AMIDES OF POLYENE MACROLIDE AUREOFACIN

SYNTHESIS AND BIOLOGICAL PROPERTIES

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Synthesis and biological properties of a number of amides of polyene macrolide antibiotic aureofacin obtained in the reaction of the antibiotic with various glycine esters are described.

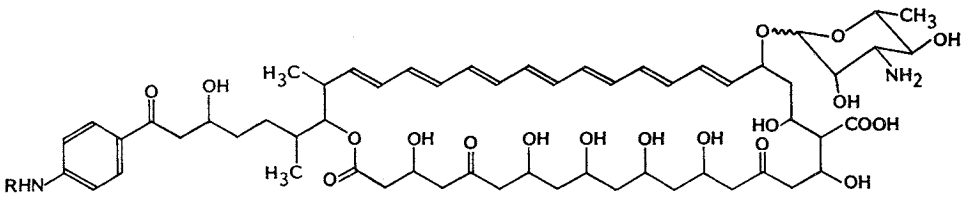
Polyene macrolides are known to be potent antifungal agents of clinical significance. Among them, amphotericin B remains one of the most important antibiotics currently available for the treatment of a wide variety of deep-seated mycotic infections in humans[1]. The clinical use of these compounds is hampered by a number of undesirable side effects, the most serious being the impairment of renal function. These side effects are consequences of poor selective toxicity of polyene macrolides. Only minimal differences exist in the antibiotics' affinities towards the animal and fungal cellular targets, membrane cholesterol and ergosterol, respectively[2].

Many attempts have been made to prepare derivatives of polyene macrolides with improved physico-chemical and biological properties[3~10]. The derivatives were obtained mainly by the modifications carried out at the amino and carboxyl groups in the molecules of all types of this class of antibiotics. It has been established that improvement of selective toxicity of polyenes may be attained by substitution at the carboxyl group resulting in the absence of carboxylate anion[11].

Continuing our studies on further improvement of biological properties of polyene macrolides *via* modification at the carboxyl group we focused our interest on the aromatic heptaene macrolides subgroup, represented by aureofacin, since it comprises polyenes with the highest antifungal activity and also the highest specificity of membrane effects related to channel formation. This specificity enables excellent recovery of animal cells from injury, expressed by the decay of channels in the repair process[12].

Aureofacin is a mixture of the closely related compounds, vacidin A and gedamycin[11]. Their

Fig. 1. Structure of aureofacin.
Vacidin A: R=H, gedamycin: R=CH_3.



structures, identical with the constitution of partricins A and B[13] are presented in Fig. 1.

We synthesized a group of aureofacin amides by reacting the antibiotic with various glycine esters to determine the influence of steric effects of substituted amide grouping on selective toxicity.

Materials and Methods

General

Aureofacin was obtained from Pharmaceutical Works Tarchomin-Polfa (Warsaw, Poland). The reactions were monitored by TLC on Kieselgel 60 plates (Merck). IR spectra were taken on UR-10 (Carl Zeiss, Jena) spectrometer in KBr; absorption bands were recorded in wave numbers (cm^{-1}). UV spectra were determined with a Beckman Model 3600 spectrophotometer in MeOH. ^1H NMR spectra were obtained on a Bruker WH-400 instrument at 400.13 MHz in DMSO-d_6 and CD_3OD solution. Chemical shifts (δ in ppm) are reported relative to internal TMS. Field desorption (FD) and fast atom bombardment (FAB) mass spectra were recorded with the Varian-MAT 711 and 311A instruments, respectively. Aureofacin modifications products were purified by counter-current distribution on a Craig apparatus using the solvent system $CHCl_3$ - MeOH - 0.5% NaCl aq solution (2:2:1).

Biological Assays

Antifungal Activity (IC_{50}): Antifungal activity was measured as the IC_{50} (concentration of compound causing 50% inhibition of cells growth) on *Saccharomyces cerevisiae* ATCC 9763 and *Candida albicans* 1440. IC_{50} values were determined by the serial-dilution method in liquid medium (Bactopeptone 1% and glucose 2% in 0.5% NaCl) inoculated to an optical density of 0.02 at 660 nm (2.2×10^5 cells/ml). Growth was monitored turbidimetrically with a colorimeter at 660 nm after 24 hours incubation at 28°C and IC_{50} values were obtained graphically from dose-response curves.

Hemolytic Activity (EH_{50}): Hemolysis experiments were carried out on human erythrocytes obtained from citrated blood. Erythrocytes were separated from plasma and buffy coat by centrifugation ($2{,}000\times g$ for 15 minutes) and washed 3 times in isotonic saline. Then the packed cells were resuspended in 250 volumes of buffered saline and 2.5 ml aliquots of this diluted suspension incubated at 37°C in a shaking water bath with increasing amounts of the compounds tested. After 30 minutes, the suspension was centrifuged and the supernatant absorbance determined at 540 nm with a colorimeter. The concentration which induced 50% hemolysis (EH_{50}) was obtained graphically from the dose-response curves.

Determination of Potassium Release (EK_{50}): Portions (2.5 ml) of cell suspension in 310 mOsmol buffered choline chloride, pH 7.4, were incubated at 37°C, in a shaking water bath with increasing amounts of the tested compounds. After 1 hour, the samples were spun and the supernatant potassium concentration was determined by flame photometer. The value for 100% of potassium release was obtained by hypotonic lysis in water.

IC_{50}, EH_{50} and EK_{50} are reported as the mean values from at least three separate experiments $\pm$ standard deviation.

Results and Discussion

Synthesis

Aureofacin derivatives were obtained by the reaction described in Fig. 2.

Amides were synthesized by the azide method using diphenyl phosphorazidate[14] (DPPA) for carboxyl group activation. The reactions were carried out in *N,N*-dimethylformamide, with triethylamine as a base. Despite polyfunctional character of the antibiotic, no protective groups were used. Following the procedure described by FALKOWSKI *et al.*[15], excess of amine component was used to avoid the intramolecular cyclization (lactam formation) or intermolecular acylation leading to antibiotic dimerization.

Fig. 2. Synthesis of aureofacin derivatives.
Y: CH_3, C_2H_5, $CH(CH_3)_2$, $C(CH_3)_3$, $CH_2C_6H_5$.

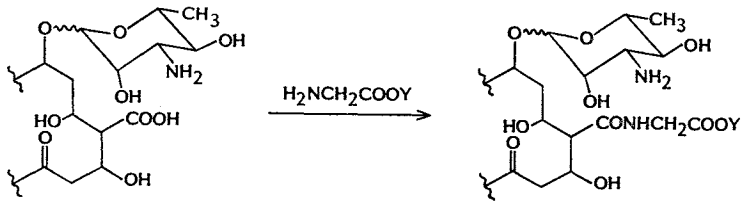


Table 1. Rf values of aureofacin and its derivatives (**1**~**9**) on silica gel TLC plates.

Compound	Y	R	A	B
Aureofacin	—	H,CH_3[a]	0.08	0.22
1	CH_3	H	0.22	0.22
2	CH_3	CH_3	0.24	0.24
3[b]	C_2H_5	H	0.28	0.26
4	$CH(CH_3)_2$	H	0.31	0.29
5	$CH(CH_3)_2$	CH_3	0.36	0.30
6	$C(CH_3)_3$	H	0.35	0.31
7	$C(CH_3)_3$	CH_3	0.40	0.33
8	$CH_2C_6H_5$	H	0.35	0.34
9	$CH_2C_6H_5$	CH_3	0.41	0.36

[a] Unresolved antibiotic complex.
[b] Corresponding gedamycin derivative was not obtained in pure form.
A: $CHCl_3$ - MeOH - H_2O (20 : 8 : 1).
B: EtOAc - AcOH - H_2O (4 : 4 : 1).

In a typical synthesis, triethylamine (1.40 ml) and DPPA (1.73 ml) were added to a cooled solution of aureofacin (2.224 g) and glycine *tert*-butyl ester dibenzenesulfimide salt (3.428 g, 4-fold molar excess) in *N*,*N*-dimethylformamide (45 ml) and the reaction was allowed to proceed for 10 hours at ice bath temperature. Crude product was precipitated with a mixture of ethyl ether and acetone (3 : 1, 500 ml). The precipitate was separated by centrifugation, washed 3 times with ethyl ether and dried *in vacuo*. Final purification of the product using the counter-current distribution (250 transfers) yielded two separated aureofacin components derivatives, vacidin A and gedamycin *tert*-butoxycarbonylmethyl-amides (**6**; 319 mg and **7**; 185 mg, respectively). ^{1}H NMR, (tetramethylsilane) amide **6**: δ 0.88 (3H, d, CH_3), 0.97 (3H, d, CH_3), 1.18 (3H, d, 5′-CH_3), 1.42 (9H, s, $COOC(CH_3)_3$), 4.46 (1H, s, 1′-H), 5.35~6.90 (polyene region), 6.55 (2H, d, Ar), 7.67 (2H, d, Ar); amide **7**: δ 0.87 (3H, d, CH_3), 0.97 (3H, d, CH_3), 1.17 (3H, d, 5′-CH_3), 1.42 (9H, s, $COOC(CH_3)_3$), 2.73 (3H, s, NCH_3), 4.45 (1H, s, 1′-H), 5.35~6.90 (polyene region), 6.52 (2H, d, Ar), 7.71 (2H, d, Ar).

Chromatographic properties of all products are given in Table 1.

The electron absorption spectra of the products tested were identical to those of the parent antibiotic, aureofacin. The IR spectra of the derivatives obtained showed bands at 1650~1645 cm^{-1}, corresponding to the secondary amides. Examination of the derivatives using the field desorption and positive ion FAB mass spectrometry ($(M+Na-H_2O)^+$ and MH^+ ions, respectively) confirmed the expected molecular masses.

Biological Activity

The synthesis and biological properties of some amides of polyene macrolides have been previously

Table 2. Biological properties of aureofacin and its derivatives (**1**~**9**).

Compound	IC_{50} (μg/ml)		EH_{50} (μg/ml)	EK_{50} (μg/ml)
	Saccharomyces cerevisiae	*Candida albicans*		
Aureofacin	0.0021±0.0011	0.0015±0.0010	0.23±0.05	0.023±0.006
VacGlyOMe (**1**)	0.0010±0.0009	0.0003±0.0001	10.5±3.0	0.028±0.002
GedGlyOMe (**2**)	0.0004±0.0002	0.0004±0.0002	8.0±2.0	0.021±0.008
VacGlyOEt (**3**)	0.0014±0.0007	0.0005±0.0002	12.0±2.0	0.035±0.005
VacGlyO*i*Pr (**4**)	0.0019±0.0003	0.0007±0.0002	2.2±0.7	0.054±0.006
GedGlyO*i*Pr (**5**)	0.0010±0.0003	0.0007±0.0004	6.0±2.0	0.035±0.005
VacGlyO*t*Bu (**6**)	0.0028±0.0009	0.0011±0.0003	2.1±0.7	0.078±0.029
GedGlyO*t*Bu (**7**)	0.0009±0.0003	0.0010±0.0005	5.0±2.5	0.042±0.005
VacGlyOBzl (**8**)	0.0025±0.0009	0.0021±0.0010	1.9±0.9	0.048±0.002
GedGlyOBzl (**9**)	0.0014±0.0003	0.0020±0.0012	4.3±1.5	0.031±0.004

Vac; Vacidin A, Ged; gedamycin, *i*Pr; isopropyl, *t*Bu; *tert*-butyl, Bzl; benzyl.

described[8,15]. However, no systematic studies with aromatic heptaenes were done. Only one compound of this group, namely aureofacin *n*-butyl amide, was described[15]. In this paper a series of amides of aureofacin components, vacidin A and gedamycin, were synthesized and their biological activities determined and the influence of steric effects at carboxyl group on selective toxicity was examined.

Biological activities of vacidin A and gedamycin derivatives examined towards ergosterol and cholesterol containing cells are presented in Table 2. With the ergosterol containing organisms, *Saccharomyces cerevisiae* and *Candida albicans*, activity was measured as IC_{50}. Activity towards human erythrocytes, the model for cholesterol containing cells was determined as EH_{50} and EK_{50}.

Generally, all compounds examined regardless of the size of the carboxyl substituent retained antifungal activity of the parent antibiotic. The differences in sensitivity of both fungi are negligible. It should be noted that gedamycin derivatives are somewhat more active when compared to vacidin A derivatives for *S. cerevisiae*. This effect is not observed with *C. albicans*.

Similarly, the biological activities of all derivatives towards human erythrocytes measured as EK_{50} were retained when compared to that of aureofacin. However, when the antifungal activity of the derivatives and their ability to induce potassium efflux from erythrocytes are compared there is a definite improvement in differential susceptibility. Derivatives exhibited a several-fold increase in the ratio of EK_{50} to IC_{50} as compared to that of aureofacin. These results corroborate the previous findings indicating that the lack of free carboxyl group in aromatic and non-aromatic heptaenes improves selective toxicity[11,16]. The hemolytic activities (EH_{50}) of the derivatives towards erythrocytes, in general, compared to that of aureofacin were reduced one order or more. Taking this effect into account the improvement of selective toxicity towards ergosterol and cholesterol containing cells is substantial. Hemolysis is one of the serious side effects encountered with animal cells and consequently must be considered in efforts to improve the selective toxicity of the polyenes.

Comparison of EH_{50} data for various amides indicates that hemolytic activity varies with substituent size, the lowest activity exhibited by the methoxycarbonyl- and ethoxycarbonylmethylamides. It can be concluded that the mentioned derivatives are optimal among the amides studied from the point of view of their biological properties. They exhibit the highest antifungal activity with simultaneous lowest ability to hemolyze erythrocytes.

It should be noted that the hemolysis data do not parallel the permeabilizing activities of these compounds. It could be assumed that although the nature of the substituent at the carboxyl group does not influence significantly channel forming ability, it does have a marked effect on the characteristics of the permeability pathway induced.

On the basis of data presented the suggestion can be made that the lack of essential influence of the size of the substituent at the carboxyl group on the ability of the active compound interaction with the membranes indicates that the carboxyl substituent is located outside that region of the molecule involved in polyene-sterol complex formation.

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