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SYNTHESIS AND *IN VITRO* CYTOTOXICITY OF (±)-ACETOMYCIN AND RELATED ANALOGS

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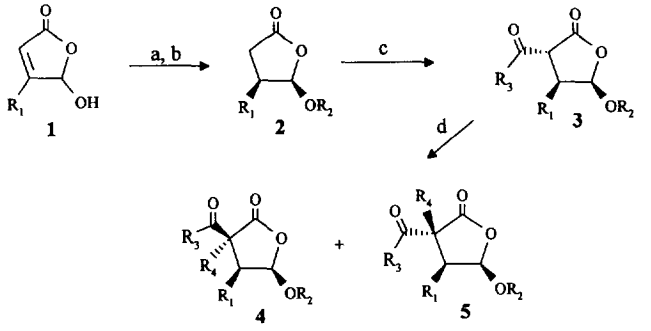
Abstract: (±)-Acetomycin and related analogs were synthesized and evaluated for their cytotoxic activity against B16 mouse melanoma cells, DA-3 mouse mammary adenocarcinoma cells, HT-29 human breast adenocarcinoma cells and MCF7-WT human colon adenocarcinoma cells. Blood stability assays were also perfomed on some of these products.

Acetomycin (**4a**), a small highly functionalized antibiotic, was initially isolated from *Streptomyces ramulosus sp.* by Prelog *et al.* in 1958.[1] The relative and absolute stereochemistry were determined many years later by X-ray analysis.[2] In 1987, acetomycin was found to be an antitumor agent (*in vitro*) against HCT-8 human colon adenocarcinoma cells, L1210 murine leukemia cells, and human tumor stem cells.[3] However, the product showed no *in vivo* activity due to rapid degradation caused by esterase mediated hydrolysis.[4] The first total synthesis was reported in 1990 by Tadano *et al.*[5] Very recently, Ziegler and Kim have reported an elegant synthesis of (-)-acetomycin using a selective Baeyer-Villiger oxidation as their key step.[6] In order to find an *in vivo* stable derivative, a short and versatile synthesis was needed. Our synthetic approach is based on a regioselective acetylation of lactone **2** (**Scheme I**). Inspired by the Ziegler synthesis, the starting materials **1**[7] were acetylated in standard conditions and stereoselectively hydrogenated[8] over palladium in the presence of triethylamine in ethyl acetate at 0°C affording the cis-substituted γ-butyrolactones **2** in 80 to 95% overall yield from **1** and ≥95% diastereoselectivity. The key step was realized by regioselectively generating the lactone enolate using 1.5 eq of LiHMDS as base at -78°C, and quenching after 1 min by rapidly adding 2 eq of an acid chloride (R_3COCl). The corresponding β-ketoesters **3** were obtained in 40 to 85% yield after flash chromatography. Enolates of **3** were treated with different electrophiles to provide mixtures of **4** and **5** in good yields. The products were separated by flash chromatography and their relative stereochemistry was easily determined by ^1H NMR.[9] Because of the anisotropic effect created by the carbonyl of the ketone side chain on C-3, the C-4 proton appears at 2.60 ppm for **4** and at 3.20 ppm for **5**.

Structure-activity relationship of (±)-Acetomycin

(±)-Acetomycin and related analogs were tested for their cytotoxic activity against B16 mouse melanoma cells, DA-3 mouse mammary adenocarcinoma cells, HT-29 human colon adenocarcinoma cells and MCF7-WT human breast adenocarcinoma cells. In general, compounds **4** (see **table I**) were slighty more active than the corresponding isomers **5**. Replacing the methyl group on C-3 (R_4) by a polar group (**4 f,g** and **5 f,g**), a hydrogen (**5e**) or by a bulkier group (**4b, d** and **5b**) resulted in a loss of activity. On the other hand, introduction

of a butanone side chain (**4c**), a benzoate group (**4h**) or a bromine (**4i**) did not affect the activity. Substituting the methyl on C-4 (R_1) by an isopropyl group (**4o** and **5o**) dramatically reduced the overall potency. Removing the carbonyl group of the acetate on C-5 (R_2) led to inactive compounds (**4n** and **5n**). Adding substituents on the methyl ketone side chain on C-3 (**4j, 5k**) did not affect the activity while replacing the acetate on C-5 by a benzoate or a pivaloate group gave slightly better compounds.



	R_1	R_2	R_3	R_4		R1	R2	R3	R4
a	Me	Acetyl	Me	Me	**i**	Me	Acetyl	Me	Br
b	Me	Acetyl	Me	$CH_2C_6H_5$	**j**	Me	Acetyl	CH_2Br	Me
c	Me	Acetyl	Me	$CH_2CH_2COCH_3$	**k**	Me	Acetyl	C_6H_5	Me
d	Me	Acetyl	Me	$CHS(CH_2)_3S$	**l**	Me	Pivaloyl	Me	Me
e	Me	Acetyl	Me	H	**m**	Me	Benzoyl	Me	Me
f	Me	Acetyl	Me	OH	**n**	Me	Et	Me	Me
g	Me	Acetyl	Me	OAc	**o**	$(Me)_2CH$	Acetyl	Me	Me
h	Me	Acetyl	Me	OBz					

Scheme I. (a) R_2COCl, pyridine, CH_2Cl_2, 0°C; (b) H_2, 10% Pd/C, Et_3N, EtOAc, 0°C (80-95% from **1**); (c) LiHMDS (1.5 eq), THF, -78°C; then R_3COCl (2 eq) (40- 85%); (d) R_4X, K_2CO_3, Acetone, reflux (R_4= Alkyl) (60-90%); NaHMDS (1.0 eq), MCPBA, benzene, r.t. (R_4= OH) (40%); NaHMDS (1.0 eq.), benzoyl peroxide, benzene, r.t. (R_4= OBz) (57%); NBS, CH_3CN, r.t. (R_4= Br) (30%); LiHMDS, 1, 3- dithienium tetrafluoroborate, THF, -78°C (R_4= $CHS(CH_2)_3S$)(86%).

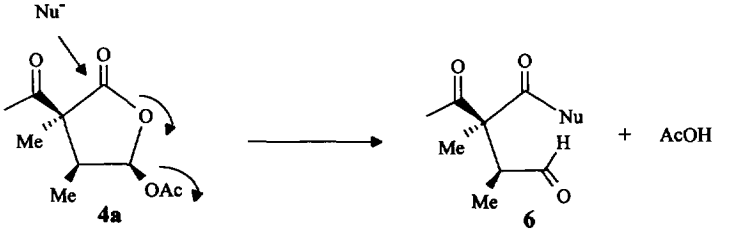
Proposed mechanism of action of acetomycin

Previous structure-activity relationship studies revealed that the lactone carbonyl plays a very important role in biological activity.[5c] Our results show that the ester functionality at C-5 is equally important since ethoxy analog **4n** is inactive. Based on SAR and known reactivity of γ-acetoxy γ-butyrolactone,[11] the mechanism of drug action (**Scheme II**) may occur via a nucleophilic attack on the lactone carbonyl producing the acyclic aldehyde **6** and 1 eq of acetic acid. Support for this hypothesis comes from the fact that analogs with a poor leaving group at C-5 were not active.

Table I. Cytotoxicity ($IC_{50}(\mu M)$) of Compounds **4** and **5** in MTT assay.[10]

Compound	B16	DA-3	HT-29	MCF7-WT
4a	4	7	5	6
4b	41	45	40	48
4c	7	8	7	6
4d	>50	>50	>50	>50
4f	>50	>50	>50	>50
4g	>50	>50	>50	>50
4h	6	3	8	22
4i	9	6	12	17
4j	6	4	6	4
4l	2	1	2	3
4m	1	1	1	3
4n	>50	>50	>50	>50
4o	>50	>50	>50	>50
5a	14	9	12	>10
5b	45	44	39	51
5e	>50	>50	>50	>50
5f	>50	>50	>50	>50
5g	>50	>50	>50	>50
5k	7	15	7	15
5l	10	4	9	7
5m	5	2	6	8
5n	>50	>50	>50	>50
5o	>50	>50	>50	>50

Moreover, the presence of bulkier groups at C-3 or C-4 decreased activity, by sterically hindering the electrophilic carbonyl group. The loss of activity for compound **5e** may be due to the presence in solution of the enol form which should reduce the lactone reactivity. The fact that various stereoisomers of acetomycin display equivalent biological activity suggests that specific binding to a receptor is not operating. *In vitro* stability



Scheme II. Possible mechanism of action of acetomycin.

assays in mouse blood[12] were performed for the most potent analogs **4l** and **4m** as well as for (±)-acetomycin (**4a**), all of which exhibited half-lives of less than 5 min.

In conclusion, a versatile synthesis of (±)-acetomycin and related analogs was developed.[13] Structure relationship studies led to a possible mechanism of action for acetomycin. The blood stability assays revealed that this class of compounds is not stable in mouse blood. Acetomycin might therefore be considered as a weak unstable alkylating agent.

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12. Samples incubated at 37°C; aliquots removed at: 0, 2, 5, 10, 15, 20 minutes; protein precipitation with acetonitrile; supernatant evaporated to dryness and reconstituted with mobile phase; injected onto an HPLC and signal monitored by UV absorbance or fluorescence. Procedure provided by Dr Robert Hart from Glaxo Inc. Research Institute NC, USA.
13. Details on this synthesis will be published elsewhere.

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