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Synthesis and Biological Evaluation of Aristolactams

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Abstract—A variety of aristolactam derivatives were synthesized and evaluated for cytotoxicity. Modulations were carried out on the phenanthrene nucleus and the lactam moiety as well. *N*-(*N*-dialkylaminoalkyl) derivatives exhibited interesting cytotoxic activity against the L1210 leukemia cell line.

Aristolactams **1** (Fig. 1) are phenanthrene lactam alkaloids structurally and biogenetically related to aporphines.[1,2] The richest source of this family of alkaloids is undoubtedly the leaves and roots of *Aristolochia* species which have been used since antiquity in obstetrics and in the treatment of snake bites.[3] Extracts of *Aristolochia* plants are still being used in the traditional medicine of certain regions in Turkey,[4] India[5] and Argentina.[6] These phenanthrene lactams are considered as the principal detoxification metabolites of aristolochic acids **2**[7] which have been implicated in an endemic renal fibrosis in young Belgian women who had followed a slimming regimen.[8]

They have also been detected in urine and faeces from mammal including humans.[9] Although the exact mode of action of aristolactams at both cellular and molecular level has not yet been elucidated, the current view is that aristolochic acids are activated by a reduction pathway induced by cytochrome P-450 and peroxidase pointing to the formation of a cyclic *N*-acylnitrenium ion embedded in an aristolactam unit with delocalized positive charge (Fig. 2).[10] It is assumed that this ionic species binds preferentially to the exocyclic amino groups of purine nucleotides by the carbon atom *ortho* to the lactam nitrogen.[11] They are also stored as *N*-glucosides[12,13] and it has been shown recently that the alkaloid aristolactam β-D-glucoside binds to DNA by the mechanism of intercalation.[14,15] Because of these findings pharmaceuticals containing aristolochic acids and aristolactams have been withdrawn from the market.

Paradoxically there is little information in the literature regarding the metabolism of aristolactams in animals and in plants, or their potential carcinogenic risks. In this report, we describe two straightforward and complementary synthetic routes to these fused lactam compounds and evaluation of their cytotoxicity. Structural

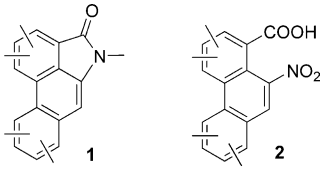

Figure 1. Aristolactams (**1**) and aristolochic acids (**2**).

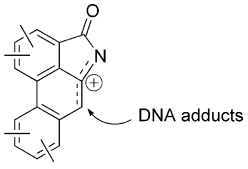

Figure 2.

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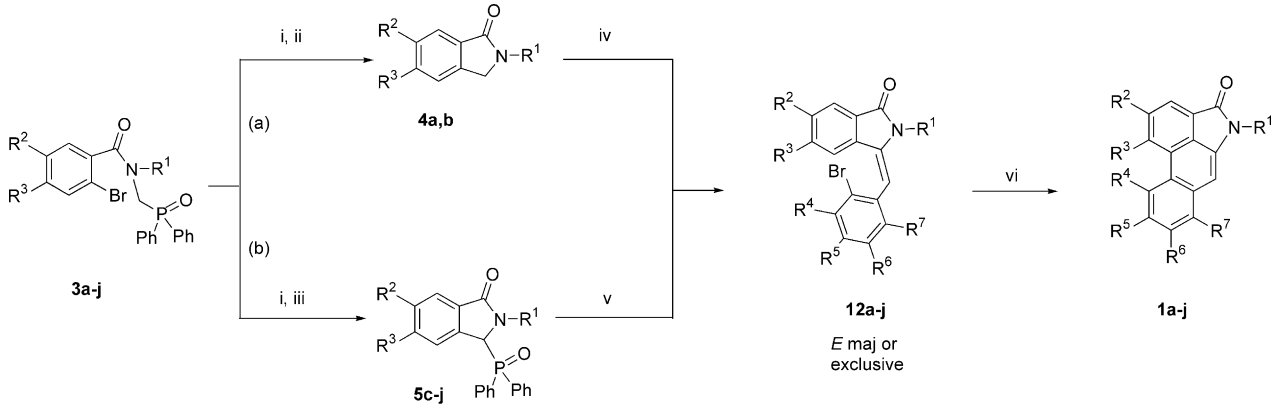
modifications focused on the phenanthrene nucleus and the lactam moiety as well and the *N*-substituted compounds synthesized by these methods were subsequently evaluated for their antiproliferative activity using the murine L1210 leukemia cell line.

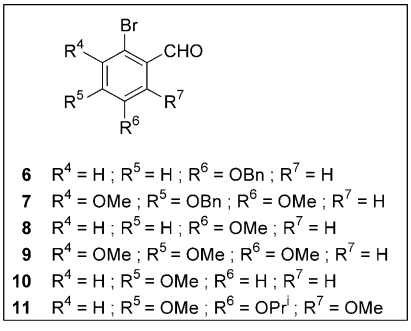
Chemistry

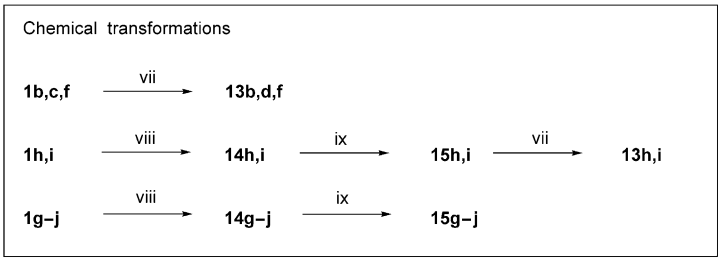
A contentious issue in the elaboration of the diversely functionalized aristolactams **1a–j** was first judging the proper strategy for the stereoselective synthesis of the parent arylmethyleneisoindolinones **12a–j** with the mandatory *E*-stereochemistry required for the radically induced cyclization leading to the target compounds (Scheme 1). For this purpose we adopted two methodologies recently developed in our laboratory which are precisely governed by the bulkiness of the substituent connected to the lactam nitrogen. Thus the (*E*)-*N*-methyl derivatives **12a**,**b** (Scheme 1, path a) were easily obtained by sequential metallation of isoindolinones **4a**,**b** followed by quenching with appropriate bromobenzaldehydes **6** or **7**, *O*-silylation in situ and ultimate benzylic deprotonation to ensure completion of the elimination reaction through an E1cb mechanism.[16,17] The bulkier substituted models **12c–j** were elaborated under the agency of the Horner process applied to the phosphorylated isoindolinones **5c–j** (Scheme 1, path b) and bromobenzaldehydes **7**.[18] Isoindolinones **4a**,**b** and **5c–j** were easily accessible by taking advantage of our newly developed aryne-mediated cyclization of the halogeno-*N*-(diphenylphosphinoylmethyl) benzamide derivatives **3a–j**.[16–18] Thus compounds **3** were exposed to potassium bis(trimethylsilyl)amide (2 equiv). Subsequent acidic treatment spared the phosphoryl unit and delivered the phosphorylated lactams **5c–j**[19,20] whilst basic work up triggered off the formation of the isoindolinones **4a**,**b** released from the phosphoryl appendage.[21] Some of the products of radically induced cyclization were subjected to further chemical transformation (Scheme 1). Thus regeneration of the formyl functionality from the diethylacetal derivatives **1g–j** furnished the formylated aristolactams **14g–j** whilst the dimethylamino derivatives **15g–j** were readily obtained by reductive amination of **14g–j**. Removal of the benzylic group in the primarily cyclized products delivered compounds **13b**,**d**,**f**,**h**,**i** equipped with a phenolic hydroxy function.

Cytotoxic Activity

The aristolactam derivatives synthesized were then tested for cytotoxic activity in vitro against L1210 murine leukemia cells. L1210 cells provided by the NCI, Frederik, USA were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% foetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL







Scheme 1. Reagents and conditions (typical yields): (i) KHMDS (2 equiv), THF, −78 °C to rt; (ii) aqueous NaOH (10%) (74–77%); (iii) aqueous NH_4Cl (10%) (53–94%); (iv) KHMDS, THF, −78 °C, then **6** or **7**, then Me_3SiCl, then KHMDS, −78 °C to rt (75–80%); (v) KHMDS, THF, −78 °C, then **7**–**11**; −78 °C to rt (64–88%); (vi) Bu_3SnH, AIBN, benzene, reflux (64–87%); (vii) $HCOONH_4$, Pd/C, MeOH (87–98%); (viii) $FeCl_3$, $6H_2O$, CH_2Cl_2–acetone, rt (76–98%); (ix) $NaBH(OAc)_3$, Me_2NH (gaz), CH_2Cl_2, rt (90–96%).

Table 1. Cytotoxicity assay results for compounds **1, 13, 14, 15**

Compd	R^1	R^2	R^3	R^4	R^5	R^6	R^7	Mp (°C)	Cytotoxicity IC_{50}, μM[a]
1a	Me	H	OBn	H	H	OBn	H	139–140	79.2
1b	Me	OMe	H	OMe	OBn	OMe	H	153–154	>10
13b	Me	OMe	H	OMe	OH	OMe	H	225–226	1.6
1c	PMB[b]	OMe	H	H	H	OMe	H	152–153	66.6
1d	PMB[b]	OBn	H	OMe	OBn	OMe	H	177–178	>100
13d	PMB[b]	OH	H	OMe	OH	OMe	H	134–135	2.6
1e	PMB[b]	OMe	H	OMe	OMe	OMe	H	114–115	>10
1f	$(CH_2)_2NEt_2$	OMe	H	OMe	OBn	OMe	H	Oil	nd
13f	$(CH_2)_2NEt_2$	OMe	H	OMe	OH	OMe	H	111–112	2.3
1g	$(CH_2)_2CH(OEt)_2$	OMe	H	H	OMe	H	H	Oil	>10
14g	$(CH_2)_2CHO$	OMe	H	H	OMe	H	H	177–178	5.2
15g	$(CH_2)_3NMe_2$	OMe	H	H	OMe	H	H	89–90	1.6
1h	$(CH_2)_3CH(OEt)_2$	H	H	OMe	OBn	OMe	H	Oil	21.2
14h	$(CH_2)_3CHO$	H	H	OMe	OBn	OMe	H	105–106	17.6
15h	$(CH_2)_4NMe_2$	H	H	OMe	OBn	OMe	H	103–104	10.8
13h	$(CH_2)_4NMe_2$	H	H	OMe	OH	OMe	H	197–198	1.8
1i	$(CH_2)_2CH(OEt)_2$	OMe	H	OMe	OBn	OMe	H	Oil	nd
14i	$(CH_2)_2CHO$	OMe	H	OMe	OBn	OMe	H	104–105	4.7
15i	$(CH_2)_3NMe_2$	OMe	H	OMe	OBn	OMe	H	Oil	nd
13i	$(CH_2)_3NMe_2$	OMe	H	OMe	OH	OMe	H	Oil	5.4
1j	$(CH_2)_2CH(OEt)_2$	OCH_2O		H	OMe	OPr^i	OMe	141–142	16.3
14j	$(CH_2)_2CHO$	OCH_2O		H	OMe	OPr^i	OMe	138–139	7.8
15j	$(CH_2)_3NMe_2$	OCH_2O		H	OMe	OPr^i	OMe	137–138	4.3

[a]nd, not determined.
[b]PMB, *para*-methoxybenzyl.

streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.[22] Cells were exposed to graded concentrations of the compounds for 48 h and results, expressed as IC_{50} (concentration which reduced by 50% the optical density of treated cells with respect to untreated controls), are reported in Table 1. In both series of *N*-alkylated models activity was not affected by the degree of substitution and by the nature of the substituents R^1–R^3 in the northern aromatic part. The replacement of the *N*-methyl group by an *N*-benzyl group on the lactam unit had no significant influence on the cytotoxic activities. Best results in these series were obtained by introduction of a phenolic hydroxy function and, in particular, introduction of an 9-hydroxy group (R^5 = OH) inserted between two methoxy phenolic groups had a favorable effect on cytotoxicities with compounds **13b** and **13d** displaying IC_{50} values of 1.6 and 2.6 μM, respectively. Incorporation of a 9-benzyloxy group (R^5 = OBn) in the structurally related parent compounds **1b** and **1d** led instead to significant loss of activity. The alkylated chain connected to the lactam nitrogen atom could be advantageously replaced by aliphatic hydrophilic side chains as in compound **14g** (compared to **1a**,**b**). Thus the *N*-dimethylaminoalkyl derivatives obtained by varying the length of the hydrocarbon segment linking the two nitrogen atoms showed significant activities since all displayed IC_{50} values in the 1.6–10 μM range. From values obtained with compounds **13h**,**i** and **15g**,**j** it is obvious that the presence of the dimethylaminoalkyl group has a marked influence on the cytototoxic activity but it is worth noting that the most potent model **15g** was not obtained by combining the presence of the phenolic hydroxy function and an aminoalkyl chain on the lactam unit (IC_{50} 2.3 and 1.8 μM for **13f** and **13h**, respectively versus 1.6 μM for **15g**).

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