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In vitro inhibitory activity against bacteria of a *fusarium* mycotoxin and new synthetic derivatives

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Summary — In vitro inhibitory activity towards Gram-positive bacteria (*Staphylococcus aureus* 209P) and Gram-negative bacteria (*Escherichia coli* K12) of a *fusarium* mycotoxin and new synthetic analogues is reported.

mycotoxin / butenolide / inhibitory activity

Résumé — Activité inhibitrice in vitro d'une mycotoxine produite par le genre *fusarium* et de nouveaux analogues synthétiques vis-à-vis de bactéries. L'activité inhibitrice in vitro d'une mycotoxine produite par le genre *fusarium* et de nouveaux analogues synthétiques, vis-à-vis de bactéries Gram + (*Staphylococcus aureus* 209P) et Gram - (*Escherichia coli* K12) est reportée.

mycotoxine / butenolide / activité inhibitrice

Introduction

Several toxic metabolites are produced by *Fusarium* species (*F nivale* [1,2,3], *F sambucinum* [4], *F tricinctum* [5],...). Among these substances, suspected to be the cause of different intoxications occasionally observed in cattle grazing on tall fescue grass [6, 7], *N*-(5-oxo-2,5-dihydro-2-furyl) acetamide: **3a** (fig 1, R = Me) is of particular importance. This mycotoxin exhibited a 43.6 ± 1.24 mg/Kg ip LD_{50} in mice [2]. This compound has been patented as a neoplasm inhibitor [8] and some structurally related substances were reported as β-adrenolytics and hypertensive agents [9] and moderate inhibitory activity against bacteria [2].

In the field of research for new antibacterial agents, we have reinvestigated the synthesis of such compounds. This was achieved by condensation of 5-ethoxyfuran-2(5*H*)-one [10] with amides, according to a modification of Gratz' synthesis [11].

The natural toxin was synthesised by condensation of 5-ethoxyfuran-2(5*H*)-one (128 mg, 10^{-3} M) and acetamide [12] (118 mg, 2×10^{-3} M) in 2 mL of concentrated aqueous HCl (80 °C, 5 min). After addition of cold water (20 mL), the reaction mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with cold water and dried over $MgSO_4$. Removing of the solvent under reduced pressure gave a white product: Mp 115 °C (ether/hexane [13], 45-50%).

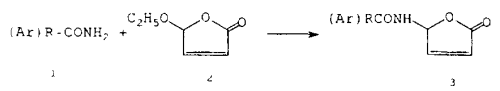

Fig 1.

Analogues of the mycotoxin were prepared by condensation of the corresponding amide (10^{-3} mol) dissolved in a minimum of acetone and 5-ethoxyfuran-2(5*H*)-one (10^{-3} mol) in 0.5 mL of concentrated aqueous HCl. After standing 24 h at 10 °C, the crude butenolide was either filtered and washed with the minimum of cold acetone or precipitated by trituration with ether after removing of the solvent under reduced pressure. The physico-chemical properties of butenolides **3a–k** are reported in table I and II.

X-ray structure

X-ray structure determination of the natural toxin **3a** has been previously reported [14] and we published [15] the crystal structure of analogue **3g** (R = 4-Cl-C_6H_4). The phenyl and the furanyl rings are planar and form a dihedral angle of 108.9°. The molecules are linked by NH···O hydrogen bonds.

Inhibitory activity against bacteria

The inhibitory activities were determined against a strain of a Gram-positive bacterium (*Staphylococcus aureus* 209P) and a strain of Gram-negative bacterium (*Escherichia coli* K12) using the nutrient agar diffusion

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Table I. Physico-chemical properties of butenolides **3a–k**.

No	*Mp* (°C)	*Yield* (%)	*Formula*	*Anal calc*			*Found*			*IR*, $\nu_{C=O}$ (cm^{-1})
				C (%)	*H (%)*	*N (%)*	*C (%)*	*H (%)*	*N (%)*	
3a	115	45	$C_6H_7NO_3$	51.05	5.00	9.93	50.98	5.12	9.88	1797, 1763
3b	192	80	$C_5H_6N_2O_3$	42.24	4.26	19.72	42.16	4.39	19.59	1810, 1755
3c	120	65	$C_{15}H_{25}NO_3$	67.37	9.43	5.24	67.21	9.60	5.19	1805, 1775
3d	103	65	$C_{20}H_{35}NO_3$	71.16	10.46	4.15	71.00	10.67	4.03	1790, 1756
3e	164	85	$C_{11}H_9NO_3$	65.01	4.47	6.90	64.90	4.59	6.80	1815, 1770
3f	163	80	$C_{12}H_{11}NO_4$	61.78	4.76	6.01	61.59	4.85	6.00	1815, 1774
3g	212	80	$C_{11}H_8ClNO_3$	55.69	3.40	5.91	55.51	3.59	5.87	1817, 1775
3h	160	80	$C_{11}H_8N_2O_5$	53.22	3.25	11.29	53.01	3.50	11.17	1818, 1775
3i	180	85	$C_{11}H_8N_2O_5$	53.22	3.25	11.29	52.99	3.40	11.15	1822, 1766
3j	163	75	$C_{11}H_7N_3O_7$	45.05	2.41	14.34	44.92	2.62	14.20	1816, 1763
3k	198	75	$C_{11}H_7N_3O_7$	45.05	2.41	14.34	44.89	2.57	14.18	1822, 1766

a: R = CH_3; **b**: R = NH_2; **c**: R = $C_{10}H_{21}$; **d**: R = $C_{15}H_{31}$; **e**: R = C_6H_5; **f**: R = (4-CH_3O)-C_6H_4; **g**: R = (4-Cl)-C_6H_4; **h**: R = (4-O_2N)-C_6H_4; **i**: R = (2-O_2N)-C_6H_4; **j**: R = (3,4-di-O_2N)-C_6H_3; **k**: R = (3,5-di-O_2N)-C_6H_3.

Table II. ^1H NMR chemical shifts of butenolides **3a–k** in DMSO-d_6, δ (ppm), J (Hz).

No/R (Ar)	*H3*	*H4*	*H5*	*J*			
				3-4	*3-5*	*4-5*	*5-NH*
3a: s: 1.90	dd: 6.32	m: 6.56	dd: 7.59	5.7	1.8	1.6	9.3
3b	dd: 6.32	m: 6.50	dd: 7.50	5.7	1.8	1.4	10.1
3c: CH_2(CO) t: 2.14 (J = 6); CH_2(β-CO) m: 1.5 (CH_2)7 se: 1.23; CH_3 t: 0.8 (J = 6)	dd: 6.32	m: 6.58	dd: 7.59	5.6	1.7	1.5	7.5
3d: CH_2(CO) t: 2.17 (J = 6); CH_2 (β-CO) m: 1.5 (CH_2)12 se: 1.32; CH_3 t: 0.92 (J = 6)	dd: 6.32	m: 6.56	dd: 7.59	6.0	1.8	1.5	9.5
3e: Ar m: 7.90–8.01, m 7.45–7.58	dd: 6.40	m: 6.80	dd: 7.60	5.7	1.8	1.6	8.7
3f: CH_3O s: 3.82; Ar dd: 7.03, dd: 7.92 (J = 6.85, 2.0)	dd: 6.44	m: 6.81	dd: 7.69	5.7	1.7	1.55	9.0
3g: Ar dd: 7.94, dd: 7.6 (J = 6.7, 2.0)	dd: 6.47	m: 6.83	dd: 7.71	5.7	1.75	1.5	9.0
3h: Ar dd: 8.35, dd: 8.12 (J = 7.2, 2.4)	dd: 6.48	m: 6.82	dd: 7.72	5.7	1.8	1.7	8.8
3i: Ar dd: 8.11, m: 7.5, m: 7.85, dd: 8.12 (J = 7.7, 2.0–7.8, 1.5)	dd: 6.46	m: 6.75	dd: 7.68	5.6	1.65	1.6	8.9
3j: Ar d: 8.7, d: 8.38, dd: 8.45 (J = 8.3, 1.5, 8.3–1.5)	dd: 6.52	m: 6.83	dd: 7.73	5.6	1.8	1.5	8.6
3k: Ar d: 9.15, d: 9.01 (J = 2.1)	dd: 6.54	m: 6.87	dd: 7.76	5.7	1.7	1.6	8.6

Table III. In vitro inhibitory activity of amides **1**, ethoxybutenolide **2** and butenolides **3**. Diameter (mm) of the inhibition zone measured after 18 h of incubation at 37 °C around wells of 6 mm filled with a solution of tested compound (400 μg) in DMSO (25 μL).

No	*1*											*2*	*3*									
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*		*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*
209P	–	*	–	–	–	–	–	19	–	35	31	39	–	–	–	–	18	–	–	15	14	27	18
K12	–	*	–	–	–	–	–	23	–	28	30	35	24	9	–	–	17	13	–	14	17	18	18

–: no inhibition zone; *: insoluble.

assay (table III). For the sake of comparison, butenolide **2** and amides **1a–k** were also evaluated in the same biological tests.

Results and discussion

The natural butenolide **3a** and analogues **3b** and **3f** are the only compounds which display a selective inhibitory activity against *E coli*. In the other cases, when activity is present, no selectivity is observed. The urea **3b** and the aliphatic analogues **3c** and **3d** are practically inactive. The amides **1a–k** and compounds **3b–k** possess a noticeable inhibitory activity when a nitro or a dinitro aromatic system is present. The most potent inhibitor appears to be the ethoxybutenolide **2**. This result suggests that the γ-lactone ring system plays a key role in the appearance of an inhibitory activity against Gram + and Gram – bacteria.

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