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Cytotoxic Michael-Type Amine Adducts of α-Methylene Lactones Alantolactone and Isoalantolactone

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Abstract—Two series of cytotoxic (IC_{50}, K562 cell line, 1–24 μM) α-aminomethyl substituted lactones **3** and **4** were prepared by stereoselective Michael-type addition of amines to alantolactone (**1**) and isoalantolactone (**2**). The lactones **1** and **2** and their amine adducts induce apoptosis and act as alkylating agents.

Different species of the genus *Inula* (Compositae) are known to be rich sources of sesquiterpenes.[1] As part of a search for anticancer agents from medicinal herbs[2] we focused our attention upon one such species, *Inula helenium* L. The roots of this plant are inexpensive and available from many herbal suppliers. The herb (also known as elecampane), used to treat bronchitis and other respiratory problems, derives its name from Helen of Troy from whose tears it is said to have sprung.[3]

Standard extraction of the dried roots of *Inula helenium* L. with methanol provided a brown extract. This methanol extract was diluted with water and partitioned between hexane, chloroform and ethyl acetate. Bioassay guided fractionation of the hexane fraction (in vitro cell cytotoxicity using the MTT assay) led to the isolation of the known sesquiterpene α-methylene lactones alantolactone (**1**) (0.112%) and isoalantolactone (**2**) (0.098%).[4] The lactones (Fig. 1) were readily identified by the comparison of their ^{1}H and ^{13}C NMR spectra with literature data.[5,6] Surprisingly, although the cytotoxicity of many naturally occurring α-methylene lactones have been reported,[7,8] this type of biological activity of **1** and **2** has received little attention.[9] The lactones possess other biological properties such as allergenic[10] and antifungal activity,[11] and are able to inhibit plant growth.[12]

We now report that the lactones exhibit good cytotoxic activity against the K562 human leukaemia cell line (**1** IC_{50} 0.7 μM; **2** IC_{50} 1.2 μM). This level of activity was sufficiently encouraging to prompt us to prepare a series of derivatives of **1** and **2**

Secondary amines react well with isoalantolactone, adding to the α-methylene lactone subunit in a conjugate manner to generate a β-amino lactone.[13–15] This reaction therefore presented us with a potentially efficient method for the preparation of a range of α-methylamino lactone derivatives for screening. We first prepared the diethylamine adduct of **2** and found that it possessed much the same activity (**4a** IC_{50} 2 μM) as its parent lactone. We therefore prepared further amine adducts of both **1** and **2**, (Scheme 1). These amine adducts **3a–c** and **4a–l** were synthesised, from **1** and **2**, respectively, by reaction with five equivalents of the appropriate amine in ethanol. The cell growth inhibition properties (against the K562 human chronic myelogenous leukaemia cell line) of these analogues are shown in Table 1. The most cytotoxic adduct of **2** was the amine **4a** (Table 1) derived from diethylamine. Indeed in both series the diethylamine derivative was the most cytotoxic of the amine adducts all of which, apart from **4j–l**, were not significantly less active than the lactones from which they were derived. Even a bulky amine such as ephedrine produces an active adduct (**4c**) indicating that the cytotoxicity is not greatly affected by the size of the compound. The adducts derived from primary amines are generally much less active. This probably reflects their poorer basicity and nucleophilicity.

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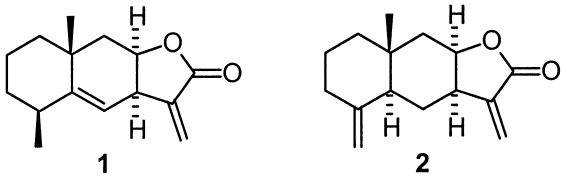
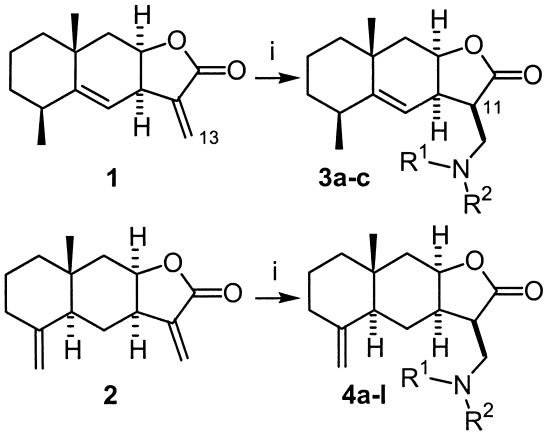


Figure 1.



Scheme 1. Reagents and conditions: (i) R^1R^2NH, EtOH, 0 °C, overnight.

Table 1. Cell growth inhibition properties of the amine-adducts[a] **3a–c** and **4a–l** against the K562 cell line

	R^1	R^2	Yield (%)	IC_{50} (μ)
3a	Et	Et	92	0.7
3b	Me	Me	61	2
3c[16]	$-(CH_2)_5-$		76	2
4a[14]	Et	Et	91	2
4b[17]	Me	Me	41	3
4c	$-CHMeCHOHPh$	Me	76	3
4d	$(CH_2)_2OH$	Me	43	4
4e[18]	$-(CH_2)_2O(CH_2)_2-$		66	5
4f[16]	$-(CH_2)_5-$		64	5
4g[19]	$-(CH_2)_4-$		79	5
4h	$(CH_2)_2Me$	$(CH_2)_2Me$	88	5
4i	$(CH_2)_3NMe_2$	H	82	6
4j	CH_2Ph	H	99	15
4k	$-(CH_2)_2NCHO(CH_2)_2-$		80	15
4l	Me	H	62	24
1	–	–	–	0.7
2	–	–	–	1.2

[a]References are provided for those adducts previously reported. In all these cases the configuration of C-11 was not defined.

X-ray crystal structure determination revealed that the configuration of the new chiral centre at C-11 in **4b** is *R* (Fig. 2). None of the other diastereoisomer (with C-11 configured *S*) was present in the crude reaction mixture. Clearly the protonation of the enolate generated in the Michael-type addition occurs from the *exo* face of the convex-shaped molecule. Other conjugate addition reactions to similar lactones have also been shown to occur with delivery of the proton with the same sense of stereoselectivity. For example, ^1H NMR analysis of the addition product of lysine derivatives and alantolactone

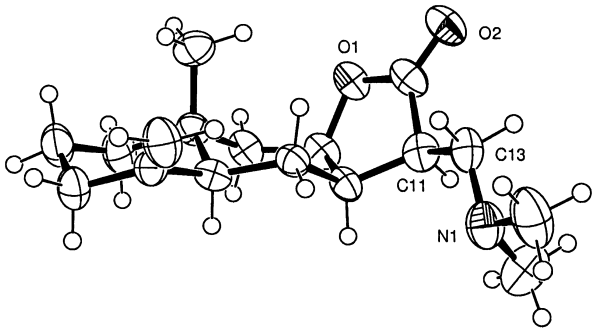


Figure 2. ORTEP view of isoalantolactone dimethylamine adduct **4b**. Anisotropic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level.

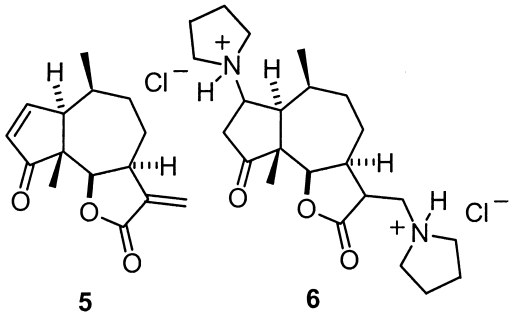


Figure 3.

reveals that the proton is delivered to the lower face.[20] Nevertheless the structure of the Michael-type adduct of **2** is the first unequivocal proof of the remarkable *exo*-selectivity. The X-ray crystal structures of the adducts **4a** and **4f** were also determined and each showed that the configuration of C-11 is also *R*.[21]

It is widely believed that α,β-unsaturated carbonyl compounds, and particularly α-methylene lactones, exert their biological effect by acting as alkylating agents. The lactones can form covalent adducts in vivo with proteins and other nucleophilic biomolecules, via a Michael-type addition of a free sulfhydryl or amine group. A thorough review of the literature revealed that similar amine adducts such as **6** (Fig. 3) derived from Ambrosin (**5**), by Michael-type addition to its α,β-unsaturated carbonyl groups did not lead to a significant loss of cytotoxicity (which also lie in the 1–15 μM range).[22] The retention of activity upon addition of the amine can be explained by the reversible nature of the Michael-type reaction. In other words, a reverse Michael-type reaction—the elimination of the amine—will provide **1** from **3** and **2** from **4**. This may explain why the activities of the majority of the amine adducts of each lactone are very similar, since the cytotoxic effect is ultimately derived from the release of the lactone.

Both isoalantolactone (**2**) and its diethylamine adduct **4a** were screened against 11 different cancer cell lines. The results, shown in Table 2, indicate that neither compound exhibits significant selective cancer cell growth inhibitory properties. This probably reflects the

Table 2. Cell growth inhibition properties of **2** and **4a**, respectively, against the Paterson Institute panel of disease-orientated tumour cell lines

Tumour cell line	**2** ID_{50} (μM)	**4a** ID_{50} (μM)
BT20 (Breast cancer)	1.2	1.2
MCF7 (Breast cancer)	0.6	0.8
COLO (Skin melanoma)	2.1	3.5
A549 (Lung cancer)	0.6	0.8
WiDR (Colon adenocarcinoma)	1.4	1.5
FF1 (Normal skin cells)	1.1	1.0
K562 (Human leukaemia)	1.2	2.0
P388 (Mouse leukaemia)	0.4	0.3
H460 (Lung cancer)	0.6	0.6
H520 (Lung cancer)	0.9	0.9

Table 3. Effect of **1**, **2**, **3a** and **4a** on the cell cycle distribution of K562 cells following treatment ($10\times IC_{50}$ for 16 h). Apoptotic cells were defined as those with DNA contents of less than cells in the G1 phase. The calculation of cell cycle parameters excludes these cells and was generated using ModFit (Verity Software), which also derives the coefficients of variation

Compound	G1 (%)	S (%)	G2/M (%)	Apoptotic (pre-G1) (%)	c.v.
Control	57	41	2	0.5	3.6
1	30	53	17	9.9	3.4
2	38	50	12	8.2	3.2
3a	38	52	10	7.3	7.0
4a	39	52	10	4.4	4.3

rather unselective nature of their proposed molecular mode of action. Nevertheless, it is encouraging to see that the compounds are able to inhibit cell growth in the sub-micromolar range against many of the cell lines.

Alantolactone (**1**), isoalantolactone (**2**) and the dimethylamine adducts **3a** and **4a** cause similar changes in cell cycle distribution. An increase in the proportion of cells in the G2/M and S phase is seen, which is consistent with alkylating activity (Table 3). Interestingly, these agents cause a significant increase in the number of cells with DNA contents of less than G1. This can be indicative of the induction of apoptosis in the K562 cell line. Again, the similarity of the effects on the cell cycle and induction of apoptosis indicates that these agents act by a common mechanism, perhaps via the parental alantolactone or isoalantolactone.

In view of the interesting activity displayed by the amine adducts and the ease of isolation of the lactones, we believe that as a class of anticancer agent they deserve further attention. The design of similar compounds that act more selectively upon cancer cells is currently in progress.

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