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Preliminary communication

New cytotoxic selenoderivatives of guaianolides

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Summary — A series of 13-phenylselenoderivatives of natural and semisynthetic guaianolides were prepared and their cytotoxicity tested *in vitro* against KB cell cultures. Generally the presence of the 13-Se-phenyl group led to an increased bioactivity (ID_{50}) supporting the hypothesis that it may act as a "masked" α-methylene-γ-lactone group.

Résumé — Nouveaux dérivés séléniés cytotoxiques des guaianolides. *Une série de 13 nouveaux dérivés séléniés des guaianolides naturels et semisynthétiques a été préparée et testée* in vitro *contre des cultures cellulaires KB. Généralement, la présence des sélénodérivés a mené à une augmentation de la bioactivité* (ID_{50}). *Les résultats obtenus confirment l'hypothèse que les produits séléniés peuvent agir biologiquement comme un groupe α-méthylène γ-lactone « masqué ».*

selenoderivatives / guaianolides / sesquiterpene lactones / cytotoxic activity

Introduction

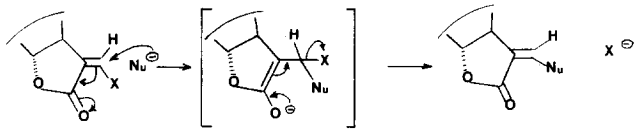
Naturally occurring unsaturated γ- and δ-lactones have attracted considerable interest in recent years because of their cytotoxic, anti-inflammatory, antibacterial, antihyperlipidemic and anti-allergic activity [1, 3].

In particular α-methylene-γ-lactone sesquiterpenes of natural, semisynthetic and synthetic origin can be considered as members of a large class of alkylating agents employed in cancer chemotherapy, and have been used as antitumour drugs in some cases [4–7].

It is well known that the alkylating nature, and hence the potential tumour-inhibiting ability of these sesquiterpene γ-lactones derives from a Michaelis-type interaction of the α, β-unsaturated lactone moiety with the nucleophile cellular components (sulphydryl enzymes) [8].

It is also widely recognized that the usefulness of most sesquiterpene lactones has been limited because of their toxicity [8]. Unfortunately, the biologically active moiety is also very reactive and hence may give rise to indiscriminate reactions towards various biological nucleophiles. Several attempts have been made to achieve lower toxicity in these compounds by improving the hydrophilicity *via* attachment to an appropriate carrier (carbohydrate or steroid moieties) and by derivatisation of α-methylene-γ-butyrolactone (sulphonate esters, silyl enol esters and others) with the aim of exploring the role of the exocyclic methylene which has a good leaving group [3, 8].

Such modification may give rise to irreversible alkylations through the replacement of the leaving group with the biological nucleophile and hence might enhance the selectivity of the α, β-unsaturated exomethylene lactone moiety [3] (see Scheme 1).



Scheme 1.

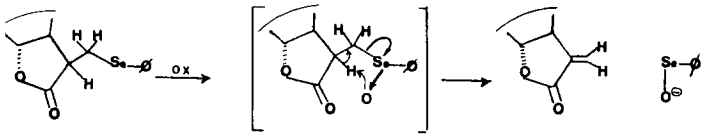
As an extensive of this hypothesis the synthesis of appropriate precursors that might act as "masked" α-methylene-δ-lactones has recently been proposed, *i.e.* a number of selenyl-δ-lactones with different lipophilic moieties; their cytotoxic activity has been screened and correlated with the hypothesis of the *in situ* generation of corresponding α-methylene lactones, probably *via* the formation of selenoxides [3]. In actual fact, the intracellular oxidation of the phenylseleno group to selenoxide seems to be particularly plausible tumour cells.

It has been supposed that cancer cells are more sensitive than normal cells to H_2O_2-mediated cytotoxicity because they lack sufficient catalase and peroxidases for enzymatic defence [9].

In addition, the high rate of pericycle-*syn*-elimination of β-H phenylselenoxide and therefore its nature as a very good leaving group, is well known in Se-organic chemistry [10].

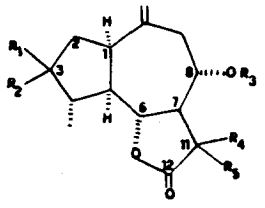
This paper deals with the preparation of a new series of

13-phenylselenoderivatives (**VII–XI**) of some natural and semisynthetic guaianolides (**I–IV** and **VI**); the *in vitro* cytotoxic activity of all the compounds (**X–XI**) against KB cell cultures is reported and some conclusions are drawn. In this case in fact, the 13-phenylselenoderivatives might act as precursors of the α,β-exomethylene-γ-lactone group undergoing Se-oxidation and retro-elimination inside the cell (see Scheme 2).

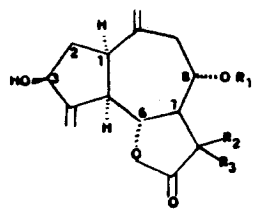


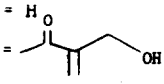
Scheme 2.

Consequently a member of the 13-phenylseleno-guaianolide family, acting as an endocellular source of its exomethylene-δ-lactone parent compound, might behave as a potential procytotoxic agent. Some interesting results on the relationship between bioactivity and chemical feature are discussed.



I	$R_1 = R_2 = O$	$R_3 = H$	$R_4 = R_5 = CH_2$
II	$R_1 = H$, $R_2 = OH$	$R_3 = H$	$R_4 = R_5 = CH_2$
III	R_1, $R_2 = O$	$R_3 = Ac$	$R_4 = R_5 = CH_2$
IV	$R_1 = H$, $R_2 = OAc$	$R_3 = Ac$	$R_4 = R_5 = CH_2$
V	$R_1 = R_2 = O$	$R_3 = H$	$R_4 = H$, $R_5 = CH_2$-OEt
VII	$R_1 = R_2 = O$	$R_3 = H$	$R_4 = H$, $R_5 = CH_2$-SePh
VIII	$R_1 = H$, $R_2 = OH$	$R_3 = H$	$R_4 = H$, $R_5 = CH_2$-SePh
IX	$R_1 = H$, $R_2 = OH$	$R_3 = Ac$	$R_4 = H$, $R_5 = CH_2$-SePh
X	$R_1 = H$, $R_2 = OAc$	$R_3 = Ac$	$R_4 = H$, $R^5 = CH_2$-SePh

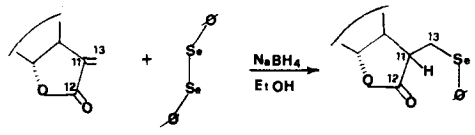


VI	$R_1 = H$	$R_2 = R_3 = CH_2$	
XI	$R_1 = H$	$R_2 = H$	$R_3 = CH_2$-SePh
XII	$R_1 =$ 	$R_2 = R_3 = CH_2$	

Chemistry

Compounds **II–IV** were prepared according to the known procedures using grosheimin **I** and cynaropicrin **XII** as starting material [11]. Compound **V** was obtained by treating **I** with NaOEt in anhydrous EtOH.

Compound **VI** was obtained by alkaline hydrolysis of **XII** [11]. Selenoderivatives **VII–XI** were prepared by the known procedure [3], appropriately modified by reacting the corresponding guaianolides with diphenyldiselenide in the presence of $NaBH_4$ in anhydrous EtOH (see Scheme 3).



Scheme 3.

Discussion

The *in vitro* cytostatic activity of compounds **I–XI** has been evaluated against KB cell cultures and is expressed as ID_{50} (see Table III).

The ID_{50} values are expressed as molar concentrations since in our opinion they are more interesting than the ID_{50} values in micrograms per millilitre of MEM, considering that there are notable differences among the molecular weights of the compounds.

All substances appeared to be very active, showing ID_{50} values much lower than the standard criteria for a significant level of activity [7]; in particular, compound **VIII** showed an uncommon cytotoxic property.

From the above results some interesting observations can be drawn about the structure–activity relationship of guaianolides **I–VI**, and the related 11-*H*-13-phenylselenoderivatives **VII–XI** (see Tables III and IV).

It can be noted that the acetylation of the C_6-OH group of grosheimin **I** increases the cytotoxicity ≈ 7-fold (M) in compound **III**, probably because of enhancement of the lipophilicity and membrane permeability. On the other hand, higher positive effect in bioactivity – a factor of about 11 – is caused by the reduction in the C_3-carbonyl of **I** to a more polar C_3-OH group in compound **II**.

In addition, comparison of the acetylated compound **IV** with the parent non-acetylated **II** shows that the acetylation of both C_3-OH and C_8-OH groups leads to a less active compound (a factor increase of about 4). Considering that the acetylation of C_8-OH should increase the bioactivity (compound **I**/**III**), one can hypothesize that the C_3-OH free group would play a positive pharmacodynamic role in this class of substances. The lowest cytotoxic activity of compound **V** may be justified by considering the presence in C_{13} of a not good-leaving ethoxyl group.

The value of ID_{50}=0.84 μg/ml for compound **VI** may be compared with ID_{50}=3.5–5.5 μg/ml reported in the literature for cynaropicrin **XII** [13]. In this case one may hypothesize that the hydrophilic property of the side-chain

Table I. ^{1}H NMR data.

	H_1	H_2 a,b	H_3	H_4	H_5	H_6	H_7	H_8	H_9 a,b	H_{11}	H_{13} a,b	H_{14} a,b	H_{15} a,b	OAc	2 H Arom.	3 H Arom. m,p	$H_{1'}$	$H_{2'}$	Solvents
Grosheimin **I**	d(8.0) 3.19 d(8.0) d(2.0)	2.55 m 2.55 m	–	2.35 m	2.25 m	d(10.0) 4.00 d(10.0)	3.07 m	3.92 m	2.88 d(6.0) d(4.0) 2.32 m	–	6.31 d(2.0) 6.38 d(2.0)	5.11 s 5.87 s	1.17 d(8.0)	–	–	–	–	–	$CDCl_3$[a]
11-*H*,-13-Se-phenyl grosheimin **VII**	3.00 m	2.60 d(14.0) d(9.0) 2.50 m	–	2.16 m**	2.25 d(9.0) d(9.0)	4.01 t(9.0)	2.50 m	3.66 m	2.67 d(6.0) d(12.0) 2.01 d(12.0) d(9.0)	3.24* m	3.43 d(5.0) 3.27 d(10.0) d(5.0)	4.80 4.60 s	1.14 d(9.5)	–	7.58 d(7.8)	7.23 m	–	–	DMSO-d_6[a]
11-*H*,13-4thoxy grosheimin **V**	d(8.0) 3.15 d(8.0) d(2.0)	2.51 m	–	2.30 m	2.45 m	d(9.5) 4.05 d(9.05)	2.20 m	3.70 m	2.85 m 2.30 m	2.85 m	4.10 d(9.0) d(3.0) 3.55 d(9.0) d(9.0)	5.10 s 4.80 s	1.24 (9.0)	–	–	–	3.65 m	1.27 t(9.0)	$CDCl_3$[a]
3-Dyhydro grosheimin **II**	–	–	3.85 m	–	–	d(10) 4.08 d(10)	–	3.60 m	–	–	6.08 d(3) 6.12 d(3)	4.98 brs 4.90 brs	1.12 d(7.5)	–	–	–	–	–	$CDCl_3$[b]
3-Dihydro, 11-*H*,13-Se-phenyl grosheimin **VIII**	1.94 m	2.14 d(6.5) d(12.0) d(5.0) 1.92 m	3.75 m	1.86 m	1.75 m	d(10.5) 3.97 d(10.5)	2.33 d(10.5) d(10.5)	3.59 m	2.68 d(11.9) d(4.0) 1.82 m	2.97 d(10.5) d(10.5) d(3.7)	3.53 d(13.2) d(3.7) 3.39 d(13.2) d(13.7)	5.03 s 4.98 s	1.21 d(6.5)	–	7.56 d(8.0)	7.28 m	–	–	$CDCl_3$[a]
8-Acetyl-grosheimin **III**	–	2.60 m	–	–	–	4.18 t(9)	5.00 m	–	–	–	6.40 d(3) 5.88 d(3)	5.12 s 4.80 s	1.20 d(8)	2.20 s	–	–	–	–	$CDCl_3$[b]
8-Acetyl, 11-*H*,13-Se-phenyl-grosheimin **IX**	–	–	–	–	–	d(9) 4.00 d(9)	–	– 4.82 m	–	–	3.43 d(3) 3.28 d(3)	5.10 s 4.80 s	1.22 d(5)	2.0 s	7.60 m	7.28 m	–	–	$CDCl_3$[b]
3-Dihydro-3,8-diacetyl grosheimin **IV**	–	–	4.60 m	–	–	d(9) 3.93 d(9)	–	4.40 m	–	–	6.12 d(3) 5.42 d(3)	4.80 brs 4.70 brs	1.10 d(7.5)	2.17 s 2.16 s	–	–	–	–	$CDCl_3$[b]
3-Dihydro-3,8-diacetyl,11-*H*,13, Se-phenyl grosheimin **X**	–	–	4.72 m	–	–	d(9) 3.98 d(9)	–	– 4.72 m	–	–	3.35-3.00 m	5.06 s 5.02 s	1.12 d(6)	2.07 s 1.92 s	7.55 m	7.28 m	–	–	$CDCl_3$[b]
8-Deacyl cyanopicrin **VI**	–	–	4.53 m	–	–	d(9) 4.18 d(9)	–	3.96 m	–	–	6.25 d(3) 6.15 d(3)	5.15 s 5.00 s	5.47 s 5.33 s	–	–	–	–	–	$CDCl_3$[b]
8-Deacyl-11-*H*,13-Se-phenyl cynaropicrin **XI**	–	–	4.66 d(9) d(9)	–	–	d(9) 4.07 d(9)	–	3.68 m	–	–	3.42 d(3)	5.00 s 4.92 s	5.25 brs	–	7.55 d(7.5)	7.20 m	–	–	CD_3OD[b]

Coupling constants in Hz are between parentheses.
[a]Spectra recorded at 400 MHz.
[b]Spectra recorded at 90 MHz.
*Collapsing to d, J=10.0 Hz by irr. at δ 2.5.
**Collapsing to q, J=9.5 Hz by irr. at δ 2.25.

Table II. ^{13}C NMR data.

Compound	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	$C_{1'}$	$C_{2'}$	OAc	C. Arom.	Solvent
Grosheimin **I**	40.40* d 45.88	43.53** t 47.08	218.67 s 218.13	47.23 d 39.29	49.80* d 48.52	83.26 d 81.99	51.11 d 49.94	73.20 d 71.71	49.16** t 42.36	145.36 s 144.36	138.73 s 137.72	170.29 s 169.6	124.52 t 123.17	114.44 t 113.81	15.02 q 14.25	–	–	–	–	Pyr DMSO
11-*H*,13-Se-phenyl grosheimin **VII**	46.43* d	48.62** t	221.66 s	38.89 d	46.56* d	83.34 d	49.89≠ d	73.66 d	43.14** t	145.15 s	50.14≠ d	176.59 s	27.69 t	113.42 t	13.85 q	–	–	–	131.24 d 129.08 d 128.81 d 126.41 d	DMSO
11-*H*,13-Ethoxy grosheimin **V**	47.09* d	47.07** t	211.11 s	39.49 d	47.11* d	83.67 d	50.67 d	74.44 d	43.72** t	146.05 s	49.05 d	176.73 s	68.09≠ t	113.76 t	14.46 q	66.44≠ t	15.35 q	–	–	DMSO
3-Dyhidro grosheimin **II**	46.84 d	38.97** t	77.52≠ d	42.92 d	51.01 d	81.60 d	53.56 d	72.78≠ d	46.56** t	144.39 s	139.76 s	171.39 s	121.79 t	114.17 t	18.33 q	–	–	–	–	Pyr
3-Dihydro,11,-*H*, 13-Se-phenyl grosheimin **VIII**	48.21* d	46.59** t	78.37 d	38.50 d	47.11* d	81.36 d	50.48 d	75.21 d	42.62** t	143.80 s	54.51 d	171.30 s	27.92 t	115.29 t	18.06 q	–	–	–	133.57 d 129.33 d 127.49 d	$CDCl_3$
8-Acetyl-grosheimin **III**	43.20* d	46.85** t	218.23 s	40.55 d	46.41* d	82.23 d	51.08 d	74.33 d	43.04** t	142.41 s	135.98 s	171.10 s	124.58 t	116.68 t	14.86 q	–	–	169.45 s 21.04 q	–	$CDCl_3$
8-Acetyl,11-*H*, 13-Se-phenyl grosheimin **IX**	43.47* d	47.08** t	218.26 s	39.45 d	46.83* d	83.26 d	50.96 d	75.84 d	43.47** t	142.76 s	44.29 d	175.36 s	28.12 t	115.99 t	14.20 q	–	–	169.66 s 20.83 q	133.37 d 129.24 d 127.53 d	$CDCl_3$
3-Dihydro,3,8-diacetyl grosheimin **IV**	43.67* d	49.77** t	79.95≠ t	35.57 d	43.54* d	80.54 d	51.57 d	74.40≠ d	40.36** t	141.50 s	137.35 s	169.7 s	122.40 s	117.06 t	18.29 q	–	–	170.86 s 169.89 s 21.08 q 22.05 q	–	$CDCl_3$
3-Dihydro-3,8-diacetyl,11-*H*,13-Se-phenyl grosheimin **X**	43.74* d	42.85** t	79.52≠ d	35.34 d	47.47* d	81.08 d	50.75• d	76.17≠ d	42.56** t	142.02 s	50.82• d	175.34 s	27.93 t	116.42 t	17.86 q	–	–	169.66 s 20.33 q	133.63 d 129.16 d 127.50 d	$CDCl_3$
S-Deacyl cynaropicrin **VI**	44.07* d	39.94** t	73.46 d	154.82 s	50.92* d	79.94 d	51.46 d	72.00 d	45.12** t	144.64 s	140.19 s	170.43 s	122.56 t	116.24 t	111.02 t	–	–	–	–	Pyr
8-Deacyl,11-*H*,13-Se-phenyl cynaropicrin **XI**	46.60* d	39.94 t	75.36≠ d	133.42 s	48.96* d	81.29 d	50.83 d	73.59≠ d	44.32** t	145.09 s	52.82 d	178.97 s	28.64 t	115.89 t	110.05 t	–	–	–	132.15 d 129.93 d 127.68 d	CD_3OD

*, **, ≠, • The assignments of these signals may be reversed.

Table III. Cytostatic activity against KB cell growth.

Compounds[a]	Drug conc. (μg/ml MEM)	% growth inhib. ±SE	Significance level (p)	ID_{50} values[b] (M)
Guaianolides				
grosheimin **I**	0.10	1.66±0.87	<0.05	
	1.00	25.27±0.61	<0.01	
	2.50	83.57±0.75	<0.01	5.6×10^{-6}
	5.00	cytolysis	–	
	10.00	cytolysis	–	
3-Dihydro grosheimin **II**	0.10	48.81±0.89	<0.01	
	0.50	53.45±0.70	<0.01	
	1.00	60.15±1.22	<0.01	4.9×10^{-7}
	2.50	68.06±0.57	<0.01	
	10.00	cytolysis	–	
8-Acetyl grosheimin **III**	0.10	39.02±0.51	<0.01	
	1.00	65.75±0.94	<0.01	
	2.50	88.47±0.33	<0.01	7.9×10^{-7}
	5.00	cytolysis	–	
	10.00	cytolysis	–	
3-dihydro-3,8-diacetyl grosheimin **IV**	0.10	1.40±0.25	ns	
	0.50	43.09±1.08	<0.01	
	1.00	52.69±1.75	<0.01	2.0×10^{-6}
	2.50	93.80±0.66	<0.01	
	10.00	cytolysis	–	
11-*H*-13-OEt grosheimin **V**	0.10	1.42±0.72	ns	
	1.00	15.69±0.65	<0.01	
	2.50	59.05±0.66	<0.01	7.8×10^{-6}
	5.00	80.80±0.50	<0.01	
	10.00	95.65±0.49	<0.01	
8-Deacyl cynaropicin **VI**	0.10	0.52±0.25	ns	
	0.50	41.99±0.67	<0.01	
	1.00	52.50±1.69	<0.01	3.2×10^{-6}
	1.25	62.34±0.76	<0.01	
	2.50	cytolysis	–	
	10.00	cytolysis	–	
11-*H*-13-Se-Phenyl derivatives				
VII	0.10	54.15±0.75	<0.01	
	1.00	66.58±0.60	<0.01	
	2.50	84.35±0.48	<0.01	2.1×10^{-7}
	5.00	89.11±0.50	<0.01	
	10.00	99.27±0.47	<0.01	
VIII	0,10	59.82±0.65	<0.01	
	1.00	78.73±0.83	<0.01	
	2.50	85.18±0.50	<0.01	4.7×10^{-8}
	5.00	90.12±0.46	<0.01	
	10.00	97.00±0.49	<0.01	
IX	0.10	8.21±2.31	<0.05	
	1.00	8.15±2.12	<0.05	
	1.25	19.08±1.16	<0.01	4.6×10^{-6}
	2.50	61.90±1.64	<0.01	
	5.00	cytolysis	–	
X	0.10	0.57±2.72	n.s	
	1.00	1.11±2.30	n.s	
	1.25	66.77±0.58	<0.01	1.2×10^{-6}
	2.50	74.37±0.94	<0.01	
	5.00	98.55±0.36	<0.01	
	10.00	cytolysis	–	
XI	0.10	49.85±1.19	<0.01	
	1.00	55.01±1.45	<0.01	
	2.50	91.94±0.36	<0.01	3.6×10^{-7}
	5.00	cytolysis	–	
	10.00	cytolysis	–	

[a]The compounds were previously dissolved in dimethyl sulfoxide.
[b]The results are expressed as molar concentrations at which cells showed a 50% growth inhibition (ID_{50}).

Table IV. *R*-values for the couples of related compounds: guaianolides / Se-derivative.

Couple	*R*
I / VII	26.7
II / VIII	10.4
III / IX	0.17
IV / X	1.7
VI / XI	8.9

$$R = \frac{ID_{50} \text{ guaianolide (M)}}{ID_{50} \text{ Se-derivative (M)}}$$

methacryl ester in C_8 can prevent the transport of **XII** into the cell.

The major activity of compound **VI** with respect to grosheimin **I** could be related both to the reduction of the C_3-carbonyl group in the molecule and probably also to the presence of a further exomethylene non-saturation in C_4.

The presence of the Se-phenyl group in compounds **VII–XI** generally leads to a notable enhancement of cytoxicity with respect to the parent substances (see *R* values in Table IV).

The highest "Se-effect" in increasing cytotoxicity is shown by the pair of compounds grosheimin **I** / Se-derivative **VII** (R=26.7), and the same trend can be observed for 3-dihydro grosheimin **II** / Se-derivative **VIII** (R=10.4), for 8-deacyl-cynaropicrin **VI** / Se-derivative *XI* (R=8.9) and also for the products **IV / X** (R=1.7). The pair of compounds 8-acetyl grosheimin **III** / Se-derivative **IX** is inconsistent with the general trend, showing a most surprising inversion of the *R*-ratio (R=0.17). Further investigation will be needed to elucidate the significance of this particular case.

It is also of interest to note that compounds **II**, **VII**, and **XI** showed very low ID_{50} values (0.1 μg / ml) and that 11-*H*-13-phenylseleno-3-dihydro grosheimin **VIII** (ID_{50}= 0.02 μg / ml=4.7×10^{-8} M) must be considered one of the most cytotoxic substances in the family of the sesquiterpene lactones and derivatives reported in the literature [7].

With the sole exclusion of the pair of compounds **III / IX**, the *in vitro* pharmacological data obtained permit us to state for the moment that the presence of the Se-phenyl group in C_{13} leads to more cytotoxic compounds, and lends support to the hypothesis outlined above, thus making it worthwhile and of interest to continue our research along these lines.

The *in vitro* assay obviously cannot be conclusive regarding antitumour effectiveness of our compounds, and so the most *in vitro* active products should be submitted for *in vivo* testing against murine experimental tumours.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot stage instrument and are uncorrected. ^{1}H NMR were recorded on Varian EP-390 90-MHz and Bruker Spectrospin 400-MHz instruments using TMS as the internal standard (chemical shifts in δ values). ^{13}C NMR spectra were determined using a Bruker WP80SY spectrometer. Mass spectra were obtained with an AEI MS 902 spectrometer.

For column chromatography, Merck silicagel 60 (230–400 mesh) was used. Reactions were monitored by TLC using Merck DC-Alufolien Kieselgel 60 F_{254}. The microanalyses were performed by the Microanalytical Laboratory of the Institute of Organic Chemistry, Pharmacy Faculty, Perugia.

Method A: 11-H-13-Se-phenyl grosheimin ***VII***

312 mg (1 mmol of diphenyldiselenide and 76 mg (2 mmol) of $NaBH_4$ were dissolved under stirring in 20 ml of anhydrous EtOH at room temperature and in N_2 atmosphere.

As soon as the yellow solution changed to decoloration 262 mg (1 mmol) of grosheimin **I** was added and, after few minutes, a white powder began to precipitate.

The mixture was stirred for a further 15 min, then the precipitate was filtered *in vacuo*, washed with cool EtOH and turned to unitary on TLC. The solution was poured into 50 ml of HCl-acidulated water and then extracted with $CHCl_3$. The chloroform extract was washed first with $NaHCO_3$, then with NaCl aqueous solutions and dried over anhydrous Na_2SO_4. The evaporation of the solvent *in vacuo* yielded a crude yellow oily residue which was purified by means of silicagel flash–CC (eluent: $CHCl_3$ $CHCl_3$ / MeOH=95 / 5). The TLC-pure fractions of 11-*H*-13-Se-phenyl grosheimin **VII** obtained proved to be TLC and spectroscopically identical to the predicted, spontaneously precipitated one. Yield = 338 mg (0.8 mmol); mp=237–240°C (from EtOH).

Anal. for $C_{21}H_{24}O_4Se$: calcd.=C 74.09; H 7.11; O 18.80; found=C 74.11; H 7.09; O 18.80. IR (ν_{max}, cm^{-1}, KBr): 3500–3300 (–OH), 2980, 1770 (γ-butyrolactone), 1740 (5-membered ring ketone), 1620, 1580, 1440, 1400, 910 (aromatic and olefine unsaturations), 1360, 1300, 1200, 1170 (C–O and C–Se–C). ^{1}H NMR (δ, ppm, DMSO–d_6, 400 MHz)=see Table I; ^{13}C NMR (δ, ppm, DMSO-d_6, 22.5 MHz)=see Table II; MS (*m/z*, I%)=422.2 (7.0)M^+ ^{82}Se, 420.6 (42.2)M^+ ^{80}Se, 418.8 (22.5)M^+ ^{78}Se, 158.0 (11.3), 93.7 (11.3), 91.6 (25.3).

3-Dihydro-11-H-13-Se-phenyl grosheimin ***VIII***

The reaction of Ph-selenization of 3-dihydro grosheimin **II** was carried out by the same procedure and stoichiometric ratios as reported in method A. Starting from 312 mg (1 mmol) of diphenyldiselenide, 76 mg (2 mmol) of $NaBH_4$ and 264 mg (1 mmol) of **III**, 340 mg (0.8 mmol) of pure **VIII** were obtained. mp=148–149°C (from ethyl ether).

Anal. for $C_{21}H_{26}O_4Se$: calcd.=C 73.66; H 7.65; O 18.69; found=C 73.35; H 7.78; O 18.87. IR (ν_{max}, cm^{-1}, $CHCl_3$): 3600 and 3400 (–OH), 3000–2860, 1770 (γ-butyrolactone), 1640, 1580, 1480–1440, 910 (olefine and aromatic unsaturations), 1350, 1290, 1165, 1140, 1070, 990 (C–O and C–Se–C). ^{1}H NMR (δ, ppm, $CDCl_3$, 400 MHz)=see Table I; ^{13}C NMR (δ, ppm, $CDCl_3$, 22.5 MHz)=see Table II. MS (*m/z*, I%)=424.3 (3.7) M^+ ^{82}Se, 422.2 (22.9) M^+ ^{80}Se, 420.4 (12.5) M^+ ^{78}Se, 192.4 (14.6), 159.0 (20.8), 158.0 (12.5), 157 (10.4), 105.5 (10.4), 91.6 (25.0).

3-Dihydro-3,8-diacetyl-11-H-13-Se-phenyl grosheimin ***X***

358 mg (1 mmol) of 3-dihydro-3-8-diacetyl grosheimin **IV** were subjected to the reaction of selenization according the same procedure of method A. About 300 mg (0.59 mmol) of pure compound **X** were obtained as an amorphous powder.

Anal. for $C_{25}H_{30}O_6Se$: calcd.=C 70.40; H 7.09; O 22.51; found =C 70.80; H 6.98; O 22.22. IR (ν_{max}, cm^{-1}, $CHCl_3$): 2940, 2990, 1770 (γ-butyrolactone), 1730 (C=O), 1645, 1570, 900 (olefine and aromatic unsaturations), 1370, 1250–X1150 and 1100–1000 (C–O and C–Se–C). ^{1}H NMR (δ, ppm, $CDCl_3$, 90 MHz)=see Table I; ^{13}C NMR (δ, ppm, $CDCl_3$, 22.5 MHz)=see Table II.

8-Acetyl-11-H-13-Se-Phenyl grosheimin ***IX***

304 mg (1 mmol) of 8-acetyl grosheimin **III** were selenized according to the same procedure as method A and yielded 210 mg (0.69 mmol) of pure compound **IX** as an amorphous powder.

Anal. for $C_{23}H_{26}O_5Se$: calcd.=C 72.23; H 6.85; O 20.92; found=C 72.05; H 6.95; O 21.0. IR (ν_{max}, cm^{-1}, $CHCl_3$): 2920, 1775 (γ-butyrolactone), 1740–1730 (C=O groups), 1640, 1580, 1450, 910 (aromatic and olefine unsaturations), 1370, 1290, 1165, 1115, 1080, 1030 (C–O and C–Se–C). ^{1}H NMR (δ, ppm, $CDCl_3$, 90 MHz)=see Table I; ^{13}C NMR (δ, ppm, $CDCl_3$, 22.5 MHz)=see Table II.

8-deacyl-11-H-13-Se-phenyl cynaropicrin **XI**
262 mg (1 mmol) of 8-deacyl cynaropicrin **VI** were selenized as reported above and yielded 335 mg (0.8 mmol) of pure compound **XI**. mp=148–50°C (from benzene).

Anal. for $C_{21}H_{24}O_4Se$: calcd.=C 74.09; H 7.11; O 18.80; found=C 74.01; H 7.28; O 18.71. IR (ν_{max}, cm^{-1}, KBr)=3400 (–OH), 2900, 1760, (γ-butyrolactone), 1580, 1440, 1400, 900, 740 (aromatic and olefine unsaturations), 1340, 1300, 1200, 1090 (C–O and C–Se–C). ^1H NMR (δ, ppm, CD_3OD, 90 MHz)=see Table I; ^{13}C NMR (δ, ppm, CD_3OD, 22.5 MHz)=see Table II.

Method B: 11-H-13-ethoxy grosheimin **V**
262 mg (1 mmol) of grosheimin **I** were added to a solution of NaOEt in 2 ml of anhydrous EtOH under stirring at room temperature and in N_2 atmosphere. After 15 min. of reaction, the solution was poured into 50 ml of HCl-acidulated water then extracted with $CHCl_3$. The chloroform extract was washed first with $NaHCO_3$, then with NaCl aqueous solutions and then dried over anhydrous Na_2SO_4. The evaporation of the solvent *in vacuo* yielded a crude amorphous residue wich was purified by means of repeated silicagel flash–CC (eluent: $CHCl_3$ $CHCl_3$/MeOH= 95/5). The TLC-pure fractions obtained were recovered as a white amorphous powder.

Anal. for $C_{17}H_{24}O_5$: calcd.=C 66.22; H 7.84; O 25.94; found=C 66.32; H 7.90; O 25.78. IR (ν_{max}, cm^{-1}, $CHCl_3$): 3350 (–OH), 2880, 1760 (γ-butyrolactone), 1740, (5-membered ring ketone), 1660, 1595, 1515, 890 (olefine unsaturation), 1150, 1080, 970 (C–O). ^1H NMR (δ, ppm, $CDCl_3$, 400 MHz)=see Table I; ^{13}C NMR (δ, ppm, DMSO–d_6, 22.5 MHz)=see Table II. MS (*m/z*, I%)=308.8 (8.11) M^+, 264.7 (13.51) M^+–OEt, 228.9 (34.23), 211.9 (27.3), 174.1 (27.9), 138.1 (26.1), 135.6 (83.8), 134.6 (100), 59.6 (39.64), 43.3 (24.3).

Pharmacology

An established cell line of human epidermoid carcinoma of the mouth (KB cells) was employed to test the cytostatic activity of the new compounds by the method of Geran *et al.* [12]. The cells were grown in 25 cm^2 tissue culture flasks with Eagle's minimal essential medium (MEM) [14] supplemented with 10% newborn calf serum, 10% non-essential amino acids and glutamine 2 mM, and buffered with TES (*N*-tris[hydroxymethyl] methyl-2-aminoethane sulphonic acid 3, mM), BES (*N,N*,bis[2-hydroxyethyl]-2-aminoethane sulphonic acid, 3 mM), HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid, 3 mM) and Tricine (*N*-tris[hydroxymethyl]methylglycine, 3 mM) [15].

For the *in vitro* cytostatic assay the cells in a logarithmic growth phase, which were re-fed 24 h before testing, were used. The cells were treated for 5 min at 37°C with 0.05% 1:250 trypsin solution and then suspended in MEM to obtain a concentration of 10^5 cells/ml. One millilitre was seeded in each Leighton tube. The samples were allowed to incubate for 24 h at 37°C. After this interval, the viable cells were attached to the bottom of the tubes. The tubes were regrouped at random and baseline was evaluated in 5 of these by counting the cells detached by trypsin solution [16]. The culture medium of the other Leighton tubes was discarded and the cells were fed with 4 ml MEM (control tubes) and with 4 ml MEM containing the compounds to be tested (treated tubes). The compounds were dissolved immediately before use in sterile dimethylsulfoxide (DMSO). Further dilutions were performed with the growth medium to the desired drug concentration. The final solvent concentration in MEM (0.5% in every tube) was previously tested by us and did not show cytotoxic effect. At least 5 concentration levels were used for each compound and each concentration value was tested in triplicate. The incubation was carried out at 37°C for 72 h, the time interval in which exponential growth occurs. Cell growth was estimated by counting the viable cells as previously described [16] . The cytostatic activity was evaluated as a percentage of cell growth inhibition in the treated tubes with respect to the controls on the basis of the formula: $100-[(T-B)/(C-B)\times 100]$, where B was the baseline and T and C were the number of viable cells, respectively, in the treated and the control tubes after 72 h of incubation. The significance of these results was evalutated by use of the t test.

The inhibition values were plotted against log D, where D is the drug concentration in micrograms per millilitre of MEM. From these curves the ID_{50} values were obtained, where ID_{50} is the concentration at which the cells showed 50% growth inhibition in relation to the control values. The ID_{50} values were expressed as molar concentrations, setting the activity threshold at 10^{-4} M, since that appears to be a fairly realistic cutoff point for most compounds [17].

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