CLAVINES AS ANTITUMOR AGENTS, 3†: CYTOSTATIC ACTIVITY AND STRUCTURE/ACTIVITY RELATIONSHIPS OF 1-ALKYL AGROCLAVINES AND 6-ALKYL 6-NORAGROCLAVINES

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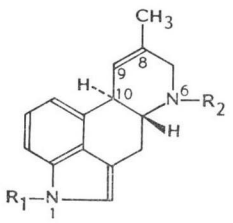
The cytostatic potential of twenty antibiotic agroclavines has been examined in the L5178y mouse lymphoma cell system. Twelve of these compounds are described for the first time. It is shown that the substituent at N-1 of agroclavine is very important whereas the substituent at N-6 is of less influence if it is not hydrogen. Incorporation studies in the presence of 1-propylagroclavine suggest that DNA synthesis in the lymphoma cells is inhibited. The effect on the corresponding [^{3}H]thymidine incorporation in murine spleen lymphocytes is comparably low. Neither a significant change of mRNA efflux nor of DNA polymerase α and β activities was caused. The mechanism of action seems to be a fundamentally new one for ergoline compounds as interactions with α-adrenoceptors, dopamine and 5-hydroxytryptamine receptors are not involved.

Clavines are alkaloids of the 8-methyl- or 8-hydroxymethylergoline type which are produced by fungi of the genera *Claviceps*, *Aspergillus* and *Penicillium* and by certain genera of the plant family *Convolvulaceae*[1]. As we were able to show, some natural clavines and their derivatives possess remarkable antibiotic activity against a series of pathogenic and nonpathogenic bacteria[2~4] and even against yeasts[4,5]. Initial work indicated that these compounds are potent cytostatic agents in the L5178y mouse lymphoma cell system[6,7]. These findings prompted us to investigate structure/activity relationships with the aim of developing the clavines as antitumor agents.

In the present contribution the molecule of the most active natural compound, agroclavine (**1**), discovered in the early fifties in wild grass ergot by ABE[8], was varied by substitution at N-1 and N-6 in order to establish homologous series for testing.

Fig. 1. Structure of agroclavines.
For $R_1 \neq H$ and $R_2 \neq CH_3$ see Table 4.



Agroclavine (**1**) $R_1=H$, $R_2=CH_3$

Materials and Methods

Agroclavine and Its Derivatives

Agroclavine (**1**) was isolated from surface cultures of *Claviceps* strain 47a. The 1-alkyl derivatives **2**~**10** (see Table 4) were prepared by alkylation of **1** with potassium and alkyl iodide in NH_3 as already

† For 2 see ref 7.

Table 1. Data on semi-synthetic 1-alkyl agroclavines.

All compounds were obtained as (−)-di-*O*,*O*′-*p*-toluoyl hydrogen tartrates; in consequence all data given in this table refer to the salt. For structure of the compounds see Table 4.

Compound	MP (°C (dec))	Yield (%)	Summation formula (molecular weight)	Calcd: / Found: C	H	N	IR ν_{max}^{KBr} (cm^{-1})
6	146~149	45	$C_{21}H_{28}N_2 \cdot C_{20}H_{18}O_8$	70.9	6.67	4.0	3450, 2920, 2490, 1720,
			(694.8)	70.8	6.87	4.2	1610, 1450, 1410
7	137~141	60	$C_{22}H_{30}N_2 \cdot C_{20}H_{18}O_8$	71.2	6.83	4.0	3450, 2920, 2510, 1720,
			(708.9)	71.1	6.77	4.1	1610, 1450, 1410
8	90~ 95	40	$C_{23}H_{32}N_2 \cdot C_{20}H_{18}O_8$	68.9	7.16	3.8	3450, 2920, 2850, 2510,
			$\cdot 1\frac{1}{2}H_2O$ (749.9)	68.8	7.13	4.2	1720, 1610, 1450, 1410
10	110~115	9	$C_{22}H_{30}N_2 \cdot C_{20}H_{18}O_8$	71.2	6.83	4.0	3430, 3050, 2950, 2870,
			(708.9)	71.0	6.77	3.8	1730, 1610, 1440, 1400

Table 2. Data on semi-synthetic 6-alkyl 6-noragroclavines.

Compounds **14~17** were obtained as free bases, compound **13** was obtained as (−)-di-*O*,*O*′-*p*-toluoyl hydrogen tartrate; in consequence the data refer to the salt for **13** and to the free base for **14~17**. For structure of the compounds see Table 4.

Compound	MP (°C (dec))	Yield (%)	Summation formula (molecular weight)	MS (M^+)	Calcd: / Found: C	H	N	IR ν_{max}^{KBr} (cm^{-1})
13	101~106 (acetone/ H_2O)	60	$C_{18}H_{22}N_2 \cdot C_{20}H_{18}O_8$ $\cdot 1\frac{1}{2}H_2O$ (679.7)	—	67.2	6.33	4.1	3570, 3430, 3250, 3050,
					67.5	6.30	3.9	2970, 2880, 2750, 2540,
								1720, 1610, 1440
14	134~137	60	$C_{19}H_{24}N_2$ (280.4)	280	81.4	8.57	10.0	3410, 3150, 3110, 3060,
					81.3	8.54	9.7	2950, 2880, 2800, 1600,
								1440
15	109~111 (EtOH/ hexane)	50	$C_{20}H_{26}N_2$ (294.5)	294	81.6	8.90	9.5	3410, 3150, 3110, 3070,
					80.8	8.79	9.2	2930, 2880, 2800, 1600,
								1440
16	115~118 (EtOH/ hexane)	50	$C_{21}H_{28}N_2$ (308.5)	308	81.8	9.15	9.1	3420, 3160, 3110, 3070,
					80.9	9.00	8.9	3020, 2950, 2890, 2850,
								2810, 1610, 1450
17	101~103 (EtOH/ hexane)	45	$C_{22}H_{30}N_2$ (322.5)	322	81.9	9.38	8.7	3420, 3150, 3110, 3070,
					80.7	9.03	8.4	3020, 2970, 2930, 2860,
								2810, 1610, 1440

Table 3. Data on semi-synthetic 1-pentyl-6-noragroclavines.

All compounds were obtained as free bases (yellowish to colorless oils); for structure see Table 4.

Compound	Yield (%)	Summation formula (molecular weight)	MS (M^+)	Calcd: / Found: C	H	N	IR ν_{max}^{KBr} (cm^{-1})
18	60	$C_{21}H_{25}N_3$ (319.5)	319	—	—	—	3410, 3070, 2960, 2930, 2870,
							2210, 1610, 1520, 1470
19	60	$C_{20}H_{26}N_2$ (294.4)	294	81.6	8.90	9.5	3440, 3080, 2970, 2930, 2870,
				81.0	9.14	9.2	1680, 1600, 1550, 1440
20	30	$C_{23}H_{32}N_2$ (336.5)	336	82.1	9.59	8.3	3430, 3070, 2970, 2930, 2870,
				81.0	9.24	8.2	1720, 1620, 1550, 1470, 1440

—: Not determined.

described previously for compounds **2**~**5** and **9**[9,10]. For data on the new compounds **6**~**8** and **10**, see Table 1. Compound **11** was obtained from **1** by means of the cyanogen bromide method[10]. The 6-alkyl 6-nor derivatives **12**~**17** were prepared by alkylation of **11** with alkyl iodide in *N,N*-dimethylformamide/K_2CO_3 as already described for **12**[10]. For data on the new compounds **13**~**17**, see Table 2. Compounds **18**~**20** were obtained by combination of these methods; for data see Table 3.

Other Materials

Concanavalin A (ConA) (No. C7275), lipopolysaccharide (LPS) (No. L4130) were from Sigma Chemical Co., St. Louis, MO, U.S.A.; [*methyl*-^{3}H]thymidine (specific activity 78 Ci/mmol), [^{3}H]uridine (generally labeled; specific activity 7.6 Ci/mmol), [^{3}H]phenylalanine (specific activity 9.3 Ci/mmol), and [^{3}H]dCTP (specific activity 30 Ci/mmol) from the Radiochemical Centre, Amersham, England.

Cell Growth Inhibition Studies

L5178y mouse lymphoma cells[11] were grown in EAGLE's minimum essential medium, supplemented with 10% horse serum in roller tube cultures[12]. Dose-response experiments were performed as described[13,14].

The purity of the ergoline compounds was higher than 98%, as determined by chromatographic methods as well as by elementary analysis. For preparation of the test solutions 10 mg of the compounds were dissolved in 0.2 g dimethyl sulfoxide; afterwards 100 ml sterilized 0.05% aqueous sodium tartrate solution (pH about 7.25) were added. Equimolar concentrations of tartrate with addition of dimethyl sulfoxide were taken as controls.

Incorporation Studies

Five ml suspensions (100,000 cells/ml) of exponentially growing L5178y cells were incubated for 24 hours with 1-propylagroclavine (**4**). During the last 2 hours of incubation the radioactively labeled precursors 10 μCi [^{3}H]thymidine (dThd), 10 μCi [^{3}H]uridine (Urd) or 10 μCi [^{3}H]phenylalanine (Phe) were added. Samples of 1 ml were analyzed for cell concentration and for acid-insoluble radioactivity[15].

Determination of Mitogenic Effects *In Vitro*

Spleen lymphocytes were prepared from 5~6 weeks old male outbread NMRI mice. The cells were dissociated mechanically be squeezing the tissue through an 80 steel mesh screen. Erythrocytes were removed from the single cell suspension by NH_4Cl-treatment. The spleen cells, containing macrophages, were suspended in RPMI 1640 medium, supplemented with 20% fetal calf serum, at a density of 1.5×10^7 cells/ml. 5×10^5 cells each were placed into a final volume of 200 μl in cups of sterile flat-bottomed microtitration plates (Costar No. 3596). The assays were cultured in a fully humidified atmosphere of 5% CO_2 and air at 37°C. Where indicated 2 μg/ml of ConA or 20 μg/ml of LPS were added to the cultures. The lymphocytes were cultivated for 72 hours; 18 hours before the end of the experiment 0.2 μCi of [^{3}H]dThd was added to each cup. Incorporation of [^{3}H]dThd into DNA was determined as described[15].

Each experiment was done in quadriplicate. The incorporation rate of [^{3}H]dThd in the absence of mitogens was $39{,}750\pm2{,}600$ cpm/5×10^5 cells$\times$18 hours and in the presence of 2 μg ConA/ml or 20 μg LPS/ml $127{,}400\pm9{,}350$ and $134{,}550\pm9{,}800$ cpm/5×10^5 cells$\times$18 hours, respectively. The ED_{50} concentrations causing a 50% reduction of [^{3}H]dThd incorporation were estimated by logit regression[14]. The blastogenic index expresses the ratio between the counts per minute of [^{3}H]dThd incorporation into lymphocytes incubated with the mitogen and incorporation with media alone.

DNA Polymerase Assay

DNA polymerase α and β were isolated from L5178y mouse lymphoma cells[16] and assayed as described[17]. Four parallel determinations were performed per value given. The standard deviations did not exceed 12%.

Measurement of RNA Efflux

RNA efflux experiments were performed with nuclei, isolated from L5178y mouse lymphoma cells. Prior to this isolation, the cells were incubated for 2 hours in the presence of [^{3}H]Urd. The isolated nuclei were incubated for up to 30 minutes in the presence of an ATP-containing medium and the agro-

Table 4. Influence of the substituent at N-1 and N-6 of agroclavine on the cytostatic activity (L5178y mouse lymphoma cell system).

Substituent	N-1-Alkyl series (N-6: CH_3)			N-6-Alkyl 6-nor series (N-1: H)		
	Compound		ED_{50} concentration (μM)	Compound		ED_{50} concentration (μM)
H	**1**	Agroclavine	6.3	**11**	6-Noragroclavine	15.8
CH_3	**2**	1-Methyl-	5.2	**1**	Agroclavine	6.3
CH_2CH_3	**3**	1-Ethyl-	2.4	**12**	6-Ethyl-6-nor-	7.1
$(CH_2)_2CH_3$	**4**	1-Propyl-	1.3	**13**	6-Propyl-6-nor-	5.3
$(CH_2)_3CH_3$	**5**	1-Butyl-	1.0	**14**	6-Butyl-6-nor-	2.9
$(CH_2)_4CH_3$	**6**	1-Pentyl-	0.87	**15**	6-Pentyl-6-nor-	5.1
$(CH_2)_5CH_3$	**7**	1-Hexyl-	1.6	**16**	6-Hexyl-6-nor-	3.4
$(CH_2)_6CH_3$	**8**	1-Heptyl-	4.4	**17**	6-Heptyl-6-nor-	3.9
$CH_2CH(CH_3)_2$	**9**	1-(2′-Methyl-propyl)-	3.2			
$(CH_2)_2C(CH_3)_3$	**10**	1-(3′,3′-Dimethylbutyl)-	3.0			

Compounds varied in positions 1 and 6		ED_{50} concentration (μM)
18	1-Pentyl-6-cyano-6-noragroclavine	4.5
19	1-Pentyl-6-noragroclavine	1.4
20	1-Pentyl-6-propyl-6-noragroclavine	1.3

clavine derivative. The RNA released during this period of time was collected by trichloroacetic acid precipitation. All experimental details were given previously[18].

Results

In the present study the cytostatic potential of twenty clavines has been examined; among them nine belong to the 1-alkyl agroclavine series and seven are 6-alkyl 6-noragroclavines, agroclavine included. The remaining compounds are 6-noragroclavine (**11**) and three clavines with structural variations in position 1 and position 6 (compounds **18**~**20**). Twelve of these ergolines are described for the first time (see Tables 1~3).

Structure/Activity Relationships

Table 4 compares the ability of the natural alkaloid agroclavine (**1**) and its derivatives to inhibit growth of L5178y mouse lymphoma cells *in vitro*. 1-Alkylation (compounds **2**~**10**) significantly augmented the cytostatic effect. Those homologs which bear a three to six carbon atoms membered straight-chain substituent at N-1 (compounds **4**~**7**) were highly effective, giving 50% inhibition at a concentration of 0.87 to 1.6 μM. Four and six membered branched-chain substituted agroclavines (compounds **9** and **10**) showed reduced activity compared with their straight-chain isomers.

Removal of the methyl group at N-6 of compound **1** resulted in a marked loss of activity (compound **11**). Re-alkylation at N-6 with C_2~C_7-membered straight-chain substituents led again to remarkably active compounds (**12**~**17**). Analogous removal of the methyl group at N-6 of compound **6** however, yielding 1-pentyl-6-noragroclavine (**19**), caused only a moderate reduction in cytostatic activity (ED_{50} concentration of **6** and **19**: 0.87 and 1.4 μM, respectively). Re-alkylation at N-6 with the

Fig. 2. Effect of 1-propylagroclavine (**4**) on the synthesis of macromolecules in L5178y mouse lymphoma cells.

Each point represents the means of counts of 6-fold samples; the standard deviations did not exceed 10%.

Incorporation of [^{3}H]dThd into DNA (△), of [^{3}H]Urd into RNA (●), and of [^{3}H]Phe into protein (○). The incorporation rates of the controls were set to 100%. ---, ED_{50} value.

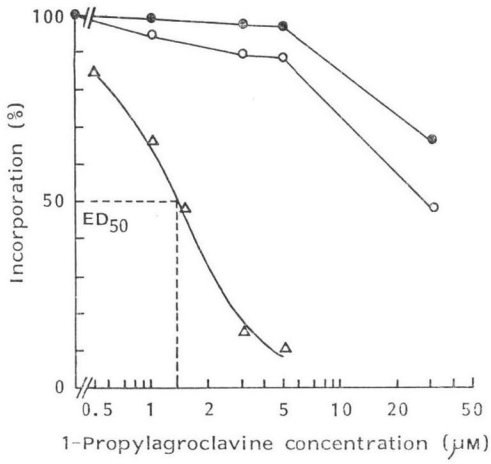
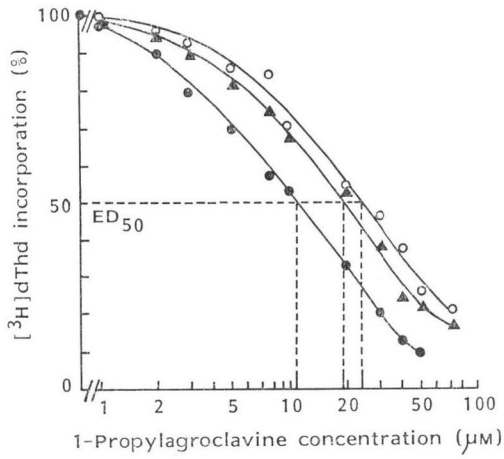


Fig. 3. Influence of 1-propylagroclavine (**4**) on the incorporation of [^{3}H]dThd into DNA of murine spleen lymphocytes.

Each value comes from 4 parallel determinations; the means are indicated and the standard deviations did not exceed 12%.

The experiments were performed in the absence of mitogens (●), or in the presence of 2 μg ConA/ml (○), or in the presence of 20 μg LPS/ml (▲). The incorporation is given in percent (controls: 100%). ---, ED_{50} value.



C_3-membered straight-chain substituent, yielding 1-pentyl-6-propyl-6-noragroclavine (**20**), had almost no effect on the proliferation inhibiting potency (ED_{50} concentration of **20**: 1.3 μM).

Influence of 1-Propylagroclavine on the Synthesis of Macromolecules in L5178y Cells

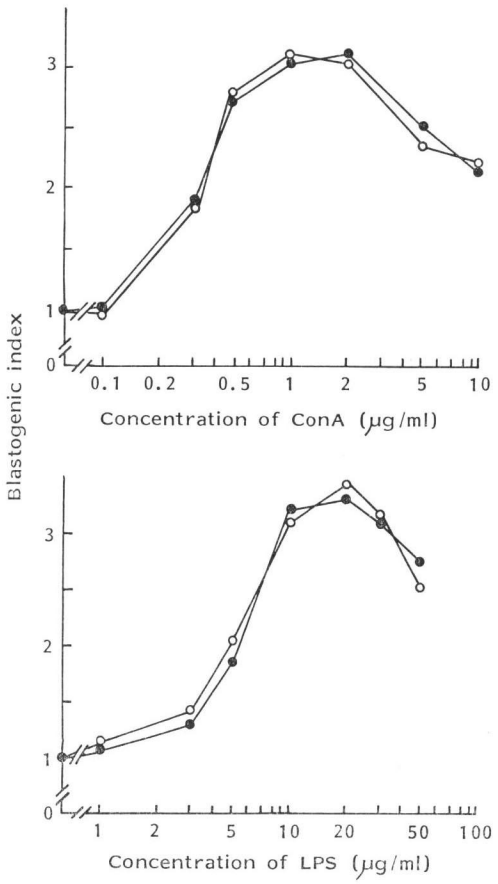
As an approximative measure of the effect of 1-propylagroclavine (**4**) on the synthesis of macromolecules in intact cell system the changes of the incorporation rates of [^{3}H]dThd into DNA, [^{3}H]Urd into RNA, and [^{3}H]Phe into protein were determined. Compound **4** inhibited strongly DNA synthesis (ED_{50} concentration 1.4 μM, 100,000 cells/ml), while RNA and protein synthesis are only slightly affected (Fig. 2). It is interesting to note that the reduction of cell growth by 50% (Table 4; 1.3 μM) occured at a concentration of the clavine derivative, which is very close to that causing a 50% reduction of DNA synthesis.

Influence of 1-Propylagroclavine on *In Vitro* Spleen Lymphocyte Blastogenesis

Compared to its influence on the [^{3}H]dThd incorporation rate in the L5178y cell system, the effect of compound **4** on [^{3}H]dThd incorporation in murine spleen lymphocytes is relatively low. As summarized in Fig. 3, a 50% reduction of the incorporation rate of lymphocytes, not stimulated with mitogens, occured at a compound concentration of 10.3±0.6 μM. ConA- and LPS-stimulated lymphocytes are even more resistant towards **4**; the ED_{50} concentrations for these cultures were determined to be 23.2±1.3 μM and 19.2±1.3 μM, respectively.

Fig. 4. *In vitro* spleen lymphocyte blastogenesis following incubation with ConA or LPS, expressed as a blastogenic index.

The experiments were performed in the presence of 0 (●) and 5 μM of 1-propylagroclavine (**4**) (○). Further details are given under "Materials and Methods".



Choosing a concentration of 5 μM, the influence of **4** was determined on the blastogenic responses of murine spleen lymphocytes on the mitogens ConA and LPS (Fig. 4). The results of the experiments revealed no significant differences between the control assays and the compound-treated cultures. Maximal blastogenic response in the studies with ConA was measured at a concentration of 2 μg/ml and with LPS at 20 μg/ml.

Effect of 1-Alkyl Agroclavines on DNA Polymerase Activity and mRNA Efflux

In a first attempt to elucidate the mode of action on enzymic level we determined the possible influence of compound **5** on DNA polymerase α and β activity. Within the concentration range 0.01 ~ 10.0 μM this alkyl agroclavine displayed no statistically significant change of the enzyme activities: Control activity of DNA polymerase α; 9.7 $\pm$ 0.8 and of DNA polymerase β; 0.9 $\pm$ 0.1 mmol of [^{3}H]dCMP incorporated into acid-insoluble material/hour $\times$ mg protein.

Compound **4** was tested for its effect on RNA efflux from isolated nuclei as described under "Materials and Methods". In the presence of 2.5 mM ATP in the incubation medium 548 $\pm$ 45 dpm of acid-precipitable radioactivity was released from 10^6 nuclei during an incubation period of 30 minutes. Addition of 0.1 ~ 10 μM of compound **4** was determined to cause no significant change of the RNA efflux rate.

Discussion

Clavine molecules possess as part of their ergoline skeleton two nitrogen atoms; the basic N-6 and the non-basic indole nitrogen N-1. The relative cytostatic activities of the various agroclavine analogs in the L5178y lymphoma system indicate that derivatization of the natural compound **1** by alkylation at N-1 may be useful depending on the chain length of the alkyl group, with increasing activity until C_5 and then with decreasing values for compounds with a substituent $> C_5$. The corresponding chain elongation of the existing methyl group at N-6 of **1** does not influence cytostatic potency to a comparable extent. It is true that C_4-alkylation at N-6 of **11** increases potency approximately 2-fold compared with **1** and more than 5-fold compared with **11**, but all members of the 6-alkyl 6-nor series (**12** ~ **17**) are less active than the $C_2 \sim C_6$-alkylated N-1-substituted compounds **3** ~ **7**. Furthermore, there are some fluctuations within the 6-alkyl 6-nor series; compound **15** (C_5) is less active than **14** (C_4), but further increase of the alkyl substituent (C_6, C_7) again leads to more active compounds (**16**, **17**). The

N-6-alkylated clavines of Table 4 (**1**, **12**~**17**) are active within a relatively narrow ED_{50} concentration range of 2.9~7.1 μM compared with their N-1-analogs (**2**~**10**), which possess a range of activities between 0.87 and 5.2 μM indicating that the substituent at N-1 is of more influence and importance.

Comparison of the influence of straight-chain substituents at N-1 with analogous branched-chain groups show that chain branching is of disadvantage; compounds **5** and **7** are 3- and 2-fold more potent than **9** and **10**, respectively.

6-Noragroclavine (**11**) with its secondary and basic N-6, an inactive compound in contrast to the tertiary and basic N-6 compounds **1** and **12**~**17**, can be transformed into a potent cytostatic agent by C_5-alkylation at N-1 (compound **19**, ED_{50} concentration: 1.4 μM), thus proving that N-6 does not necessarily have to be tertiary. N-6 does not even need basic properties; the 6-cyano-6-nor derivative **18** is also active (ED_{50} concentration: 4.5 μM) in spite of the fact that the cyano substituent causes the loss of N-6 basicity. But **18** is C_5-alkylated at N-1, too; the corresponding compound not substituted at N-1 (6-cyano-6-noragroclavine) is inactive.

In conclusion the combination of these results with those of previously published papers[6,7,19] now allows a generalization with respect to the influence of the substituent at the non-basic N-1 (indole nitrogen) of clavines; 1-alkylation, especially C_3~C_5, does not only increase the potency of already active clavines but also transforms inactive clavines into cytostatically very active compounds, neutralizing the negative influence of the 8-hydroxymethyl substituent compared with the 8-methyl substituent[7], of the secondary state of N-6 in 6-norclavines compared with tertiary 6-alkyl 6-norclavines (see also ref 19), and of the N-6 cyano group compared with N-6 alkylated analogs.

Like 1-propylfestuclavine[6], 1-propylagroclavine (**4**) was also shown to be a potent and preferential inhibitor of DNA synthesis. It has now been clarified that this inhibition is not due to a direct effect on DNA polymerase α or β. Preliminary studies to determine the target, which causes the reduction of DNA synthesis, suggest that agroclavine (**1**) interferes with the state of organization of the nuclear reticulum (unpublished). RNA synthesis and nucleo-cytoplasmic efflux of mRNA are not affected at cytostatic concentrations.

It is known that natural clavines interact, like the lysergic acid amides, with dopamine (DA) and 5-hydroxytryptamine (5-HT) receptors and with a lower affinity even with α-adrenoreceptors[20]. Thus an obvious mechanism of action could involve the interaction with one of these receptors for the inhibition of proliferation, too. Nevertheless there is evidence indicating that this does not seem to be the case.

The following facts led us to the assumption that a mechanism involving interaction with DA receptors is improbable: (1) DA itself was not active in our cell system (ED_{50} concentration: 13.2 μM); (2) sulpiride, a DA antagonist, was also inactive (33.3 μM); (3) the combination of sulpiride and compound **4** did not show any change in the cytostatic activity compared with **4** alone; (4) simple lysergic acid amides (lysergic acid amide, isolysergic acid amide, LSD, methylergometrine) are well known as DA agonists and antagonists[20], but they were not cytostatic; (5) on the other hand it is true that agroclavine also shows dopaminergic properties, but N-1-substituted ergolines, *e.g.* methysergide and methergoline, do not[20,21] (N-1-alkylated agroclavines, however, are very potent cytostatic agents); (6) agroclavine is less active as an inhibitor of prolactin release than elymoclavine[21,22], but elymoclavine is cytostatically inactive. From all this evidence we conclude that the agroclavines are not working by the same mechanism as the ergoline compounds which are useful against prolactin-dependent tumors, *e.g.* bromocriptine, namely *via* DA receptors.

The following facts led us to the assumption that a mechanism of action involving interactions with 5-HT receptors is also improbable though even 1-alkyl agroclavines are 5-HT antagonists[23]: (1) Serotonin itself was absolutely inactive (ED_{50} concentration: >60 μM); (2) simple lysergic acid amides are well known as both 5-HT agonists and antagonists[20], but they were inactive as cytostatics.

Similar considerations lead us to conclude that interactions with α-adrenoreceptors are also not involved. Simple lysergic acid amides and even the ergopeptine type alkaloid ergotamine are cytostatically inactive or weakly active (ergotamine: ED_{50} concentration 11.3 μM, ergometrine >30 μM) though interacting with α-adrenoreceptors and DA/5-HT receptors.

Finally the fact, that cytostatically active clavines also show antibiotic properties against bacteria

and yeasts, could be further evidence against the participation of catecholamine or serotonin receptors in the mechanism of action; as far as we know these receptors do not exist in microorganisms.

The cytostatic potential of the agroclavines is relatively high; under otherwise identical conditions well-known cytostatic antibiotics like bleomycin, doxorubicin (adriamycin), and daunorubicin (daunomycin) reduce cell proliferation by 50% at 1.0, 1.2 and 1.9 μM, respectively.

Of potential importance for future application is the finding that 1-propylagroclavine (4) causes a 50% inhibition of the [^{3}H]dThd incorporation rate in murine spleen lymphocytes only at concentrations as high as 10~23 μM, depending on the presence of mitogens. In comparison, this inhibition was caused by the same compound in the L5178y cell system at 1.4 μM.

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