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Synthesis and biological evaluation of amino analogs of Ludartin: Potent and selective cytotoxic agents

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ABSTRACT

Diverse amino analogs of Ludartin, a cytotoxic guaianolide and a position isomer of an anticancer drug, Arglabin were prepared through Michael type addition at its highly active α-methylene-γ-lactone motif. The semisynthetic derivatives were subjected to sulphorhodamine B cytotoxicity assay against a panel of four different human cancer cell lines viz. lung (A-549), leukemia (THP-1), prostate (PC-3) and colon (HCT-116) to look into structure–activity relationship. Few of the analogs displayed potent selective cytotoxicity compared to the parent molecule-Ludartin (**1**). (11*R*)-13-(Diethyl amine)-11,13-dihydroludartin (**6**) and (11*R*)-13-(piperidine)-11,13-dihydroludartin (**10**) showed almost same cytotoxicity against leukemia cell lines (THP-1) as that of parent molecule-Ludartin, but were more active against colon (HCT-116) cancer cells. (11*R*)-13-(Morpholine)-11,13-dihydroludartin (**11**) displayed selectively better cytotoxicity against Leukemia cancer cells (THP-1) exhibiting IC_{50} of 2.8 μM. (11*R*)-13-(6-Nitroindazole)-11,13-dihydroludartin (**17**) was four times more potent than Ludartin with selective cytotoxic effects against prostate cancer cells (2.2 μM) while as (11*R*)-13-(6-nitroindazole)-11,13-dihydroludartin (**18**) exhibited three-fold selective cytotoxicity for Lung (A-549) cancer cell lines exhibiting IC_{50} of 2.6 μM.

Sesquiterpene lactones (SLs) display a wide array of biological activities like antiviral, antibacterial, antiulcer, cytotoxic, anti-inflammatory, antifungal and effects on the central nervous system and cardiovascular system.[1] The unique chemical properties of SLs like presence of alkylating center reactivity (α-methylene-γ-lactone), lipophilicity along with molecular geometry and electronic features make them highly bioactive with in the living systems.[2] However the clinical translation of these lactones is hampered due to their nonselective binding at undesired targets via the highly reactive Michael acceptor, that is, α-methylene-γ-lactone.[3] The high lipophilicity allows their facile penetration via the cell membranes increasing their cytotoxicity in vitro, but higher lipophilicity dictates their lower drug bioavailability in vivo.[2] These queries have been addressed by synthetic chemists and one such approach has been the amino-prodrug approach, where in the aza-Michael adducts display the enhanced aqueous solubility, improved pharmarmacokinetic potential and even retention or augmentation in bioactivity.[3] These amino based Michael adducts have been prepared for various bioactive sesquiterpene lactones such as alantolactone,[4] costunolide,[5] parthenolide,[6–9] α-santonin,[10] helenalin[11] and ambrosin[12] and effective structure–activity relationships have been developed. It is however assumed that the amino analogs serve as prodrugs which in presence of thiol groups of proteins, enzymes and glutathione release the amine group to generate the parent enone motif that defines their mechanism of action at the desired targets.[9] Approaches have been made to devise such synthetic methods that make SLs highly target specific to cancerous cells while sparing the normal cells. At present the SL drugs in clinical trials are artemisinin from *Artemisia annua* L., thapsigargin from *Thapsia* and parthenolide from *Tanacetum parthenum* and/or many of their synthetic derivatives.[2]

Ludartin, a bioactive natural product of the widely distributed class of guaianolides was previously isolated as a mixture along with 11,13-dihydroderivative from *A. caruthii*[13] and then in pure form from *Stevia yaconensis* var. *subeglandulosa*[14] and from *A. filatovae*.[15] The heart of this molecule consists of a cycloheptane ring with five contiguous stereocenters, to which two five membered rings are *trans*-annulated. One of the five membered rings is a γ-butyrolactone with an exocyclic double bond. Ludartin shows gastric cytoprotective effect[16] and also inhibits aromatase enzyme which is involved in hormone-dependent breast cancer.[17] So far there are no reports on the SAR studies of this molecule, and keeping in view the bioactivity of the molecule along with the literature precedent that reductive amination at exocyclic double bond of a cytotoxic agent can enhance selectivity for malignant cell lines,[18]

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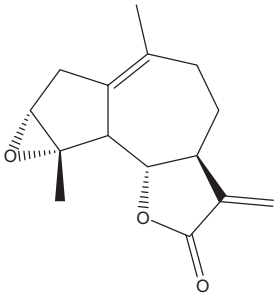
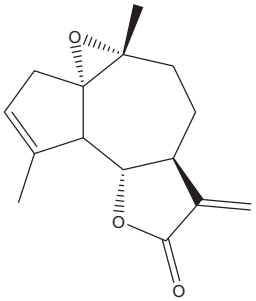
we designed the synthesis of various Michael adducts at the highly electrophilic α-methylene-γ-lactone moiety with the hope to develop analogs with enhanced bioavailability, lesser toxicity and better activity. This however would constitute an important step towards the rationalization of lead properties of Ludartin (**1**).



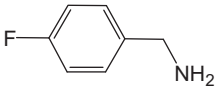
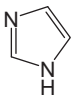
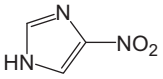
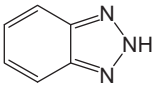
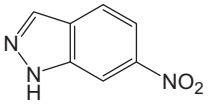
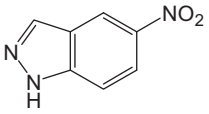
Ludartin (**1**)



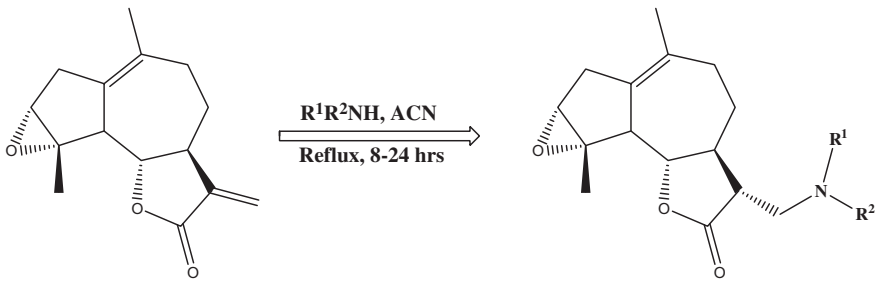
Arglabin (**19**)

Our synthetic efforts were mainly focussed towards Michael addition at exocyclic double bond of α-methylene-γ-lactone motif (Scheme 1). Therefore Ludartin was subjected to synthetic modifications (Michael addition) using a diversity of amines as Michael donors. Different analogs were prepared by refluxing a solution of Ludartin (**1**) in acetonitrile and an appropriate amine for 8–24 h[19] (Scheme 1). The reaction was completely clean furnishing products with satisfactory yields (70–92%) (Table 1). Unlike primary amines cyclic secondary amines like pyrollidine, piperidine, morpholine reacted quickly without using any base. On the other hand azole type Michael donors like imidazole, benzotriazole, required DBU as base for the successful completion of the reaction owing to lesser reactivity of *N*-containing heterocycles. A library of 17 analogs was prepared whose formation could easily be confirmed by the disappearance of two diagnostic proton resonances at δ 5.38 ppm (d, J = 3.5 Hz) and δ 6.21 ppm (d, J = 3.5 Hz) of α-methylene-protons (13-H_2) of Ludartin (**1**). Since the molecule Ludartin in itself bears five contiguous chiral centers (C-3–7), the Michael addition creates one more chiral center at C-11 position whose configuration was determined on the basis of correlations deduced from NOESY experiment. In the NOE experiment of compound **9**, the NOESY correlation observed between H-6 and H-11 unambiguously established the H_7–H_{11} anticonfiguration (Fig. 1). Since the configuration of already existing stereocenter at C-7 is *S*, the stereocenter at C-11 was unambiguously put as *R*, hence staying in tune with the literature precedent[4,6,18,20] that the Michael addition at exocyclic double bond of sesquiterpene lactones proceeds with high diastereoselectivity at position-11 giving preferentially the less hindered *R* isomer.

Table 1
Preparation of different amino analogs of Ludartin (**1**)

S. no	R^1R^2HN[a]	Product	Yield[b] (%)
1		**2**	80
2		**3**	76
3		**4**	64
4		**5**	72
5		**6**	67
6		**7**	78
7		**8**	80
8		**9**	90
9		**10**	92
10		**11**	90
11		**12**	73
12		**13**	64
13		**14**	60
14		**15**	66
15		**16**	75
16		**17**	78
17		**18**	84

[a] Here R^1R^2NH refers to any primary, secondary or heteroaromatic amine.
[b] Refers to the yield after isolation of products.



Ludartin (1) **17 amino Ludartin analogs**

Scheme 1.