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SYNTHESIS AND MUTAGENIC ACTION OF TETRAHYDROPYRANYL- AND TETRAHYDROTHIOPYRANYL PYRAZOLES AND PYRIMIDINES

R. S. Vartanyan, R. S. Shaginyan, Zh. V. Kazaryan, G. M. Paronikyan, T. P. Sarkisyan, and S. A. Vartanyan

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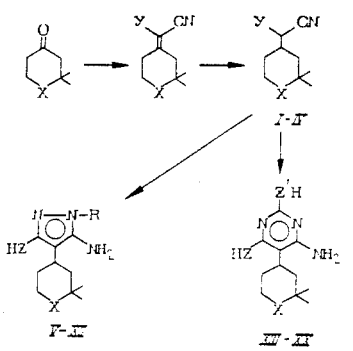
Derivatives of pyrazoles and pyrimidines are generally recognized to be of considerable interest because of their biological action, and they are widely used in medicine as therapeutic drugs. Moreover, compounds with diversified biological properties have been found among the bicyclic derivatives of tetrahydropyran [1]. In that connection we felt it would be of interest to synthesize the tetrahydropyranyl- and tetrahydrothiopyranyl-substituted derivatives of pyrazoles and pyrimidines. The indicated bicyclic compounds with C–C linked rings can be obtained by using the functional substituted tetrahydropyrans and tetrahydrothiopyrans in function position 4 that are capable of heterocyclization. Selected for this purpose were the β-carethoxynitrile and β-dinitrile groups. We then devised a method for synthesizing the tetrahydropyranyl- and tetrahydrothiopyranyl substituted esters of cyanoacetic acid (I, II) and malonic acid dinitriles (III, IV) which were obtained for the first time by the sodium borohydride reduction of the appropriate ilidene derivatives [3]. Compounds I-IV were reacted with 1,2- and 1,3-binucleophiles, i.e., hydrazine, phenylhydrazine, urea, and thiourea, in order to synthesize the target pyrazoles and pyrimidines (V-XX).



X = O (I, III, V, V , IX, X, XIII, XIV, XVII, XVIII), S (II, IV, VII, VIII, XI, XII, XV, XVI, XIX, XX); Y = COOEt (I, II), CN (III, IV); Z = O (V—VIII, XIII—XVI), NH (IX—XII, XVII—XX); Z^1 = O (XIII, XV, XVII, XIX), S (XIV, XVI, XVIII, XX); R = H (V, VII, IX, XI), Ph (VI, VIII, X, XII).

The IR spectra of the resultant pyrazoles V-XII exhibited absorption bands at 1530 and 1560 cm^{-1} that are characteristic of the aromatic pyrazole ring, at 1600 cm^{-1} which is characteristic of an aromatic benzene ring (for compounds VI, VII, X, and XII), at 1630 cm^{-1}

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which is characteristic of deformation vibrations of the NH_2 group. Bands were also observed at 3200, 3300, and 3420 cm^{-1} (NH_2, NH, OH).

Absorption bands were found in the IR spectra of the pyrimidines XIII-XX at 1270 cm^{-1} (C=S), 1670-1680 cm^{-1} (C=O), 1670 and 1700 cm^{-1} (C=O) for compounds XV and XVII as well as at 3170-3180, 3240, 3330, and 3550 cm^{-1} (NH and NH_2) which indicated that they exist in the oxo-, thio-, oxothio-, and dioxo- forms.

Biological tests of the synthesized pyrazoles V-XII indicated that they do not exhibit analgesic, antipyretic, antiinflammatory or diuretic activity. They did exhibit, however, weak local anesthetic properties. These were substances among those compounds, however, which exhibited mutagenic activity against the threonine locus of E. coli and the lysine locus of actinomycetes. Antimutagenic activity was exhibited in a number of the pyrimidine series compounds.

EXPERIMENTAL (CHEMICAL)

GLC was performed on a Khrom-4 chromatograph (Czechoslovakia) on glass packing columns in which the liquid phase was a 5% silicone KhE-60 solution on N-AW chromaton silanized by HMDS. TLC was performed on Silufol UV-254 plates. Iodine vapor was used for developing. The IR spectra were recorded on a UR-20 instrument (GDR), the PMR spectra were recorded on a Varian T-60 spectrometer (USA) with TMS as the internal standard. Mass spectra were recorded on a MKh-1320 (USSR) with direct feeding of the sample into the ionization chamber.

Ethyl [2,2-Dimethyl-4-tetrahydro(thio)pyranyl]cyanoacetates I, II) and Dinitrile [2,2-Dimethyl-4-tetrahydro(thio)pyranyl]malonates (III, IV). A 1.9 g (0.05 mole) portion of sodium borohydride was added in small portions to a solution of 0.1 mole of the corresponding (2,2-dimethyl-4-tetrahydropyralylidene or tetrahydrothiopyranylidene)cyanoacetate or malonodinitrile in 100 ml of ethyl alcohol so that the reaction mixture temperature did not exceed 20°C. The mixture was stirred for 1 h at room temperature and the alcohol was distilled off. A 100 ml portion of water was added to the residue which was then extracted with ether. After the residue was dried with magnesium sulfate and the solvent was distilled off, the residue was vacuum distilled. The constants of the resultant compounds are given in Table 1.

1-Substituted-3-amino-(oxy)-5-amino-4-[2,2-dimethyl-4-tetrahydro(thio)pyranyl]pyrazoles (V-XII). A mixture of 0.01 mole of the corresponding substituted cyanoacetate ester I, II or malonodinitrile III, IV and 0.01 mole of phenylhydrazine or an 85% aqueous solution of hydrazine hydrate was boiled for 1.5-2 h and cooled, after which 10-15 ml of ether was added. The precipitated crystals were filtered and washed with ether. The constants of the synthesized compounds are given in Table 2.

4-Amino-6-amino-(oxy)-2-mercapto(oxy)-5-[2,2-dimethyl-4-tetrahydro(thio)pyranyl]pyrimidines (XIII-XX). A mixture of 0.01 mole of an appropriate substituted cyanoacetate ester I, II or dinitrile malonate III, IV, 0.01 mole of urea or thiourea, and a sodium alcoholate solution obtained from 0.01 mole of sodium in 10 ml of methanol or ethanol, was evaporated to dryness on a water bath. The residue was dissolved in 10 ml of hot water to which 0.6 ml of glacial acetic acid was then added. The precipitated crystals were filtered off, washed with water, and dried in a vacuum dessicator. The constants of the synthesized compounds are given in Table 2.

EXPERIMENTAL (BIOLOGICAL)

The genetic action of the pyrazole and pyrimidine derivatives was studied by the dose-effect method on biochemical strains of E. coli P-678 thr^- and Actinomyces rimosus 22 lys^-. The mutagenic and antimutagenic activity of the compounds under study was assayed by the frequency at which revertants from the auxotrophic to prototrophic state were encountered along the loci responsible for the threonine and lysine synthesis. The mutagenic action of all 16 compounds was tested at a dose of 100 mmole and the test culture was treated for 120 min. The antimutagenic action was studied at a dose of 10 mmole at an exposure time of 10 min [4]. Spontaneously occurring mutations were used as the control.

The effect that these same compounds had on UV-induced mutations was examined on the same test objects. UV irradiation was accomplished by a BUF-30 bacteriocidal lamp at a distance of 60 cm from the light emission source at room temperature with constant stirring for 90 sec. The experiments were performed in a darkroom in red light [2]. When the test

TABLE 1. Physicochemical Properties of Compounds I-IV

Compound	Yield, %	bp, °C/ mm Hg	n_D^{20}	Found, %				Empirical formula	Calculated, %				IR spectrum ν, cm^{-1}	PMR spectrum (in CCl_4), δ, ppm
				C	H	N	S		C	H	N			
I	62,7	144—145/4	1,4640	64,38	8,15	6,33	—	$C_{12}H_{19}NO_3$	63,98	8,50	6,22	—	1765 (C=O), 2265 (C≡N),	3,35 d, J = 6 Hz (CH—CN)
II	61,8	156—157/3	1,4990	59,97	8,13	6,11	13,14	$C_{12}H_{19}NO_2S$	59,73	7,94	5,80	13,29	1765 (C=O), 2265 (C≡N)	3,43 d, J = 6 Hz (CH—CN)
III	62,3	132—133/2	—	67,76	8,12	15,63	—	$C_{10}H_{14}N_2O$	67,39	7,92	15,72	—	2260 (C≡N)	3,65 d, J = 6 Hz (CH—CN)
IV	58,2	98—99 (mp)	—	61,64	7,40	14,63	16,23	$C_{10}H_{14}N_2S$	61,86	7,26	14,42	16,50	2260 (C≡N)	3,73 d, J = 6 Hz (CH—CN)

TABLE 2. Physicochemical Properties of Compounds V-XX

Compound	Yield, %	mp, °C	R_f	Found, %				M*	Empirical formula	Calculated, %				M
				C	H	N	S			C	H	N	S	
V	70,0	225—6	0,56[a]	57,09	8,43	19,88	—	211	$C_{10}H_{17}N_3O_2$	56,85	8,11	19,89	—	211
VI	68,0	238—9	0,43[b]	66,70	7,40	14,80	—	287	$C_{16}H_{21}N_3O_2$	66,88	7,37	14,62	—	287
VII	60,2	132—3	0,63[a]	52,70	7,23	18,61	14,30	227	$C_{10}H_{17}N_3OS$	52,83	7,54	18,48	14,10	227
VIII	63,3	208—9	0,55[c]	63,71	7,08	13,67	10,22	303	$C_{16}H_{21}N_3OS$	63,33	6,98	13,85	10,57	303
IX	62,4	182—3	0,52[b]	57,20	8,70	26,70	—	210	$C_{10}H_{18}N_3O$	57,12	8,63	26,64	—	210
X	66,7	203—4	0,49[a]	67,50	7,70	19,90	—	286	$C_{16}H_{22}N_3O$	67,10	7,74	19,56	—	286
XI	73,9	172—3	0,60[a]	53,20	7,55	24,77	13,80	226	$C_{10}H_{18}N_3S$	53,06	7,96	24,75	14,16	226
XII	56,7	183—4	0,61[c]	63,40	7,60	18,68	10,40	302	$C_{16}H_{22}N_3S$	63,54	7,33	18,52	10,60	302
XIII	50,0	347—8	0,49[d]	54,88	6,81	17,42	—	239	$C_{11}H_{17}N_3O_3$	55,22	7,16	17,56	—	239
XIV	64,7	313—4	0,56[d]	52,02	6,35	16,30	12,10	255	$C_{11}H_{17}N_3O_2S$	51,74	6,71	16,46	12,56	255
XV	51,6	307—8	0,52[d]	52,06	6,95	16,84	12,80		$C_{11}H_{17}N_3O_2S$	51,74	6,71	16,46	12,56	255
XVI	67,0	288—9	0,55[d]	48,84	6,10	15,10	23,29	271	$C_{11}H_{17}N_3OS_2$	48,68	6,31	15,48	23,63	271
XVII	61,5	Decomposition over 380	0,46[d]	55,79	7,84	23,72	—		$C_{11}H_{18}N_4O_2$	55,45	7,61	23,51	—	238
XVIII	63,0	323—4	0,65[d]	52,00	6,99	21,95	12,20	254	$C_{11}H_{18}N_4OS$	51,94	7,13	22,02	12,61	254
XIX	60,0	Decomposition over 300	0,53[d]	51,69	6,91	21,57	12,60		$C_{11}H_{18}N_4OS$	51,94	7,13	22,02	12,61	254
XX	54,4	309—10	0,69[d]	48,70	6,40	20,76	23,35	270	$C_{11}H_{18}N_4S_2$	48,86	6,71	20,72	23,71	270

M value given (mass-spectrometrically).

[a] In a 1:3 DMPa—methanol system.

[b] In a 2:1 acetone—hexane system.

[c] In a 1:1 acetone—hexane system.

[d] In a 7:2:5 butanol—ethanol—25% NH_4OH system.

TABLE 3. Effect of Test and Control Compounds on Cell Survival Rate and Mutations in the Test Objects

Compound	E. coli P-678 thr⁻			Actinomyces rimosus 222 lys⁻		
	survival rate, %	revertant encounter rate per 10^6 survived cells		survival rate, %	revertant encounter rate per 10^5 survived cells	
		abs.	% of control		abs.	% of control
VI	8.5	78±9,2	1 300	30	8,8±0,9	220
IX	10	36±4.2	600	36	6±0,5	150
X	11	45±6.5	750	55	6,7±0,8	166
XI	5	672±55	11 200	6,6	332±41	8300
XII	26	30±3,5	500	33	9±0,85	225
EI	0.1	219±18,6	36 500	1	344±38,5	8100
Control	100	6±0,55	100	100	4±0,6	100
XIII	81	11,4±1,3	162	86	4,4±0,5	87*
XIV	108	4±0,3	57	104	2,9±0,25	57
XV	125	5,5±0,45	78	84	4,75±0,6	95*
XVI	94	5,25±0,45	75	100	7,5±0,6	150
XVIII	79	9,1±0,8	130	83	3,8±0,4	75
MEA	96	3,78±0,5	54,1	45,4	2,64±0,35	52.7
Cystamine	97	6,5±0.7	93*	106	2,65±0,25	53
Control	100	7±0.8	100	100	5±0,45	100
XIV	82	3,75±0,4	75	86	3,2±0,4	80
MEA	180	1,03±0,1	20,5	175	2,34±0,3	58,4
Cystamine	86	5,9±0.7	118	127	3±0,45	74,2
Control (UV-irradiation)	100	5±0,6	100	100	4±0,45	100

*Data statistically uncertain.

cultures were exposed to combined action, one type of treatment was directly followed by the other (protector + UV irradiation). The microorganisms were treated with the test compounds at a dose of 10 mmole for 10 min. The frequency at which mutations took place during the UV-light treatment of the objects was used as the control [5]. The known mutagen ethyleneimine (EI) and the protectors 2-mercaptoethylamine (MEA) and cystamine were used as the positive controls.

The experimental results were evaluated by counting the number of revertant colonies in the control and experimental versions in relation to the cell survival rate in each version. The averaged results cited in Table 3 were obtained after three to four repetitions of the experiments in which three dishes were taken for each experimental version. The truth of the induced reverse mutations was verified by a triple passage onto a minimal medium.

The results of our study of the test compounds' mutagenic action against the threonine locus of E. coli showed that only certain derivatives of tetrahydropyranyl- and tetrahydrothiopyranyl pyrazoles exhibit mutagenic activity (see Table 3). Notable activity was exhibited by compound XI which induced mutations 112 times greater than did the control (spontaneous mutation). Compound XI from among the pyrazole derivatives also exhibited moderate mutagenic activity against the lysine locus of actinomycetes in that it induced mutations 83 times greater than the control.

A few of the tetrahydropyranyl- and tetrahydrothiopyranyl pyrimidine derivatives exhibited antimutagenic activity. With respect to the E. coli threonine locus a reliable level of protector activity was exhibited by compounds XIV, XVI, and XV. At a high bacterial cell survival rate the mutations induced by those compounds were 43%, 25%, and 22% less than the spontaneously occurring mutations in the control versions. Compounds XIV and XVII exhibited notable antimutagenic action with respect to the lysine locus of actinomycetes in that they induced 43% and 25% less mutations respectively than the control.

With respect to antimutagenic activity, the test compounds exhibited activity that was either equal to or less than that shown in the positive controls.

We also examined the effect that tetrahydropyranyl- and tetrahydrothiopyranyl pyrimidines and the control protectors had on UV-induced mutations in the test objects. These

experiments showed that of all the examined compounds only compound XIV, i.e., 6-amino-2-mercapto-4-oxy-5-(2,2-dimethyl-4-tetrahydropyranyl)pyrimidine, most strongly suppressed spontaneous mutation in the test cultures (see Table 3) in which case it exhibited a protective action whereby it reduced the number of mutations resulting from UV rays by 25% and 20% in comparison to the control (see Table 3).

Thus, the variable degree of observed mutagenic and antimutagenic activity would seem to warrant further study among the tetrahydropyranyl- and tetrahydrothiopyranyl substituted pyrazole and pyrimidine derivatives for the purpose of finding highly active mutagens and antimutagens.

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SYNTHESIS AND ANTI-MONOAMINE OXIDASE ACTIVITY OF N^1-(4-PYRIMIDINOYL)-N^2-BENZYLHYDRAZINES

P. I. Vainilavichyus, V.-S. M. Rochka, G. D. Myakushkene, N.-D. I. Lautsyuvene, and R. Yu. Savitskene

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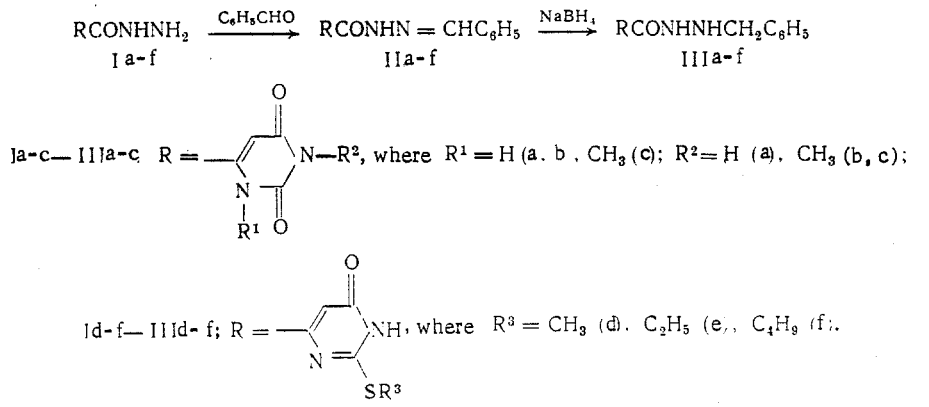
Comparison of the therapeutic activity of monoamine oxidase (MAO) inhibitors and other antidepressants shows that with certain forms of depression MAO inhibitors are highly effective [5]. Therefore, despite the possible side effects [5], MAO inhibitors belonging to various classes of organic compounds continue to be of interest to researchers.

It was reported [1, 9] that arylidenehydrazides of 4-pyrimidinecarboxylic acids in experiments in vitro have anti-MAO activity. In order to investigate new MAO inhibitors we carried out the synthesis of the benzylidenehydrazides of 4-pyrimidinecarboxylic acids (II) and studied the possibility of reducing them with sodium borohydride to the corresponding hydrazines (III).



V. Kapsukas Vil'nyus University. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 22, No. 4, pp. 421-424, April, 1988. Original article submitted December 9, 1986.

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