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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF N-PYRIMIDINYLSUCCINAMINIC ACIDS AND SUCCINIMIDES

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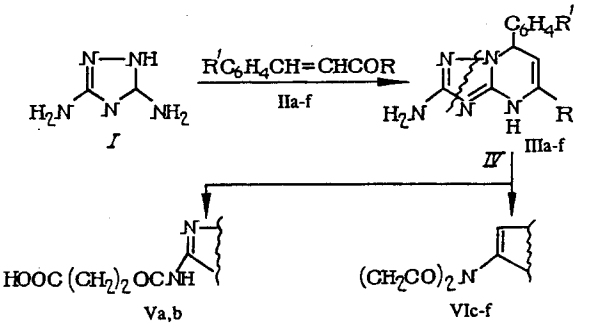
Interest in dihydroazolo[1.5-a]pyrimidines as biologically active compounds has arisen relatively recently [1-5]. This is primarily because of the difficulty in obtaining these substances, most of which have previously been regarded only as possible intermediates in the synthesis of their heteroaromatic analogs. Among the dihydroazolopyrimidines thus far prepared, compounds have been found which have vasodilator activity, and some aspects of coronary action have been studied in detail [1-4]. Antimicrobial and antiviral activities have been noted in a number of nitroazolo[1,5-a]pyrimidines [5,6]. We report here the synthesis of new aromatic substituted dihydroazolopyrimidines, for the purposes of carrying out pharmacological studies.

3,5-Diamino-1,2,4-triazole (I) was condensed with α,β-unsaturated ketones (IIa-f) in dimethylformamide (DMF) to yield 2-amino-substituted dihydro-1,2,4-triazolo[1,5-a]pyrimidines (IIIa-f).



II, III, V, VI: R=Me (a, b), Ph (c, d), 4-$CH_3C_6H_4$ (e), 4-ClC_6H_4 (e); R'=H (a, c, e), 4-Cl (b, f), OMe (d).

The resulting compounds were identified by spectral methods (Table 1). The IR spectra of the compounds synthesized showed valent vibrations of the C=C bond in the region 1652-1680 cm^{-1}. The electron spectra were characterized by the presence of a weak absorption band with λ_{max} = 276-288 nm and were generally analogous to the spectra of aromatic substituted 4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidines. The 6,7-dihydroform absorption typical of imines in the longer-wavelength region was absent. Thus, these results show that compounds IIIa-f had the 4,7-dihydro structure both in the solid state and in solution.

The low water solubility of 2-amino-4,7-dihydro-1,2,4-triazolo-[1,5-a]pyrimidines significantly reduces their biological availability. At the same time, the presence of a 2-amino group in these compounds opens up the possibility for their chemical modification. We were able to acylate compounds IIIa-f. Succinic anhydride (IV) was selected as the acylating agent, because the succinamide fragment, significantly increasing solubility and decreasing toxicity, is itself a pharmacophore group [8].

Acylation was carried out either by boiling the initial substances in pyridine or by fusion at a temperature of 150-170°C in the absence of solvent. In the first case, the reaction was directed towards the formation of N-triazolopyrimidinyl-substituted

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TABLE 1. Properties of Compounds IIIa-f, Va,b and VIc-f

Compound	Melting temperature, °C	IR spectrum, ν_{max}, cm^{-1}, in KBr		UV spectrum, λ_{max}, nm ($\varepsilon \times 10^{-3}$)	Yield, %
		C=C	C=O		
IIIa	305—7	1683		274 (4,5)	80
IIIb	240—1	1674		276 (2,5)	70
IIIc	244—5	1669		290 (3,1)	72
IIId	238—40	1662		278 (9,2)	87
IIIe	247—9	1668		287 (3,8)	80
IIIf	220—2	1654		288 (2,1)	68
Va	230—1	1670 (plateau)	1718*	289 (2,4)	73
Vb	203—4	1668 (plateau)	1720*	264 (1,9)	77
VIc	256—8	1652	1728	288 (2,3)	92
VId	202—4	1682	1735	230 (25,6)	87
VIe	245—7	1656	1730	294 (2,6)	91
VIf	289—90	1682	1735	290 (2,3)	93

*Bands for the amide of I and the amide of II, and of their associated COOH− and NH-groups were located in the regions 1687, 1548, 2850-3300 (Va) and 1705, 1540, 2850-3350 cm^{-1} (Vb) respectively.

succinaminic acids (Va, b), and the second reaction involved cyclization to form the corresponding succinimide derivatives (VIc-f). Acylation at the imino group did not occur.

The structures of V and VI were distinguished on the basis of differences in their IR spectra. Thus, compounds Va, b were characterized by intense absorption at 1720 cm^{-1} (the C=O group of the COOH fragment) and 1705, 1540 cm^{-1} (amides of I and II). Carbonyl group absorption bands in compound VI were present in the region 1730-1735 cm^{-1}. In addition, compounds Va, b had good solubility in alkaline aqueous solutions.

EXPERIMENTAL (CHEMICAL)

IR spectra were taken on a Specord IR-75 in KBr tablets. UV spectra were recorded using a Specord M-40 spectrometer in alcohol.

Chromatographic monitoring of reactions and product purity was carried out by TLC using Silufol UV-254 plates in systems consisting of benzene:methanol (10:1) and benzene:hexane:chloroform (5:2:3), with detection under UV light. The characteristics of the compound synthesized are shown in Table 1.

The results of elemental analysis agreed with expected values.

2-Amino-3-methyl-7-(2-chlorophenyl)-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidine (IIIa)

A mixture of 0.2 g (2 mmol) of 3,5-diamino-1,2,4-triazole and 0.4 g (2 mmol) of 4-chlorobenzal acetone in 1 ml of DMF was boiled for 30 min, until formation of a precipitate. The mixture was cooled, the precipitate was washed with benzene, and 0.39 g (70% yield) of product was collected by filtration. This was crystallized from a mixture of benzene and DMF (1:1). The same method was used to prepare compounds IVb-f.

N-(5-Methyl-7-(4-chlorophenyl)-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidin-2-yl)succinaminic Acid (Va)

A solution of 2.6 g (10 mmol) of amine IIIa and 1.3 g (12 mmol) of succinic anhydride in 10 ml of pyridine was boiled for 30 min, mixed with 50 ml of water, and acidified with HCl (1:1) to a pH of 2-3. Acid Va was collected by filtration, which yielded 2.1 g (65%). The same method was used to prepare compound Vb.

TABLE 2. Comparison of the Analgesic Activity and Toxicity of Compounds Va,b, VIc-f and Voltaren (n = 10)

Compound	Number of spasms (at a dose of 10 mg)	% reduction	ED_{50}		LD_{50} in mice (p.o.)		Therapeutic index	
			mg/kg	in comparison with Voltaren	mg/kg	in comparison with Voltaren	LD_{50}/ED_{50}	in comparison with Voltaren
Va	28,3 (23—33)*	37,1			2430±225	6,4		
Vb	14,0 (8—22)**	57,8	9,0	2,25	1770±261	4,7	196,6	2,07
VIc	35,0 (12—54)*	37,0			2025±294	5,3		
VId	26,0 (21—33)*	39,2			2100±228	5,5		
VIe	59,6 (47—68)*	1,6			1800±261	4,7		
VIf	13,0 (13—16)**	60,8	8,0	2,0	1620±268	4,3	202,5	2,12
Voltaren	At a dose of 5 mg/kg							
	22,2 (9—33)	63,4	4,0	—	380±18	—	95	—
Control	60,6 (54—68)	—	—	—	—	—	—	—

Notes. $^{*}p < 0.05$; $^{**}p < 0.02$ in comparison with controls.

N-(5,7-Diphenyl-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidin-2-yl)succinimide (VIc)

A mixture of 2.9 g (10 mmol) of amine IIIb and 1.2 g (12 mmol) of succinic anhydride was heating to melting and kept at 150-170°C for 10 min. The mixture was cooled, 20 ml of water was added, and 3.5 g (yield = 92%) of succinimide VIc was collected by filtration. The same method was used to prepare compounds VId-f.

EXPERIMENTAL (BIOLOGICAL)

The biological activities of N-acyl derivatives of 2-amino-4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidines were studied by measuring their analgesic action and acute toxicity.

Acute p.o. toxicity of compounds was determined using mice of both sexes (18-20 g) [9]. LD_{50} values were calculated by an express method of probit analysis as described by V. V. Prozorovskii [10, 11].

Analgesic activity was studied using a model of chemical pain induction using acetic acid spasms [11] in rats. Experiments were carried out using white Wistar rats (80-120 g), with 10 animals in each group.

Spasms were elicited by i.p. dosage with 1% acetic acid at a dose of 1 ml per 100 g body weight. Compounds Va,b and VIc-f were given 30 min before acetic acid at doses of 2.5, 5, and 10 mg/kg. The number of spasms was recorded 15 min after acetic acid for a period of 30 min. Reductions in the number of spasms, in comparison with control animals, was a measure of analgesic activity. The reference agent was Voltaren, used at a dose of 5 mg/kg. The results are shown in Table 2. Results were analyzed statistically using the non-parametric Wilcoxon–Mann–Whitney test [12].

The data presented in Table 2 show that compounds Va,b and VIc-f were of low toxicity in the classification system of K. K. Sidorov [13]. Studies showed that N-acyl-substituted dihydroazolopyrimidines had analgesic activity, which was most pronounced in compounds Vb and VIf. Compounds VIc and VId were less active. Imide VIe had no analgesic activity. Comparison of the doses of compounds Vb and VIf producing toxic and analgesic effects showed them to have wider therapeutic ratios than compounds VIc and VId.

However, the compounds did not have greater analgesic activity than the reference compound Voltaren. Analysis of structure-activity relationships among azolopyrimidinylsuccinimide derivatives studied showed that introduction of 4-chlorophenyl substituents into the pyrimidinyl fragment of the molecule led to increases in the analgesic activity, which was accompanied by some increase in the toxicity of these compounds.

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