DRUG SYNTHESIS METHODS AND MANUFACTURING TECHNOLOGY

STUDY OF THE PRODUCTS OF REACTION OF 5-AZAURACIL WITH MALONDIAMIDE AND AROMATIC C-NUCLEOPHILES

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As is known, hexamethylmelamine has been used in the USA for the treatment of lung carcinoma [1]. Another 1,3,5-triazine derivative, 5-azacytidine, was used for the treatment of acute lymphoblastic leukemia [2]. Apparently, the antitumor activity of some 1,3,5-triazine derivatives is related to the fact that these compounds, being antimetabolites of pyrimidine bases, are capable of accumulating in tumor cells and damaging these cells. At the same time, there are well-known chemical transformations of 5-azauracils with the formation of various pyrimidine derivatives. For example, 1,3-dimethyl-5-azauracil interacting with fluoracetamide in the presence of lithium diisopropylamide converts into the well-known antitumor drug 5-fluorouracil [3]. 5-Carboxamido and 5-cyano substituted uracil derivatives were obtained via interactions of 1,3-dimethyl-5-azauracil with malonamide and cyanamide, respectively, in the presence of sodium ethylate [4].

Previously, it was reported in a brief communication [5] that 5-azauracil (I), interacting with 1-phenyl-3-methyl-5-pyrazolone (IIa) without charge activation of the reagents, converted into a derivative of dipyrazolylmethane (IIIa) and biuret (IV). The same paper reported on the formation of stable 6-indolyl adducts of 5-azauracil.

In continuation of the investigation of chemical transformations of 5-azauracil, we have studied the products of conversion of this compound upon interaction with some β-dicarbonyl compounds representing heterocyclic and aromatic C-nucleophiles.

It was found that 5-azauracil (I) can smoothly react not only with 1-phenyl-3-methyl-5-pyrazolone (IIa), but with some other 1-phenyl-5-pyrazolone derivatives (IIb – IId) as well. In these reactions, 5-azauracil acts as the donor of a one-carbon fragment; therefore, I can be used in preparative chemistry for the synthesis of dipyrazolylmethane derivatives III (see scheme and Table 1). The reaction of 5-azauracil with indoles (V) proceeds differently. This interaction (like that with pyrazolones) requires no charge activation of the reagents. The reaction products represent stable σ-adducts of 6-indolyl and 5-azauracil (VIa and VIb), the structure of which was confirmed by the data of ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry [5].

In this study, we have synthesized for the first time a σ-adduct of 5-azauracil and β-dicarbonyl compound. Heating 5-azauracil in butanol with malonamide (VII) leads to the formation of 1,2,3,4,5,6-hexahydro-6-(dicarbaminomethyl)-1,3,5-triazine-2,4-dione (VIII). The character and positions of proton signals in the ^{1}H NMR spectrum of this compound confirm the proposed structure. Indeed, the doublet due to proton of the malonamide fragment is observed at 3.43 ppm; H-6 proton of triazine is manifested by a multiplet at 4.8 ppm (the splitting is caused by spin–spin coupling with the neighboring NH protons of the triazine ring and with proton of the malonamide fragment). The 1- and 5-NH signals are observed in the region of 7.6 – 7.7 ppm. A broad singlet due to 3-NH proton is observed at 9.28 ppm. The singlets due to amino groups of the malonamide fragment are manifested at 7.27 and 7.30 ppm.

It is interesting to note that adducts VI and VIII are formed even when reagents are mixed in DMSO at room temperature. The NMR spectrum measured upon keeping the mixture at this temperature reveals an increase in the adduct concentration.

An original transformation was effected by interaction of 5-azauracil with *o*-phenylenediamine (IX): performed in the presence of hydrochloric acid, this reaction yielded 1,2,3,4,5,6-hexahydro-6-(3′,4′-diaminophenyl)-1,3,5-triazine

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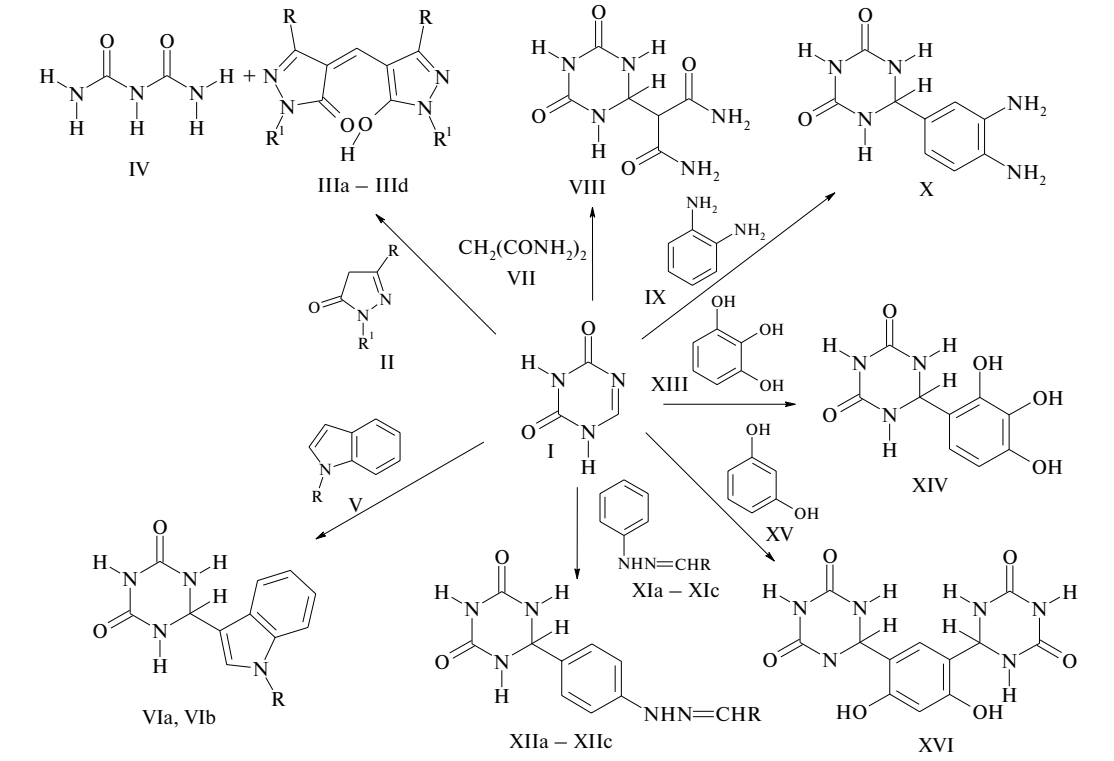


IIa – IId, IIIa – IIId: R' = Ph, R = CH_3 (a); R' = R = Ph (b); R' = Ph—NO_2-*para*, R = CH_3 (c); R' = Ph, R = Ph—NO_2-*para* (d).

VIa, VIb: R = H (a); R = CH_3 (b).

XIIa – XIIc: R = Ph—OCH_3-*para* (a); R = Ph—Cl-*para* (b); R = Ph—NO_2-*para* (c).

-2,4-dione (X). The ^{1}H NMR spectrum of this compound contains a singlet at 5.2 ppm due to H-6 atom of the triazine ring. The pattern of spin – spin splitting of the signal related to aromatic protons of the *o*-phenylene fragment corresponds to the 1,2,4,4-substituted phenyl (Fig. 1).

The reactions of 5-azauracil with phenylhydrazones (XIa – XIc) proceed under analogous conditions and smoothly yields the corresponding 6-substituted 1,2,3,4,5,6-hexahydro-1,3,5-triazine-2,4-diones (XIIa – XIIc). The ^{1}H NMR spectra of compounds XII also display the signals due to H-6protons at sp^3-hybridized carbon atom (5.4 – 5.5 ppm), while the presence of two pairs of equivalent protons in the aromatic ring is indicative of the *p*-addiction of the phenylhydrazone fragment.

The access to another series of derivatives was opened by the transformations of 5-azauracil via reactions with polyphenols. Even short-time heating of 5-azauracil with pyrogallol (XIII) in the presence of hydrochloric acid leads to the formation of 1,2,3,4,5,6-hexahydro-6-(2′,3′,4′-trihydroxyphenyl)-1,3,5-triazine-2,4-dione (XIV). The interaction of I with resorcinol (XV) proceeds differently and yields 2,4-bis(1′,2′,3′,4′,5′,6′-hexahydro-1′,3′,5′-triazine-2′,4′-dion-6′-yl)-1,5-dio(XVI).

Structures of the phenolic derivatives of 5-azauracil were unambiguously established with the aid of ^{1}H NMR spectroscopy. The spectra of compounds XIV and XVI contain a characteristic signal from the H-6 proton of the triazine cycle (5.0 – 5.7 ppm), which is characteristic of all the obtained σ-adducts. The presence of two doublets from the aromatic *o*-protons is indicative of the site of attachment of the pyrogallol nucleus to 5-azauracil. The spectrum of resorcinol derivative XVI exhibits two singlets of *p*-protons of the aromatic nucleus and the doubled number of protons in the triazine cycle (Fig. 2).

The pattern of mass-spectrometric fragmentation of the adducts (Fig. 3) reveals both common characteristic decay pathways and certain special features for various derivatives. One common feature of the σ-adducts is the decay of the triazine nucleus. All the adducts studied yield ions with a mass of $[M–86]^+$ or $[M–H–86]^+$, which correspond to the detachment of two carbamide fragments. It is interesting to note that no decays of this type take place in the initial (unsibstituted) 5-azauracil.

The mass spectra of compounds possessing less intense peaks of molecular ions (indolyl, diaminophenyl, and hydrazinophenyl derivatives) also display intense peaks of $[M–H]^+$. In the case of indolyl and diaminophenyl derivatives, decay with the detachment of carbamide groups takes place after proton detachment. In other cases, the carbamide groups are probably detached from molecular ions.

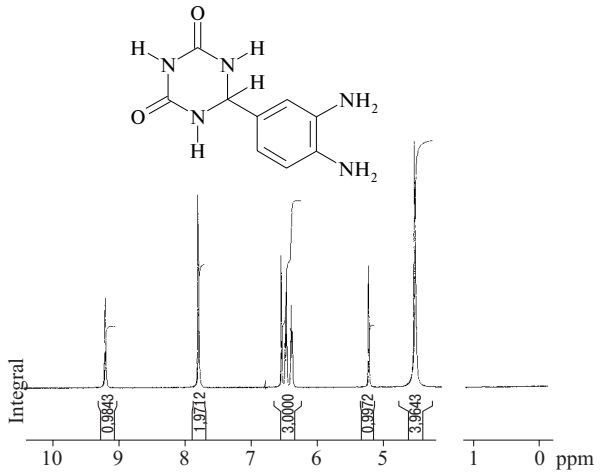


Fig. 1. ^{1}H NMR spectrum of 1,2,3,4,5,6-hexahydro-6-(3,4-diaminophenyl)-1,3,5-triazine-2,4-dione (X).

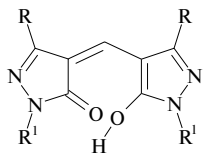
Fig. 2. ^{1}H NMR spectrum of 2,4-bis(1,2,3,4,5,6-hexahydro-1,3,5-triazine-2,4-dion-6-yl)-1,5-dioxybenzene (XVI).

Although the mass spectra of pyrogallol (XIV) and malonamide (VIII) derivatives exhibit no peaks of molecular ions, there are signals (m/z = [M–86]$^+$) confirming the decay of σ-adducts. At the same time, the spectra of these σ-adducts display intense peaks of the ions with masses corresponding to the initial components. This fact indicates that, under the conditions of mass-spectroscopic measurements, the adducts are subject to (impact or thermal) dissociation into initial components.

Thus, depending on the nature of nucleophiles, 5-azauracil can be used either as a donor of a one-carbon fragment (e.g., for the synthesis of di(pyrazolylmethane) or as a "trap" for β-dicarbonyl compounds, indoles, aromatic amines, hydrazines, or phenols.

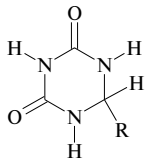
The obtained 5-azauracil derivatives offer a unique example of stable crystalline σ-adducts. These adducts can be useful as model intermediate compounds for studying reactions of the nucleophilic displacement of hydrogen or the transformation (metabolism) of 5-azauracil derivatives into other heterocyclic systems. At the same time, 6-aryl-5-azauracils bearing amino, hydrazino, or oxy groups in the aromatic ring represent convenient substrates for saturation with various biologically active fragments. For example,

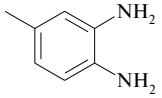
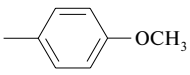
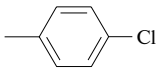
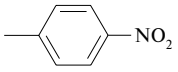
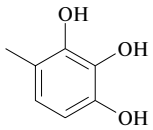
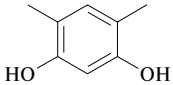
TABLE 1. Yields and Physicochemical Characteristics of Dipyrazolylmethane Derivatives (IIIa – IIId)



Compound	R′	R	Empirical formula	Yield, %	M.p., °C
IIIa	Ph	CH_3	$C_{21}H_{18}N_4O_2$	70 – 75	168 – 170
IIIb	Ph	Ph	$C_{31}H_{22}N_4O_2$	45 – 50	240 – 241
IIIc	$C_6H_4NO_2$-*p*	CH_3	$C_{21}H_{16}N_6O_6$	30 – 35	> 300
IIId	Ph	$C_6H_4NO_2$-*p*	$C_{31}H_{20}N_6O_6$	35 – 40	277 – 279

TABLE 2. Yields and Physicochemical Characteristics of 5-Azauracil σ



Compound	R	Empirical formula	Yield, %	M.p., °C
VIII	$CH(CONH_2)_2$	$C_6H_9N_5O_4$	30 – 35	220 – 221
X		$C_9H_{11}N_5O_2$	30 – 35	> 300
XIIa		$C_{17}H_{17}N_5O_3$	50 – 55	275 – 277
XIIb		$C_{16}H_{14}ClN_5O_2$	55 – 60	273 – 275
XIIc		$C_{16}H_{14}N_6O_4$	40 – 45	284 – 286
XIV		$C_9H_9N_3O_5$	50 – 55	215 – 217
XVI		$C_{12}H_{12}N_6O_6$	30 – 35	260 – 262

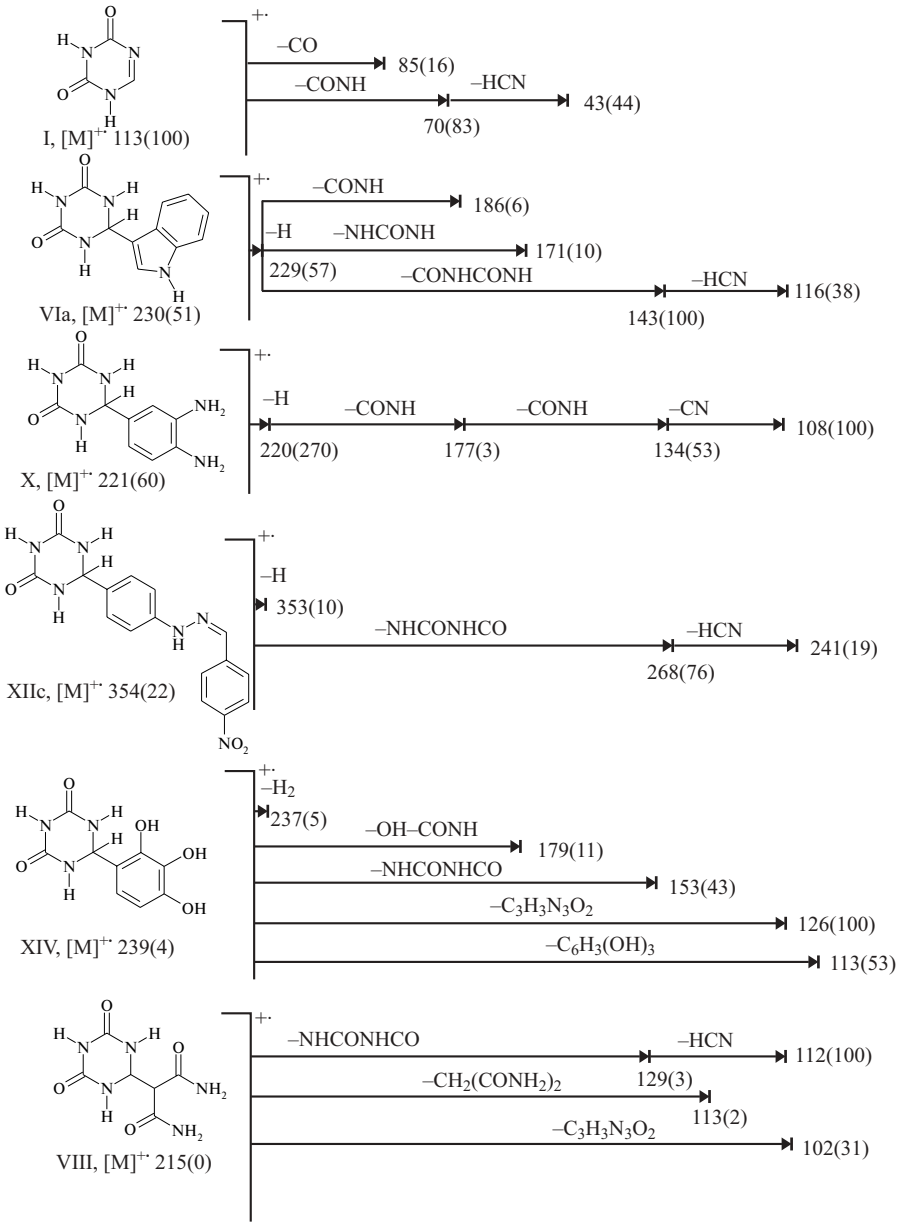


Fig. 3. The main pathways for the decay of σ-adducts.

these adducts can be used for the delivery of boron to tumor cells [6] within the framework of boron neutron capture therapy.

EXPERIMENTAL PART

The ^{1}H NMR spectra were measured on a Bruker 80 spectrometer (Germany). The chemical shifts were measured relative to TMS as the internal standard. The mass spectra were recorded on a Varian MAT-311A instrument operating at an accelerating voltage of 3 kV, cathode emission current of 300 μA, ionizing energy of 75 eV, and ion source temperature of 200°C. The data of elemental analyses (C, H, N) agree with the results of calculations using the empirical formulas (Tables 1 and 2).

Dipyrazolylmethane derivatives IIIb – IIId were synthesized similarly to compound IIIa as described in [5].

1,2,3,4,5,6-Hexahydro-6-(dicarbaminomethyl)-1,3,5-triazine-2,4-dione (VIII). A mixture of 5-azauracil (2.0 mmole) and malondiamide (2.0 mmole) in 10 ml of butanol was boiled for 3 – 4 h and cooled to room temperature. The precipitate of compound VIII was separated by filtration and recrystallized (Table 2). ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 3.43 (d, 1H, J 9.0 Hz, CH malonamide), 4.80 (m, 1H, H-6 triazine), 7.6 – 7.7 (m, 1H, NH triazine), 9.28 (bs, 1H, 3-NH triazine). A method for the synthesis of

6-indolyl derivatives of 1,2,3,4,5,6-hexahydro-1,3,5-triazine-2,4-dione (VIa, VIb) was described in [5],

Reaction of 5-azauracil with aromatic C-nucleophiles. A mixture of 5-azauracil (1.0 mmole) and the corresponding C-nucleophile (1.0 mmole) in 5 ml of ethanol with 0.2 ml of concentrated HCl was boiled for 1 h and cooled to room temperature. The precipitate of compound X · HCl was dissolved on heating in a minimum amount of water and treated with a saturated aqueous solution of sodium acetate until reaching neutral pH. This solution was cooled for 1.5 – 2 h on an ice bath. The precipitate was separated by filtration and recrystallized from water (Table 2). The products XIIa – XIIc and XIV were purified by reprecipitation from DMF with water. The resorcinol derivative XIV was reprecipitated from DMSO with water.

The ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm):

Compound X: 4.5 (s, 4H, 2 NH_2), 5.2 (s, 1H, H-6), 6.4 (d, 1H, J 7.8 Hz, CH arom.), 6.5 (d, 1H, J 7.8 Hz, CH arom.), 6.6 (s, 1H, CH arom.), 7.8 (s, 2H, 2 NH), 9.2 (s, 1H, NH).

Compound XIIa: 3.8 (s, 3H, OCH_3), 5.4 (s, 1H, H-6), 6.9 (s, 2H, J 8.3 Hz, CH arom.), 7.1 (d, 2H, J 8.7 Hz, CH arom.), 7.2 (d, 2H, J 8.3 Hz, CH arom.), 7.6 (d, 2H, J 8.7 Hz, CH arom.), 7.8 (s, 1H, CH=N), 7.9 (s, 2H, 1-NH, 5-NH), 9.3 (s, 1H, NH), 10.2 (s, 1H, NH).

Compound XIIb: 5.4 (s, 1H, H-6), 7.1 (s, 2H, J 7.9 Hz, CH arom.), 7.2 (d, 2H, J 7.9 Hz, CH arom.), 7.4 (d, 2H, J 8.3 Hz, CH arom.), 7.7 (d, 2H, J 8.3 Hz, CH arom.), 7.8 (s, 1H, CH=N), 8.0 (s, 2H, 1-NH, 3-NH), 9.3 (s, 1H, NH), 10.5 (s, 1H, NH).

Compound XIIc: 5.5 (s, 1H, H-6), 7.2 (s, 2H, J 8.3 Hz, CH arom.), 7.3 (d, 2H, J 8.3 Hz, CH arom.), 7.9 (d, 2H, J 8.5 Hz, CH arom.), 8.0 (s, 1H, CH=N), 8.1 (s, 2H, 1-NH, 5-NH), 8.2 (d, 2H, J 8.5 Hz, CH arom.), 9.3 (s, 1H, NH), 11.0 (s, 1H, NH).

Compound XIV: 5.6 (s, 1H, H-6), 6.3 (d, 2H, J 7.8 Hz, CH arom.), 6.5 (d, 1H, J 7.8 Hz, CH arom.), 7.6 (s, 2H, 1-NH, 3-NH), 8.4 (bs, 1H, OH), 8.6 (s, 1H, OH), 9.2 (bs, 2H, NH, OH).

Compound XVI: 5.6 (s, 1H, H-6, H-6′), 6.4 (s, 1H, CH arom.), 7.1 (s, 1H, CH arom.), 7.6 (s, 4H, 1-NH, 5-NH, 1′-NH, 5′-NH), 9.2 (s, 2H, 3-NH, 3′-NH), 9.8 (s, 2H, 2 OH).

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