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Synthesis and Antibacterial Effect of Derivatives of 5-(3,4,5--Trimethoxybenzyl)-pyrimidine, -Tetrahydropyrimidine, -Hexahydropyrimidine and -Hydantoin

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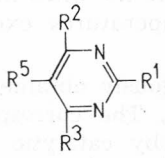
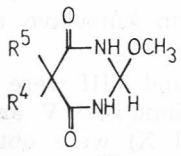
Pyrimidine derivatives I (R^1, R^2, R^3 = Cl, Cl, Cl; Cl, OCH_3, Cl; OCH_3, OCH_3, Cl; OCH_3, OCH_3, OCH_3; NH_2, OCH_3, Cl; OCH_3, NH_2, OCH_3; NH_2, H, H; H, NH_2, H; H, OH, OH; H, Cl, Cl; H, NH_2, NH_2 resp.), hexahydropyrimidine-4,6-diones II and III (R^4 = CH_3, C_2H_5, C_3H_7, $CH_2CH = CH_2$, C_4H_9), tetrahydropyrimidine-4,6-diones IV (R^4 the same) and hydantoins V (R^4 the same) were synthesized.



I



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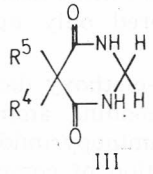

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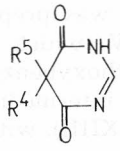

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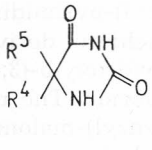
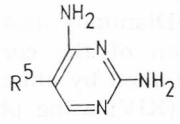


V



VI

R^5 = 3,4,5-trimethoxybenzyl

In vitro antibacterial activity of these compounds was tested against some bacteria strains and compared with that of the well known bacteriostatic trimethoprim VI.

The activity of compound I (R^1 = H, $R^2 = R^3$ = OH) was higher than that of trimethoprim against Sarcina lutea ATCC 9341, Klebsiella pneumoniae ATCC 10031 and Pseudomonas aerugiosa NCTC 10490 while the compounds of group IV acted also against Corynebacterium xerosis NCTC 9755, E. coli ATCC 10536 and Shigella flexneri.

INTRODUCTION

Continuing our research on the antibacterial activity of 5-(3,4,5-trimethoxybenzyl)-pyrimidines[1] and -barbiturates[2], we have synthesized new groups of substituted pyrimidines, hexahydropyrimidines, tetrahydropyrimidines and hydantoins and have tested their antibacterial effect. It is known[3,4] that 3,4,5--trimethoxybenzyl group represents a substantial subunit in the broad-spectrum antibacterial drug trimethoprim and, therefore, it was interesting to study its analogous compounds following the variation of the heterocyclic moiety. On the other hand, variation of the heterocyclic moiety in this structure seemed to us a worthwile research objective, since substituted heterocycles themselves possess various biological activities[5,6].

Thus, in the group of 2,4,6-trisubstituted 5-(3,4,5-trimethoxybenzyl)-pyrimidines (I—X) we have prepared various chloro, methoxy and amino derivatives. In the group of 5-(3,4,5-trimethoxybenzyl)-hexa- (XXIa—XXIh) and -tetrahydropyrimidine-4,6-diones (XVIIIa—XVIIIe) we have prepared 5-alkyl-, 5-alkenyl- or 5-(3,4,5-trimethoxybenzyl)-derivatives, and the analogous series of 2-methoxyhexahydropyrimidin-4,6-diones (XVIIa—XVIIe). Analogously substituted hydantoine derivatives (XIXa—XIXe) have been also prepared.

RESULTS

Synthesis of New Compounds

Mono-, di- and trimethoxyderivatives of 5-(3,4,5-trimethoxybenzyl)-pyrimidine (II—IV) were obtained by methoxylation of 2,4,6-trichloro derivate (I). As expected[7], mono and disubstitution occurred easily while the third methoxy group entered only upon heating in autoclave at temperatures exceeding 100 °C.

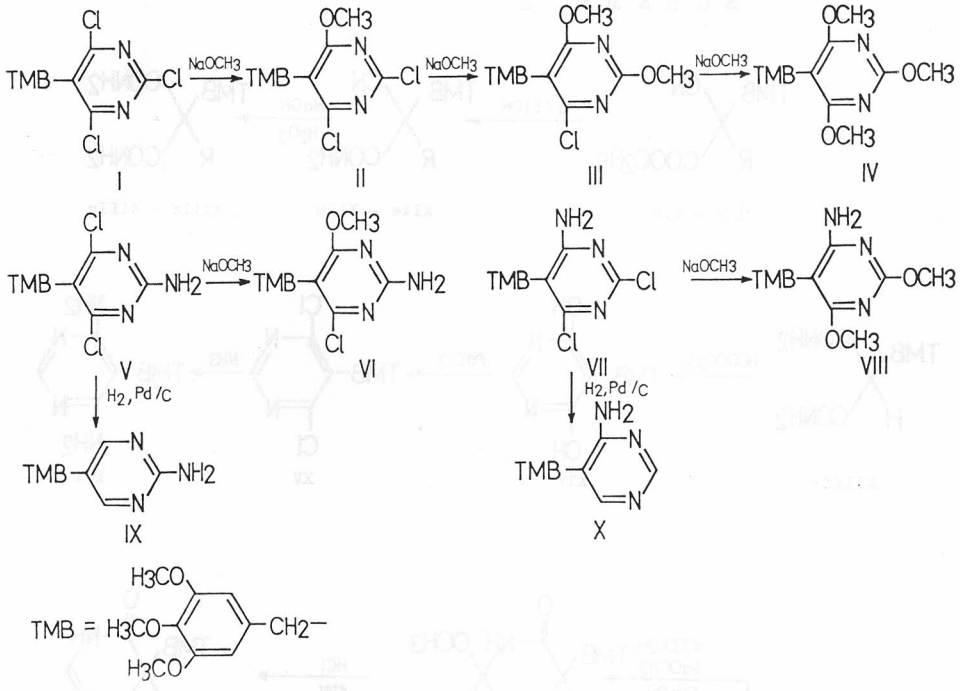
Amino-methoxy derivatives VI and VIII were analogously obtained from the corresponding amino-chloro-pyrimidines[1] V and VII. The corresponding 2- and 4-aminopyrimidines (IX and X) were obtained by catalytic hydrodehalogenation of compounds V and VII, using hydrogen in the presence of Pd/C.

4,6-Diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine (XVI) was prepared by amination of the corresponding 4,6-dichloro derivative XV, which in turn was obtained by chlorination of 4,6-dihydroxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine (XIV) using phosphorous oxychloride. The necessary intermediate XIV was obtained from 2-(3,4,5-trimethoxybenzyl)-malondiamide (XIIIf), with ethylformate in the presence of sodium methylate.

To obtain 5-substituted derivatives of 2-methoxy-5-(3,4,5-trimethoxybenzyl)-hexahydropyrimidin-4,6-dione (XVIIa—XVIIe), the corresponding tetrahydropyrimidines (XVIIIa—XVIIIe) and hydantoins (XIXa—XIXe), 2-substituted esters of 2-(3,4,5-trimethoxybenzyl)-cyanoacetic acid (XIa—XIh) were used as the starting compounds.

Compounds XIa—XIh were first transferred into the corresponding cyanoamides XIIa—XIIh then into the malondiamides (XIIIa—XIIIg) by partial hydrolysis with sodium hydroxyde and hydrogen peroxide in aqueous solution (Scheme 2). By cyclization of 2-substituted derivatives of 2-(3,4,5-trimethoxybenzyl)-malondiamide (XIIIa—XIIIe) with ethylformate and sodium methylate in methanol, the corresponding 2-methoxyhexahydropyrimidine-4,6-diones

SCHEME 1



(XVIIa—XVIIe) were prepared. Elimination of methanol with hydrogen chloride in dry ether from compounds XVIIa—XVIIe afforded the corresponding tetrahydropyrimidine-4,6-diones (XVIIIa—XVIIIe). Malondiamides XIIIa—XIIIe reacted with sodium hypochlorite in an alkaline medium to give 5-substituted derivatives of 5-(3,4,5-trimethoxybenzyl)-hydantoin (XIXa—XIXe) (Scheme 2).

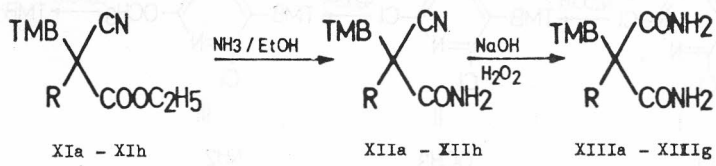
5-(3,4,5-Trimethoxybenzyl)-hexahydropyrimidine-4,6-dione and its 5-substituted derivatives (XXIa—XXIh) were prepared by reductive desulfuration of the corresponding thiobarbituric acids[2] (XXa—XXh) with the nascent hydrogen (Scheme 2).

Antibacterial Activity

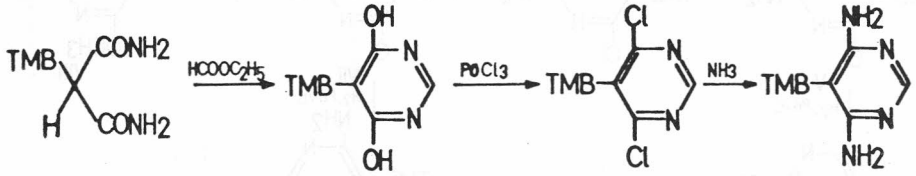
Antibacterial activity of the new compounds and some intermediates in their preparation, was tested applying the standard disk-diffusion method and dilution method[8,9]. The results obtained by disk-method are presented in Table I. The activity was tested on a few strains of Gram-positive and Gram-negative bacteria and compared to that of well known bacteriostatic trimethoprim, inhibitor of enzyme dihydrofolatereductase (DHFR)[4].

Compound I showed the antibacterial spectrum similar to that of trimethoprim, but the level of activity was substantially lower, with the exception of the comparable activities against *Brucella bronchiseptica* ATCC 4617 and *Staphylococcus aureus* of the animal origin. Compounds II—IV exhibited antibacterial activity against a number of bacterial strains at the concentra-

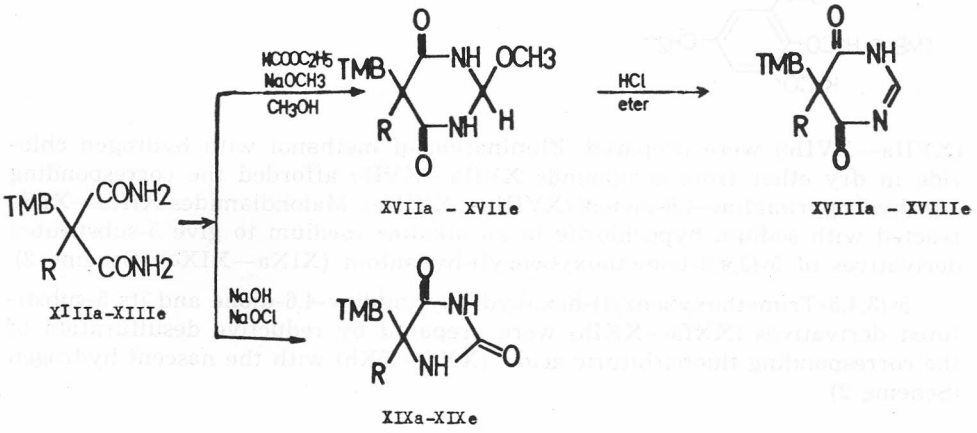
S C H E M E 2

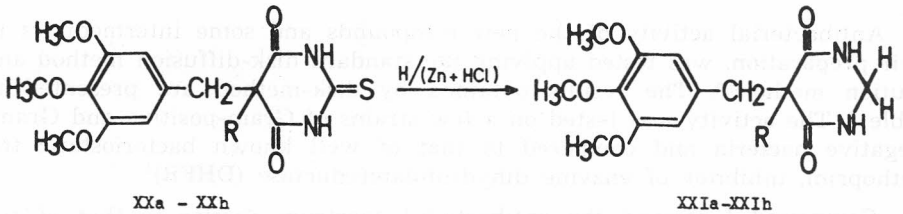


XIa - XIh XIIa - XIIh XIIIa - XIIIg



XIIIf XIV XV XVI





XXa - XXh XXIa-XXIh

	R
a	CH_3
b	C_2H_5
c	$n\text{-}C_3H_7$
d	$-CH_2-CH=CH_2$

	R
e	$n\text{-}C_4H_9$
f	H
g	TMB*
h	$iso\text{-}C_3H_7$

TMB* = 3,4,5-trimethoxybenzyl

TABLE I

The Minimum Active Quantities, Expressed in μg per Disk

	I	II	III	XIV	XVIIb	XVIIIa	XVIIIb	XVIIId	XIXa	TMP[a]
Gram-positive bacterial strains										
Streptococcus faecalis ATCC 8043	>200	>300	>300	>300	100	>300	>300	>300	300	300
Staphylococcus aureus ATCC 6538-P	100	25	25	25	0.2	25	>300	>300	>300	1
Sarcina lutea ATCC 9341	50	300	300	0.1	—	0.1	1	1	150	1
Bacillus subtilis ATCC 6633	50	>300	>300	>300	0.2	500	25	50	>300	0.1
Corynebacterium xerosis NCTC 9755	50	300	300	1	0.2	25	0.1	0.1	50	0.1
Gram-negative bacterial strains										
Brucella bronchiseptica ATCC 4617	0.2	—	—	—	2	—	—	—	—	0.2
Escherichia coli ATCC 10536	>200	>300	50	1	200	0.1	0.1	0.1	150	1
Shigella flexneri* II-1819/C	—	>300	>300	50	—	50	0.1	1	150	1
Klebsiella pneumoniae ATCC 10031	50	25	25	0.1	—	0.1	0.1	0.1	25	1
Pseudomonas aeruginosa NCTC 10490	100	300	50	0.1	50	0.1	0.1	0.1	150	25

* human clinical isolate

[a] TMP = trimethoprim

tion of 25—300 μg/disk while trimethoprim acted against the same bacteria already at the concentration of 0.1—25 μg/disk.

Compound V acted only against the strain *Streptococcus pyogenes* of the animal origin, and in the quantity of 20 μg/disk.

Compound IX was completely inactive. Compounds XVIIa, XVIIc—XVIIe acted against *Staphylococcus aureus, Bacillus subtilis* and *Corynebacterium xerosis* similarly as trimethoprim, while they exhibited a much lower activity against *Escherichia coli* (200 μg/disk) than trimethoprim (1 μg/disk).

The activity of the group of compounds XVIIIa, XVIIIb and XVIIId against Gram-negative bacteria is very high, e. g. compound XVIIIb acted against *Escherichia coli, Shigella flexneri, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* already in the quantity of 0.1 μg/disk, while trimethoprim acted on the same bacteria in the quantity of 1μg, 25 μg/disk.

Activity of compound XVIIIb on the strains *Sarcina lutea* and *Corynebacterium xerosis* is comparable to that of trimethoprim, while its activity against other strains was somewhat lower.

Compounds XVIII generally exhibited a rather low activity, while compound XIXa was active only against *Klebsiella pneumoniae* at the level of 25 μg/disk. Compound XIV exhibited interesting results, i. e. against three strains of bacteria (*Sarcina lutea, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) its activity was higher than for trimethoprim, while against other strains (*Bacillus subtilis* and *Shigella flexneri*) the activity was lower. Compounds XVIIa, XVIIc—XVIIe, XVIIIc, XVIIIe and XXI were tested by the dilution method. Some of the results obtained are presented in Table II.

Compounds from group XVII acted in somewhat lower concentrations against Gram-negative bacteria (50—75 μg/ml) than against Gram-negative bacteria (75 μg/ml), while trimethoprim, used for comparison purposes, displayed an action within the range from 10 to 100 μg/ml. The best action shown by compound XVIId. Against *Staphylococcus aureus, Micrococcus flavus, Sarcina lutea* and *Pseudomonas aeruginosa* it acted in a concentration of 50 μg/ml and against other tested strains in a concentration of 75 μg/ml. Against these strains trimethoprim acted in concentrations of 50, 10, 10 and 50 μg/ml, respectively.

Compound XVIIIe showed a somewhat better effect than compound XVIIIc (50—75 μg/ml), which is within the limits of trimethoprim action. Only against *Micrococcus flavus* and *Sarcina lutea* trimethoprim showed a better action (10 μg/ml) than compound XVIIIe.

Compounds from group XXI acted against the strain *Streptococcus haemolyticus* of human origin in a concentration of 10 μg/ml, and on other strains in a concentration of 75—200 μg/ml, weaker than trimethoprim.

DISCUSSION

The results obtained show that 3,4,5-trimethoxybenzyl derivatives of chlorinated pyrimidines possess a somewhat lower antibacterial effect than trimethoprim and that no significant altering of the activity takes place if chlorine is substituted either by methoxy or amino group. Derivatives with one or two amino groups in positions 2, 4 and 6 of the pyrimidine ring prooved to be inactive. Derivatives with hydroxy groups in positions 4 and 6 of the pyrimidine ring were found significantly active against *Sarcina lutea, Kleb-*

TABLE II

Determination of Minimum Inhibitory Concentrations (MIC) by Dilution Method (Results Expressed in μg/ml)

Bacteria strains	XVIIa	XVIIc	XVIId	XVIIe	XVIIIc	XVIIIe	TMP[a]
Gram-positive							
Streptococcus faecalis ATCC 8043	75	75	75	75	100	75	100
Staphylococcus aureus ATCC 6538-P	75	75	50	75	100	75	50
Micrococcus flavus ATCC 10240	75	50	50	50	100	50	10
Sarcina lutea ATCC 9341	50	50	50	75	50	50	10
Bacillus cereus variatis mycoides ATCC 11778	75	75	75	75	100	75	75
Bacillus subtilis ATCC 6633	75	75	75	75	100	75	50
Gram-negative							
Pseudomonas aeruginosa ATCC 9027	50	50	50	50	100	50	50
Escherichia coli 113-3 DAVIS ATCC 11105	75	75	75	75	100	75	50
Escherichia coli — human isolate	75	75	75	75	100	75	50
Klebsiella pneumoniae — human isolate	75	75	75	75	50	75	50

[a] TMP = trimethoprim

siella pneumoniae and *Pseudomonas aeruginosa*, and some other strains. Our findings described here are in accordance with previous results[3], where only compounds with the free position 6 of the pyrimidine ring proved to be active. An apparent exception to this rule is the 4,6-dihydroxypyrimidine derivative XIV.

The activity of substituted hexahydropyrimidine-4,6-diones (XXIa—XXIh) is also lower than that of trimethoprim for all the strains tested but *Streptococcus haemolyticus* of human origin. The activity is noticeably increased on introduction of 2-methoxy group in hexahydropyrimidine-4,6-dione derivatives (XVIIa—XVIId). The effect of these compounds against *Staphylococcus aureus* is higher than that of trimethoprim, while against *Bacillus subtilis* and *Corynebacterium xerosis* they act at almost the same level.

Tetrahydropyrimidine-4,6-diones (XVIIIa—XVIIIe) could be regarded as the most active of all the tested compounds. Their activity against *Sarcina lutea* and *Corynebacterium xerosis* is comparable to that of trimethoprim, while against *Escherichia coli, Shigella flexneri, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* their activity is even higher.

Hydantoin XIXa proved to be less active than trimethoprim. Among Gram-positive bacteria we found *Sarcina lutea* and *Corynebacterium xerosis* to be very sensitive to the compounds from the group of tetrahydropyrimidine--4,6-diones (XVIIIb and XVIIId) and 4,6-dihydroxypyrimidine (XIV). Besides, selective action of 2-methoxyhexahydropyridine-4,6-dione (XVIIb) against *Staphylococcus aureus* and *Bacillus subtilis* was observed.

In the group of Gram-negative bacteria, *Escherichia coli, Shigella flexneri, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are sensitive to tetrahydropyrimidine-4,6-diones (XVIIIa, XVIIIb and XVIIId) and 4,6-dihydroxypyrimidine (XIV). Other bacteria have been found resistant to most of the tested compounds.

EXPERIMENTAL

Chemistry

2,6-Dichloro-4-methoxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine (II)

Solution of 3.63 g (0.01 mol) of 2,4,6-trichloro-5-(3,4,5-trimethoxybenzyl)-pyrimidine[1] (I) and 0.54 g (0.01 mol) of sodium methylate in 10 ml of methanol is heated for 1 h at reflux temperature and then stirred for additional 4 hrs at room temperature. Methanol is evaporated to dryness, the residue suspended in 30 ml water and the formed crystals sucked off. 3.13 g (87.4%) of crude 2,6-dichloro-4-methoxy--5-(3,4,5-trimethoxybenzyl)-pyrimidine is obtained, and recrystallized from diluted ethanol for analysis purposes.

6-Chloro-2,4-dimethoxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine (III)

Solution of 3.63 g (0.01 mol) of 2,4,6-trichloro-5-(3,4,5-trimethoxybenzyl)-pyrimidine (I) and 1.08 g (0.02 mol) of sodium methylate in 20 ml of methanol is heated for one hour at reflux temperature and then stirred for additional 4 hrs at room temperature. Methanol is evaporated to dryness, the residue suspended in 30 ml water and the formed crystals sucked off. 3.4 g (96.0%) of crude 6-chloro-2,4-dimethoxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine is obtained and then recrystallized from diluted ethanol for analysis purposes.

2,4,6-Trimethoxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine (IV)

Solution of 3.6 g (0.01 mol) of 2,4,6-trichloro-5-(3,4,5-trimethoxybenzyl)-pyrimidine (I) and 1.78 g (0.033 mol) of sodium methylate in 30 ml of methanol is heated in an autoclave for 4 hrs at 100 °C. Methanol is evaporated to dryness, the residue suspended in 30 ml water and the crystals are separated by filtration. 3.42 g (97.7%) of crude 2,4,6-trimethoxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine is obtained and recrystallized from diluted ethanol for analysis purposes.

2-Amino-6-chloro-4-methoxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine (VI)

Mixture of 3.4 g (0.01 mol) of 2-amino-4,6-dichloro-5-(3,4,5-trimethoxybenzyl)-pyrimidine[1] (V), 0.54 g (0.01 mol) of sodium methylate and 10 ml of methanol is heated for 1 h at reflux temperature and then stirred for additional 4 hrs at room temperature. Methanol is evaporated, the residue suspended in 30 ml water and the formed crystals sucked off.

2.79 g (82.4%) of crude 2-amino-6-chloro-4-methoxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine is obtained and recrystallized from diluted ethanol for analysis purposes.

4-Amino-2,6-dimethoxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine (VIII)

Mixture of 3.4 g (0.01 mol) of 4-amino-2,6-dichlor-5-(3,4,5-trimethoxybenzyl)-pyrimidine[1] (VII), 1.08 g (0.02 mol) of sodium methylate and 20 ml of methanol is heated for 1 h at reflux temperature and then stirred for additional 4 hrs at room temperature. Methanol is evaporated, the residue suspended in 30 ml water and the formed crystals sucked off.

2.75 g (82.5%) of crude 4-amino-2,6-dimethoxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine is obtained and then recrystallized from diluted ethanol for analysis purposes.

2-Amino-5-(3,4,5-trimethoxybenzyl)-pyrimidine (IX)

Solution of 3.43 g (0.01 mol) of 2-amino-4,6-dichlor-5-(3,4,5-trimethoxybenzyl)-pyrimidine[1](V) and 0.8 g (0.02 mol) of sodium hydroxide in 80 ml of 80% ethanol in the presence of 1 g of 5% Pd/C is hydrogenated in the hydrogenation apparatus of the Paar firm under hydrogen pressure of 4 bars and a temperature of 70 °C. After consumption of an equivalent quantity of hydrogen (0.02 mol), the catalyzer is sucked off, the filtrate is evaporated to dryness under lowered pressure, the residue suspended in 20 ml water and the formed crystals sucked off.

2.7 g (98.0%) of crude 2-amino-5-(3,4,5-trimethoxybenzyl)-pyrimidine is obtained and recrystallized from diluted ethanol for analysis purposes.

4-Amino-5-(3,4,5-trimethoxybenzyl)-pyrimidine (X)

Solution of 3.43 g (0.01 mol) of 4-amino-2,6-dichloro-5-(3,4,5-trimethoxybenzyl)-pyrimidine[1](VII) and 0.8 g (0.02 mol) of sodium hydroxide in 80 ml of 80% ethanol is hydrogenated in the hydrogenation apparatus of the Paar firm in the presence of 1 g of 5% Pd/C under hydrogen pressure of 4 bars and temperature of 70 °C until the consumption of an equivalent quantity of hydrogen (0.02 mol). The catalyzer is sucked off, the filtrate evaporated to dryness under lowered pressure, the residue suspended in 20 ml water and the formed crystals sucked off. 2.68 g (97.5%) of crude 4-amino-5-(3,4,5-trimethoxybenzyl)-pyrimidine is obtained and recrystallized from diluted ethanol for analysis purposes. Elemental analyses for new compounds are given in Table III.

2-Substituted 2-(3,4,5-trimethoxybenzyl)-cianoacetamide Derivatives (XIIa—XIIh)

Into the ethanol solution (150 ml) of one of the esters of 2-substituted 2-(3,4,5-trimethoxybenzyl)-cianoacetic acid[2] (XIa—XIh. 0.05 mol) gaseous ammonia is introduced until saturation and the obtained reaction mixture is stirred for 5 hrs at

room temperature. Upon ethanol evaporation and residue recrystallization from ethanol, the corresponding amides of 2,2-disubstituted cianoacetic acid XIIa—XIIh are obtained in the yield of 64.9—90.5%. Analyses data concerning these compounds are given in Table IV.

2-Substituted 2-(3,4,5-trimethoxybenzyl)-malondiamide Derivatives (XIIIa—XIIIg)

To 70 ml of 15% sodium hydroxide water solution (0.3 mol), 20 ml of 33% hydrogen peroxide (0.21 mol) and 0.04 mol of the corresponding cianoacetamide (XIIa—XIIg) are added. The obtained reaction mixture is stirred for 6 hours at a temperature of 60 °C and then cooled to 0 °C. The formed crystals are sucked off and recrystallized from ethanol and after that 2,2-disubstituted malondiamides XIIIa—XIIIg in the yield of 62.6—74.5% are obtained. Analysis data concerning these compounds are given in Table IV.

4,6-Dihydroxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine (XIV)

To the solution of 1.2 g (0.022 mol) of sodium methylate in 30 ml of methanol, 1.6 ml (0.022 mol) of ethylformate and 5.6 g (0.02 mol) of 2-(3,4,5-trimethoxybenzyl)-malondiamide (XIIIf) are added. The formed reaction mass is stirred for 6 hours at reflux temperature, methanol is evaporated, the residue dissolved in cold water (20 ml) and neutralized with diluted hydrochloric acid until pH 6. The formed crystals are sucked off and recrystallized from methanol and after that 4,6-dihydroxy-5-(3,4,5-trimethoxybenzyl)-pyrimidine (XIV, 3.4 g, 58.5%) with m. p. 242—244 °C is obtained.

4,6-Dichloro-5-(3,4,5-trimethoxybenzyl)-pyrimidine (XV)

Mixture of 2.9 g (0.01 mol) of compound XIV, 18.4 g (0.12 mol) of phosphoroxychloride and 1.7 g (0.014 mol) of *N,N*-dimethylaniline in 25 ml of benzene is heated for 2 hours at reflux temperature. Reaction mixture is then evaporated to dryness under lowered pressure, ice-water (25 ml) is added to the residue and stirred for an additional half hour. The formed crystals are sucked off and recrystalized from isopropanole and after that 4,6-dichloro-5-(3,4,5-trimethoxybenzyl)-pyrimidine (XV, 2.7 g, 82,1%) with m. p. 111—112 °C is obtained.

4,6-Diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine (XVI)

Mixture of compound XV (2.7 g, 0.0082 mol) and conc. ammonium water solution (24 ml) is heated at 130 °C for 6 hours in an autoclave under stirring. After cooling to room temperature, the residue is sucked off and washed with water. 4,6-Diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine (XVI, 2.1 g, 88.3%) is obtained which is after recrystallization from 50% ethanol melted at 265—266 °C.

TABLE III

2,4,6-Trisubstituted 5-(3,4,5-trimethoxybenzyl)-pyrimidine Derivatives

Comp.	M.p./°C	Formula	Calculated/%			Found/%		
			C	H	N	C	H	N
II	83—84	$C_{15}H_{16}Cl_2N_2O_4$	50.19	4.48	7.80	50.16	4.40	8.10
III	99—100	$C_{16}H_{19}ClN_2O_5$	54.18	5.40	7.88	54.12	5.10	8.14
IV	99—100	$C_{17}H_{22}N_2O_6$	58.28	6.33	7.99	58.26	6.24	8.20
VI	188—189	$C_{15}H_{18}ClN_3O_4$	53.04	5.33	12.36	52.81	5.04	12.30
VIII	189—190	$C_{16}H_{21}N_3O_5$	57.30	6.31	12.53	57.22	6.02	12.70
IX	145—146	$C_{14}H_{17}N_3O_3$	61.08	6.22	15.27	60.94	6.40	15.06
X	141—142	$C_{14}H_{17}N_3O_3$	61.08	6.22	15.27	61.01	5.92	15.52
XI	242—244	$C_{14}H_{12}N_2O_5$	57.53	5.52	9.59	57.42	5.79	9.70
XII	111—112	$C_{14}H_{14}Cl_2N_2O_3$	51.08	4.29	8.51	51.34	4.58	8.74
XIII	265—266	$C_{14}H_{13}N_4O_3$	57.92	6.25	19.30	58.12	6.40	19.47

TABLE IV

2-Substituted-2-(3,4,5-trimethoxybenzyl)-cianoacetamides (XIIa—XIIb) and -malondiamides (XIIIa—XIIIg)

Comp.	R	M.p./°C	Formula	Calculated/%			Found/%		
				C	H	N	C	H	N
XIIa	CH_3	145—147	$C_{14}H_{18}N_2O_4$	60.24	6.52	10.07	60.36	6.36	10.32
XIIb	C_2H_5	138—139	$C_{15}H_{20}N_2O_4$	61.63	6.90	9.58	61.38	7.15	9.85
XIIc	C_3H_7	116—117	$C_{16}H_{22}N_2O_4$	62.72	7.24	9.14	62.58	6.95	9.13
XIId	$—CH_2—CH=CH_2$	127—129	$C_{16}H_{20}N_2O_4$	63.14	6.62	9.21	63.06	6.92	9.50
XIIe	C_4H_9	103—105	$C_{17}H_{24}N_2O_4$	63.73	7.55	8.74	63.47	7.60	8.83
XIIf	H	128—130	$C_{13}H_{16}N_2O_4$	59.08	6.10	10.60	59.01	5.88	10.68
XIIg	—TMB*	150—151	$C_{23}H_{28}N_2O_7$	62.15	6.35	6.30	61.90	6.46	6.53
XIIh	i-C_3H_7	141—142	$C_{16}H_{22}N_2O_4$	62.72	7.24	9.14	62.80	7.50	9.35
XIIIa	CH_3	184—185	$C_{14}H_{20}N_2O_5$	56.74	6.80	9.45	56.52	6.59	9.54
XIIIb	C_2H_5	187—189	$C_{15}H_{22}N_2O_5$	58.05	7.15	9.03	58.14	7.06	9.28
XIIIc	C_3H_7	200—201	$C_{16}H_{24}N_2O_5$	59.24	7.46	8.64	59.19	7.17	8.80
XIIId	$—CH_2—CH=CH_2$	197—198	$C_{16}H_{22}N_2O_5$	59.61	6.88	8.69	59.62	6.69	8.91
XIIIe	C_4H_9	234—235	$C_{17}H_{26}N_2O_5$	60.34	7.74	8.28	60.08	7.45	8.44
XIIIf	H	223—224	$C_{13}H_{18}O_5N_2$	55.31	6.43	9.92	55.10	6.26	10.18
XIIIg	—TMB*	193—194	$C_{23}H_{30}N_2O_8$	59.73	6.54	6.06	59.92	6.71	6.24

* TMB— = 3,4,5-trimethoxybenzyl-

5-Substituted 2-methoxy-5-(3,4,5-trimethoxybenzyl)-hexahydropyrimidine-4,6-dione Derivatives (XVIIa—XVIIe)

Into 30 ml of anhydrous methanol 1.2 g (0.02 mol) of sodium methylate is dissolved and then 1.6 ml (0.022 mol) of ethylformate and 0.02 mol of the corresponding malondiamide (XIIIa—XIIIe) are added. The obtained reaction mixture is stirred for 6 hours at reflux temperature, methanol is evaporated, the residue suspended in 20 ml of cold water and neutralized with diluted hydrochloric acid. The formed crystals are sucked off and recrystallized from methanol. In this way 5-substituted derivatives of 2-methoxy-5-(3,4,5-trimethoxybenzyl)-hexahydropyrimidin-4,6-dione (XVIIa—XVIIe) are obtained in 64.1—75.5% yield (Table V).

5-Substituted 5-(3,4,5-trimethoxybenzyl)-tetrahydropyrimidine-4,6-dione Derivatives (XVIIIa—XVIIIe)

Corresponding 2-methoxy-5,5-disubstituted derivatives of hexahydropyrimidine-4,6-dione (XVIIa—XVIIe, 0.015 mol) are dissolved in ether (50 ml), cooled to 0—5 °C, saturated with gaseous hydrogen chloride and the obtained reaction mixture is stirred for an additional hour at the same temperature. The formed crystals are sucked off, washed with ether and recrystallized from ethanol. 5,5-Disubstituted derivatives of tetrahydropyrimidin-4,6-dione (XVIIIa—XVIIIe) in the yield of 81.5—89.3% are obtained (Table V).

5-Substituted 5-(3,4,5-trimethoxybenzyl)-hydantoin Derivatives (XIXa—XIXe)

In 80 ml of sodium hypochlorite water solution containing 8.2 g (0.11 mol) NaOCl, 4.16 g (0.1 mol) of sodium hydroxide are dissolved and then 0.03 mol of the corresponding 2-substituted 2-(3,4,5-trimethoxybenzyl)-malondiamide (XIIIa—XIIIe) is added. After dissolving, the reaction mixture is stirred for 5 hours at 50 °C, cooled to room temperature and acidified to pH 6 with 10% hydrochloric acid. The formed crystals are sucked off and recrystallized from ethanol and after that the corresponding 5-substituted 5-(3,4,5-trimethoxybenzyl)-hydanoin derivatives (XIXa—XIXe) in 44.2—51.2% yield are obtained (Table V).

5,5-Disubstituted hexahydropyrimidine-4,6-dione Derivatives (XXIa—XXIh)

Mixture of the corresponding 5-substituted 5-(3,4,5-trimethoxybenzyl)-thiobarbituric acid[2] (XXa—XXh, 0.006 mol), ethanol (40 ml) and zink in powder form (11.0 g, 0.167 gat) is heated to 35 °C and while at that temperature 50 ml (0.32 mol) of 22% hydrochloric acid is added dropwise. Reaction mixture is evaporated until 40 ml volume and poured into 100 ml water. The formed crystals are sucked off, washed with 10% sodium bicarbonate solution and recrystallized from isopropanol. Pure 5-substituted derivatives of 5-(3,4,5-trimethoxybenzyl)-hexahydropyrimidine-4,6-dione (XXIa—XXIh) are obtained, the elemental analyses for which are given in Table VI.

Biology

Antibacterial activity of the tested compounds has been examined applying the standard disk-diffusion method and the dilution method described earlier[8,9]. Prior to testing the compounds were dissolved in *N,N*-dimethylformamide, and the required concentrations were prepared by dilution phosphate buffer pH 8.

Two groups of bacteria strains were used: standard strains from the international collection of test-cultures and some clinical isolates of human origin (location Zagreb).

The test results were after 18—20 hours incubation in a thermo-chamber at the air temperature of 37 °C. Minimal inhibitory concentrations (M I C) for the dilution method are expressed in μg/ml and minimal active quantities (for the disk method) are given in μg per disk. The antibacterial activity of the new compounds has been compared to that of trimethoprim and is presented in Tables I and II.

TABLE V

5-Substituted-5-(3,4,5-trimethoxybenzyl)-2-methoxyhexahydropyrimidine-4,6-diones (XVIIa—XVIIe), -tetrahydropyrimidine--4,6-diones (XVIIIa—XVIIIe) and -hydantoines (XIXa—XIXe)

Comp.	R	M.p./°C	Formula	Calculated/%			Found/%		
				C	H	N	C	H	N
XVIIa	CH_3	154—160	$C_{16}H_{22}N_2O_6$	56.79	6.55	8.28	56.65	6.29	8.48
XVIIb	C_2H_5	130—135	$C_{17}H_{24}N_2O_6$	57.94	6.87	7.95	57.72	6.78	8.10
XVIIc	C_3H_7	140—143	$C_{18}H_{26}N_2O_6$	59.00	7.15	7.65	59.11	7.31	7.88
XVIId	$—CH_2—CH=CH_2$	190—197	$C_{18}H_{24}N_2O_6$	59.33	6.64	7.69	59.44	6.70	7.63
XVIIe	C_4H_9	175—177	$C_{19}H_{28}N_2O_6$	59.88	7.42	7.36	60.03	7.61	7.60
XVIIIa	CH_3	316—318	$C_{15}H_{18}N_2O_5$	58.81	5.92	9.15	58.60	6.07	9.40
XVIIIb	C_2H_5	284—285	$C_{16}H_{20}N_2O_5$	59.99	6.29	8.75	60.18	6.36	8.86
XVIIIc	C_3H_7	293—294	$C_{17}H_{22}N_2O_5$	61.06	6.63	8.38	60.84	6.52	8.16
XVIIId	$—CH_2—CH=CH_2$	258—260	$C_{17}H_{20}N_2O_5$	61.43	6.07	8.43	61.19	5.80	8.44
XVIIIe	C_4H_9	264—265	$C_{18}H_{24}N_2O_5$	62.05	6.94	8.04	61.97	7.21	8.16
XIXa	CH_3	186—189	$C_{14}H_{18}N_2O_5$	57.14	6.16	9.52	57.01	6.32	9.65
XIXb	C_2H_5	183—185	$C_{15}H_{20}N_2O_5$	58.43	6.54	9.09	58.35	6.57	9.33
XIXc	C_3H_7	179—182	$C_{16}H_{22}N_2O_5$	59.61	6.88	8.69	59.83	6.62	8.79
XIXd	$—CH_2—CH=CH_2$	197—200	$C_{16}H_{20}N_2O_5$	59.99	6.29	8.75	60.19	6.02	8.46
XIXe	C_4H_9	153—154	$C_{17}H_{24}N_2O_5$	60.70	7.19	8.33	60.78	7.21	8.25

TABLE VI

5-Substituted-5-(3,4,5-trimethoxybenzyl)-hexahydropyrimidine-4,6-dione Derivatives (XXIa—XXIh)

Comp.	R	M.p./°C	Formula	Calculated/%			Found/%		
				C	H	N	C	H	N
XXIa	CH_3	224—225	$C_{15}H_{20}N_2O_5$	58.43	6.54	9.09	58.19	6.40	9.27
XXIb	C_2H_5	260—264	$C_{16}H_{22}N_2O_5$	59.61	6.88	8.69	59.39	6.72	8.99
XXIc	C_3H_7	249—250	$C_{17}H_{24}N_2O_5$	60.70	7.19	8.33	60.45	7.30	8.25
XXId	$—CH_2—CH=CH_2$	250—252	$C_{17}H_{22}N_2O_5$	61.06	6.63	8.38	60.80	6.59	8.22
XXIe	C_4H_9	249—250	$C_{18}H_{26}N_2O_5$	61.70	7.48	8.00	61.52	7.24	8.29
XXIf	H	231—233	$C_{14}H_{18}N_2O_5$	57.13	6.13	9.52	57.36	6.10	9.81
XXIg	—TMB*	301—304	$C_{24}H_{30}N_2O_8$	60.75	6.37	5.90	60.50	6.40	6.09
XXIh	i-C_3H_7	271—273	$C_{17}H_{24}N_2O_5$	60.70	7.19	8.33	60.72	7.32	8.57

* TMB- = 3,4,5-trimethoxybenzyl-

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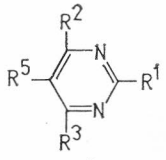
SAŽETAK

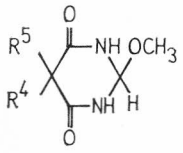
Sinteza i antibakterijsko djelovanje nekih derivata pirimidina i hidantoina

N. Kujundžić, K. Kovačević, M. Jakovina i B. Glunčić

Sintetizirani su derivati pirimidina I (R^1, R^2, R^3 = Cl, Cl, Cl; Cl, OCH_3, Cl; OCH_3, OCH_3, Cl; OCH_3, OCH_3, OCH_3; NH_2, OCH_3, Cl; OCH_3, NH_2, OCH_3; NH_2, H, H; H, NH_2, H; H, OH, OH; H, Cl, Cl; H, NH_2, NH_2 resp.), heksahidropirimidin-4,6-diona II i III (R^4 = CH_3, C_2H_5, C_3H_7, CH_2—CH=CH_2, C_4H_9), tetrahidropirimidin-4,6-diona IV (R^4 isti) i hidantoina V (R^4 isti).



I



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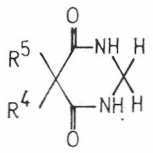

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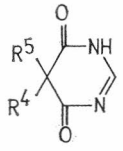

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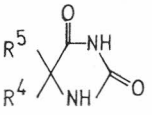

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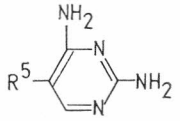

VI

R^5 = 3,4,5-trimethoxybenzyl

Spojevima je ispitano antibakterijsko djelovanje in vitro prema različitim sojevima bakterija i uspoređeno s djelovanjem poznatog bakteriostatika trimetoprima VI.

Spoj I (R^1=H, R^2=R^3=OH) aktivniji je nego trimetoprim prema Sarcina lutea ATCC 9341, Klebsiella pneumoniae ATCC 10031 i Pseudomonas aeruginosa NCTC 10490, dok su spojevi iz grupe IV aktivniji i prema bakterijama spojeva Corynebacterium xerosis NCTC 9755, E.coli ATCC 10536 i Shigella flexneri.