Synthesis of New Derivatives of 4-Amino-2,4-dihydro-1,2,4-triazol-3-one as Potential Antibacterial Agents

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Thirty new 2-substituted-4-amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-ones and ten 2-substituted-5-alkyl or aryl-4-(5-nitro-2-furfurylidene)amino-2,4-dihydro-1,2,4-triazol-3-ones were synthesized and characterised by their sharp melting points, elemental analysis, ir and ^{1}H nmr spectra. These new derivatives of 5-nitro-2-furaldehyde were screened for their antibacterial activities. Most of the compounds showed good activity against one test organism, *Staphylococcus aureus*. For a few compounds, C.M.I. ranged from 4 to 8 μg/ml (higher results than nitrofurantoin).

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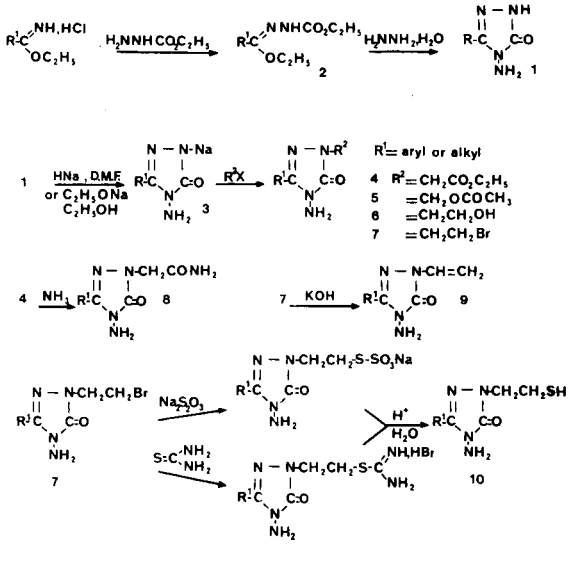
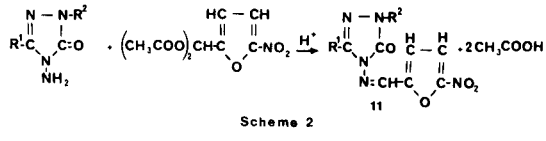
In a previous paper, we reported the synthesis and antibacterial activity of several series of 4-(5-nitro-2-furfurylideneamino) and 4-(5-nitro-2-thenylideneamino)-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-ones, substituted in the 2-position [1]. In order to extend our investigation on structure-activity relationships with regard to substitution in the 2-position, we prepared some new derivatives of 4-amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-ones **1**. These heterocycles **1** required as starting material were prepared by the reaction of hydrazine hydrate on the ethyl ester carbethoxyhydrazones **2**, in *n*-butyl alcohol under reflux for 30 hours [2]. The compounds **3** (sodio derivatives of **1**) were prepared by the reaction of sodium hydride on compounds **1** in anhydrous dimethylformamide as the solvent at 60-80° for 2 hours (method A) or by the reaction of sodium ethoxide on compounds **1** in absolute ethyl alcohol (method B). The 2-substituted derivatives of **1** (esters **4** and **5**, alcohols **6** and halides **7**) were synthesized by the reaction of the appropriate halide on the sodio derivatives **3**.



Scheme 1

By the action of ammonia on esters **4** in absolute ethyl alcohol for 7 days at room temperature amides **8** were obtained in good yields. Acids corresponding to esters **4** were not available because a resinification occured when their sodium salts were acidified. Vinylic compound **9** was prepared by the action of potassium hydroxide on halides **7** in ethyl alcohol-water (1/1) for 19 hours at reflux. The thiols **10** were synthesized by two methods. By the reaction of sodium thiosulfate on halides **7** the corresponding "Bunte" salts were formed. Next, they were hydrolyzed by hydrochloric acid solution in order to give the thiols **10**. The second method consisted of the action of thiourea on halides **7** with the formation of isothiouronium bromide salts of **7**. These compounds were hydrolyzed into thiols **10** in acid medium [3] [4]. The physical properties of compounds **4-10** are given in Table I.



Scheme 2

The condensation of 2-bis(acetoxy)methyl-5-nitrofuran with some esters **4**, alcohols **6** or amides **8** in appropriate solvents (*n*-propyl alcohol, ethyl alcohol or methyl alcohol) at reflux in the presence of hydrochloric acid for 2 hours gave the corresponding 4-(-5-nitro-2-furfurylideneamino) derivatives. This condensation was not possible with the thiols **10** because resinification occured during the reaction.

The propyl esters **11d**, **11f** and **11j** were obtained by a transesterification with *n*-propyl alcohol from the corres-

Table I

Physical Data of 2-Substituted-4-amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-ones

Compound No.	R_1	R_2	Yield % (Method)	Mp, °C	Solvent [a]	Molecular Formulae	Microanalysis Found C	Found N	Calcd. C	Calcd. N
4a	CH_3	CH_2CO_2Et	50 (A) 55 (B)	116	a	$C_7H_{12}N_4O_3$	41.8	28.1	42.0	28.0
4b	C_2H_5	CH_2CO_2Et	55 (A)	85	a	$C_8H_{14}N_4O_3$	45.0	26.4	44.8	26.2
4c	*iso*-C_3H_7	CH_2CO_2Et	25 (A)	109	b	$C_9H_{16}N_4O_3$	47.4	24.7	47.4	24.5
4d	C_6H_5	CH_2CO_2Et	41 (A)	141	b	$C_{12}H_{14}N_4O_3$	54.8	21.4	55.0	21.4
4e	$p(CH_3O)C_6H_4$	CH_2CO_2Et	58 (A) 60 (B)	150	a-c	$C_{13}H_{16}N_4O_3$	53.5	19.2	53.4	19.2
4f	$p(Cl)C_6H_4$	CH_2CO_2Et	62 (A)	161	a	$C_{12}H_{13}ClN_4O_3$	48.9	18.6	49.0	18.6
5	C_6H_5	$(CH_2)_2OCOCH_3$	25 (A)	126	b	$C_{12}H_{14}N_4O_3$	54.8	21.6	55.0	21.4
6a	CH_3	$(CH_2)_2OH$	20 (B)	100	d	$C_5H_{10}N_4O_2$	38.0	35.5	38.0	35.4
6b	C_2H_5	$(CH_2)_2OH$	20 (B)	103	e	$C_6H_{12}N_4O_2$	41.6	32.8	41.8	32.6
6c	C_6H_5	$(CH_2)_2OH$	42 (B)	139	d-c	$C_{10}H_{12}N_4O_2$	54.6	25.4	54.5	25.4
6d	$p(CH_3O)C_6H_4$	$(CH_2)_2OH$	30 (A)	147	f	$C_{11}H_{14}N_4O_3$	52.9	22.6	52.8	22.4
7a	CH_3	$(CH_2)_2Br$	45 (A)	108	g	$C_5H_9BrN_4O$	27.2	25.4	27.1	25.5
7b	C_2H_5	$(CH_2)_2Br$	61 (A)	85	g-h	$C_6H_{11}BrN_4O$	30.8	23.8	30.6	23.8
7c	*n*-C_3H_7	$(CH_2)_2Br$	40 (A)	88	c	$C_7H_{13}BrN_4O$	33.5	22.3	33.7	22.5
7d	*iso*-C_3H_7	$(CH_2)_2Br$	45 (A)	100	h	$C_7H_{13}BrN_4O$	33.6	22.7	33.7	22.5
7e	C_6H_5	$(CH_2)_2Br$	54 (A)	136	b	$C_{10}H_{11}BrN_4O$	42.4	19.7	42.4	19.7
7f	$p(CH_3O)C_6H_4$	$(CH_2)_2Br$	51 (A)	140	b	$C_{11}H_{13}BrN_4O_2$	42.0	18.0	42.2	17.9
7g	$p(Cl)C_6H_4$	$(CH_2)_2Br$	55 (A)	155	b	$C_{10}H_{10}BrClN_4O$	37.7	17.9	37.8	17.9
8a	CH_3	CH_2CONH_2	87	167	i	$C_5H_9N_5O_2$	35.2	41.1	35.1	40.9
8b	C_2H_5	CH_2CONH_2	62	169	i	$C_6H_{11}N_5O_2$	39.0	37.8	38.9	37.8
8c	$p(CH_3O)C_6H_4$	CH_2CONH_2	83	206	i	$C_{11}H_{13}N_5O_3$	50.1	26.7	50.1	26.6
8d	$p(Cl)C_6H_4$	CH_2CONH_2	95	233,5	i	$C_{10}H_{10}ClN_5O_2$	44.9	26.1	44.9	26.1
9	C_6H_5	$CH=CH_2$	32	160	c	$C_{10}H_{10}N_4O$	59.6	28.0	59.4	27.7
10a	CH_3	$(CH_2)_2SH$	45	95	g	$C_5H_{10}N_4OS$	34.7	32.1	34.5	32.2
10b	C_2H_5	$(CH_2)_2SH$	65	100	c	$C_6H_{12}N_4OS$	38.1	29.9	38.3	29.8
10c	*n*-C_3H_7	$(CH_2)_2SH$	63	101	c	$C_7H_{14}N_4OS$	41.6	27.7	41.6	27.7
10d	*iso*-C_3H_7	$(CH_2)_2SH$	57	77	c	$C_7H_{14}N_4OS$	41.5	27.7	41.6	27.7
10e	C_6H_5	$(CH_2)_2SH$	84	104	d	$C_{10}H_{12}N_4OS$	50.9	23.8	50.8	23.7
10f	$p(CH_3O)C_6H_4$	$(CH_2)_2SH$	62	137	f	$C_{11}H_{14}N_4O_2S$	49.9	23.7	49.6	23.7
10g	$p(Cl)C_6H_4$	$(CH_2)_2SH$	65	130	d	$C_{10}H_{11}ClN_4OS$	44.4	21.0	44.3	20.7

[a] Solvents: (a) ethylacetate, (b) ethanol, (c) cyclohexane, (d) benzene, (e) butyl acetate, (f) methanol, (g) ether, (h) petroleum ether 30-60°, (i) acetonitrile, (j) propanol, (k) chloroform, (l) 1-butanol.

Table II

Physical Data of 2-Substituted-4-(5-nitro-2-furfurylidene)amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-ones **11**

Compound No.	R_1	R_2	Yield %	Mp, °C	Solvent [a]	Molecular Formulae	Microanalysis Found C	Found N	Calcd. C	Calcd. N
11a	CH_3	CH_2CO_2Et	73	152	b	$C_{12}H_{13}N_5O_6$	44.8	21.5	44.6	21.7
11b	CH_3	$(CH_2)_2OH$	76	163	b	$C_{10}H_{11}N_5O_5$	42.5	24.8	42.7	24.9
11c	C_2H_5	CH_2CO_2Et	95	123	b	$C_{13}H_{15}N_5O_6$	46.1	21.0	46.3	20.8
11d	C_2H_5	CH_2CO_2n-Prop	47	98	j	$C_{14}H_{17}N_5O_6$	47.6	20.1	47.9	19.9
11e	C_2H_5	$(CH_2)_2OH$	69	129	b	$C_{11}H_{13}N_5O_5$	44.9	23.9	44.7	23.7
11f	C_6H_5	CH_2CO_2n-Prop	38	120	j	$C_{18}H_{17}N_5O_6$	54.9	17.7	54.1	17.5
11g	C_6H_5	$(CH_2)_2OH$	83	141	k	$C_{15}H_{13}N_5O_5$	52.5	20.5	52.5	20.4
11h	$p(CH_3O)C_6H_4$	CH_2CO_2Et	78	148	b	$C_{18}H_{17}N_5O_7$	52.1	17.0	52.0	16.9
11i	$p(CH_3O)C_6H_4$	$(CH_2)_2OH$	82	154	j	$C_{16}H_{15}N_5O_6$	51.4	18.7	51.5	18.7
11j	$p(Cl)C_6H_4$	CH_2CO_2n-Prop	41	138	b	$C_{18}H_{16}N_5O_6Cl$	49.6	16.3	49.8	16.1

[a] Solvents: see Table I.

Table III

IR and ^{1}H NMR Spectral Data of Compounds **4-11**

Compound No.	IR (Potassium bromide)	^{1}H NMR (Solvent) (TMS = 0 ppm)
4a	3300-3200 (NH_2), 1720 (CO ester) 1690 (ring C=O)	(Deuteriochloroform), 1.3 (t, 3H), 2.25 (s, 3H) 4.25 (q, 2H), 4.5 (m, 4H)
4b	3300-3200 (NH_2), 1720 (CO ester) 1680 (ring C=O)	(Deuteriochloroform) 1.2 (m, 6H), 2.5 (q, 2H) 4.2 (q, 2H), 4.5 (s, 2H), 5.3 (2H)
4c	3300-3200 (NH_2), 1730 (CO ester) 1690 (ring C=O)	(Deuteriochloroform), 1.4 (m, 9H), 3.2 (m, 1H) 4.25 (q, 2H), 4.5 (s, 2H), 4.6 (s, 2H)
4d	3300-3200 (NH_2), 1745 (CO ester) 1700 (ring C=O)	(Deuteriochloroform), 1.3 (t, 3H), 4.3 (q, 2H) 4.7 (m, 4H), 7.5-8.2 (m, 5H)
4e	3300-3200 (NH_2), 1740 (CO ester) 1680 (ring C=O)	(DMSO-d_6), 1.3 (t, 3H), 3.85 (s, 3H), 3.9 (q, 2H) 4.7 (s, 2H), 5.7 (s, 2H), 7.2-7.7 (m, 4H)
4f	3300-3200 (NH_2), 1730 (CO ester) 1700 (ring C=O)	(DMSO-d_6), 1.28 (t, 3H), 4.2 (q, 2H), 4.7 (s, 2H) 5.6 (s, 2H), 7.4-8.1 (m, 4H)
5	3300-3200 (NH_2), 1730 (CO ester) 1700 (ring C=O)	(Deuteriochloroform), 2, (s, 3H), 4 (m, 4H), 5 (s, 2H) 7.2-8 (m, 5H)
6a	3400-3100 (NH_2, OH), 1700 (CO)	(DMSO-d_6), 2.25 (s, 3H), 3.7 (s, 4H), 4.4 (s, 1H) 5.2 (s, 2H)
6b	3400-3100 (NH_2, OH), 1700 (CO)	(DMSO-d_6), 1 (t, 3H), 2.4 (q, 2H), 3.7 (s, 4H) 4.6 (s, 1H), 5.2 (s, 2H)
6c	3450-3100 (NH_2, OH), 1700 (CO)	(DMSO-d_6), 2.5 (m, 1H), 3.8 (t, 2H), 4.2 (t, 2H) 5.2 (s, 2H), 7.4-8.2 (m, 5H)
6d	3400-3150 (NH_2, OH), 1700 (CO)	(DMSO-d_6), 3.4 (s, 3H), 3.8 (m, 4H), 4.8 (s, 1H) 5.4 (s, 2H), 7-7.9 (2d, 4H)
7a	3280-3170 (NH_2), 1670 (CO)	(DMSO-d_6), 1.1 (t, 2H), 2.15 (s, 3H), 3.8 (t, 2H) 4.35 (s, 2H)
7b	3270-3170 (NH_2), 1670 (CO)	(DMSO-d_6), 1.1 (m, 5H), 2.5 (q, 2H), 3.8 (t, 2H) 4.35 (s, 2H)
7c	3260-3160 (NH_2), 1670 (CO)	(DMSO-d_6, 1.1 (m, 7H), 2.5 (t, 2H), 3.8 (t, 2H) 4.8 (s, 2H)
7d	3260-3160 (NH_2), 1670 (CO)	(DMSO-d_6), 1.2 (m, 8H), 3 (m, 1H), 3.8 (t, 2H) 4.45 (s, 2H)
7e	3285-3150 (NH_2), 1660 (CO)	(DMSO-d_6), 1.1 (t, 2H), 3.8 (t, 2H), 4.8 (s, 2H) 7.5-8 (m, 5H)
7f	3280-3140 (NH_2), 1660 (CO)	(Deuteriochloroform), 3.8 (s, 3H and t, 2H) 4.15 (t, 2H), 5.5 (s, 2H), 7.1-8 (m, 4H)
7g	3240-3140 (NH_2), 1660 (CO)	(Deuteriochloroform), 3.9 (t, 2H), 4.25 (t, 2H) 5.5 (s, 2H), 7.5-8.1 (m, 4H)
8a	3310, 3220, 3150 (NH_2, $CONH_2$) 1695 (ring C=O), 1660 (CO amide)	DMSO-d_6), 2.3 (s, 3H), 4.2 (s, 2H), 5.2 (s, 2H) 7.3 (d, 2H)
8b	3380, 3300, 3200 (NH_2, $CONH_2$) 1700 (ring C=O), 1675 (CO amide)	(DMSO-d_6), 1.15 (t, 3H), 2.5 (q, 2H), 4.2 (s, 2H) 5.2 (s, 2H), 7.3 (d, 2H)
8c	3360, 3320, 3180 (NH_2, $CONH_2$) 1690 (ring C=O), 1675 (CO amide)	(DMSO-d_6), 3.8 (s, 3H), 4.3 (s, 2H), 5.4 (s, 2H) 7.2-7.9 (m, 4H + d, 2H)
8d	3420, 3290, 3200 (NH_2, $CONH_2$) 1700 (ring C=O), 1670 (CO amide)	(DMSO-d_6), 4.3 (s, 2H), 5.5 (s, 2H), 7.2-8 (m, 4H + d, 2H)
9	3300-3000 (NH_2), 1660 (CO) 1580 (C=C)	(DMSO-d_6), 4.7 (s, 2H), 4.8 (d, 1H), 5.5 (d, 1H) 7.1 (q, 1H), 7.5-8.3 (m, 5H)
10a	3280, 3160 (NH_2), 2520 (SH) 1660 (CO)	(Deuteriochloroform), 2.2 (s, 3H), 2.9 (m, 3H) 3.9 (m, 4H)
10b	3300, 3180 (NH_2), 2500 (SH) 1660 (CO)	(Deuteriochloroform), 1.2 (t, 3H), 2.5 (m, 4H) 2.8 (t, 1H)
10c	3300, 3200 (NH_2), 2510 (SH) 1660 (CO)	(DMSO-d_6), 1.15 (t, 3H), 1.6 (m, 2H), 2.5 (m, 4H) 2.8 (t, 1H), 3.85 (t, 2H), 5.25 (s, 2H)
10d	3300, 3180 (NH_2), 2500 (SH) 1660 (CO)	(DMSO-d_6), 1.15 (d, 6H), 2.4-3 (m, 4H), 3.8 (t, 2H), 5.2 (s, 2H)
10e	3320, 3200 (NH_2), 2510 (SH) 1690 (CO)	(DMSO-d_6), 2.5 (t, 1H), 2.9 (m, 2H), 4 (m, 4H) 7.7-8.2 (m, 5H)

Table III, Continued

Compound No.	IR (Potassium bromide)	^{1}H NMR (Solvent) (TMS = 0 ppm)
10f	3300, 3180 (NH_2), 2520 (SH) 1670 (CO)	(DMSO-d_6), 2.5 (t, 1H), 2.8 (m, 2H), 3.8 (s, 3H) 3.9 (t, 2H), 5.5 (s, 2H), 7.1-8 (m, 4H)
10g	3300, 3180 (NH_2), 2520 (SH) 1680 (CO)	(DMSO-d_6), 2.5 (t, 1H), 2.85 (m, 2H), 4 (t, 2H) 5.6 (s, 2H), 7.55-8.15 (m, 4H)
11a	1740 (CO ester), 1700 (ring C=O)	(DMSO-d_6), 1.25 (t, 3H), 2.3 (s, 3H), 4.2 (q, 2H) 4.65 (s, 2H), 7.5 and 7.8 (2d, 2H), 9.8 (s, 1H)
11b	3430 (OH), 1700 (CO)	(DMSO-d_6), 2.3 (s, 3H), 3.8 (s, 4H), 4.4 (s, 1H), 7.45 and 7.7 (2d, 2H), 9.7 (s, 1H)
11c	1730 (CO ester), 1700 (ring C=O)	(DMSO-d_6), 1.25 (t, 6H), 2.75 (q, 2H), 4.2 (q, 2H), 4.6 (s, 2H), 7.5 and 7.8 (2d, 2H), 9.7 (s, 1H)
11d	1700 (CO)	(DMSO-d_6), 1.1 (m, 6H), 1.65 (q, 2H), 2.7 (q, 2H), 4.15 (t, 2H), 4.7 (s, 2H), 7.6 and 7.9 (2d, 2H) 9.75 (s, 1H)
11e	3460 (OH), 1700 (CO)	(DMSO-d_6), 1.3 (t, 3H), 2.7 (q, 2H), 3.75 (s, 4H), 4.3 (s, 1H), 7.5 and 7.8 (2d, 2H), 9.8 (s, 1H)
11f	1730 (CO ester), 1680 (ring C=O)	(DMSO-d_6), 0.9 (t, 3H), 1.6 (q, 2H), 4.1 (t, 3H), 4.8 (s, 2H), 7.7 (m, 7H), 9.7 (s, 1H)
11g	3400 (OH), 1670 (CO)	(DMSO-d_6), 3.4 (t, 2H), 3.9 (m, 2H), 4.9 (t, 1H) 7.6-8.2 (m, 7H), 10.1 (s, 1H)
11h	1730 (CO ester), 1710 (ring C=O)	(DMSO-d_6), 1.25 (t, 3H), 3.85 (s, 3H), 4.2 (q, 2H), 4.8 (s, 2H), 7.2-7.8 (m, 6H), 9.85 (s, 1H)
11i	3360 (OH), 1690 (CO)	(DMSO-d_6), 3.9 (t, 4H), 4.9 (s, 1H), 7-7.6 (m, 6H), 9.7 (s, 1H)
11j	1740 (CO ester), 1700 (ring C=O)	(DMSO-d_6), 0.9 (t, 3H), 1.7 (q, 2H), 4.15 (t, 2H), 4.8 (s, 2H), 7.6-8 (m, 6H), 9.8 (s, 1H)

Table IV

Antibacterial Activity of 2-Substituted-4-(-5-nitro-2-furfurylidene)amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-ones **11**

Compound No.	R_1	R_2	*Staph. aureus* 209P MIC g/ml	*Staph. aureus* 209P MBC g/ml	*E. coli* 7624 MIC g/ml	*E. coli* 7624 MBC g/ml
11a	CH_3	CH_2CO_2Et	16	64	64	128
11b	CH_3	$(CH_2)_2OH$	8	32	32	64
11c	C_2H_5	CH_2CO_2Et	16	128	128	256
11d	C_2H_5	CH_2CO_2n-Prop	32	64	128	256
11e	C_2H_5	$(CH_2)_2OH$	16	32	64	64
11f	C_6H_5	CH_2CO_2n-Prop	8	16	256	256
11g	C_6H_5	$(CH_2)_2OH$	8	8	128	128
11h	$p(CH_3O)C_6H_4$	CH_2CO_2Et	16	64	256	256
11i	$p(CH_3O)C_6H_4$	$(CH_2)_2OH$	4	4	256	256
11j	$p(Cl)C_6H_4$	CH_2CO_2n-Prop	8	16	256	256
sodium Fusidate			0.125	0.125	256	256

ponding ethyl esters. The physical properties and yields for these compounds **11** are given in Table II.

All the newly synthesized compounds gave satisfactory spectral data and the structures were assigned on the basis of elemental analyses, ms, ir (potassium bromide) and ^{1}H nmr spectral data which is given in Table III.

Microbiology.

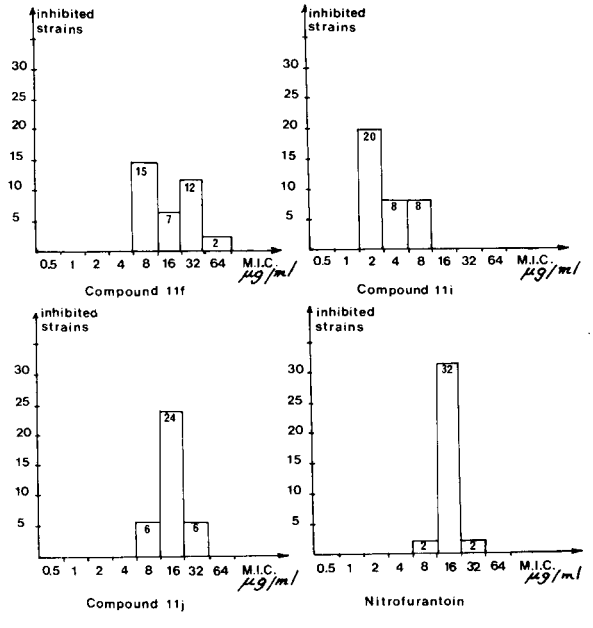
The new compounds **11** reported herein were screened for their antimicrobial activity (only compounds soluble in aqueous medium). One gram positive (*Staphylococcus aureus* 209P), and two grams negative strains (*Escherichia coli*, Pasteur Institut 7624 and *Pseudomonas aeruginosa*, C. Bernard hospital, Paris, $P_{11}C_{794}$) were selected. The tests were performed on Mueller Hinton medium using a dilution technique on analytical microplates Dynatech [1]. The compounds were first solubilized in DMSO and then the dilutions were made in the Mueller Hinton medium with an interval ranging from 256 to 0.125 micrograms per one milliliter of broth (with 0.5% DMSO by volume). A 24 hours broth culture was used as inoculum, which contained 10^6 cells per milliliter. Results were recorded as micrograms/milliliter, and the lowest concentration of substance

which inhibited growth of bacteria after a 24 hour incubation period at 37° was designated as the minimal inhibitory concentration (MIC) and after a 48 hour incubation period at 37° was designated as the minimal bactericide concentration (MBC). The sodium fusidate [5] was the reference product (the nitrofurantoin [6] was too insoluble at the highest concentration in the Mueller Hinton medium, therefore it could not be used as reference). The results are summarized in Table IV.

Antibacterial activity was exhibited only against gram positive bacteria by all the compounds. A very slight activity against gram negative bacteria was only found with aliphatic derivatives. The most interesting antibacterial activity against *Staphylococcus aureus* obtained with the aromatic compounds and when the N_2-substituant was an 2-hydroxy ethyl group (compounds **11g** and **11i**). Therefore, three compounds **11f**, **11i** and **11j** were selected and tested against thirty one strains of *Staphylococcus aureus* coagulase positive, four strains of *Staphylococcus aureus* coagulase negative from the C. Bernard hospital, Paris) and *Staphylococcus aureus* 209P. A solid medium (trypto caseine soja) was used for that screening. Sodium fusidate and nitrofurantoin were the two reference products. The range of dilutions were from 64 to 0.5 μg/ml, except for Nitrofurantoin which was 32 to 0.25 μg/ml because of its insolubility. The results are summarized in Scheme 3. Compound **11i** showed a better activity against *Staphylococcus aureus* than commercial nitrofurantoin.

Scheme 3

Antibacterial activity of compounds 11f, 11i, 11j, and nitrofurantoin against 36 strains of Staphylococcus aureus. [a]



[a] The M.I.C. of sodium fusidate is 1μg ml for the 36 strains of Staphylococcus aureus.

EXPERIMENTAL

Melting points were determined with a Buchi oil heated apparatus and are uncorrected. Infrared (ir) spectra were recorded with a Perkin Elmer 337 spectrophotometer using potassium bromide discs unless otherwise stated. Nuclear magnetic resonance (nmr) were determined in the solutions stated with tetramethylsilane as the internal reference and recorded in 60 MHz on a Varian T60 spectrometer.

4-Amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-ones **1**.

These compounds were synthesized by a method described in previous papers [1] [2].

4-Amino-5-alkyl or aryl-2-carbethoxymethyl-2,4-dihydro-1,2,4-triazol-3-ones **4a-f**, 2-(2-Acetoxyethyl)-4-amino-5-phenyl-2,4-dihydro-1,2,4-triazol-3-one **5**, 4-Amino-5-alkyl or aryl-2-(2-hydroxyethyl)-2,4-dihydro-1,2,4-triazol-3-ones **6a-d**, 4-Amino-5-alkyl or aryl-2-(2-bromoethyl)-2,4-dihydro-1,2,4-triazol-3-ones **7a-g**.

Method A.

Sodium hydride (80% dispersion in mineral oil, 0.13 moles) was placed in dry dimethylformamide (200 ml). Sodium hydride was used after washing twice with hexane (20 ml). The slurry was cooled to 10° and the suitable 4-amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-one (0.1 mole) was added in small portions with stirring. After the addition, the slurry was stirred at room temperature for 30 minutes, then for 2 hours at 70-80°. The reaction mixture was cooled to 40-50° and the appropriate halide was added in one portion with stirring (ethyl bromoacetate 0.13 moles for esters **4a-f**, 2-chloroethyl acetate, 0.13 moles for ester **5**, 2-chloroethanol, 0.2 moles for alcohols **6a-d**, and 1,2-dibromoethane, 0.3 moles for bromides **7a-g**). The reaction mixture was stirred at 70-80° for 8 hours. The solvent was evaporated *in vacuo* and the resulting residue was dissolved in hot dry ethanol (compounds **4**, **5**, **6**), or in chloroform (compounds **7**). After filtration, the solvent was evaporated. The crude product was recrystallized from the appropriate solvent (see Table I).

Method B.

To a solution of sodium ethoxide (0.1 mole) in dry ethanol (100 ml), suitable 4-amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-one (0.1 mole) was added. The mixture was stirred at reflux for 2 hours. After addition of the halide the same procedure of method A was used. Esters **4** and **5** were chromatographed on silicagel using ether-pentane, 95/5, before recrystallization.

(5-Alkyl or aryl-4-amino-2,4-dihydro-3-oxo-1,2,4-triazol-2-yl)acetamides **8a-d**.

A solution of the appropriate ester **4** (0.1 mole) in ethanol (200 ml) was saturated with dry ammonia at room temperature. The reaction mixture was allowed to stand 5 days at 0°. The resulting precipitate was collected by filtration. The solvent was evaporated and the crude product was recrystallized from acetonitrile.

4-Amino-5-phenyl-2-vinyl-2,4-dihydro-1,2,4-triazol-3-one (**9**).

A solution of potassium hydroxide (0.1 mole), ester **7e** (0.1 mole) in ethanol/water (1/1, 60 ml) was stirred at reflux for 20 hours. After cooling the crude product **9** precipitated. It was collected by filtration and recrystallized from cyclohexane.

4-Amino-5-alkyl or aryl-2-(2-mercaptoethyl)-2,4-dihydro-1,2,4-triazol-3-ones **10a-g**.

Synthesis *via* "Bunte Salt".

A mixture of suitable halide **7** (0.015 mole), anhydrous sodium thiosulfate (0.02 mole) and ethanol/water (1/1, 100 ml) was stirred at reflux for 7 hours (aromatic halides **7e-g**) or for 12 hours (aliphatic halides **7a-d**). The solvent was evaporated *in vacuo* and the resulting solid was washed twice with ether. The Bunte salt was placed in ethanol (50 ml) and concentrated hydrochloric acid (5 ml) was added. The reaction mixture was stirred 1 hour at reflux. The solvent was evaporated *in vacuo* and the

residue was washed with water (10 ml) and recrystallized from the appropriate solvent (for thiols **10e-g**). The residue was dissolved in ether and after filtration the solvent was evaporated. The crude product was recrystallized from appropriate solvent (for thiols **10a-d**).

Synthesis with Thiourea.

A mixture of halide **7e** (0.01 mole, 2.85 g), thiourea (0.01 mole, 0.75 g) in ethanol (25 ml) was stirred at reflux for 20 hours. After cooling a solution of sodium hydroxide (0.02 mole, 0.8 g) in water (5 ml) was added dropwise at 0°. After addition the reaction mixture was stirred for 1 hour at reflux. After cooling at 0° the solution was acidified with 20% hydrochloric acid and water (50 ml) was added. The mixture was allowed to stand overnight at 0°. The resulting precipitate was filtered, washed with water, dried and recrystallized from benzene, yield 1.7 g (71%).

2-Substituted-4-(-5-nitro-2-furfurylidene)amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-ones **11a-j**. General Procedure.

A solution of 2-substituted-4-amino-5-alkyl or aryl-2,4-dihydro-1,2,4-triazol-3-one (0.01 mole), 5-nitro-2-furaldehyde diacetate (0.01 mole, 2.43 g) and concentrated hydrochloric acid (0.5 ml) in 1-propanol (40 ml) was stirred at reflux for 2 hours. After cooling at 0°, the precipitated yellow solid was filtered, washed with ethanol (10 ml) then with water (10 ml), dried and recrystallized from the appropriate solvent. Compounds **11a,c,h** were obtained when ethanol was used as the solvent in the reaction. When 1-propanol was used with esters **4b,d,f**, transesterification was occured during the condensation with 5-nitro-2-furaldehyde diacetate and compounds **11d,f,j** were obtained.

REFERENCES AND NOTES

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[5] Sodium fusidate: sodium 3α,11α,16β-trihydroxy-29-*nor*-8α,9β,13α,14β-dammara-17(20),24-dien-21-oate 16-acetate; Fucidin from Laboratoires Léo.

[6] Nitrofurantoin: 1-[(5-nitrofurfurylidene)amino]hydantoin (from Laboratoires Oberval).