2,3-Disubstituted 4-Thiazolidones (I-IV). A mixture of 0.01 mole azomethine base, 0.01 mole thioglycolic acid, and 30 ml nonaqueous benzene is placed in a round-bottomed flask equipped with a Dean–Stark apparatus and heated in a boiling water bath for 20-40 h. Excess benzene is driven off and the residue is dried in air and recrystallized from ethanol.

Formyldithienyl Mercaptals (V-VI). Excess thioglycolic acid (0.03-0.05 mole) is added to 0.01 mole azomethine base. The reaction mixture is heated to 40°C and crystalline precipitates settle out of the transparent solution. The crystals are filtered off and washed with water to neutral according to litmus tests. The residue is dried in air and recrystallized from ethanol.

The qualitative determination of nitrogen in compounds I-VI was conducted at the Institute of Organic Synthesis of the Academy of Sciences of the Latvian SSR under the direction of V. É. Égert, Candidate in Chemistry, to whom the authors wish to extend their thanks.

LITERATURE CITED

1. H. Erlenmeyer and V. Oberlin, Helv. Chim. Acta, 30, 1329 (1947).
2. A. R. Surrey, J. Am. Chem. Soc., 69, 234 (1947).
3. A. R. Surrey, J. Am. Chem. Soc., 74, 3450 (1952).
4. G. W. Stacy and R. J. Morath, J. Am. Chem. Soc., 74, 3385 (1952).
5. G. Fenech, Ann. Chim. (Roma), 50, 413 (1960).
6. K. I. Vakhreeva, M. G. Viderker, and P. I. Buchin et al., Khim.-Farm. Zh., 1, 24 (1972).
7. K. I. Vakhreeva, A. E. Lipkin, T. O. Ryskina et al., Khim.-Farm. Zh. 3, 24 (1973).
8. Method of Experimental Chemotherapy [in Russian], Moscow (1972).

SYNTHESIS AND BIOLOGICAL PROPERTIES OF PYRIMIDO[4.5-b][1.4]-THIAZINE DERIVATIVES

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In recent years considerable research has been conducted in the search for folic acid (FA) antagonists which show selective toxicity toward tumor cells and are capable of exerting a therapeutic effect in cases of resistance to aminopterin and methotrexate. Such research was furthered by the discovery of a type of enzymatic reaction involving FA and its antagonists. It has been established that the principal factor in the conversion of FA in an organism is its reduction to tetrahydrofolic acid (FH_4) which occurs through the action of the enzyme dihydrofolate reductase (DFR). FH_4 fulfills the important role of transferring one-carbon moieties in the synthesis of purines, thymidylates, and amino acids.

The basis for the therapeutic and toxic activity of aminopterin and methotrexate lies in blocking the DFR and through this interrupting the synthesis of many metabolites, above all, thymidylic acid and purinyl nucleic acids [1], which the tumor cell requires in greater than normal amounts due to its pattern of explosive growth.

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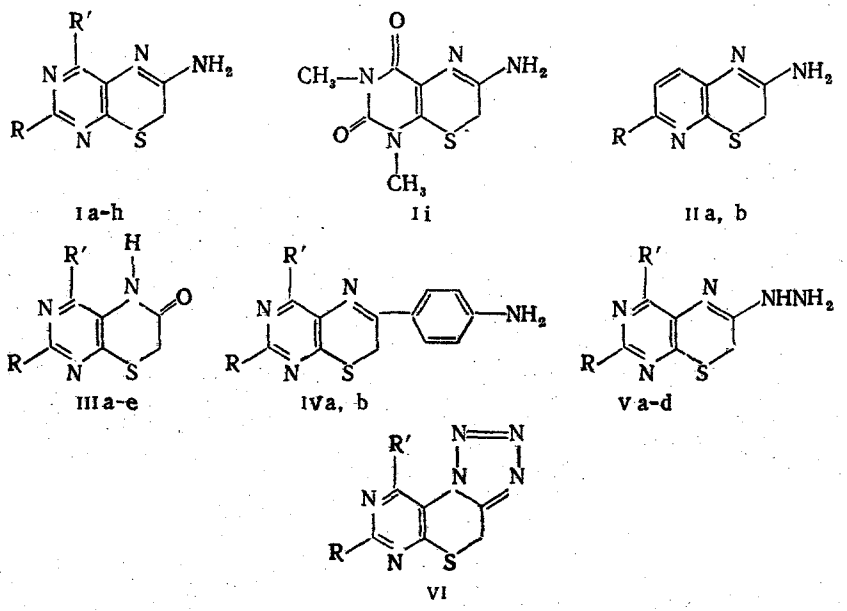


Ia-h Ii IIa, b

IIIa-e IVa, b Va-d

VI

We have synthesized various derivatives of pyrimidino[4.5-b][1.4]thiazine (I, III-IV) in a search for new FA antagonists having lower toxicity than aminopterin and methotrexate. The 6-aminopyrido[2.3-b][1.4]thiazines (II) were also prepared for the comparative study of biological activity.

The 6-aminopyrimido[4.5-b]- and 6-aminopyrido[2.3-b][1.4]thiazines (Ia-i, IIa,b, IIIa,b) were synthesized by the reaction of 5-amino-6-mercaptopyramidines or 2-mercapto-3-aminopyridines with chloroacetonitrile [2,3]. Treatment of the 6-aminopyrimido[4,5-b][1,4]thiazines with hydrazine hydrate gave 6-hydrazinopyrimido[4.5-b][1.4]thiazines (Va-d). The diglucose derivative (Vd) was obtained from the interaction of 4,6-dihydrazinopyrimido[4.5-b][1.4]thiazine (Vc) with glucose, and the tetrazolo[1.2-f]pyrimido[4.5-b][1.4]thiazines (VI) were obtained by treating compounds (Va,b) with nitrite in the presence of a mineral acid. The 6-hydroxypyrimido[4.5-b][1.4]thiazines (IIIa-e) were synthesized from the reaction of 5-amino-6-mercaptopyrimidines with α-halogen acids [4]. p-Aminochloroacetophenone was used as the carbonyl component [5] in the preparation of 6-(p-aminophenyl)pyrimido[4.5-b][1.4]thiazines (IVa,b).

The compounds of the pyrimidothiazine series differ from the pteridine FA antagonists by the presence of a 1,4-thiazine ring instead of a pyrazine ring and can be considered as the thio analogs of simple pteridines. Biochemical studies show that the changes made in the pteridine molecule lead to the appearance of an inhibitory activity toward DFR [6] (see Table 1). The inhibitory properties of these compounds are associated with the presence of the pyrimidothiazine bicyclic ring and are enhanced if the pyrimidine and thiazine portions of the molecule contain amino, hydroxy, or methoxy groups. The effect of the pyrimido[4.5-b]-[1.4]thiazine derivatives on the grafted tumors of animal tissues and on normal (chick embryo heart) and tumorous (sarcoma 45) tissue cultures was studied, and it was observed that the 6-aminopyrimidothiazines and pyrimidothiazine-6-ones (I-III) possessed antitumor activity in *vivo* and *in vitro*. These same compounds show an inhibitory effect of DFR. Of the 6-aminopyrimido[4.5-b][1.4]thiazines, 4-methoxy-6-aminopyrimido[4.5-b][1.4]thiazine hydrochloride (Ia) has the greatest antitumor activity. This compound slows the growth of sarcoma AK and sarcoma 37 by 56-77% and of lymphoid leukosis NK/Ly and Erlich's ascites carcinoma by 69-78%. Methotrexate has a similar activity toward these strains of tumors. If the methoxy group of position 4 in compound I is replaced by an amino, methylamino, or dimethylamino group (compounds Ib, c, e), the antitumor activity decreases or completely disappears. The presence of a methylthio or methyl group in position 4 of compounds (Id, i) has an analogous effect on the antitumor activity.

Nearly all of the studied 6-aminopyrimido[4.5-b][1.4]thiazines, with the exception of compounds Ig and Ih, have low toxicity. On intravenous introduction in mice, their LD_{100} was in the range 200-300 mg/kg. A significantly high toxicity was observed for the 6-aminopyrimidothiazines having an amino group in position 2. Thus, 2-amino-4-methyl and 2,4-diamino derivatives (Ig,h) are 10-20 times more toxic than compounds Ia-e. Disruption of

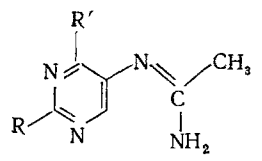
TABLE 1. Biological Activity Data for Pyrimido[4.5-b][1.4]-thiazine Derivatives

Compound	R	R′	LD_{100}, mg/kg	Antitumor activity	DFR inhibition, %		Concentration, mg/ml					
							10^{-3}		$5\cdot10^{-4}$		$2\cdot10^{-4}$	
							suppressed growth of tissue cultures, %					
					$1\cdot10^{-5}$ M	$1\cdot10^{-6}$ M	chick embryo heart	sarcoma 45	chick embryo heart	sarcoma 45	chick embryo heart	sarcoma 45
Methotrexate	—	—	5	++	$I_{50}=1\cdot10^{-8}$M		100	100	74	88	50	50
Ia	H	CH_3O	250	++	56	37	58	100	17	87	0	82
Ib	H	$NHCH_3$	350	+	60	0	46	40	0	0	—	—
Ic	H	$N(CH_3)_2$	250	—	0	—	38	60	0	0	—	—
Id	H	SCH_3	300	+	—	27	73	100	—	—	38	85
Ie	H	NH_2	200	+	33	21	50	83	—	—	30	0
If	H	Cl	—	—	80	35	not studied					
Ig	NH_2	NH_2	100÷200	—	96	37	72	100	—	—	34	63
Ih	NH_2	CH_3	15	—	0	—	42	60	0	0	—	—
Ii	—	—	500	—	0	—	58	70	30	40	0	0
IIa	Cl	H	45	—	0	0	not studied					
IIb	CH_3O	H	15	—	37	0	not studied					
IIIa	NH_2	OH	>500	+	—	53	—	—	33	78	0	35
IIIb	OH	OH	>500	+—	—	42	0	0	—	—	—	—
IIIc	H	OH	>500	+—	—	52	50	60	—	—	32	30
IIId	H	CH_3O	>500	+—	56	43	30	40	—	—	20	30
IIIe	NH_2	CH_3	>500	—	—	—	not studied					
IVa	H	CH_3O	400	++	—	—	100	100	50	100	0	0
IVb	NH_2	CH_3	150	+	—	—	54	100	—	—	50	90
Va	NH_2	CH_3	200	+—	—	—	40	58	0	0	—	—
Vb	H	SCH_3	750	—	—	—	not studied					
Vc	H	$NHNH_2$	>500	—	—	—	100	100	—	—	78	50
Vd*			<500	—	—	26	0	100	—	—	0	50

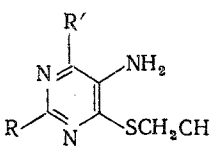
*4,6-diglucosyl derivative of Vc.

the aromaticity of the pyrimidine ring in 6-aminopyrimidothiazines by introduction of substituents in positions 1 and 3 (compound Ii) leads to loss of antitumor activity. 6-Aminopyrimido[2,3-d][1.4]thiazines, being de-aza analogs of compounds (I), are characterized by increased toxicity and the absence of antitumor activity (compounds IIa,b).

The pyrimidothiazin-6-ones (IIIa-e) have lower toxicity than the 6-aminopyrimidothiazines; their LD_{100} during per os introduction in mice was greater than 500 mg/kg. Compounds of this group have lower antitumor activity than 6-aminopyrimidothiazines. Some suppression of tumor growth was observed for 2-amino-4-hydroxy- and 2,4-dihydroxypyrimido[4.5-b][1.4]-thiazin-6-ones (IIIa,b). These compounds also show a marked inhibitory effect on DFR. Cleavage of the thiazine ring in 6-aminopyrimidothiazines with preservation of the amidine fragment (type VII compounds) or sulfur (type VIII compounds) leads to a significant decrease or complete loss of the carcinolytic properties as well as the ability to inhibit DFR.



VII



VIII

The data obtained indicate a dependence of biological activity in the studied group of compounds not only on the nature of substituents on the pyrimidine and thiazine rings but also on the presence of a pyrimidinothiazine system per se.

Among the 6-hydrazino derivatives of pyrimidothiazine (Va-d), antitumor activity was observed in compound Va which has an amino group in position 2 and a methyl group in position 4. The glucose derivative (Vd) and tetrazolo[1.2-f]pyrimido[4.5-b][1.4]thiazines (VI) ob-

tained from 6-hydrazinopyrimidothiazines do not have enhanced antitumor activity. Compounds in which the amino group in position 6 is separated from the thiazine ring by a benzene ring (IVa,b) are significantly less toxic than 6-aminopyrimidothiazines, while their antitumor activity is maintained at approximately the same level.* The relationship established between the structure and biological activity of pyrimido[4.5-b][1.4]thiazine derivatives is supported also in tests on cultures of tumorous and normal tissues. The highest cytostatic activity was observed for 6-aminopyrimidothiazines (Ia-h), pyrimidothiazin-6-ones (IIIa-e), 6-(p-aminophenyl)pyrimidothiazines (IVa,b), and 6-hydrazino derivatives (Vc,d), i.e., in basically the same compounds which showed antitumor activity in tests on animals and which inhibited DFR. However, no correlation was observed between *in vitro* and *in vivo* tests for 4,6-dihydrazinopyrimidothiazine (Vc) and its diglucose derivative (Vd). Although these compounds show activity in tests on tissue culture, they exhibit no antitumor activity in tests on grafted tumors in animals. It is of interest to note that certain of the 6-aminopyrimidothiazine and pyrimidothiazine-6-one derivatives (Ia,c,e; IIIa,c), in contrast to methotrexate, suppress the growth of tumorous tissue culture more strongly than the growth of normal tissues.

Thus, the biological study of pyrimido[4.5-b][1.4]thiazine derivatives shows that they have antitumor activity in tests *in vitro* as well as *in vivo* and that they also inhibit enzymes of folic acid exchange. On comparison of the inhibitory effect of pyrimido[4.5-b]-[1.4]thiazine derivatives on DFR with their antiblastic activity *in vivo*, a correlation between these properties was exhibited in 80% of the cases. On the basis of this, it can be concluded that for this class of compounds a study of the suppressive action on DFR *in vitro* can be used for the preliminary selection of substances having antitumor activity.

The most active in this group of substances is the 4-methoxy-6-aminopyrimido[4.5-b][1.4]-thiazine hydrochloride. On closer biological study of this compound [7], which we named tomizin, it was observed that it has a different spectrum of antitumor activity than methotrexate, inhibits enzymes of folic acid exchange, is less toxic, and exhibits weakly expressed cumulative properties. Such data permitted recommendation of tomizin for clinical testing in diseases where antimetabolites of folic acid exchange (hemoblastosis, chorioepithelioma of the uterus, etc.) are used, in particular, in those cases where resistance to methotrexate occurs.

EXPERIMENTAL

Chemical

2,4,6-Triaminopyrimido[4.5-b][1.4]thiazine (Ig). This compound was obtained according to the method of [3] via the reaction of 2,4,5-triamino-6-mercaptopyrimidine with chloroacetonitrile. Yield 51%, mp > 300°C (from water). Found, %: C 36.64, H 4.12, S 16.20. $C_6H_5N_6S$. Calculated, %: C 36.71, H 4.1, S 16.34.

1,3-Dimethyl-2,4-dihydroxy-6-amino-1,2,3,4-tetrahydropyrimido-[4.5-b][1.4]triazine (Ii). Obtained according to the method of [3] by the reaction of 1,3-dimethyl-2,4-dihydroxy-1,2,3,-4-tetrahydro-5-amino-6-mercaptopyrimidine with chloroacetonitrile. Yield 59%, mp 235-237°C (from water). Found, %: C 42.45, H 4.66. $C_8H_{10}N_4SO_2$. Calculated, %: C 42.46, H. 4.45.

4-Chloro-6-aminopyrimido[4.5-b][1.4]thiazine (IIa). A solution of 1.5 g (0.007 mole) 4-chloro-5-amino-6-(cyanomethylthio)pyrimidine in benzene is added to a suspension of sodium methylate obtained from 0.36 g (0.015 mole) sodium hydride and 0.6 ml methanol in boiling benzene. The reaction mixture was heated at boiling for 10 min and cooled, and the precipitate was filtered off and washed with water. Yield 1.2 g IIa (80%), mp 208°C. Found, %: C 35.82, H 2.85, Cl 17.67, N 28.00, S 16.22. $C_6H_5ClN_4S$. Calculated, %: C 35.91, H 2.51, Cl 17.67, N 27.92, S 15.98.

2,4-Dihydroxypyrimido[4.5-b][1.4]thiazin-6-one (IIIb). Chloroacetic acid (0.6 g) was added to a solution of 1 g 2,4-dihydroxy-5-amino-6-mercaptopyrimidine and 1.7 g potassium hydroxide in 50 ml water. The mixture was heated in a water bath for 1 h. The solution was acidified with hydrochloric acid, and the precipitate was filtered off. Yield 1.1 g (88%),

*Determination of the activity of compounds IVa,b and Va-d with respect to DFR was unsuccessful as they entered into reaction with the reagents used in the method.

mp > 300°C (reprecipitation from aqueous sodium hydroxide with hydrochloric acid). Found, %: C 35.97, H 2.82, N 21.47, S 15.61. $C_6H_5N_3O_3S$. Calculated, %: C 36.18, H 2.53, N 21.1, S 16.1.

4-6-Diglycosylhydrazinopyrimido[4.5-b][1.4]thiazine (Vd). A mixture of 1 g 4,6-dihydrazinopyrimido[4.5-b][1.4]thiazine and 1.7 g glucose in 70 ml methanol was heated at boiling for 2 h. The reaction mixture was filtered, the solvent driven off, and the residue dissolved in 70 ml methanol and filtered. The filtrate was dissolved in 70 ml benzene and evaporated without heating to one-third of the original volume. The precipitate was filtered off. Yield 1.5 g (51%), mp 157-160°C. Found, %: C 39.70, H 5.70, N 18.75, S 5.68. $C_{16}H_{29}N_7O_{10}S$. Calculated, %: C 40.37, H 5.46, N 18.3, S 5.98.

Biological

The antitumor activity of the compounds was studied using white mice and rats with grafted tumors according to method [4]. The following tumor strains were used: on rats — sarcoma M-1, Jensen 536, ascites hepatoma; on mice — sarcoma AK, 37, Ehrlich's ascites carcinoma, lympholeukosis NK/Ly, leukoses La, and L1210 (on mice of lines S57V1 and DVA 2). The preliminary study was conducted on no less than three strains. The preparations were introduced intraperitoneally in an isotonic solution of sodium chloride or internally (in a starchy paste), depending on solubility, once every 24 hours for a period of 5-9 days. Treatment of the solid tumors was begun on the third to fourth day after grafting; treatment of ascitic tumors and leukoses was begun on the day following grafting. The antitumor activity of the preparations was determined from the inhibition index (I) (in %) calculated from the equation:

$$I, \% = \frac{W_c - W_t}{B} \cdot 100,$$

where W_c and W_t are the weight of the tumor (or amount of ascites) in the control and test, respectively. In tests with acites tumors or leukoses the influence of the preparations on the life span of the animals was also determined. The magnitude of the toxic action of the preparations was determined from the growth coefficient C_g, which was calculated from the equation:

$$C_g = \frac{(B_1 - A_1) \cdot C_2 \cdot 100}{C_1 (B_2 - A_2)} - 100,$$

where C_1 and C_2 are the average weight of the animals of the test and control groups at the beginning of testing; B_1 and B_2 are the same average weights at the end of the testing; and A_1 and A_2 are the average weight of the tumors in the test and in the control.

The comparative cytostatic action of the compounds on the initial tumor (sarcoma 45) and normal (chick embryo heart) tissue cultures was studied in tests *in vitro*. Cultivation was conducted on plasmoids in Carroll flasks. The preparations were dissolved in a liquid culture medium (80% medium No. 199, 20% livestock serum) in concentrations 10^{-3}, 5×10^{-4}, 2×10^{-4}, and 10^{-4} mg/ml and added 2.5 ml at a time to the flask. Three to four flasks were used for each concentration. The action of the preparations was determined from the change in the areas of the growth zones after 72- and 96-h incubation at 37°C. The index (in %) of culture growth inhibition was calculated from the equation:

$$I = \frac{S_c - S_t}{S_c} \cdot 100,$$

where S_c and S_t are the area of the growth zone in the control and test group, respectively.

The inhibitory action of pyrimidothiazine derivatives was studied on DFR enzyme preparations and on DFR chromatographed on DEAE cellulose [6]. The obtained data were subjected to statistical treatment by the Student—Fisher method [8].

LITERATURE CITED

1. T. S. Safonova, Vses. Khim. Obshch., 18, 567 (1973).
2. T. S. Safonova, M. P. Nemeryuk, L. N. Myshkina et al., Khim. Geterotsikl. Soedin., 7, 944 (1972).

3. M. P. Nemeryuk, T. S. Safonova, Khim. Geterotsikl. Soedin., 2, 192 (1975).
4. T. S. Safonova and M. P. Nemeryuk, Khim. Geterotsikl. Soedin., 5, 714 (1966).
5. T. S. Safonova and M. P. Nemeryuk, Khim. Geterotsikl. Soedin., 4, 735 (1968).
6. N. A. Ryabokon', N. A. Andreeva, M. P. Nemeryuk et al., Khim. Farm. Zh., 4, 15 (1975).
7. A. S. Sokolova, N. A. Ryabokon', Yu. A. Ershova et al., Vopr. Onkol., 11, 48 (1975).
8. V. A. Chernov, in: Methods of Experimental Chemotherapy [in Russian], Moscow (1971), pp. 357-403.

SYNTHESIS AND PHARMACOLOGICAL AND ANTIOXIDATIVE PROPERTIES OF 1,4-DIHYDROPYRIDINE DERIVATIVES

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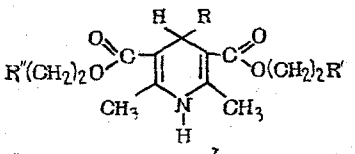
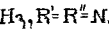
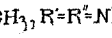
UDC 547.827

Highly effective coronary dilators [1] and hypotensive agents [2] have been found among the 4-aryl- and 4-heteroaryl-1,4-dihydropyridines. The 4-aryl-1,4-dihydropyridines, having aminoester groups in positions 3 and 5 and exhibiting such biological properties were prepared via cyclization of the corresponding starting components in several variations of the Hantzsch reaction [3]. The 4-alkyl and 4-unsubstituted 1,4-dihydropyridines have been studied relatively little in this respect, although they are of interest as potential antioxidants [4, 5].



I

a) R=H, R'=R''=Cl
b) R=CH_3, R'=H, R''=Cl
c) R=CH_3, R'=R''=Cl
d) R=CH_3, R'=H, R''=$N(CH_2)_2O(CH_2)_2$
e) R=CH_3, R'=H, R''=$N(C_2H_5)_2$
f) R=CH_3, R'=H, R''=$HNCH(CH_3)CH_2C_6H_5$
g) R=CH_3, R'=H, R''=$N(CH_2)_4$
h) R=H, R'=R''=CN
i) R=CH_3, R'=R''=$HNCH(CH_3)CH_2C_6H_5$
j) R=CH_3, R'=R''=$N(CH_2)_5$
k) R=CH_3, R'=R''=$N(CH_2)_4$
l) R=CH_3, R'=R''= 
m) R=CH_3, R'=R''= 

The aim of the present study is the synthesis and study of the pharmacological and antioxidative properties of 4-unsubstituted and 4-alkyl-1,4-dihydropyridines with positions 3 and 5 occupied by ester groups containing β-substituted (chlorine, amine radicals, cyano) ethyl groups.

The dichloro derivative Ia was obtained by a modified Hantzsch synthesis [6] from β-chloroethyl acetoacetate and urotropine in the presence of ammonium acetate. Ic was similarly obtained from β-chloroethyl acetoacetate and aldehyde ammonia. The monochloro derivative Ib was synthesized from ethyl β-aminocrotonate and β-chloroethyl ethylideneacetoacetate. Ih was formed from condensation of β-cyanoethyl acetoacetate with urotropine according to method [6].

The dichloro derivative Ia, which is unsubstituted in position 4, did not give the expected substances on interaction with amines, whereas heating Ib,c, with certain primary and secondary amines in dimethylformamide or a mixture of dimethylformamide with benzene led to the formation of amino derivatives of type I, which were isolated and characterized as the hydrochlorides. These were hygroscopic, weakly stable substances.

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