SYNTHESIS OF NEW 5-AMINOOROTIC ACID DERIVATIVES AS ANTIMICROBIAL AGENTS

Meriem EL KOLLI[1], Abdallah MAHAMOUD[1], Adama COULIBALY[2], Jacqueline CHEVALIER[2], Andre CREMIEUX[2], and Jacques BARBE[1]

[1]*GERCTOP-URA CNRS 1411, Faculté de pharmacie, 27, boulevard Jean Moulin, 13385 Marseille Cedex 5, France*

[2]*MICRAAM, Faculté de pharmacie, 27, boulevard Jean Moulin, 13385 Marseille Cedex 5, France*

ABSTRACT: Reaction between 5-Aminoorotic acid and alkyl or aryl halides in dimethylformamide gave esters and/or 1-alkyl substituted orotates depending upon experimental conditions. These compounds were converted into the corresponding 5-acylamino derivatives. The later derivatives were tested in vitro against gram+ and gram- bacteria. The great majority of them show significant growth inhibitory effects. Moreover, some others are specific inhibitors for gram+ bacteria.

INTRODUCTION

The biosynthesis of nucleotides is managed by several enzymes including dihydroorotase and dihydroorotate dehydrogenase which are involved in the synthesis of orotic acid from the carbamoyl aspartic acid, in procaryotic cells.

With respect to this, we intended to prepare some compounds able to inhibit the biosyntheses concerned with the aim to used them as antimicrobial agents.

RESULTS AND DISCUSSION

According to the literature, the 5-aminoorotic acid **1** reacts with arylisothiocyanates (1), N-arylthioureas (1) or aroyl halides (2) to give 5-acylamino derivatives, and reacts with alkylamines and/or arylamines to give the 2,4-disubstituted-5-amino-pyrimidine-4-carboxylic acid after conversion into the 2,4-dichloro-orotic acid.

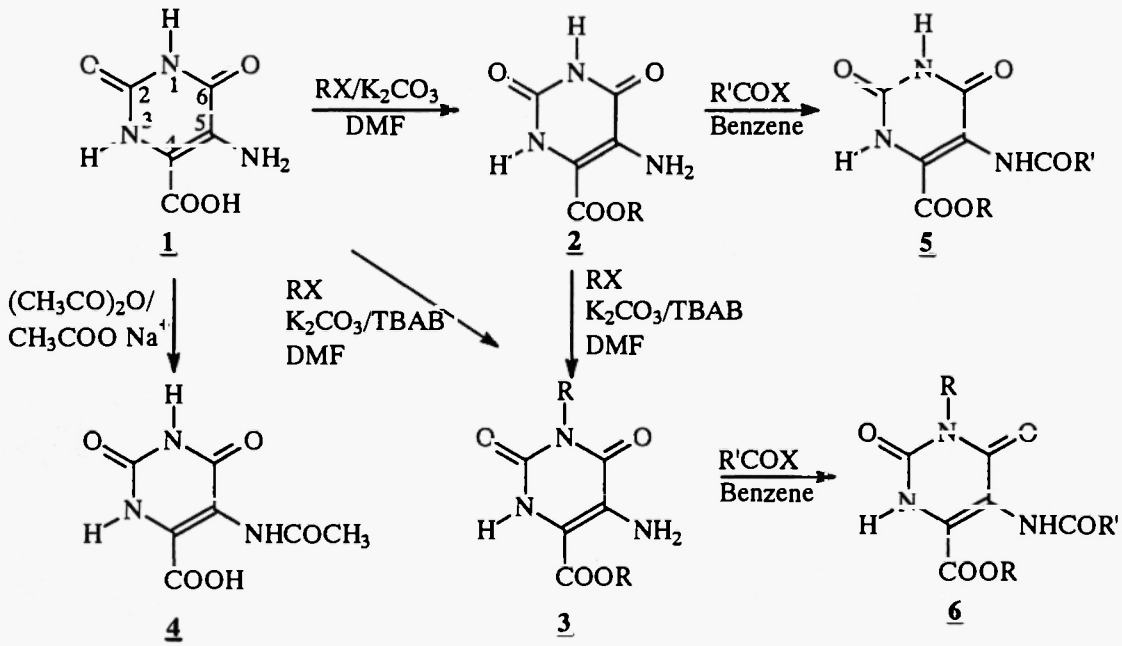
In contrast alkylation, esterification and acylation at positions 1, 4 and 5 to give 1,4,5-substituted derivatives, have never been investigated till now.

With the aim to prepare such compounds (scheme), a mixture of **1** and alkyl halide was heated in dimethylformamide at 110°C in the presence of potassium carbonate. In doing that, 5-amino-4-carboxylates orotic acid esters **2** are isolated in good yields as cristalline solids whilst the 1-alkyl-5-

amino-4-carboxylates orotic acid esters **3** are obtained when the solution in dimethylformamide of 5-aminoorotic acid **1** (or 5-amino-4-carboxylate-orotic acid ester **2**) react with alkyl halide in the presence of potassium carbonate and tetrabutylammonim bromide (TBAB) at 65°C. However, yields are rather poor in this case. Added to this, it must be noted that methyl iodide, benzyl bromide and dialkylaminoalkyl chloride led only to the ester **2** in working under the conditions mentioned above.

By the way, the 5-acetylaminoorotic acid **4** is obtained in a rather good yield (75%) when 5-aminoorotic acid **1** reacts with acetic anhydride in the presence of sodium acetate at 140°C for 30 minutes.

Finally, compounds **2** and **3** react with acyl halides (and monohaloacyl halides) in benzene at 100°C to give respectively 5-acylamino substituted orotic acid derivatives **5** or the 1-alkyl-5-acylamino substituted ones **6**.



Scheme. General synthetic pathways

Antimicrobial activity of 5-acylaminoorotate derivatives was evaluated in vitro against gram negative and gram positive bacteria namely, *E. coli* CIP 54127 and *S. aureus* CIP 53124.

Results are gathered in table I.

On the one hand, it must be noted that **4** and **5l** are quite ineffective versus both strains whilst other derivatives are good inhibitors of bacteria growth whatever the strain tested may be.

On the other hand, **5m**, **5n**, **6a**, **6b** are selective inhibitors for gram+ bacteria.

Thus, there are promising results in this series. However, water solubility of compounds must be optimized before activity be evaluated in vivo.

Table I. Antimicrobial activity of selected 5-acylaminoorotic acid derivatives at the 1mM concentration.

Compounds	R	R'	% of growth inhibition	
			E. coli CIP 54127	*S. aureus* CIP 53124
4	-	-	0	2
5a	CH_3	CH_2Br	86	75
5b	C_2H_5	CH_2Br	85	70
5c	C_2H_5	CH_3	85	76
5i	C_5H_{11}	CH_2Br	77	97
5k	C_6H_{13}	CH_2Br	76	89
5l	C_6H_{13}	CH_3	6	2
5m	C_6H_{13}	CH_2Cl	5	97
5n	C_6H_{13}	$(CH_2)_2Br$	0	78
6a	C_3H_7	CH_2Br	13	99
6b	C_5H_{11}	CH_2Br	0	99

EXPERIMENTAL

1- Chemistry

^1H and ^{13}C NMR spectra were recorded on a Brucker ARX200 spectrometer. Chemical shifts (δ) are given with reference to the TMS used as internal standard. Melting points were determined with a Buchi-Tottoli apparatus and are given uncorrected. Elemental analyses agree within ± 0.4% of the theoretical values. IR were recorded on a FT57 Biorad spectrometer.

General procedures

***5-amino-4-carboxylates orotic acid derivatives* 2 :**

Alkyl halide is added to a suspension of 5-aminoorotic acid (5.8 mmol) and potassium carbonate (5.8 mmol) in dimethylformamide (15 ml). The mixture is heated at 110°C for 4-5h with stirring. After cooling, the mixture is poured out into water. The precipitated is filtered off, washed with water and recrystallized from ethanol. Compounds **2a** and **2f** are obtained according to the procedure described for compounds **3**.

***1-alkyl-5-amino-4-carboxylates orotic acid derivatives* 3 :**

The 5-aminoorotic acid (5.8 mmol) is dissolved in 100 ml of dimethylformamide at 110°C. After cooling, alkyl halide (6 mmol), potassium carbonate (5.8 mmol) and TBAB (1.6 mmol) are added to the solution.
Stirring is continued for several hours at 65°C. After cooling, the mixture is filtered and the crude is separated according to the following :

3a. The filtrate is poured into the chloroform and the precipitate is washed with ether before to be recrystallized from ethanol.

Table II. Chemical and Physical Data of 4-carboxylates orotic acid (**2**-**6**)

compounds	R	R'	MW	Mp (°C)	^{1}H NMR (δ, ppm)^1
2a	CH_3	-	185.14	290	11.53 (s, 1H, NH) ; 9.70 (s, 1H, NH) ; 6.00 (s, 2H, NH_2) ; 3.80 (s, 3H, CH_3)
2b	CH_2CH_3	-	199.17	310	11.53 (s, 1H, NH) ; 9.69 (s, 1H, NH) ; 6.02 (s, 2H, NH_2) ; 4.24 (qd, 2H, CH_2, J = 7.08 Hz) ; 1.28 (t, 3H, CH_3, J = 7.08 Hz)
2c	$(CH_2)_2CH_3$	-	213.19	218	11.54 (s, 1H, NH) ; 9.73 (s, 1H, NH) ; 6.02 (s, 2H, NH_2) ; 4.16 (t, 2H, OCH_2, J = 6.6 Hz) ; 1.79 (m, 2H, CH_2) ; 0.91 (t, 3H, CH_3, J = 7.3 Hz)
2d	$(CH_2)_4CH_3$	-	241.25	204	11.52 (s, 1H, NH) ; 9.72 (s, 1H, NH) ; 6.02 (s, 2H, NH_2) ; 4.20 (t, 2H, OCH_2, J = 6.6Hz) ; 1.67 (m , 2H, CH_2) ; 1.32 (m, 4H, CH_2) ; 0.89 (t, 3H, CH_3, J = 7.1Hz)
2e (4)	$(CH_2)_5CH_3$	-	255.27	190	11.54 (s, 1H, NH) ; 9.71 (s, 1H, NH) ; 6.02 (s, 2H, NH_2) ; 4.20 (t, 2H, OCH_2, J = 6.6 Hz) ; 1.67 (m, 2H, CH_2) ; 1.29 (m, 6H, CH_2) ; 0.86 (t, 3H, CH_3, J = 6.4 Hz).
2f	$(CH_2)_2N(C_2H_5)_2$	-	270.29	180	11.15 (s, 1H, NH) ; 9.79 (s, 1H, NH) ; 6.15 (s, 2H, NH_2) ; 4.50 (urs, 2H, OCH_2) ; 3.40 (urs, 2H, NCH_2) ; 3.20 (qd, 4H, NCH_2, J = 7 Hz) ; 1.12 (t, 6H, CH_3, J = 7 Hz).
3a (5)	$(CH_2)_2CH_3$	-	255.27	180	10.04 (s, 1H, NH) ; 6.03 (s, 2H, NH_2) ; 4.17 (t, 2H, OCH_2, J = 6.65 Hz) ; 3.78 (t, 2H, NCH_2, J = 7.35 Hz) ; 1.70 (m, 2H, CH_2) ; 1.54 (sep, 2H, CH_2, J = 7.35Hz) ; 0.92 (t, 3H, CH_3, J = 7.35 Hz) ; 0.85 (t, 3H, CH_3, J = 7.35 Hz)
3b	$(CH_2)_5CH_3$	-	311.38	172	7.82 (s, 1H, NH) ; 5.64 (s, 2H, NH_2) ; 4.33 (t, 2H, OCH_2, J = 6.71 Hz) ; 3.95 (t, 2H, NCH_2, J = 7.5 Hz) ; 1.66 (m, 4H) ; 1.31 (m, 12H) ; 0.89 (m, 6H, CH_3).
4	-	-	213.15	305	11.05 (s, 1H, NH) ; 9.80 (s, 1H, NH) ; 8.90 (s, 1H, NH) ;1.87 (s, 3H, CH_3)
5a	CH_3	CH_2Br	306.07	232	11.73 (s, 1H, NH) ; 11.18 (s, 1H, NH) ; 9.83 (s, 1H, NH) ; 4.04 (s, 2H, CH_2) ; 3.74 (s, 3H, CH_3)
5b	CH_2CH_3	CH_2Br	320.10	232	11.72 (s, 1H, NH) ; 11.17 (s, 1H, NH) ; 9.80 (s, 1H, NH) ; 4.19 (qd, 2H, OCH_2, J = 7.10 Hz) ; 4.04 (s, 2H, $COCH_2$) ; 1.24 (urs, 3H, CH_3)
5c	CH_2CH_3	CH_3	241.20	258	11.62 (s, 1H, NH) ; 11.03 (s, 1H, NH) ; 9.31 (s, 1H, NH) ; 4.17 (urs, 2H, OCH_2) ; 1.94 (s, 3H, $COCH_3$) ; 1.22 (t, 3H, CH_3, J = 7.10 Hz).
5d	CH_2CH_3	CH_2Cl	275.65	250	11.71 (s, 1H, NH) ; 11.16 (s, 1H, NH) ; 9.69 (s, 1H, NH) ; 4.24 (s, 2H, CH_2Cl) ; 4.18(qd, 2H, CH_2, J = 7.1 Hz) ; 1.23 (t, 3H, CH_3, J = 7.1 Hz)
5e	CH_2CH_3	$(CH_2)_2Br$	334.13	246	11.63 (s, 1H, NH) ; 11.08 (s, 1H, NH) ; 9.48 (s, 1H, NH) ; 4.18 (qd, 2H, CH_2, J = 7.06 Hz) ; 3.61 (t, 2H, CH_2, J = 6.3 Hz) ; 2.90 (t, 2H, CH_2, J = 6.3 Hz) ; 1.22 (t, 3H, CH_3, J = 7.06 Hz)
5f (5)	$(CH_2)_2CH_3$	CH_2Br	334.13	218	11.72 (s, 1H, NH) ;11.18 (s, 1H, NH) ; 9.79 (s, 1H, NH) ; 4.10 (t, 2H, OCH_2, J = 6.7 Hz) ; 4.04 (s, 2H, CH_2) ; 1.67 (m , 2H, CH_2) ; 0.88 (t, 3H, CH_3, J = 7.4 Hz)
5g	$(CH_2)_3CH_3$	CH_3	269.26	240	11.63 (s, 1H, NH) ; 11.03 (s, 1H, NH) ; 9.31 (s, 1H, NH) ; 4.12 (t, 2H, CH_2, J = 6.4 Hz) ; 1.93 (s, 3H, CH_3) ; 1.58 (m , 2H, CH_2) ; 1.33 (m, 2H, CH_2) ; 0.88 (t, 3H, CH_3, J = 7.3 Hz)
5h	$(CH_2)_5CH_3$	CH_2Cl	303.70	226	11.71 (s, 1H, NH) ; 11.17 (s, 1H, NH) ; 9.67 (s, 1H, NH) ; 4.23 (s, 2H, CH_2Cl) ; 4.14(urs, 2H, CH_2) ; 1.59 (m, 2H, CH_2) ; 1.34 (m, 2H, CH_2) ; 0.87 (urs, 3H, CH_3).

TableII. (continued)

compounds	R	R'	MW	Mp (°C)	^{1}H NMR (δ, ppm)[1]
5i	$(CH_2)_4CH_3$	$(CH_2)_2Br$	361.02	228	11.66 (s, 1H, NH) ; 11.09 (s, 1H, NH) ; 9.47 (s, 1H, NH) ; 4.13 (t, 2H, OCH_2, J = 6.3 Hz) ; 3.61 (t, 2H, CH_2, J = 6.1 Hz) ; 2.89 (t, 2H, CH_2, J = 6.1 Hz) ; 1.59 (m, 2H, CH_2); 1.32 (m, 2H, CH_2) ; 0.87 (t, 3H, CH_3, J = 7.1Hz)
5j	$(CH_2)_4CH_3$	CH_2Br	362.11	224	11.71 (s, 1H, NH) ; 11.16 (s, 1H, NH) ; 9.77 (s, 1H, NH) ; 4.13 (t, 2H, OCH_2, J = 6.7 Hz) ; 4.04 (s, 2H, $COCH_2$) ; 1.65 (m, 2H, CH_2) ; 1.29 (m, 4H, CH_2) ; 0.89 (t, 3H, CH_3, J = 6.7 Hz)
5k (5)	$(CH_2)_5CH_3$	CH_2Br	376.11	210	11.62 (s, 1H, NH) ; 11.08 (s, 1H, NH) ; 9.69 (s, 1H, NH) ; 4.30 (t, 2H, OCH_2, J = 6.7 Hz) ; 3.94 (s, 2H, $COCH_2$) ; 1.50 (m, 2H, CH_2) ; 1.16 (m, 6H, CH_2) ; 0.76 (t, 3H, CH_3, J = 6.7 Hz)
5l	$(CH_2)_5CH_3$	CH_3	297.11	232	11.64 (s, 1H, NH) ; 11.04 (s, 1H, NH) ; 9.33 (s, 1H, NH) ; 4.11 (t, 2H, OCH_2, J = 6.5 Hz) ; 1.93 (s, 3H, CH_3) ; 1.58 (m, 2H, CH_2) ; 1.26 (m, 8H, CH_2) ; 0.86 (urs, 3H, CH_3)
5m	$(CH_2)_5CH_3$	CH_2Cl	331.61	206	11.72 (s, 1H, NH) ; 11.19 (s, 1H, NH) ; 9.70 (s, 1H, NH) ; 4.23 (s, 2H, $COCH_2$) ; 4.13 (t, 2H, OCH_2, J = 6.6 Hz) ; 1.61 (m, 2H, CH_2) ; 1.26 (m, 6H, CH_2) ; 0.86 (t, 3H, CH_3).
5n	$(CH_2)_4CH_3$	$(CH_2)_2Br$	390.23	239	11.66 (s, 1H, NH) ; 11.10 (s, 1H, NH) ; 9.48 (s, 1H, NH) ; 4.12 (t, 2H, OCH_2, J = 6.6 Hz) ; 3.75 (t, 2H, CH_2, J = 6.4 Hz); 2.89 (t, 2H, CH_2, J = 6.4 Hz) ; 1.56 (m, 2H, CH_2) ; 1.26 (m, 6H, CH_2) ; 0.86 (t, 3H, CH_3, J = 6.7 Hz).
6a (7)	$(CH_2)_4CH_3$	CH_2Br	376.11	190	11.46 (s, 1H, NH) ; 9.83 (s, 1H, NH) ; 4.11 (t, 2H, OCH_2, J = 6.7 Hz) ; 4.05 (s, 2H, $COCH_2$) ; 3.75 (t, 2H, NCH_2, J = 7.3 Hz) ; 1.62 (m, 4H, CH_2) ; 0.89 (t, 3H, CH_3, J = 7.4Hz) ; 0.85 (t, 3H, CH_3, J = 7.6Hz).
6b	$(CH_2)_4CH_3$	CH_2Br	432.11	185	11.46 (s, 1H, NH) ; 9.84 (s, 1H, NH) ; 4.14 (t, 2H, OCH_2, J = 6.6 Hz) ; 4.05 (s, 2H, $COCH_2$) ; 3.77 (t, 2H, NCH_2, J = 7.1 Hz) ; 1.62 (m, 2H, CH_2) ; 1.52 (m, 2H, CH_2) ; 1.28 (m, 8H, CH_2) ; 0.86 (urs, 6H, CH_3).

[1]in DMSO-d_6 as soivent, apart from **3b** ($CDCl_3$).

3b. The filtrate is poured into water. The precipitate is washed several times with water and recrystallized from ethanol

***5-acetylaminoorotic acid* 4** :

Sodium acetate (0.2 g) is added to a suspension of 5-amino orotic acid **1** (1 g ; 5.8 mmol) in acetic anhydride (0.6 ml). The mixture is refluxed at 140°C for 30min before to be cooled. The precipitate is filtrated and just washed with hot methanol.

***5-acylamino-4-carboxylates orotic acid* 5** :

The reagent is added in excess to a mixture of compound **2** (2 mmol) in benzene (50 ml). The mixture is heated at 100°C with stirring for 48h.

After filtration, the residue is washed with ether, and recrystallized from ethanol.

***1-alkyl-5-acylamino-4-carboxylates orotic acid derivatives* 6** :

Monohaloacetic acid halide (4 mmol) is added to a solution of **3** (2 mmol) in benzene (25 ml). Mixture is heated at 100°C for 3h. Solvent is evaporated to dryness and the residue is washed with water before to be recrystallized from ethanol.

2- Biology

At first, compounds were dissolved in pure DMSO at 100mM concentration. Solutions were then diluted at 1/50 with sterile distilled water. Assays were achieved with these aqueous dimethylsulfoxide solutions at 2mM drug concentration while a 2% DMSO-water mixture was used as control.

After 48 hours of incubation, at 37°C, absorbances were measured at λ = 623nm and the percent of growth inhibition was expressed as :

$$\frac{OD(control) - OD(assay)}{OD(control)} \times 100$$

ACKNOWLEDGMENTS

Authors are greateful to Michèle MARTINO and Michèle DANI for their valuable technical assistance.

REFERENCES AND NOTES

(1) B. V. Golomolzin and G. M. Anoshina, Khim. Farm. Zh. 10, 17 (1975)

(2) Z. Machon and R. Jasztold-Howorko, Pol. J. Pharmacol. Pharm. 28, 61 (1976)

(3) R. Jasztold-Howorko, Z. Machon, M. Wilimowski,W. Wojewodzki, J. Barczynska, L. Kedzierska, K. Orzechowska-Juzwenko, E. Dus, M. Rutkowska and A. Szelag, J. Pol. Pharmacol. Pharm. 44, 393 (1992)

(4) ^{13}C NMR chemical shifts for **2e** ($CDCl_3$) are : 162.25 (COO) ; 160.66 (C-6) ; 147.34 (C-2) ; 129.67 (C-5) ; 108.62 (C-4) ; 66.55 (OCH_2) ; 25.61 (OCH_2CH_2) ; 31.42 (OCH_2CH_2) ; 28.64 (CH_2) ; 25.61 (CH_2) ; 22.56 ($OCH_2CH_2CH_2$) ; 14.01 (CH_3)

(5) ^{13}C NMR chemical shifts for **3a** (DMSO-d_6) are : 162.74 (COO) ; 160.28 (C-6) ; 147.65 (C-2) ; 129.23 (C-5) ; 107.22 (C-4) ; 66.75 (OCH_2) ; 42.22 (NCH_2) ; 21.46 (OCH_2CH_2); 20.52 (NCH_2CH_2) ; 11.26 (CH_3) ; 10.44 (CH_3)

(6) IR spectroscopic bands for **5k** are : 3279cm^{-1} (NH) ; 3225cm^{-1} (NH) ; 3171cm^{-1} (NH) ; 1732cm^{-1} (carbonyl ester) ; 1730-1643cm^{-1} (carbonyl groups and C_4=C_5), and ^{13}C NMR chemical shifts (DMSO-d_6) are : 165.32 ($COCH_2Br$) ; 161.54 (COO) ; 160.70 (C-6) ; 149.39 (C-2) ; 136.05 (C-5) ; 110.44 (C-4) ; 66.18 (OCH_2) ; 31.00 (CH_2) ; 28.97 ($COCH_2$) ; 27.80 (CH_2) ; 25.017 (CH_2) ; 22.083 (CH_2) ; 14.01 (CH_3)

(7) ^{13}C NMR chemical shifts for **6a** (DMSO-d_6) are : 165.42 ($COCH_2Br$) ; 160.68 (COO) ; 160.59 (C-6) ; 149.30 (C-2) ; 134.74 (C-5) ; 109.83 (C-4) ; 67.64 (OCH_2) ; 42.21 (NCH_2); 28.93 ($COCH_2Br$) ; 21.30 (OCH_2CH_2) ; 20.37 (NCH_2CH_2) ; 11.28 ($OCH_2CH_2CH_3$) ; 10.31 ($NCH_2CH_2CH_3$)

Received November 13, 1996