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5-Arylideneaminouracils: I. Synthesis and Relations between Physicochemical Parameters and Biological Activity

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Abstract—A number of 5-arylideneaminouracils were synthesized by condensation of 5-aminouracil with substituted aromatic aldehydes. Correlation analysis according to Hantzsch revealed strong effects of the lipophilicity parameter and hydration energy of these compounds on their biological activity.

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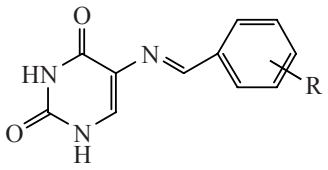
More than 30 years ago, 5-arylideneaminouracils of the general formula **I** were synthesized for the first time with a view to obtain potential antitumor agents [1]. It was presumed that hydrolysis of these compounds under physiological conditions should release active aldehyde which is capable of acting as alkylating agent. The other hydrolysis product is 5-aminouracil which is known as antimetabolite. However, the goal was not reached: the synthesized compounds showed no antitumor activity.



I

Cherayath et al. [2] and Enrique et al. [3] synthesized palladium complexes from Schiff bases **I** derived from 5-aminouracil and benzaldehyde and from 5-aminouracil and salicylaldehyde, which were found to exhibit germicidal activity. Seitembetov [4] reported on the synthesis of Schiff bases from substituted 2-hydroxybenzaldehydes and pronounced antioxidant activity of some of the obtained compounds. Despite already known physiological properties of 5-arylideneaminouracil derivatives and their accessibility from the preparative viewpoint, interest in these compounds has not been exhausted. In 1998 we patented structurally related compounds possessing antimicrobial and antiviral properties [5].

5-Arylideneaminouracils **Ia**–**Iw** were synthesized by reaction of 5-aminouracil with the corresponding substituted benzaldehydes in aqueous ethanol (1 : 1). The products separated from the reactant mixture on heating and were isolated as colorless or brightly colored crystalline substances whose purity was checked by thin-layer chromatography. All compounds **Ia**–**Iw** melted above 300°C; their yields, R_f values, and elemental analyses are collected in Table 1. The structure of 5-arylideneaminouracils **Ia**–**Iw** was confirmed by the ^{1}H NMR spectra which characteristically contained a singlet at δ 9.3–9.7 ppm from the CH=N proton, a singlet at δ 7.8–8.1 ppm from the 6-H proton in the pyrimidine ring, and a degenerate signal from two NH protons (average chemical shift δ 11.1–11.5 ppm). Uracils **Ia**–**Iw** displayed in the IR spectra an absorption band due to stretching vibrations of the azomethine CH=N bond (1665 cm^{-1}), a broad band in the region 3560–3320 cm^{-1} due to OH stretching vibrations (in the spectra of salicylaldehyde derivatives), NH band in the region 3200–2965 cm^{-1}, carbonyl absorption bands at 1650–1630 cm^{-1}, and C–Cl band at 800–700 cm^{-1} (in the spectra of chloro-substituted compounds). In the UV spectra of **Ia**–**Iw**, absorption maxima at λ 280–300 nm were present due to electron transitions in the uracil and aryl fragments. In addition, an absorption band with its maximum at λ 400 nm was observed in the visible region; this band characterizes the entire conjugation system.

5-Arylideneaminouracils showed *in vitro* strong antiviral activity against herpes simplex and various mycobacteria; they were also found to act as interferon

Table 1. Yields, R_f values, and elemental analyses of 5-arylideneaminouracils **Ia**–**Iw**

Comp. no.	R	Yield, %	R_f	Found, %			Formula	Calculated, %		
				C	H	N		C	H	N
Ia	H	77	0.50	61.8	4.21	19.4	$C_{11}H_9N_3O_2$	61.4	4.22	19.5
Ib	4-Cl	81	0.48	52.7	3.13	16.7	$C_{11}H_8ClN_3O_2$	52.9	3.23	16.8
Ic	3-Cl	82	0.47	52.8	3.31	16.4	$C_{11}H_8ClN_3O_2$	52.9	3.23	16.8
Id	2-Cl	90	0.51	53.1	3.25	16.9	$C_{11}H_8ClN_3O_2$	52.9	3.23	16.8
Ie	4-Br	92	0.51	44.8	2.70	14.2	$C_{11}H_8BrN_3O_2$	44.9	2.74	14.3
If	4-OH	66	0.44	57.3	3.89	18.1	$C_{11}H_9N_3O_3$	57.1	3.92	18.2
Ig	2,4-$(OH)_2$	72	0.52	53.5	3.62	17.0	$C_{11}H_9N_3O_4$	53.4	3.67	17.0
Ih	2-OH-5-Br	94	0.53	42.7	2.56	13.2	$C_{11}H_8BrN_3O_3$	42.6	2.60	13.6
Ii	2-OH-5-Cl	85	0.47	50.0	3.00	15.6	$C_{11}H_8ClN_3O_3$	49.7	3.04	15.8
Ij	4-F	92	0.52	56.4	3.39	18.1	$C_{11}H_8FN_3O_2$	56.7	3.46	18.0
Ik	2,4-Cl_2	86	0.51	46.5	2.43	14.7	$C_{11}H_7Cl_2N_3O_2$	46.5	2.48	14.8
Il	2-OH-3,5-Cl_2	92	0.52	44.3	2.54	14.2	$C_{11}H_7Cl_2N_3O_3$	44.0	2.35	14.0
Im	2-OH-3,5-Br_2	90	0.54	33.8	1.82	11.0	$C_{11}H_7Br_2N_3O_3$	34.0	1.81	10.8
In	4-OCH_3	58	0.48	58.7	4.50	17.0	$C_{12}H_{11}N_3O_3$	58.8	4.52	17.1
Io	4-OH-3-OCH_3	60	0.49	54.9	4.14	16.0	$C_{12}H_{11}N_3O_4$	55.2	4.24	16.1
Ip	4-NO_2	79	0.51	50.8	3.19	21.4	$C_{11}H_8N_4O_4$	50.8	3.10	21.5
Iq	3-NO_2	81	0.52	50.7	3.11	21.6	$C_{11}H_8N_4O_4$	50.8	3.10	21.5
Ir	2-NO_2	80	0.49	50.7	3.09	21.7	$C_{11}H_8N_4O_4$	50.8	3.10	21.5
Is	2-OH-5-NO_2	78	0.42	47.5	2.95	19.9	$C_{11}H_8N_4O_5$	47.8	2.92	20.3
It	2-OH-5,6-benzo	65	0.51	63.9	3.94	14.7	$C_{15}H_{11}N_3O_3$	64.0	3.94	14.9
Iu	2,3:5,6-dibenzo	71	0.45	71.8	4.21	13.2	$C_{19}H_{13}N_3O_2$	72.4	4.16	13.3
Iv	2-OH-3,5-I_2	92	0.49	27.3	1.42	8.70	$C_{11}H_7I_2N_3O_3$	27.4	1.46	8.70
Iw	4-$N(CH_3)_2$	45	0.56	60.4	5.38	21.9	$C_{13}H_{14}N_4O_2$	60.5	5.46	21.7

inductors, i.e., these compounds are capable of enhancing immunity of warm-blooded organisms.

The azomethine fragment in organic compounds is highly reactive due to the presence of nearby nucleophilic and electrophilic centers. This is especially important for protonation processes which often occur in biogenic media. Elucidation of the role of the azomethine bond and other structural fragments of 5-arylideneaminouracils in their antiviral and antimicrobial activity is necessary for target-oriented synthesis of compounds with required properties.

Correlation analysis is one of the main tools in chemical studies, specifically in organic chemistry. It is used to solve various problems, but the most important application of correlation analysis is concerned with studies on intramolecular interactions. Correlation analysis operates with an extended system of parameters which may be regarded as real factors affecting the chemical behavior and physical properties of compounds. Substituent effect on a reaction center should be related to some intrinsic parameters of that substituent, which does not depend on the reaction series. The set of available parameters, which can be used to construct an appropriate mathematical model for the effect of organic compounds on various biological species, is fairly broad. Theoretically, any parameter characterizing electronic, steric, or hydrophobic effect on the reaction center of a biological target may be involved.

With a view to optimize biological activity of 5-arylideneaminouracils, in the present work we selected the following parameters: (1) lipophilicity parameter log P which characterizes the solubility ratio of a compound in water and octan-1-ol; (2) energy of hydration E_{hydr}; (3) molar area S and volume V that characterize steric effect of a biologically active molecule on its interaction with biological target; and (4) the constant $\Sigma\sigma_{IR}$ proposed in the present work, which reflects joint inductive and resonance effects of substituents in the aromatic fragment of 5-arylideneaminouracils (Table 2).

Table 2. Parameters of 5-arylideneaminouracils **Ia**–**Iw** for structure–biological activity correlations according to Hantzsch

Comp. no.	log (1/C_{50})			log P	E_{hydr}, kJ mol^{-1}	S, Å^2	V, Å^3	$\Sigma\sigma_{IR}$
	MB[a]	HSV-1[b]	CA[c]					
Ia	–	3.41	–	–0.03	9.46	247	374	0
Ib	–	3.48	–	0.68	9.46	261	397	–0.061
Ic	3.95	–	–	0.68	9.50	261	397	–0.080
Id	3.98	3.48	–	0.68	9.33	250	384	–0.061
Ie	3.97	3.72	–	0.95	9.41	270	409	–0.053
If	3.44	3.62	–	1.30	13.8	252	381	0.153
Ig	–	–	–	2.62	16.0	253	386	0.615
Ih	4.09	3.75	–	2.28	11.8	272	414	–0.008
Ii	3.72	3.68	3.72	2.00	11.8	263	402	0.006
Ij	–	–	–	0.30	9.50	250	378	–0.055
Ik	4.01	–	–	1.38	9.12	267	414	–0.243
Il	4.08	3.78	4.08	2.71	11.6	274	424	–0.302
Im	4.15	4.07	3.89	3.26	11.5	292	448	–0.318
In	–	2.99	–	–0.09	9.66	271	416	0.062
Io	–	3.02	–	1.23	11.8	281	431	0.243
Ip	4.00	3.62	3.72	–3.75	20.7	263	406	0.121
Iq	4.00	3.32	–	–3.75	16.9	265	401	0.106
Ir	4.02	3.42	3.72	–3.75	12.4	259	395	0.121
Is	4.00	3.89	3.74	–2.43	20.7	269	392	–0.099
It	4.03	–	–	1.92	10.9	265	426	0.169
Iu	–	–	3.80	1.21	7.95	282	473	–0.018
Iv	4.29	–	4.19	4.19	11.4	305	469	–0.259
Iw	–	3.01	–	0.42	5.06	281	438	0.174

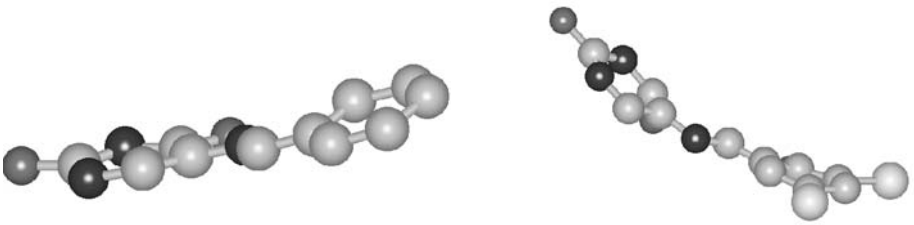
[a] Pathogenic mycobacteria *M. smegmatis*. [b] Herpes simplex HSV-1. [c] Yeast-like fungus *Candida albicans*.

Optimization according to the Fletcher–Reeves method showed that molecules of 5-arylideneaminouracils have almost planar structure (Fig. 1). Therefore, our choice of the parameters S and V may be regarded as proper. The use in multiparameter correlation analysis of so-called cross terms (the parameter σ_{IR} may be regarded as such cross term) is dictated by the formalism of the theory of interactions.

$$Y = y_0 + a\sigma_I + b\sigma_R + c\sigma_{IR}.$$

Here, Y is a dependent quantity which determines the rate of chemical reaction, and σ_I and σ_R are constants that quantitatively characterize inductive and resonance effects of a substituent on reaction center. The parameter σ_{IR} must be taken into account to achieve appropriate description of the inductive and resonance effects both on reaction center and on each other.

It should be noted that in our case the cross term is not the product of similar constants of different substituents but the product of different constants of the same substituent. We proceeded from the undeniable fact that the division of constants σ into



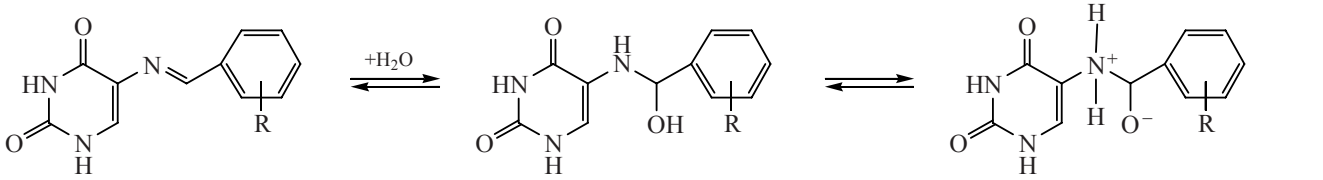
Ia **Im**

Fig. 1. Optimized structures of the molecules of 5-benzylideneaminouracil (**Ia**) and 5-(3,5-dibromo-2-hydroxybenzylideneamino)-uracil (**Im**) according to quantum-chemical calculations.

inductive and resonance components is quite arbitrary, for it is impossible to isolate these components of substituent electronic effect in the pure form in a chemical reaction. On the whole, the problem related to orthogonalization of inductive and resonance parameters is intrinsic to the correlation analysis [6]. The term σ_{IR} may be regarded as a parameter that ensures (as a first approximation) quantitative estimation of the overall effect of substituents on the rate of chemical reactions. The above stated makes it possible to use the cross term for description of reactions involving formation of highly ordered transition states [7]. Undoubtedly, reactions of 5-arylideneaminouracils with biological targets belong just to interactions of such type.

The $\Sigma\sigma_{IR}$ values were calculated by the formulas $\Sigma\sigma_{IR} = \Sigma(\sigma_R^0\sigma_m^0)$ and $\Sigma\sigma_{IR} = \Sigma(\sigma_R^0\sigma_p^0)$ for *meta* and *para* substituents, respectively; here, σ_m^0, σ_p^0, and σ_R^0 are, respectively, the inductive constants for *meta* and *para* substituents and resonance constant; their values were taken from [6].

Schiff bases in a biological medium are capable of forming hydrophilic structures. Hydrolysis of Schiff bases is a two-step process including addition of water molecule at the double C=N bond with the formation of carbinol–amine intermediate. At physiological pH value, the rate-determining step is just hydration of the C=N bond. Therefore, the energy of hydration was also taken as parameter for correlation analysis. We believe that the parameter E_{hydr} is very important for simulation of reactions in biological media; it should quantitatively characterize the ability of arylideneaminouratcls to react with water molecules.



I

One of the most important parameters used in structure–biological activity correlations is the lipophilicity parameter log *P*. In fact the ratio of solubilities of a compound in water and lipids largely determines the level of its biological activity. In the present work we examined the relation between log *P* values of 5-arylideneaminouracils **I** and their biological activity against yeast-like fungi, mycobacteria, and herpes simplex (HSV-1) with a view to find optimal structural parameters ensuring enhanced biological activity of the examined compounds.

Table 3 contains the parameters of correlations like $\log(1/C_{50}) = f[(\log P)^n]$ for different kinds of biological activity of 5-arylideneaminouracils **I** (C_{50} is a minimal concentration of a compound, inducing 50% inhibition of growth of microorganisms). The experimental data were satisfactorily described by the classical Hantzsch formula. It should be noted that the correlation coefficients and mean-square deviations found for the activity of substituted aminouracils **I** with respect to *Candida albicans* and HSV-1 are fairly poor. The correlations $\log(1/C_{50})$–$\log P$ for mycobacteria are more distinct (Fig. 2).

The obtained correlations $\log(1/C_{50}) = f[(\log P)^n]$ allowed us to draw unambiguous conclusions only on $\log P$ value for potential antimicrobial and antiviral 5-arylideneaminouracils **I**. The minimal activity *in vitro* is observed for compounds with a $\log P$ value close to –1, i.e., for those less soluble in lipids than in water (Fig. 2). On the other hand, both increase and decrease

Table 3. Parameters of correlations $\log(1/C_{50}) = a_0 + a_1 \log P + a_2(\log P)^2 + a_3(\log P)^3$

Microorganism	a_0	a_1	a_2	a_3	r	s
Candida albicans	3.70	0.0462	0.0143	–	0.877	0.100
	3.74	0.0133	0.0112	0.00256	0.892	0.103
HSV-1	3.21	0.124	0.0409	–	0.784	0.221
	3.17	0.202	0.0409	–0.00725	0.794	0.227
M. smegmatis	3.96	0.00748	0.0107	0.00147	0.986	0.018

in the lipophilicity parameter should lead to enhanced biological activity.

The above correlations do not provide exhaustive description of the effect of physicochemical parameters on biological activity of arylideneaminouracils **I**. We also tried to draw correlations with account taken of electronic and steric effects of substituents on the reaction center and of the energy of hydration of biologically active molecules.

The following correlations were found for *in vitro* inhibition of yeast-like fungus *Candida albicans*:

$$\log (1/C_{50}) = 3.82 + 0.0389 \log P - 0.135 \Sigma \sigma_{IR};$$
$$R = 0.784, s = 0.129;$$

$$\log (1/C_{50}) = 3.65 + 0.0389 \log P + 0.0139(\log P)^2 - 0.00363 E_{hydr}; R = 0.879, s = 0.109;$$

$$\log (1/C_{50}) = 3.70 + 0.0448 \log P + 0.0130(\log P)^2 - 0.088 \Sigma \sigma_{IR}; R = 0.882, s = 0.107;$$

Mycobacterium smegmatis:

$$\log (1/C_{50}) = 3.91 + 0.0289 \log P + 0.0114(\log P)^2 + 0.00298 E_{hydr}; R = 0.976, s = 0.0228;$$

$$\log (1/C_{50}) = 3.94 + 0.0251 \log P + 0.0120(\log P)^2 - 0.0267 \Sigma \sigma_{IR}; R = 0.973, s = 0.0243;$$

herpes simplex HSV-1:

$$\log (1/C_{50}) = 0.75 + 0.158 \log P - 0.0071(\log P)^2 - 0.555 \Sigma \sigma_{IR} + 0.228 E_{hydr}; R = 0.901, s = 0.595.$$

These correlations suggest higher biological activity of compounds with larger log *P* values and having electron-withdrawing substituents in the aromatic fragment.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded from solutions in DMSO-d_6 on a Bruker spectrometer (200 MHz; Bruker–Franzen Analytik GmbH Version: 950801.1 software); the chemical shifts were determined relative to the residual proton signals of the solvent. The UV spectra were measured on an SF-26 spectrophotometer (MicroCal Origin Version 3.0 software) from solutions in 50% ethanol with a concentration of 10^{-4} M. The IR spectra were recorded in KBr on a Specord M-80 spectrometer. The purity of the synthesized compounds was checked by thin-layer chromatography on Silufol UV-254 using carbon tetrachloride–propan-2-ol (9 : 1) as eluent.

Quantum-chemical calculations were performed using HyperChem™ Release 6.03 for Windows Molecular Modeling System assuming the temperature 310 K.

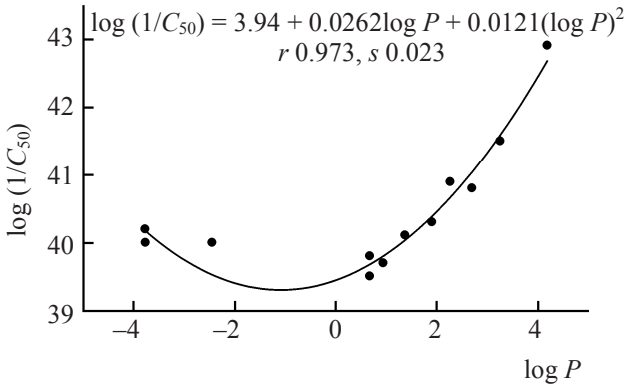


Fig. 2. Plot of log (1/C_{50}) (*M. smegmatis*) of 5-arylideneaminouracils **I** versus lipophilicity parameter log *P*.

Antibacterial and antiviral activity of 5-arylideneaminouracils was studied using standard strains of microorganisms from the Microbiology Department, Pavlov St. Petersburg State Medical University.

5-(3,5-Dichloro-2-hydroxybenzylideneamino)-pyrimidine-2,4(1*H*,3*H*)-dione (II). 5-Aminouracil, 1.27 g, was dissolved in 150 ml of water on heating under stirring, and a solution of 1.91 g of 3,5-dichlorosalicylaldehyde in 50 ml of ethanol was added dropwise. A bright orange solid began to separate almost immediately. The mixture was heated at the boiling point for 1 h under stirring, stirred for 1 h at room temperature, and left overnight. The precipitate was filtered off, washed with warm water and alcohol, and dried. Yield 2.76 g (92%). The other 5-arylideneaminouracils were synthesized in a similar way.

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