CH_2)_2$), 93.21 (C5), 151.47 (C2), 159.14 (C6), 164.16 (C4). ^{15}N-NMR (50 MHz) (DMSO-d_6, δ, ppm): 134.8 (N1), 155.9 (N3). Anal. calc. for $C_7H_8N_2O_2$, %: C, 55.26; H, 5.30; N, 18.41. Found, %: C, 55.24; H, 5.29; N, 18.44. Cyclopropyluracil 1 g (6.58 mmol) was methylated with 0.62 mL (6.58 mmol) of dimethyl sulfate without solvent (Bram et al., 1985). After recrystallization from ethanol, the purity was 98% (by HPLC).



The yield was 0.87 g as white crystals, m. p. 178-180°C (sub.); ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS, δ, ppm): 0.91-0.96 and 1.04-1.10 (m, 2H и 2H, cycloprop. CH_2), 1.61-1.68 (m, 1H, cycloprop. $\underline{C}H(CH_2)_2$), 3.275 (s, 3H, CH_3), 5.426 (s, 1H, C(5)H), 10.653 (s, 1H, N(1)H). ^{13}C-NMR (125 MHz) (DMSO-d_6/TMS, δ, ppm): 8.76 (CH_2), 12.86 ($\underline{C}H(CH_2)_2$), 26.88 (CH_3), 95.49 (C5), 153.14 (C2), 156.45 (C6), 163.65 (C4). Anal. calc. for $C_8H_{10}N_2O_2$, %: C, 57.82; H, 6.07; N, 16.86. Found, %: C, 57.83; H, 6.09; N, 16.83.

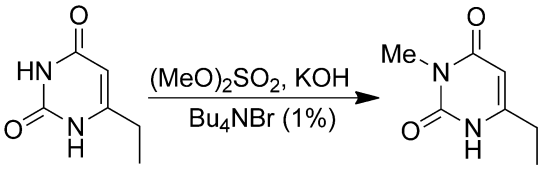
6-Ethyluracil. Synthesized from 4.2 g (69.4 mmol) of urea and 5.0 g of mL (34.7 mmol) of ethyl 3-oxopentanoate (Huckin and Weiler, 1974) in the presence of 40.0 mmol of sodium ethoxide in 30 mL of ethanol (Anderson et al., 1945). After recrystallization from water, the purity was 99% (by HPLC).



The yield was 0.99 g. White crystals m. p. 309-310°C [lit. 308-311°C (Basnak and Farkas, 1976)]. ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS, δ, ppm): 1.103 (t, J=7.5 Hz, 3H, CH_3), 2.306 (quin, J=7.5 Hz, 2H, CH_2), 5.327 (s, 1H, CH), 10.826 (s, 1H, N(1)H), 10.913 (s, 1H, N(3)H). ^{13}C-NMR (125 MHz) (DMSO-d_6/TMS, δ, ppm): 11.48 (CH_3), 24.96 (CH_2), 97.00 (C5), 151.63 (C2), 157.87 (C6), 164.25 (C4). ^{15}N-NMR (50 MHz) (DMSO-d_6, δ, ppm): 135.4 (N1), 156.9 (N3). Anal. calc. for $C_6H_8N_2O_2$, %: C, 51.42; H, 5.75; N, 19.99. Found, %: C, 51.43; H, 5.77; N, 19.97.

3-Methyl-6-ethyluracil. Methylation of 500 mg (3.57 mmol) of 6-ethyluracil 0.34 mL (3.60 mmol) with dimethyl sulfate without solvent (Bram et al.,

1985). After recrystallization from ethanol, the purity was 97% (by HPLC).



The yield was 450 mg. White crystals. ^{1}H-NMR (500 MHz) (DMSO-d_6/TMS, δ, ppm): 1.115 (t, J=7.5 Hz, 3H, CH_3), 2.325 (quin, J=7.5 Hz, 2H, CH_2), 3.099 (s, 3H, CH_3), 5.459 (s, 1H, CH), 11.091 (s, 1H, N(1)H). ^{13}C-NMR (125 MHz) (DMSO-d_6/TMS, δ, ppm): 11.49 (CH_3), 24.83 (CH_2), 26.18 (N(3)CH_3) 96.44 (C5), 151.67 (C2), 155.94 (C6), 163.14 (C4). ^{15}N-NMR (50 MHz) (DMSO-d_6, δ, ppm): 134.7 (N1), 152.3 (N3). Anal. calc. for $C_7H_{10}N_2O_2$, %: C, 54.54; H, 6.54; N, 18.17. Found, %: C, 54.53; H, 6.56; N, 18.15.

Cell culture, cytotoxicity and cell proliferation

The study was performed using the cell culture of fetal calf lung cells (LCs) immortalized by infection with bovine leukemia virus. After immortalization LCs had the doubling time 8-10 h. For immortalization fetal calf lung cells were isolated as described previously (Mirchamsy et al., 1976). The obtained cell line expresses a number of lung specific receptors and maintains lung-specific metabolism as they can be productively infected with bovine herpes virus type 1 causing infectious bovine rhinotracheitis, bovine parainfluenza virus 3 and bovine respiratory syncytial virus. The use of bovine cell lines allows obtaining the most native culturing condition in the presence of fetal calf serum.

The cells were cultured in DMEM medium supplemented with 10% fetal calf serum at 37°C and 5% CO_2. The tested compounds were dissolved in a mixture of DMSO and 96% ethanol in a ratio (1:1). The test substances were added to the cell culture medium. The mixture of DMSO/ethanol without any compounds was added in the control experiments.

After 24 h of cultivation, the cell layer was assessed using an inverted microscope according to the following parameters: percentage of surface coverage, cell shape, number of cell aggregates, number of floating cells. Cells were counted in the Goryaev chamber.

Trypan Blue staining (0.1% solution) assessed the number of living and dead cells. The influence of the studied compounds on the cultural and morphological properties of cells was determined taking into account the following parameters: the coefficient of viability - the ratio of living cells to their total number, expressed in%; proliferation index - the ratio of the number of grown cells to the number of seeded cells; cytotoxicity index - the ratio of living cells remaining after exposure to a compound to the number of living cells in the control (Novikov and Geisman, 2014; Stevaert and Naesens, 2016; Bhat, 2017).

The experiments were carried out within 24 h in connection with the study of the effect of studied compounds on proliferative activity. During this time, the main period of cell proliferation occurs, and the calculations will be more correct for assessing the effect of the compounds.

Statistical analysis

All experiments were performed minimum twice in triplicates. The numeric results in tables and in figures are presented as mean ± standard error and one-way analysis of variance (ANOVA), followed by the Tukey's multiple comparison test. P values less than 0.05 were considered as indicative of significance. SPSS software was used for data analysis. The mentioned in the text rise or drop of the measured indexes represents % to respective values from control.

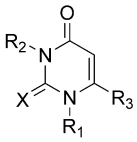
RESULTS AND DISCUSSION

The effect of compounds **1-9** (Fig. 1, Table 2) on the proliferation of fetal calf lung cells (LC) *in vitro* was studied. Compound **1** at a concentration of 0.1 mM does not significantly affect the viability of LC. An increase in the concentration of test compounds to 1 mM leads to a decrease in the viability coefficient by 6% (**5**) - 25% (**8**, **9**). A further increase in the concentration of up to 10 mM decreases the viability by 34-50%.

Table 2. Characteristics of culturally morphological parameters of the cell culture of fetal calf lung cells under the influence of the studied compounds (exposition 24 h).

No.	Concentration (mM)	Vitality coefficient (%)	Proliferation index	Cytotoxic index (%)	Cell death (%)
Control	0.0	88.8 ± 2.2	0.80 ± 0.07	0.0 ± 0.4	11.1 ± 0.4
6-Methyluracil (**1**)	0.1	80.0 ± 2.6	0.60 ± 0.01	10.0 ± 0.7	13.9 ± 0.6
	1.0	75.0 ± 1.9	0.00 ± 0.07	63.0 ± 3.0	25.0 ± 0.5
	10.0	40.0 ± 2.1	0.00 ± 0.04	75.0 ± 8.0	60.0 ± 0.0
2	0.1	87.8 ± 1.9	0.64 ± 0.02	10.0 ± 0.5	12.1 ± 0.5
	1.0	80.0 ± 1.6	0.00 ± 0.08	50.0 ± 2.0	20.0 ± 0.3
	10.0	40.0 ± 2.0	0.00 ± 0.07	75.0 ± 3.0	60.0 ± 0.5
3	0.1	87.6 ± 2.1	0.64 ± 0.09	10.0 ± 1.0	12.0 ± 0.6
	1.0	80.0 ± 1.8	0.00 ± 0.07	50.0 ± 2.0	20.0 ± 0.5
	10.0	40.0 ± 2.0	0.00 ± 0.08	75.0 ± 7.0	60.0 ± 0.8
4	0.1	88.4 ± 1.8	0.72 ± 0.02	10.0 ± 1.0	12.1 ± 0.5
	1.0	72.2 ± 1.7	0.00 ± 0.05	68.0 ± 7.0	27.8 ± 0.9
	10.0	40.0 ± 2.0	0.00 ± 0.07	75.0 ± 8.0	60.0 ± 2.0
5	0.1	88.9 ± 1.6	0.80 ± 0.03	0.0 ± 0.7	10.8 ± 0.4
	1.0	83.3 ± 1.7	1.40 ± 0.08	25.0 ± 5.0	16.7 ± 0.6
	10.0	57.7 ± 2.1	0.04 ± 0.02	63.0 ± 7.0	42.3 ± 0.5
6	0.1	89.1 ± 2.0	0.84 ± 0.04	0.0 ± 1.0	11.1 ± 0.7
	1.0	80.0 ± 1.7	1.00 ± 0.07	0.0 ± 2.0	20.0 ± 0.7
	10.0	56.0 ± 2.0	0.00 ± 0.08	65.0 ± 5.0	44.0 ± 0.8
7	0.1	88.6 ± 1.7	0.76 ± 0.02	0.0 ± 1.0	10.9 ± 0.4
	1.0	75.0 ± 1.8	0.60 ± 0.09	25.0 ± 3.0	25.0 ± 1.3
	10.0	44.0 ± 2.1	0.00 ± 0.07	73.0 ± 8.0	56.0 ± 1.5
8	0.1	88.4 ± 1.8	0.72 ± 0.02	10.0 ± 1.0	11.4 ± 0.5
	1.0	66.6 ± 1.6	0.20 ± 0.08	50.0 ± 6.0	33.3 ± 0.9
	10.0	52.0 ± 2.0	0.00 ± 0.08	68.0 ± 7.0	48.0 ± 0.7
9	0.1	88.1 ± 2.7	0.68 ± 0.02	24.0 ± 2.0	11.2 ± 0.7
	1.0	66.5 ± 1.9	0.20 ± 0.01	60.0 ± 5.0	33.6 ± 1.4
	10.0	52.0 ± 2.1	0.00 ± 0.01	72.0 ± 7.0	48.2 ± 1.5

Each value represents the mean ± SEM of six dimensions; values are statistically significant at $p<0.05$ when compared to control. The control experiments were carried out in the same condition, but without compound.



		R_1	R_2	X	R_3
1	6-Methyluracil	H	H	O	CH_3
2	6-iso-Propyl-2-thiouracil	H	H	S	$CH(CH_3)_2$
3	6-tret-Butyl-2-thiouraci	H	H	S	$C(CH_3)_3$
4	1-Methyl-6-aminouracil	CH_3	H	O	NH_2
5	3-Methyl-6-cyclopropyluracil	H	CH_3	O	
6	1-Butyl-6-methyluracil	Bu	H	O	CH_3
7	6-Ethyluracil	H	H	O	CH_2CH_3
8	3-Methyl-6-ethyluracil	H	CH_3	O	CH_2CH_3
9	3-Butyl-6-methyluracil	H	Bu	O	CH_3

Figure 1. Studied compounds.

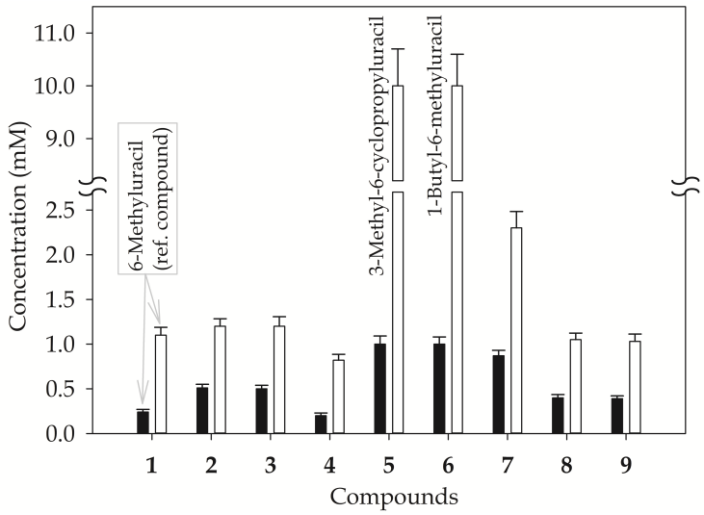


Figure 2. The proliferative effect (□ – IC_{50}, mM) and the maximum tolerated dose (■ – MTD, mM) of the compounds **1-9** against fetal calf lung cells by incubating cells for 24 h (values are statistically significant at $p<0.05$ when compared to control).

Compounds **5**, **6**, **7** at 0.1 mM do not affect proliferative activity. The proliferation index reduces to 25% in the presence of the remaining compounds. An increase of the concentration of test compounds leads to a decrease of the proliferation index by 25-100%. With the exception of **5** and **6** in the presence of which there is an increasing of the proliferation index by 75% and 25%, respectively. In all cases at a concentration of 10 mM, a decrease in the proliferation index by 100% observed.

Compounds in a dose of 0.1 mM practically do not affect the cytotoxic index. An increase in the concentration of compounds up to 1 mM increases the cytotoxic index by 25% to 67%, compound **6** does not affect its values and **5** decreases by 25%. When the concentration increased to 10 mM, the cytotoxic index increases for all compounds by 62-75%.

Compounds **5-8** at 0.1 mM do not affect cell death, **1-4** increase by 9-25%. An increase in the concentration of up to 1 mM leads to an increase in the percentage of cell death in the presence of compounds by 50% to 200%. When using compounds at a concentration of 10 mM, the percentage of cell death increases significantly.

As can be seen, the maximum tolerated dose (MTD) of compounds for the LC line depends on the chemical structure of the test compounds. So, for 6-methyluracil (**1**), which has a broad spectrum of therapeutic effect MTD is equal to 0.24 mM. For all other compounds, with the exception of 1-methyl-6-aminouracil, MTD is significantly higher. The MTD value for 3-methyl-6-cyclopropyluracil and 1-butyl-6-methyluracil is 4 times higher. Differences in MTD between these compounds and 6-methyluracil are significant at a confidence level of $p<0.05$. For the same compounds, the highest IC_{50} value is more than 10 mM and the proliferation index increased by 75% and 25%, respectively. In other words, for 3-methyl-6-cyclopropyluracil and 1-butyl-6-methyluracil are found as the most tolerable and cell propagation inductive compounds (Fig. 2).

CONCLUSIONS

The maximum tolerated dose (MTD) of compounds for the lung cells line depends on the chemical structure of the test compounds. So, for 6-methyluracil, which has a broad spectrum of therapeutic effect MTD is equal to 0.24 mM. For all other compounds, with the exception of 1-methyl-6-aminouracil, MTD is significantly higher. The

MTD value for 3-methyl-6-cyclopropyluracil and 1-butyl-6-methyluracil is 4 times higher, then for 6-methyluracil. Differences in MTD between these compounds and 6-methyluracil are significant at a confidence level of $p<0.05$. For the same compounds, the highest IC_{50} value is more than 10 mM and the proliferation index is increased by 75% and 25%, respectively. In other words, for 3-methyl-6-cyclopropyluracil and 1-butyl-6-methyluracil are found as the most tolerable and active in cell propagation.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found at https://jppres.com/jppres/pdf/vol9/jppres20.944_9.3.357.suppl.pdf

CONFLICT OF INTEREST

Authors declare that this manuscript does not have any conflict of financial interests (political, personal, religious, ideological, academic, intellectual, commercial, or otherwise) for its publication.

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REFERENCES

Anderson GW, Halverstadt IF, Miller WH, Roblin Jr RO (1945) Studies in hemotherapy. X. Antithyroid compounds. Synthesis of 5- and 6-substituted 2-thiouracils from β-oxoesters and thiourea. J Am Chem Soc 67: 2197–2200.

Basnak I, Farkas J (1976) The synthesis of 5-cyclopropyluracil. Collect Czech Chem Commun 41: 311–316.

Basnak I, Farkas J (1979) Synthesis of uracils substituted in the position 5 or 5,6 with alkyl or cycloalkyl groups and their UV spectra. Collect Czech Chem Commun 44: 2426-2437.

Bhat AR (2017) Biological activity of pyrimidine derivatives: A review. Org Med Chem I J 2: 555581.

Bram G, Decodts G, Bensaïd Y, Farnoux CC, Galons H, Miocque M (1985) N-Alkylation of pyrimidine and purine derivatives (uracils, xanthines, adenine) using solid/liquid phase-transfer catalysis without solvent. Synthesis 1985: 543–545.

Brulikova L, Hlavac J (2011) Synthesis, reactivity and biological activity of 5-alkoxymethyluracil analogues. Beilstein J Org Chem 7: 678–698.

Gimadieva AR, Myshkin VA, Mustafin AG, Chernyshenko YuN, Borisova NS, Zimin YuS, Abdrakhmanov IB (2014) Preparation and antihypoxic activity of complexes of uracil derivatives with dicarboxylic acid. Pharm Chem J 48: 93–96.

Golubyatnikova LG, Khisamutdinov RA, Grabovskii SA, Kabal'nova NN, Murinov YI (2017) Complexes of palladium(II) and platinum(II) with 6-tert-butyl-2-thiouracil. Russ J Gen Chem 87: 117–121.

Gordon GM, Lagier AJ, Ponchel C, Bauskar A, Itakura T, Jeong S, Patel N, Fini ME (2016) A cell-based screening assay to identify pharmaceutical compounds that enhance the regenerative quality of corneal repair. Wound Rep Reg 24: 89–99.

Huckin SN, Weiler L (1974) Alkylation of dianions of β-keto esters. J Am Chem Soc 96: 1082–1087.

Kabal`nova NN, Grabovskiy SA, Andriayshina NM, Egorov VI, Valiullin LR, Nabatov AA, Murinov YI (2017) The impact of 5-substituted uracil derivatives on immortalized embryo lung cells. Lett Drug Des Discov 14: 1409–1414.

Kato T, Chiba T, Shimizu T, Takahashi H (1981) Studies on ketene and its derivatives. CII. Reaction of diketene with cyanamide derivatives. Chem Pharm Bull 29: 862–866.

Kumar S, Deep A, Narasimhan B (2019) A review on synthesis, anticancer and antiviral potentials of pyrimidine derivatives. Curr Bioact Compd 15: 289–303.

Lagoja IM (2005) Pyrimidine as constituent of natural biologically active compounds. Chem Biodivers 2: 1–50.

Li D, Zhan P, De Clercq E, Liu X (2012) Strategies for the design of HIV-1 non-nucleoside reverse transcriptase inhibitors: lessons from the development of seven representative paradigms. J Med Chem 55: 3595–3613.

Mirchamsy H, Bahrami S, Kamali M, Hazrati A, Shafyi A, Mahinpour M (1976) Development of a diploid cell line from fetal calf lung for virus vaccine production. Dev Biol Stand 37: 53–57.

Novikov MS, Geisman AN (2014) Methods of synthesis of 6-substituted uracil derivatives – the structural base of antiviral agents. Chem Heterocycl Compd 49: 1426–1450.

Paknikar SK, Fondekar KPP (2001) Synthesis of 7-hydroxy-4-isopropyl-6-methylcoumarin: A bisnorsesquiterpene. J Indian Inst Sci 81: 175–179.

Pałasz A, Ciez D (2015) In search of uracil derivatives as bioactive agents. Uracils and fused uracils: Synthesis, biological activity and applications. Eur J Med Chem 97: 582–611.

Prekupec S, Makuc D, Plavec J, Kraljevic S, Kralj M, Pavelic K, Andrei G, Snoeck R, Balzarini J, De Clercq E, Raić-Malić S, Mintas M (2005) Antiviral and cytostatic evaluation of the novel 6-acyclic chain substituted thymine derivatives. Antivir Chem Chemother 16: 327–338.

Ragavan RV, Kumar KM, Vijayakumar V, Sarveswari S, Ramaiah S, Anbarasu A, Karthikeyan S, Giridharan P,

Kumari NS (2013) β-Keto esters from ketones and ethyl chloroformate: a rapid, general, efficient synthesis of pyrazolones and their antimicrobial, *in silico* and *in vitro* cytotoxicity studies. Org Med Chem Lett 3: 1–15.

Riss TL, Moravec RA (2004) Use of multiple assay endpoints to investigate the effects of incubation time, dose of toxin, and plating density in cell-based cytotoxicity assays. Assay Drug Dev Technol 2: 51–62.

Rosenfeldt FL, Richards SM, Lin Z, Pepe S, Conyers RAJ (1998) Mechanism of cardioprotective effect of orotic acid. Cardiovasc Drugs Ther 12: 159–170.

Shaker RM, Elrady MA, Sadek KU (2016) Synthesis, reactivity, and biological activity of 5-aminouracil and its derivatives. Mol Divers 20: 153–183.

Sharma V, Chitranshi N, Agarwal AK (2014) Significance and biological importance of pyrimidine in the microbial world. Int J Med Chem 2014: 202784.

Stevaert A, Naesens L (2016) The influenza virus polymerase complex: An update on its structure, functions, and significance for antiviral drug design. Med Res Rev 36: 1127–1173.

Vlassa M, Barábas A (1980) One-step synthesis of 3-oxo-4,4-dimethylpentanoic esters from pivaloyl chloride and esters of malonic acid. J Prakt Chemie 322: 821–825.

Wang Z, Tang J, Salomon CE, Dreis CD, Vince R (2010) Pharmacophore and structure-activity relationships of integrase inhibition within a dual inhibitor scaffold of HIV reverse transcriptase and integrase. Bioorg Med Chem 18: 4202–4211.

Yan M, Ma S (2012) Recent advances in the research of heterocyclic compounds as antitubercular agents. ChemMedChem 7: 2063–2075.

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Contribution	Grabovskiy SA	Kabal'nova NN	Andriayshina NM	Egorov VI	Valiullin LR	Nabatov AA	Raginov IS	Murinov YI
Concepts or ideas	x	x					x	x
Design	x	x					x	x
Definition of intellectual content	x	x	x	x	x	x	x	x
Literature search	x	x					x	x
Experimental studies	x		x	x	x	x	x	
Data acquisition	x				x		x	
Data analysis	x						x	
Statistical analysis							x	
Manuscript preparation	x	x	x	x	x	x	x	x
Manuscript editing	x	x					x	
Manuscript review	x	x	x	x	x	x	x	x

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