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SYNTHESIS AND ANTITUMOR ACTIVITY OF 5-MERCAPTO-9-ETHOXYCARBONYL-1,2-DIHYDRO-3H-PYRIMIDO-[5,4-e]PYRROLIZINE DERIVATIVES

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It is generally known that a number of pyrrolo[3,2-d]pyrimidine derivatives have been found to possess pronounced antitumor activity [2]. We have recently demonstrated that enamines obtained by reacting N-cyanomethylpyrrolid-2-one diethylacetal (I) with compounds containing an active methyl ring are easily cyclized by the Thrope–Ziegler method into derivatives of 5-cyano-6-aminopyrrolizidine [1]. The presence of cyano and amino groups in adjacent positions in these compounds assures that the pyrimidine ring will be closed with the formation of condensed pyrrolo[3,2-d]pyrimidines.

Thus, the purpose of the present work is the synthesis and study of the antitumor activity of pyrimido[5,4-e]pyrrolizines whose principlal heterocyclic fragment is the bicyclic pyrrolo[3,2-d]pyrimidine.

The starting compound selected for this synthesis was 1-cyanomethyl-2-(2-ethoxycarbonyl-2-cyano)methylene pyrrolidine (II) which was obtained from acetal (I) and cyanoacetic ester.

The primary approach to the synthesis of pyrimido[5,4-e]pyrrolizine derivatives was based on treating enamine II with ammonium hydrosulfide which is accompanied by the conversion of one cyano group into a thioamide group. The IR spectrum of the resultant compound exhibited an absorption band of the conjugate cyano group at 2200 cm^{-1}. At the same time it is known [3] that a cyano group absorption band does not show up in the cyanomethyl substituent in these kinds of derivatives. This indicates that the cyanomethyl group has indeed been converted into a thiocarbamide group with the formation of 1-thiocarbamoylenamine (III). This compound has also been obtained by another method in which enamine II is treated with HCl in formic acid followed by thionylation of the resultant 1-carbamidomethyl-2-(2'-ethoxycarbonyl-2'-cyano)methylenepyrrolidine (IV) with Lawesson's Reagent (5) [5]. The IR spectrum of compound IV also exhibits an intense cyano group absorption band in the enamine β-position at 2210 cm^{-1}. Selective transformation of the 1-cyanomethyl group of enaminonitriles into a 1-thiocarbamoylmethyl group was observed for dinitrile (VI). In this case, however, there is a spontaneous cyclization into a pyrrolizidine derivative (VIIa). A similar cyclization into pyrrolizines (VIIb, c) is observed when compounds III and IV are reacted with sodium ethylate. Thus, the aggregate of the cited data indicates that under selective conditions the conversion of the CN-group in 1-cyanomethyleneaminonitriles II and IV takes place selectively in the 1-cyanomethyl substituent without affecting the CN groups in the β-position of the enamines. This kind of selectivity is due to the conjugation of the enamine β-cyano group to the electron donor N atom which diminishes its ability to react with nucleophilic reagents. The lowered reactivity of β-substituents in enamines has been observed previously as well [7].

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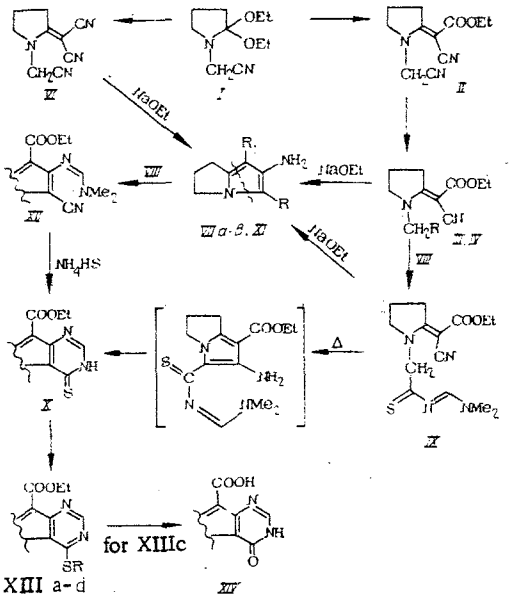
TABLE 1. Physicochemical Properties of the Synthesized Compounds

Compound	mp., °C	Yield	Found, %				Empirical formula	Calculated, %			
			C	H	N	S		C	H	N	S
II	97—8	68	60,47	5,78	19,31	—	$C_{11}H_{13}N_3O_2$	60,26	5,98	19,17	—
III	153—5	78*	51,86	5,97	16,83	12,42	$C_{11}H_{15}N_3SO_2$	52,15	5,97	16,59	12,66
IV	172—3	84	55,66	6,22	17,80	—	$C_{11}H_{15}N_3O_3$	55,68	6,37	17,71	—
VI	59—61	72	63,18	4,63	32,59	—	$C_9H_8N_4$	62,78	4,68	32,54	—
VIIa	>260	79	52,30	4,80	27,13	15,36	$C_9H_{10}N_4S$	52,41	4,89	27,16	15,54
VIIb	231—3	37*	52,22	6,20	16,58	12,43	$C_{11}H_{15}N_3SO_2$	52,15	5,97	16,59	12,66
VIIc	229—30	53	55,30	6,27	17,80	—	$C_{11}H_{15}N_3O_3$	55,68	6,37	17,71	—
IX	130—2	83	54,50	6,67	18,53	10,36	$C_{14}H_{20}N_4SO_2$	54,52	6,54	18,17	10,40
X	>260	61**	54,88	5,01	16,05	12,30	$C_{12}H_{13}N_3SO_2$	54,74	4,98	15,96	12,18
XI	246—7	92	60,38	6,11	19,30	—	$C_{11}H_{13}N_3O_2$	60,26	5,98	19,17	—
XII	146—9	94	61,10	6,65	20,36	—	$C_{14}H_{18}N_4O_2$	61,30	6,61	20,42	—
XIIIa	177—9	89	56,14	5,46	15,30	11,37	$C_{13}H_{15}N_3SO_2$	56,30	5,45	15,15	11,56
XIIIb	119—21	96	54,94	5,52	12,31	9,20	$C_{16}H_{19}N_3SO_4$	55,00	5,48	12,03	9,18
XIIIc	236—8	81	52,29	4,77	13,12	9,95	$C_{14}H_{15}N_3SO_4$	52,32	4,71	13,08	9,98
XIIId	158—60	85	64,60	5,43	11,79	9,19	$C_{19}H_{19}N_3SO_2$	64,57	5,42	11,89	9,07
XIV	>260	67	54,72	4,18	19,08	—	$C_{10}H_9N_3O_3$	54,81	4,11	19,18	—

Note. One asterisk represents method A, two represent method B. Compounds II, VI, and XII were crystallized from isopropanol, VIIa-c and XIIIc from aqueous DMFA, III, IV, IX, and XIIIa from alcohol, XIIIb and XIIId from 50% alcohol, X and XIV from DMFA, and XI from a 1:1 mixture of DMFA and MeCN.



R = H (XIIIa), CN (VIIa), COOEt (VIIb, c, XI, XIIIb), COOH (XIIIc), Ph (XIIId); R^1 = CN (XI), $CSNH_2$ (VIIa, b), $CONH_2$ (VIIc).

The reaction between the thiocarbamoyl derivative of III and dimethylformamide diethylacetal (VIII) proceeds smoothly as is generally characteristic of thioamides [6]. In addition, there is a high yield of thioacylamidine (IX). Cyclization of the latter in the presence of sodium alcoholate results in the elimination of the amidine group and a 40% yield of compound VIIb. When thioacylamidine IX is heated without a catalyst at 140°C, not only is the pyrrole ring closed, but subsequent cyclization takes place with the formation of 9-ethoxycarbonyl-1,2,5,6-tetrahydro-3H-pyrimido[5,4-e]pyrrolizine-5-thione (X). However, this process is accompanied by an intensive resinification of the reaction mixture, and the yield of tricyclic X is insignificant (7%).

Consequently, we studied another approach to the synthesis of pyrimidopyrrolizine X. Thus, the cyclization of compound II in the presence of sodium tert-butylate resulted in a high yield of 5-cyano-6-amino-7-ethoxycarbonyl-1,2-dihydro-3H-pyrrolizine (XI) whose condensation with the acetal of VIII smoothly leads to the amidine (XII). When the latter is treated with ammonium hydrosulfide the 5-cyano group is converted to a thiocarbamide group with the subsequent splitting off of dimethylamine and the formation of tricylic X. Alkylation of pyrimidopyrrolizine X by alkyl halides in an alkaline medium proceeds, as one might expect, at the thione group with the formation of the S-alkyl derivatives (XIIIa-d). The UV spectra of these compounds are quite similar (Table 3) and exhibit a hypsochromic shift in the maximum longwave absorption in comparison to the original thio derivatives of X to which the thione structure might be attributed. Also indicative of the S-substitution of the tricyclic X compound during alkylation is the hydrolysis of XIIIc in an acid medium which results in the formation of 9-carboxy-1,2,5,6-tetrahydro-3H-pyrimido[5,4-e]pyrrolizin-5-one (XIV).

EXPERIMENTAL CHEMICAL

IR spectra were recorded on a Perkin-Elmer instrument, and UV spectra were recorded on a EPS-3T spectrometer in ethanol. Mass spectra were obtained on a Varian MAT-112 spectrometer (ionization chamber temperature 180°C, ionizing electron energy 70 eV). Melting points were determined on a Boetius type heating stand.

1-Cyanomethyl-2-(2'-cyano-2'-ethoxycarbonyl)methylenepyrrolidine (II). A mixture of 9.9 g (50 mmole) of I and 5.7 g (50 mmole) of cyanoacetic ester was kept at a temperature of 5-7°C for 2 h after which ether was added and compound II was filtered off. $M^{+\cdot}$ 219.

1-Carbamidomethyl-2-(2'-cyano-2'-ethoxycarbonyl)methylenepyrrolidine (IV). Dry HCl was passed into a solution of 5.6 g (23.7 mmole) of II in 50 ml of 99.7% formic acid at a temperature of 0°C for 2 h. The reaction mass was then vacuum-evaporated, the residue was triturated with isopropanol, and compound IV was filtered off. $M^{+\cdot}$ 237.

TABLE 2. IR Spectra of Synthesized Compounds (ν_{max}, cm^{-1})

Compound	NH_2, NH	C≡N	C=O
II	—	2190	1700
III	3140,3310	2200	1690
IV	3200, 3370	2210	broad1690
VI	—	2210	—
VIIa	3220—3280, 3360, 3440	2200	—
VIIb	3260, 3400, 3445, 3505	—	1680
VIIc	3250, 3310, 3360, 3480	—	1650, 1670
IX	—	2190	1690
X	3140	—	1700
XI	3220, 3340, 3430	2190	1675
XII	—	2200	1700

TABLE 3. UV Spectra of Compounds X and XIIIa-d

Compound	λ_{max}, nm (lg ε)
X	230 (4,39), 272 (4,53), 336 (4,59)
XIIIa	216 (4,29), 233 (4,31), 249 (4,38), 295 (4,53)
XIIIb	216 (4,28), 249 (4,47), 293 (4,51)
XIIIc	205 (3,98), 249 (4,47), 295 (4,54)
XIIId	208 (4,42), 218 (4,44), 240 (4,4), 295 (4,19)

1-Thiocarbamidomethyl-2-(2'-cyano-2'-ethoxycarbonyl)methylenepyrrolidine (III). A. A 28-ml portion of a 10% NH_4HS solution (50 mmole) was added to a suspension of 1.1 g (5 mmole) of II in 50 ml of a 50% aqueous alcohol. The mixture is agitated at room temperature for 24 h. The resultant precipitate was filtered off and washed with water, which yielded the final product III. $M^{+\cdot}$ 253.

B. A mixture of 1 g (4.2 mmole) of IV and 0.84 g (2.1 mmole) of Lawesson's Reagent in 30 ml of dry benzene was heated at 70°C for 3 h. The reaction mixture was then cooled, and the resultant precipitate was filtered off and recrystallized from aqueous alcohol. Compound III was obtained at a 56% yield.

1-Cyanomethyl-2-(2,'2'-dicyano)methylenepyrrolidine (VI) was obtained in the same manner as compound II from compound I and dinitrile malonate. $M^{+\cdot}$ 172.

5-Thiocarbamido-6-amino-7-cyano-1,2-dihydro-3H-pyrrolizine (VIIa) was obtained in the same manner as III (method A) from compound VI. $M^{+\cdot}$ 206.

5-Carbamido-6-amino-7-ethoxycarbonyl-1,2-dihydro-3H-pyrrolizine (VIIc). A 6.64-g (28 mmole) portion of IV was added to a solution of EtONa prepared from 1 g of Na and 100 ml of abs. alcohol. The mixture was agitated for 1 h at room temperature, then boiled for 10 min, and then cooled. The resultant precipitate was filtered off and washed with alcohol, which yielded the final product VIIc. $M^{+\cdot}$ 237.

5-Thiocarbamido-6-amino-7-ethoxycarbonyl-1,2-dihydro-3H-pyrrolizine (VIIb). A. This compound was obtained in the same manner as VII from compound III. $M^{+\cdot}$ 253.

B. A solution of EtONa prepared from 0.1 g of Na and 5 ml of alcohol was added to a solution of 0.5 g (16 mmole) of IV in 15 ml of abs. alcohol at 50°C. The mixture was agitated at room temperature for 30 min and cooled, and the resultant precipitate was filtered off and recrystallized from a 1:1 mixture of DMFA and abs. alcohol. The yield of VIIb was 40%.

1-(N,N-Dimethylaminomethylene)thiocarbamidomethyl-2-(2'-cyano-2'-ethoxycarbonyl)methylenepyrrolidine (IX). A 1.2-g (7.5 mmole) portion of VIII was added to a suspension of 3.8 g (15 mmole) of III in 20 ml of abs. alcohol. The mixture was agitated at room temperature for 30 min after which another 1.47 g (10 mmole) of VIII were added. The mixture was then agitated for 1 h and the resultant precipitate was filtered off, yielding the final product IX. $M^{+\cdot}$ 308.

TABLE 4. Antitumor Properties of Compounds X and XIIIa-d

Compound	Tumor strain	Single therapeutic dose, mg/kg	T, %	α	K_g
X	Jensen's sarcoma	100	22	$0,9<\alpha<0,95$	3,0
XIIIa	Jensen's sarcoma	100	16	$0,8<\alpha<0,9$	—3,0
XIIIb	Jensen's sarcoma	100	24	$0,95<\alpha<0,98$	2,0
XIIIc	Jensen's sarcoma	100	0	$\alpha<0,1$	—2,0
XIIId	Jensen's sarcoma	100	17	$0,8<\alpha<0,9$	—3,9
X	Melanoma B-16	250*	21	$0,7<\alpha<0,8$	—4,0
XIIIa	Melanoma B-16	250*	46	$0,99<\alpha<0,999$	—5,0
XIIIb	Melanoma B-16	250*	50	$0,99<\alpha<0,999$	—3,0
XIIIc	Melanoma B-16	250*	21	$0,7<\alpha<0,8$	—3,0
XIIId	Melanoma B-16	250*	46	$0,99<\alpha<0,999$	—13,0
XIIIa	Carcinoma 755	125*	12		—3,0
XIIIb	Carcinoma 755	250*	34	$0,9<\alpha<0,95$	3,0
XIIId	Carcinoma 755	250*	13		0
XIIIa	Lewis' bronchial carcinoma	150*	21		5,1
XIIIb	The same	300*	0		—5,9
XIIId	The same	300	43	$\alpha>0,999$	0

*Lethal dose = 500 mg/kg.

5-Cyano-6-amino-7-ethoxycarbonyl-1,2-dihydro-3H-pyrrolizine (XI). A 6.6-g (30 mmole) portion of II was added to a solution of sodium tert-butylate prepared from 0.1 g of Na and 35 ml of tert-butanol. The mixture was boiled with stirring for 4 h, then vacuum evaporated, and the residue was washed with alcohol and then filtered, yielding the final product XI. $M^{+\cdot}$ 219.

5-Cyano-6-(N,N-dimethylaminomethylene)amino-7-ethoxycarbonyl-1,2-dihydro-3H-pyrrolizine (XII). A 43-g (290 mmole) portion of VIII was added dropwise over a period of 1.5 h to a solution of 50 g (228 mmole) of XI in 350 ml of DMFA at a temperature of 100°C. The mixture was then heated for an additional 30 min at the same temperature. The reaction mixture was then evaporated and the residue was triturated with isopropanol, yielding the final product XII. $M^{+\cdot}$ 274.

9-Ethoxycarbonyl-1,2,5,6-tetrahydro-3H-pyrimido[5,4-e]pyrrolizine-5-thione (X). A. A 1.54-g portion (5 mmole) of IX was heated for 2 min at 140°C, then cooled and recrystallized from DMFA with charcoal. The yield of X was 7%. $M^{+\cdot}$ 263.

B. A 100-ml portion (150 mmole) of a 10% NH_4HS solution was added to a suspension of 19.8 g (72 mmole) of XII in 80 ml of 50% aqueous alcohol, and kept at room temperature for 24 h. The precipitate was filtered and washed with water and alcohol, yielding the final product X.

5-Methylmercapto-9-ethoxycarbonyl-1,2-dihydro-3H-pyrimido[5,4-e]pyrrolizine (XIIIa). A solution of 0.36 g (9 mmole) of NaOH in 40 ml of abs. alcohol was added to a suspension of 2.37 g (9 mmole) of X in 40 ml of abs. alcohol, and agitated at room temperature for 15 min. The mixture was then boiled for another 15 min after which 1.28 g (9 mmole) of MeI were added. The mixture was then vacuum evaporated and the residue was washed with water and filtered, yielding the final product XIIIa. $M^{+\cdot}$ 277.

5-Ethoxycarbonylmethylmercapto-9-ethoxycarbonyl-1,2-dihydro-3H-pyrimido[5,4-e]pyrrolizine (XIIIb) was obtained in the same manner as XIIIa from compound X and monochloroacetic ethylate. Following evaporation, the residue was triturated with hexane. $M^{+\cdot}$ 349.

5-Carboxymethylmercapto-9-ethoxycarbonyl-1,2-dihydro-3H-pyrimido[5,4-e]pyrrolizine (XIIIc). A 2.63-g (10 mmole) portion of X was added to a solution of 6 g (63 mmole) of monochloroacetic acid in 40 ml of water and boiled for 3 h. The mixture was then cooled and brought up to pH 5.0 with 40% NaOH. The precipitate was then filtered, yielding product XIIIc. $M^{+\cdot}$ 321.

5-Benzylmercapto-9-ethoxycarbonyl-1,2-dihydro-3H-pyrimido[5,4-e]pyrrolizine (XIIId) was obtained in the same manner as XIIIb from compound X and benzyl chloride. $M^{+\cdot}$ 353.

9-Carboxy-1,2,5,6-tetrahydro-3H-pyrimido[5,4-e]pyrrolizin-5-one (XIV). A 6.4-g (20 mmole) portion of XIIIc was added to 40 ml of 6 N HCl and boiled for 6 h. The resultant precipitate was then filtered, yielding the product XIV. $M^{+\cdot}$ 219.

The physical properties, yields, and elemental analysis data of the compounds are given in Table 1. IR spectral data for compounds II, III, IV, VI, VIIa-c, and IX-XII are given in Table 2, and UV spectral data for compounds X and XIIIa-d are given in Table 3.

EXPERIMENTAL BIOLOGICAL

Five experiments were conducted in order to study the antitumor activity of the synthesized compounds on 60 mongrel male rats with Jensen's sarcoma, 162 BDF_1 male mice with melanoma B-16, carcinoma 755, and Lewis' bronchial carcinoma. The compounds were administered daily in a 10% polyvinylpyrrolidone solution for 11 days, beginning 24 h after the tumor transplant. The animals were killed by ether 24 h after the last administration of the compounds. Tumor mass and changes in the animals' body mass were evaluated throughout the experiment. Determinations were made of the index of tumor growth inhibition (T, %) and the body growth coefficient (Kg) which reflected changes in the experimental animals' body mass during the treatment in comparison to the control animals' mass. A positive Kg indicated a greater mass increment during the experiment than in the control, and a negative Kg indicated a smaller mass than in the control [4].

The cited data showed (Table 4) that the compounds under study are nontoxic when administered IP one time. The lethal dose is greater than 500 mg/kg. However, compound XIIIa exhibits cumulative toxicity, i.e., the animals died after three administrations at a dose of 250 mg/kg. Compounds which showed no activity against melanoma B-16 in the mice were not studied any further (X, XIIIc). Compounds XIIIa, b, and d were found to exhibit antitumor activity in the mice with melanoma B-16 (T = 40-50%). Compound XIIIb was also active against carcinoma 755 (T = 34%), and compound XIIId was also active against Lewis' bronchial carcinoma (T = 43%). The results obtained indicate that the antitumor compounds in this series may be quite promising.

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