Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 13, pp. 1649-1654, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00277-1

CYTOTOXIC ACTIVITY OF *NEO*-CLERODANE DITERPENOIDS

Marina Gordaliza*[1], José M. Miguel del Corral[1], María Luz de la Puente[1], María Dolores Garcia-Grávalos[2] and Arturo San Feliciano[1]

[1]*Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Salamanca Avenida Campo Charro s/n, E-37007 Salamanca, Spain. Fax: 34 23 294515. E-mail: mliza@gugu.usal.es*

[2]*PharmaMar S.A. Calera 3, E-28760 Tres Cantos (Madrid), Spain.*

Abstract: Several *neo*-clerodane diterpenoids isolated from extracts of roots and aerial parts of *Linaria saxatilis* var. *glutinosa*, as well as some semisynthetic derivatives, exhibited *in vitro* growth inhibition against different neoplastic cell cultures. A number of substances showed IC_{50} values under 1μM. © 1997 Elsevier Science Ltd.

During the last years our research group has been actively involved in the phytochemical and structural research on new bioactive products obtained from natural sources, since the promising role that certain secondary metabolites play in the treatment of human cancer is becoming more generally recognized.[1]

Thus, two varieties of plants belonging to the genus *Linaria* (Fam. Schrophulariaceae), *Linaria saxatilis* var. *saxatilis* and *Linaria saxatilis* var. *glutinosa* were selected for such studies, due to the variety of biological activities and therapeutical uses reported for other species of this genus.[2-4] As the result of that research we reported the isolation, structure assignment and chemical elucidation of a large number of *neo*-clerodane diterpenoids obtained from root and aerial part extracts.[5-9] In earlier work in these laboratories, a selection of those natural products, representatives of the various structural classes, was tested for cytotoxic activity against a series of cancer cells cultured *in vitro*, providing results of interest. This findings prompted us to start a semisynthesis program around these structures, for comparative purposes. Herein we describe the results of this study, which is the first report on the potential cytotoxic activity of *neo*-clerodane derivatives.

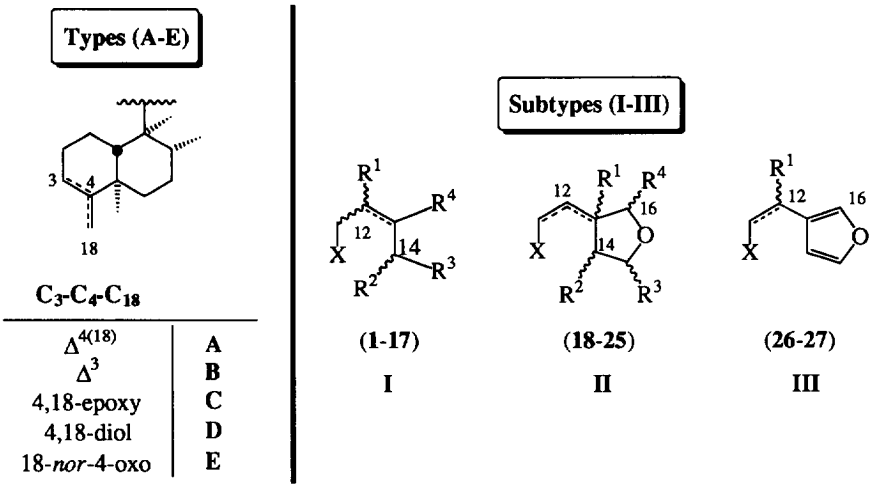
CHEMISTRY

All the compounds evaluated, those natural products isolated from the roots and aerial parts of *Linaria saxatilis* var. *glutinosa*, as well as most of the semisynthetic derivatives, have been already described in previous communications of the series dedicated to this species[7-9], including structure assignment and chemical correlation. The synthesis of these compounds which have not been reported before, is briefly described in this paper.

Due to reasons of synthetic convenience, major components isolated from the aerial parts, such as *E*-isolinaridial (**2**)[8] or triacetate of isolinaritriol (**9**)[9] were used as starting units for the semisynthetic work, the derivatives being prepared by slightly modifying the side-chain and/or the *exo*-cyclic $\Delta^{4(18)}$ double bond, while conserving the *neo*-clerodane moiety. Transformations were carried out following conventional and simple methods with the aim of also applying these conditions to other minor natural products isolated from the extracts, since a larger number of analogues could lead to a better understanding of the structure-activity relationship.

In order to simplify the discussion, the compounds tested were classified into the types **A-E** according to the structural arrangement of their decaline moiety: type **A**, with a $\Delta^{4(18)}$ *exo*-cyclic double bond; type **B**, their *endo*-cyclic Δ^3 analogue; type **C**, having an epoxide function at 4,18 position; type **D** the corresponding 4,18 diols and type **E**, the C-18 degraded ketone; and into the subtypes **I-III** depending on the nature of the side chain: subtype **I**, for compounds having a polyfunctionalized, but open, side chain; subtype **II** for compounds having a tetrahydrofuran moiety defined by the oxygen bridge between positions C-15 and C-16 of the *neo*-clerodane structure, and subtype **III** for the aromatic derivatives (Scheme I, Table I).

Scheme I: Types and subtypes of *neo*-clerodanes being evaluated.



Initial structural analysis, with the aim of improving on the potency, was focussed on the decaline fragment. We were particularly interested in making changes at the $\Delta^{4(18)}$ *exo*-cyclic double bond, since its easy functionalization would allow the preparation of a number of derivatives.

The varied biological activities shown, in our preliminary studies, by Δ^3*neo*-clerodane diterpenoids isolated from the roots of the same species,[7] prompted us to force isomerisation of the $\Delta^{4(18)}$ *exo*-cyclic double bond. Such derivatives were synthesized by treatment of the corresponding $\Delta^{4(18)}$ starting material with a mixture of AcOH-H_2SO_4-H_2O.[5,8] No improvement of the yield was obtained by using P_2I_4.[10] As a result of this study, compound **14**, among other was prepared and evaluated for biological activity.

Replacement of the $\Delta^{4(18)}$ *exo*-cyclic double bond by an epoxide group was successfully carried out by oxidation of the $\Delta^{4(18)}$ *neo*-clerodanes **9**, **18** and **19** with MCPBA/$NaHCO_3$ and dichloromethane as solvent,[8] to yield compounds **15** (α:β 1:6), **24** (β) and **25** (α:β 1:3), respectively.[11] Epoxide ring cleavage of compound **15** by oxidation with periodic acid[5] led to the expected 4,18-dihydroxy derivative **16** and the 18-*nor*-*neo*-clerodane **17** (previously isolated as a natural component of the aerial parts).[5,9]

We next turned our attention to modify the side chain. In recent publications, several methylketones have been reported to present interesting biological properties,[12] and it is also well known that condensation of aldehydes with diazomethane leads to this kind of structure.[13] Thus, condensation of the readily available *E*-isolinaridial (**2**) with diazomethane resulted in the formation of the methylketone **3**, as a major product, in which the condensation had taken place on the C-15 carbonyl group.[14]

Table 1: Structures of the *neo*-clerodane diterpenoids evaluated in this study.

Compound	X	$C_{11}/C_{12}/C_{13}$	R^1	R^2	R^3	R^4
1	A	Δ^{12} (*Z*)	H	H	CHO	CHO
2	A	Δ^{12} (*E*)	H	H	CHO	CHO
3	A	Δ^{12} (*E*)	H	H	CHO	$COCH_3$
4	A	Δ^{12} (*E*)	H	α-OAc	CHO	CHO
5	A	Δ^{12} (*E*)	H	H	CH_2OH	CH_2OH
6	A	Δ^{12} (*E*)	H	H	CH_2OAc	CH_2OAc
7	A	Δ^{12} (*E*)	H	H	OH	$CH(OH)CH_2OH$
8	A	Δ^{12} (*E*)	H	H	OAc	$CH(OAc)CH_2OAc$
9	A	Δ^{13} (*E*)	OAc	H	CH_2OAc	CH_2OAc
10	A	Δ^{13} (*E*)	OH	H	CH_2OAc	CH_2OAc
11	A	Δ^{13} (*E*)	OAc	H	CH_2OH	CH_2OAc
12	A	Δ^{13} (*E*)	OAc	H	CH_2OAc	CH_2OH
13	A	Δ^{13} (*E*)	OH	H	CH_2OH	CH_2OH
14	B	Δ^{13} (*E*)	OAc	H	CH_2OAc	CH_2OAc
15	C	Δ^{13} (*E*)	OAc	H	CH_2OAc	CH_2OAc
16	D	Δ^{13} (*E*)	OAc	H	CH_2OAc	CH_2OAc
17	E	Δ^{13} (*E*)	OAc	H	CH_2OAc	CH_2OAc
18	A	Δ^{12} (*Z*)		H	α(β)-OAc	β(α)-OAc
19	A	Δ^{12} (*Z*)		β-OAc	α-OAc	β-OAc
20	A	Δ^{12} (*Z*)		β-OH	α-OAc	β-OAc
21	A	Δ^{11} (*E*)	OH	H	OAc	OAc
22	B	12(13)β-epoxy		H	β-OAc	β-OAc
23	B	Δ^{12} (*Z*)		β-OAc	α-OAc	β-OAc
24	C	Δ^{12} (*Z*)		H	α(β)-OAc	β(α)-OAc
25	C	Δ^{12} (*Z*)		β-OAc	α-OAc	β-OAc
26	A		β-OH			
27	A	Δ^{11} (*E*)				

Since the lipophilic/hydrophilic balance could play an important role on the bioactivity of these compounds, we focussed on the modification of the polarity of several derivatives. The simplest way to address this point could be the synthesis of a series of derivatives differing in the position and number of the hydroxyl groups around the side chain. Studies of the extract from the aerial parts of the plant, had led to the isolation of a series of three diacetate-monoalcohols,[9] the fact that those natural products only differed by the position of the hydroxyl group at C-12, C-15 and C-16 allowed to see the influence of this factor on the activity. For the evaluation of the second factor a series of polyhydroxylated analogues were prepared by reduction with LAH, in dry ether, of the aldehyde **2** and the natural tetrahydrofuran derivative **19** to afford diol **5** and triol **7** respectively, and direct saponification of the triacetate derivative **9**, with NaOH/MeOH, to yield triol **13**. Analysis of the biological results obtained showed that a high increase of the hydrophilicity led to a decrease of

the potency. In order to confirm this relationship, the hydroxyl groups of diol **5** and triol **7** were masked by acetylation with Ac_2O-Py under standard conditions, affording diacetate **6** and triacetate **8**, respectively.

During previous work on antifeedant properties of *neo*-clerodane derivatives, we found that compounds containing a furan system seemed to have a wide range of activities.[15] In view of the efficacy of these kind of agents, we also considered of interest to prepare similar compounds by modification of the side chain. As a result, compounds **26** and **27** were synthesized by oxidation of isolinaritriol (**13**) with MnO_2 and pyrolysis of the tetrahydrofuran derivative **18**, respectively.

Finally, we have included in the study, a minor natural component, compound **21**, which possesses a different structural feature from all those above described, a *trans* double bond located at C_{11}.[9]

BIOACTIVITY

Compounds were tested against cell cultures of P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and, in most cases, also against Mel-28 malign human melanoma.[16] Table II shows the IC_{50} (μM) values for each compound.

Table II : *In vitro* cytotoxic activities for compounds **1-27** (IC_{50} μM).

Compound	P-388	H-549	HT-29	MEL-28
1	3.3	3.3	3.3	3.3
2	3.3	3.3	1.6	-
3	1.6	1.6	1.6	1.6
4	2.8	2.8	5.6	-
5	3.3	3.3	3.3	3.3
6	3.1	3.1	3.1	3.1
7	15.5	15.5	3.7	15.5
8	22.3	22.3	22.3	22.3
9	11.0	11.0	11.0	-
10	2.5	2.5	2.5	2.5
11	1.2	1.2	1.2	1.2
12	2.5	2.5	2.5	2.5
13	31.0	31.0	31.0	31.0
14	11.0	11.0	11.0	11.0
15	11.0	22.0	33.0	-
16	20.7	> 40	> 40	> 40
17	5.6	11.0	22.4	22.4
18	> 50	> 50	> 50	> 50
19	0.5	0.5	0.2	-
20	2.4	2.4	2.4	-
21	2.4	2.4	2.4	2.4
22	0.5	0.5	0.5	-
23	0.4	0.4	0.4	-
24	1.1	1.1	1.1	-
25	0.5	0.5	0.5	-
26	6.6	6.6	8.3	8.3
27	8.8	16.6	16.6	-

(-): not tested

From the analysis of these results the following observations can be made:

- Practically all the compounds tested are cytotoxic, their potencies ranging from medium ($IC_{50} \approx 0.2\mu M$) to low ($IC_{50} > 50\mu M$).

- The selectivity against the different kinds of neoplasms is very low. Only compound **7** showed a certain degree of selectivity towards the multi-drug-resistant HT-29 system, which proved to be 4 times more sensible to this compound than the other neoplastic cell lines, though without great efficacy. Similar behaviour was observed for compound **17** towards murine leukemia P-388, although in a lower degree.

- Relating to the structure of the bicyclic fragment, the comparison of derivatives identically or similarly substituted at the side chain, indicates that compounds belonging to types **A**, **B** or **C** display similar potencies, as it can be observed, for instance, for compounds **9**, **14**, and **15**, having the same substitution pattern at the side chain. However, one significative exception is represented by compounds **18** and **24**, which have the same side chain, being the latter one (type C) substantially more potent. A similar deduction can be extended to categories **D** and **E** by observing results for compounds **16** (**D**), and **17** (**E**), and although these compounds are less potent than those having the same side chain, corresponding to types **A** (**9**), **B** (**14**), and **C** (**15**), their potencies change less than one order of magnitude.

- Relating to the structure of the side chain, the comparison of potencies for compounds **1-17** (subtype **I**), with those for **18-25** (subtype **II**) and **26-27** (subtype **III**) containing a similar bicyclic system, clearly shows that the tetrahydrofuran derivatives (subtype **II**) are more potent than the open-chain (subtype **I**) or the furan (subtype **III**) derivatives.

- Within the tetrahydrofuran series (subtype **II**) with a different substitution at C-14 (compounds **18-20**), analogue **20** having a free hydroxyl group, was found to show greater potency than the deacetylated derivative (**18**) but lower cytotoxicity compared to that found for the acetylated member of the series (**19**).

- The geometry of the Δ^{12} double bond does not seem to affect the activity significantly as it can be seen by comparison of the *cis* and *trans* dialdehydes **1** and **2**. The isomerisation of the *Z* (**1**) into the *E* (**2**) isomer upon assay conditions, is one possible reason for this result.

- A comparison of structures **5-13** (subtype **I**) allows us to see the effect of the hydroxyl groups at the side chain. It seems clear that the presence of three acetate groups or three hydroxyls diminishes the cytotoxicity with respect to that of compounds containing one hydroxyl (triacetates **8**, **9** and triols **7**, **13** *versus* diacetate monoalcohols **10**, **11** and **12**). In contrast, similar activity was found for diol **5** and its corresponding diacetate **6**. This fact indicates the preference for a medium degree of polarity of the side chain for activity within this subtype.

CONCLUSIONS

The SAR presented herein does give an idea of the requirements for the decaline ring in combination with the side chains.

As deduced from the data, most of the compounds inhibited the proliferation of all the cell types assayed, with IC_{50} values in the μM range, showing similar responses for all the neoplastic systems. Two compounds, **7** and **17** were found to present selectivity against HT-29 and murine leukemia P-388 respectively.

Within the series presented, components containing a tetrahydrofuran ring at the side chain (**19** from aerial parts and **22** and **23** from roots) were the most potent and selective (selectivity index > $40\mu g/mL$ compared with their cytotoxicity against cells CV-1) antineoplastic drugs at the concentration tested.

Most of the semisynthetic derivatives assayed were less active against the cancer cell lines that their natural parent compounds. Nevertheless, a few analogues presented appreciable activity against representative cancer cells, as in the case of the tetrahydrofuran-4(18)-epoxy derivatives (compounds **24** and **25**, belonging to type **C**) which exhibited activity greater than or equal to their corresponding $\Delta^{4(18)}$ unsaturated precursors (**18** and **19**, type **A**). A possible explanation for the overall low activity of the derivatives containing a polyhydroxylated side chain could point to the importance of the lipophilic/hydrophilic balance in these kind of structures.

Further work on the inhibition of enzymes involved in cellular growing and reproduction, as well as of the synthesis of proteins and nucleic acids, is currently in progress in our laboratory.

ACKNOWLEDGMENTS

Financial support for this work came from Spanish DGICYT (PB 93-0616) and Junta de Castilla y León (Consejería de Educación y Cultura, 35/94 and 57/96)

REFERENCES and NOTES

1.- Pettit, G. R.; Pierson, F. H.; Herald, C. L. In *"Anticancer Drugs from Animals, Plants and Microorganisms* John Wiley & Sons, New York, N. Y.,1994.
2.- Pahlow, M.; In *"El gran libro de las plantas medicinales"* ; 4th. ed.; Everest S.A.: Madrid, 1979; p. 273.
3.- Kitagawa, I.; Tani, T.; Akita, K.; Yosioka, I. *Chem. Pharm. Bull* . **1978**,*21*, 1973.
4.- Dobrescu, D.; Cristea, A.; Susanu, M. *Farmacia* **1985**, *33*, 215.
5.- San Feliciano, A.; Barrero, A. F., Miguel del Corral, J. M.; Gordaliza, M.; Medarde, M. *Tetrahedron* **1985**, *41*, 671.
6.- San Feliciano, A.; Barrero, A. F.; Miguel del Corral, J. M.; Gordaliza, M.; Medarde, M. *An. Quim.* **1985**, *81C*, 244.
7.- San Feliciano, A.; Gordaliza, M.; Miguel del Corral, J. M.; de la Puente, M. L. *Phytochemistry* **1993**, *33*, 631.
8.- San Feliciano, A.; Gordaliza, M.; Miguel del Corral, J. M.; de la Puente, M. L. *Tetrahedron* **1993**, *49*, 9067.
9.- Gordaliza, M.; Miguel del Corral, J. M.; de la Puente, M. L.; San Feliciano, A. *Phytochemistry* **1994**, *36*, 1453.
10.-a) Denis, J.N.; Magnane, R.; Van Eenoo, M.; Krief, A. *Nouv. J. Chem.* **1979** *3*, 745. b) Shibata, K.; Takegawa, S.; Koizumi, N.; Yamakoshi, N.; Shimazawa, E. *Chem. Pharm. Bull.* **1992**, *40*, 935.
11.- The $\Delta^{4(18)}$ epoxide derivatives are reported for the first time, being completely characterized by spectroscopic and analytical methods in our laboratories.
12.- a) Braekman, J. C.; Daloze, D.; Kaisin, M.; Moussiaux, B. *Tetrahedron* **1985**, *41*, 4603. b) Nakagawa, M.; Hamamoto, Y.; Ishihama, M.; Hamasaki, S.; Endo, M. *Tetrahedron Lett.* **1987**, *28*, 431.
13.- a) Gustche, C. D. The reaction of diazomethane and its derivatives with aldehydes and ketones. In *Organic reactions* , Adams, R. Ed.; John Wiley & Sons, New York, N. Y.,1954; Vol. VIII, p 364-429. b) Black, T. H. *Aldrichim. Acta* **1983**, *16*, 36.
14.- Gordaliza, M.; Miguel del Corral, J. M.; de la Puente, M. L.; San Feliciano, A. Unpublished results
15.- Merrit, A. T.; Ley, S. V. *Nat. Prod. Reports* **1992**, 243.
16.- Cells were seeded into 16 mm wells (multidishes NUNC 42001) at concentrations of $1x10^4$ (P-388), 2 x 10^4 (A-549, HT-29 and MEL-28) cells/well, respectively, in 1 mL aliquots of MEM10FCS medium containing the compound to be evaluated at the concentrations tested. In each case, a set of control wells was incubated in the absence of sample and counted daily to ensure the exponential growth of cells. After four days at 37ºC, under a 10% CO_2, 98% humid atmosphere, P-388 cells were observed through inverted microscopy and the degree of inhibition was determined by comparison with the controls, whereas A-549, HT-29 and MEL-28 were stained with crystal violet before examination.

(*Received in Belgium* 4 *March* 1997; *accepted* 23 *May* 1997)