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Tricyclic 1, 2, 4-triazine bearing heterosystem: directed synthesis of new bioactive compounds

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A new series of tricyclic heteroaromatic compounds was prepared by cyclization of N2-substituted-6-bromo-3-oxo(amino)-1,2,4-triazin-5(4H)-ones with 2-aminobenzothiol. The structure of bioactive tricyclic glycosides, obtained earlier by the simplified silylic method, was confirmed as well as expedience and adequacy of this method for directed glycosylation of triazine bearing tricyclic bases.

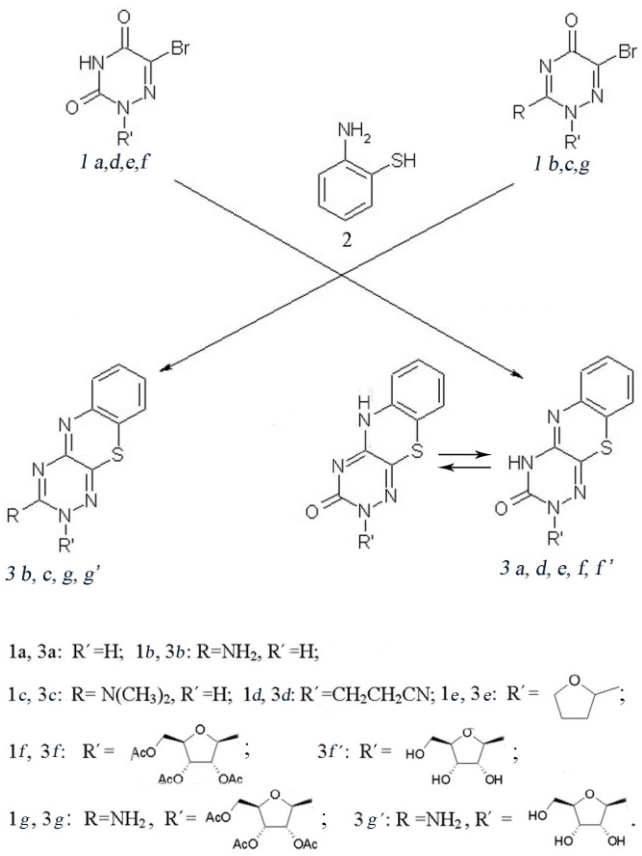
Keywords: condensed 1,2,4-triazine, tricyclic nucleoside analogues.

Introduction. The present work is a continuation of our study on synthesis and biological properties of a number of derivatives of tricyclic heteroaromatic system, which contains bioactive 1,2,4-triazine, to create compounds with useful properties as well as to establish the influencing of structural alteration on their biological activity.

The compounds from the 1st series (tricyclic heterobases and their glycosidic derivatives) have shown antiviral activity, in particular, towards the herpes simplex virus type 2 (HSV-2) and Epstein-Barr virus [1, 2]. The glycosides have been synthesized by the direct ribosylation of tricyclic heterobases using the silylic method [1].

The aim of this work is to extend the range of condensed triazine-containing heterosystems using another synthetic approach, where triazine fragment is represented by N2-alkyl-, N2-tetrahydrofuranyl- and N2-ribofuranosyl-derivatives of 6-bromo-3-oxo(amino)-1,2,4-triazin-5-one, as a result ensuring production of compounds with the beforehand preassigned structures.

Material and methods. The parent compounds for condensed N2-triazine derivatives synthesis - N2-substituted 6-bromo-1,2,4-triazine-3,5(2H,4H)-diones (1a, d – f) as well as 3R-6-bromo-1,2,4-triazine-5(4H)-ones (1b, c, g) were produced by the described methods [1, 3 – 6]. Triacetate N2-D-ribofuranosyl-3-amino-6-bromo-1,2,4-triazine-5-one (1g) was synthesized by the method [7]. The reagents and solvents from "UkrOrgSyntez" (Ukraine) and "Fluka" (Switzerland) were used . The reaction behavior and a purity of the compounds obtained were monitored by thin-layer chromatography (TLC) on the plates from "Merck" (Germany) in the solvent system chloroform/methanol (9:1) (A) or hexane/ethyl acetate (1:1) (B). The solvent system isopropanol/toluene/ammonium hydroxide (3:2:1) and concentration gradient of methanol in chloroform were used for a preparative chromatography. Additionally glycosilated derivatives were visualized on TLC using a specific color reaction



Scheme. Synthetic route to condensed triazine N2-derivatives

with Dishe's reagent (0.5% solution of cysteine hydrochloride in 3N of sulfuric acid). The ^{1}H-NMR spectra were recorded using the spectrometer "Mercury-400" ("Varian", USA) in DMSO-d6, with tetramethylsilane as an internal standard. The UV-absorption spectra were recorded by the spectrometer "Shimdzu UV-3100" (Japan). Melting points (M.p.) were determined with the Boetius micro melting point apparatus (Germany).

Chemical synthesis. The derivatives of condensed triazine (Scheme, compounds 3a-e, table 1) have been synthesized by the method, described in the previous work [1].

*The synthesis of N2- -D-ribofuranoside of condensed triazine (*3f', 3g'). To a suspension of 1.0 mmol N2-(2',3',5'-tri-0-acetyl)-ribofuranoside of corresponding 6-bromo-triazine (1f, 1g) in 5 ml of ethanol/dimethylformamide mixture were added 0.12 ml (1.5 mmol) of pyridine and 0.15 ml (1.5 mmol) of 2-aminobenzothiol. The reaction mixture was heated several hours at 100 ^{0}C, monitoring process by TLC. After a standard treatment the reaction mixture product (oily) was purified on the silica gel chromatographic column. The obtained glycoside triacetate was crystallized from ethanol. Deacylation of glycoside derivatives was carried out for 20 hours with aqueous-alcoholic solution of ammonia under room temperature.

Physicochemical characteristics of the glycosides produced are represented in the tables 2 and 3.

*The synthesis of N2-(2',3',5'-tri-0-acetyl- -D-ribofuranosyl)-3-amino-6-bromo-1,2,4-triazine-5(4H)-one (*1g). To the suspension of of 3-amino-6-bromotriazine-5(2H)-one (1b) (900 mg, 2 mmol) and tetraacetylribose (700 mg, 2.2 mmol) in 15 ml of absolute acetonitrile were added 0.4 ml (3.2 mmol) of trimethylchlorosilane, 0.34 ml (1.6 mmol) of hexamethylsilazane and 0.3 ml (3.2 mmol) of tin chloride. The reaction mixture was stirred in argon atmosphere under the room temperature for 4 hours. Deposition was removed, filtrate was vaporized down to oily residue. The latter was solved in 70 ml of chloroform, flushed and after chloroform removal applied onto the silica-gel column for chromatographic purification. Analytically pure acylated N2-glycoside was obtained after reprecipitation from ethylacetate (the characteristics are represented in table 2.)

Results and discussion. The condensed heterocyclic systems, based particularly on pyrimidines, imidazoles, triazines, take a significant place in studies, dedicated to the development of chemical methods for production of bioactive polycyclic heterosystems, related to the natural antibiotics and alkaloids, which have antitumor and antiviral activity [8-10] and are also important for the construction of diagnostic fluorescence probes [11-14]. Our previous work demonstrated that the most effective procedure for forming a linear triazine-containing system appeared to be the annelation of 1,2,4-triazine derivatives by 2-aminobenzothiol. Heteroaromatic bases, obtained by this method, were used for the synthesis of their nucleoside analogs.

The compounds structure was determined by physical and chemical approaches. However, sometimes the analytical characteristics are not enough to inter-

Table 1.
Physicochemical data of new condensed triazine derivatives ***3a - e***

Compound	Yield, %	M.p., (°C)	^{1}H-NMR (DMSO-d_6 /TMS) (ppm)				UV, $\lambda_{макс.}$, nm (ethanol)
			Protones of the triazine ring			Other protons of hetero system	
			N_2H	N_4H	NH_2		
3a	98	346-350	12,08 s	11,12 s	-	7,18 - 6,92 (m, 4H, Ph)	208, 240, [269]*, 376
3b	77	295-297	-	-	6,23 s	6,24 - 6,90 (m, 4H, Ph), 10,05 (s, 1H, NH)	213, 242, [303]*, 388
3c	79	250- 253	11,98 s	-	-	7,18 - 7,09 (m, 4H, Ph), 3,19 (s, 6H, (CH3)2)	219, 254, [303]*, 391
3d	50	315-317	-	11,33 s	-	7,10 - 6,98 (m, 4H, Ph), 4,06 (d, 2H, CH2); 2,86 (m, 2H, CH2)	209, 241, [269]*, 386
3e	47	237-239	-	11,31 s	-	7,12 - 6,94 (m, 4H, Ph); 6,21 (dd, 1H, Fur); 3,92 - 3,70 (m, 2H, Fur); 2,21- 1,90 (m, 4H, Fur)	209, 241, [269]*, 385

Protones of cycles: Ph – phenyl, Fur - tetrahydrofuranyl; * - concealed maximum

Table 2.
Comparison of physicochemical characteristics of the 1,2,4-triazine tri-0-acetate ribofuranosides and 1,2,4-triazinebearing tricyclic

Compound	Yield, %	M.p., (C)	^{1}H-NMR (DMSO-d_6 /TMS) (ppm)			R_f*
			Protones of the triazine ring		Protones of the carbohydrate fragment	
			N_4H	NH_2		
1f	87	70–73	12,73 s(1H)	-	6,08 (d, 1H, H-1 , J=3,6 Hz; 5,50 (dd, 1H, H-2 , J=3,5; 3,6 Hz); 5,34 (t, 1H, H-3); 4,31 (d, 1H, H-4); 4,26 (m, 1H, H-5); 4,08 (dd, 1H, H-5); 2,10 (s, 3H, CH3CO-2), 2,07 (s, 6H, CH3CO- 3 та - 5)	0,31
3f	50	91–93	11,57s(1 H)	-	6,06 (d, 1H, H-1 , J=4,0 Hz); 5,46 (dd, 1H, H-2 , J =4,0; 8,0 Hz); 5,32 (m, 1H, H-3); 4,29 (m, 1H, H-4); 4,22 (m, 1H, H-5); 4,06 (m, 1H, H-5); 2,09 (dd, 9H, CH3CO- 2 , -3 та -5)	0,45
1g	67	82–85	-	7,67 (2H)	6,04 (d, 1H, H-1 ,J =2,4 Hz); 5,56 (dd, 1H, H-2 , J= 2,4; 2,0 Hz); 5,33 (t, 1H, H-3); 4,32 (m, 1H, H-4); 4,27 (m, 1H, H-5); 4,08 (m, 1H, H-5); 2,11(s, 3H, CH3CO -2); 2,08 (s, 3H, CH3CO -3); 2,04 (s, 3H, CH3CO -5)	0,39
3g	47	110–112	-	7,23 (2H)	5,86 (d, 1H, H-1 , J =2,0 Hz); 5,47 (dd, 1H, H-2 , J =2,4; 2,0 Hz); 5,26 (t, 1H, H-3); 4,28 (m, 1H, H-4); 4,11 (t, 1H, H-5); 4,07 (m, 1H, H-5); 2,10 (s, 3H, CH3CO- 2); 2,06 (s, 6H, CH3CO- 3 та -5)	0,73

* R_f in system of solvents: 2-propanol – toluene – 25% NH_4OH (3:2:1)

pret unambiguously the structural modifications of heterosystem , *e.g.* in the case of its glycosylation.

Therefore, this work in addition to obtaining novel compounds with substituents in various positions of triazine cycle was aimed to confirm the earlier produced tricyclic glycosides structures using proper azanucleosides (1f, g) with fixed position of sugar moiety upon synthesis.

The choice of compounds 1a, 1d - f and 3-amino-replaced triazine (1b, c, g) is determined by the presence of two vicinal electrophylic centers (carbon atoms in positions 5 and 6) in the structure of

Table 3.
Comparison of the physicochemical characteristics of the condensed 1,2,4-triazine N2- -ribofuranosides have been synthesized by

Com-po und	M.p., (?C)	1H-NMR (DMSO-d6 /TMS) (ppm)	UV, макс., nm (ethanol)	Rf **
IУ (ref.1)	236-238	11,36 (s, 1H, NH); 7,11-7,00 (m, 4H, Ph); 5,86 (d, 1H, H-1); 4,98 (d, 1H, OH-2); 4,78 (d, 1H, OH- 3); 4,40 (t, 1H, OH- 5); 4,15 (dd, 1H, H- 2); 3,97 (dd, 1H, H- 3); 3,77 (dd, 1H, H- 4), 3,50 (m, 1H, H - 5); 3,41 (m, 1H, H - 5);	240, [266]*, 384	0,64
3f '	236-239	11,18 (br.s, 1H, NH); 7,12-6,99 (m, 4H, Ph); 5,86 (d, 1H, H-1); 4,98 (dd, 1H, OH-2); 4,78 (t, 1H, OH- 3); 4,40 (m, 1H, OH- 5); 4,15 (dd, 1H, H- 2); 3,97 (dd, 1H, H- 3); 3,77 (dd, 1H, H- 4), 3,50 (m, 1H, H - 5); 3,41 (m, 1H, H - 5);	241, [267]*, 385	0,64
УIII (ref.1)	224-226	7,19 (br.s, 2H, NH2); 7,10 -6,88 (m, 4H, Ph); 5,46 (d, 1H, H-1), 5,12 (dd, 1H, OH-2), 4,87 (t, 1H, OH- 3); 4,77 (dd, 1H, OH- 5); 4,29 (m, 1H, H- 2); 3,96 (m, 1H, H- 3); 3,82 (m, 1H, H- 4); 3,53 (m, 1H, H -5); 3,46 (m, 1H, H -5)	251,5; [303]*, 383	0,54
3g'	226-228	7,13 (br.s, 2H, NH2); 7,14 -6,82 (m, 4H, Ph); 5,45 (d, 1H, H-1), 5,11 (m, 1H, OH-2), 4,86 (t, 1H, OH- 3); 4,76 (m, 1H, OH- 5); 4,27 (m, 1H, H- 2); 3,95 (m, 1H, H- 3); 3,80 (m, 1H, H- 4); 3,52 (m, 1H, H -5); 3,44 (m, 1H, H -5)	252, [303]*, 383	0,54

* concealed maximum; ** R_f in system of solvents: n-butanol - acetic acid – water (5:2:3)

triazine heterocycle. These atoms are consistently connected with oxygen and halogen atoms.

The process of condensed triazine formation (as well as pyrimidine) starts from substitution of bromine atom for aryl fragment. The further intramolecular cyclization of arylation product [6-phenylthio-triazin-3,5(2H,4H)-dione] leads to the expected linear system formation [1,8].

Considering a more pronounced aromatic character of triazine bases in comparison with pyrimidine bases [15], we predicted that the synthesis of condensed system to involve N2-substituted derivatives would not require an additional activation of reactive centers in a triazine ring and proceed with a satisfactory yield. This assumption appeared true only partially.

The heterosystem formation using azanucleosides (1f, 1g) occurred with some complication because of strict requirements for reaction, contrary annelation of non-glycosylated triazines (1a - e), with formation of side products.

The purification of acylated glycosides and furanyl derivative of condensed triazine was carried out by the column chromatography on the silica gel with further crystallization. The yield made up 45-50%. The compounds 3f, 3g having fixed position of sugar moiety were produced in this way. The NMR spectra, correlating with current proton signals in the spectra of initial acylglycosides 1f, 1g and tricyclic aglycones 1a, 1b (table 1, 2), indicate this definitely.

The most typical are the signals of carbohydrate fragment (the location of anomeric proton and proton singlet of acetyl groups with total intensity 9H) and signals of phenyl nucleus in region 7.0 - 6.8 ppm for both compounds. The 3f-compound is distinguished by the signal of cyclic nitrogen proton at triazine ring in region 11.57 ppm whereas 3g-glycoside is characteristic by the proton signal of exoaminogroup to be located at 7.23 ppm.

An important information was also received owing to the analysis of electronic absorption spectra of the synthesized compounds. The absorption curves of the unprotected glycosidic derivatives were comparable with the same spectra for the corresponding tricyclic aglycones thus suggesting a weak effect of the glycosidic residues on the chromophoric system of condensed triazines.

The comparison of experimentally determined physico-chemical and spectral characteristics of condensed triazine deacylated glycosides (3f ‘, 3g’) with those obtained via an independent synthetic procedure (tricyclic glycosides IV and VIII [1]) is presented in table 3. It is worth noting that collation of ^{1}H-NMR

spectra revealed convincing coincidence in position and form for signals from carbohydrate fragment and triazine ring of aglicones to indicate entire structural identity between compound pairs .

The evaluation of other analytical properties of these compounds let us to claim that all of them have the structure of N2-glycosidic derivatives.

The proposition in paper [1] concerning glycosilation of N5-position in triazine ring was based on quantum-chemical calculations for the reaction-competent nucleofilic centers in original heterosystem III and significant discrepancy between ^{1}HNMR data and shift of anomeric proton in compounds III and IV. Additionally it should be noted that prototropic tautomerism for a synthesized triazin-containing compound remains unexplored, but it seems necessary to consider the availability of tautomers. The data of quantum-chemical calculations demonstrate that nitrogen atom charges in N2 and N5 tricyclic system positions differ from the charge at nitrogen atom N5 in tiazine ring exceeding that of at nitrogen atom (N2) in triazine cycle. That's why by analogy with glycosilation of other condensed system derivatives (guanine and aloxasine) it seems reasonable to suppose that N5-glycoside may present a final kinetic product of the reaction, but upon strict synthesis conditions involved (~100°C, active catalyst, time length) there is preferentially generated the energetically most advantageous product of thermodynamic control with glycoside bond in N2-position.

Hence, the overall structural identity between tricyclic nucleoside samples, obtained by two synthetic approaches, provides every reason to believe that under the conditions of simplified silyl condensation technique as a major regioisomer the N2-riboside may be generated.

Pilot investigation into the synthesized compounds action on HCV replicon (Ph.D. S.L.Rybalko from the L.V.Gromashevskii Institute of Epidemiology and Infectious Diseases of Academy of Medical Sciences of Ukraine) brought out the effective inhibition of hepatitis C virus by some condensed triazine derivatives at the level of clinic preparation, rybavirin. The materials of biological research will be presented in a separate publication.

Conclusion. The using of a triazine bases with fixed substituents at N2- position including the ribose residue too for generating new biologically active triazine-bearing derivatives allows to avoid the problem of structural uncertainty in the synthesized compounds and to provide the functionalization of the triazine fragment in tricyclic base in the alternative way. Such approach proved the adequacy of the simplified silylic condensation for the directed glycosilation of tricyclic triazine-bearing bases.

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Трициклічна 1,2,4-триазинвмісна гетеросистема: спрямований синтез нових біологічно активних сполук

Резюме

Циклізацією N2-заміщених 6-бром-3-оксо(аміно)-1,2,4-триазин-5(4H)-онів з орто-амінотіофенолом одержано і досліджено нову серію трициклічних гетероароматичних сполук. Підтверджено структуру біологічно активних трициклічних глікозидів, нещодавно синтезованих спрощеним методом силільної конденсації, та доведено доцільність і адекватність останнього для направленого глікозилювання триазинвмісних трициклічних основ.

Ключові слова: конденсований 1,2.4-триазин, трициклічні аналоги нуклеозидів.

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Трициклическая 1,2,4-триазинсодержащая гетеросистема: направленный синтез новых биологически активных соединений

Резюме

Циклизацией N2-замещенных 6-бром-3-оксо(амино)-1,2,4-триазин-5-онов с орто-аминотиофенолом синтезирована и исследована новая серия трициклических гетероароматических соединений. Подтверждена структура биологически активных трициклических гликозидов, полученных ранее упрощенным методом силильной конденсации, и доказана целесообразность и адекватность использования последнего для направленного гликозилирования триазинсодержащих трицикличных оснований.

Ключевые слова: конденсированный 1,2,4-триазин, трициклические аналоги нуклеозидов.

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