Diastereoselective Synthesis of 1,10β-Epoxy-11*R*,13-dihydroamino Analogs of Ludartin as Anti-breast Cancer Agents

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Abstract

Diverse 11*R*,13-dihydroamino and 1,10β-epoxy-11*R*,13-dihydroamino analogs of ludartin were synthesized using a highly diastereoselective Michael addition and epoxidation reaction. The semisynthetic analogs were characterized using rigorous spectral data analysis. All the compounds were subjected to sulphorhodamine B cytotoxicity screening against a panel of three breast cancer cells, viz., T47D, MCF-7, and MDA-MB 231. Among the synthesized analogs, compounds **11, 19**, and **20** proved to be active against the breast cancer cell lines. Compound **11** represents an epoxy analog of arglabin (**5**), which is a noteworthy and clinically significant antitumor agent and exhibited an IC_{50}s of 0.75 µM and 1.20 µM against MCF-7 and MDA MB-231 cell lines, respectively. Compounds **19** and **20** displayed selectivity among the three breast cancer cell lines and were active against MCF-7 cell lines only displaying IC_{50}s of 1.00 and 1.21 µM, respectively. This study provides initial structure-activity relationship data on ludartin and its analogs against breast cancer cell lines based on the previous literature reports of ludartin as an aromatase inhibitor.

Key words

ludartin · amino analogs · epoxy amino analogs · breast cancer · cytotoxicity

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Leaving apart the cancers of skin, breast cancer is the most frequently occurring cancer in women [1]. It ranks second as a cause of tumor-related death only after lung cancer. Presently, it is envisaged that one in eight American women will develop breast cancer sometime during her life. Two-thirds of breast cancer tumors are hormone dependent, requiring estrogen to flourish [2]. An effective approach to treat such hormone-dependent cancer involves interfering with hormone production. Aromatase, or estrogen synthase, has always been considered the most promising target for the endocrine treatment of breast cancer [3]. Among all inhibitors, nonsteroidal inhibitors have proved to be the best therapeutic agents, as they reversibly inhibit the enzyme [2,4]. Aromatase inhibitors (AIs) are further divided into categories based on the order in which they were discovered or synthesized: first-, second-, and third-generation AIs. Currently, the third generation aromatase inhibitors are triazole-derived AIs and are approved as front-line therapy for early and advanced cases of breast cancer in postmenopausal women [1,5,6]. Brueggemeier and Cruz [1] framed a list of 65 molecules with potent aromatase inhibitory activity, among which the four sesquiterpene lactones, designated **1, 2, 3**, and **4** (**Fig. 1**), display strong aromatase inhibition. The research group opined that the triazole-based compounds display a potent inhibitory effect. Taking a cue from this literature, we previously synthesized the 1, 2, 3 triazole-based analogs [7] of this molecule to develop more potent and less toxic compounds, and screened them against breast cancer cells. Among all of the synthesized compounds, only one compound, namely, (11*R*)-13-[1-(3,4-dimethylphenyl) [1–3] triazol-4-ylmethylamine]-11*R*,13-dihydroludartin, displayed a potent effect with an IC_{50} of 8.5 µM and the parent ludartin molecule displayed an IC_{50} of 0.5 µM. Based on the close resemblance of ludartin (**3**) to arglabin (**5**) (position isomers), we also synthesized the amino analogs of ludartin [8], wherein compound **9** displayed a selective and potent biological effect, and, finally, we also developed a route for the hemisynthesis of arglabin from ludartin [9]. Arglabin, along with its dimethylamine analog (11*R*)-13-(dimethylamine)-11,13-dihydroarglabin (**6**), is an approved anticancer agent in several countries for the treatment of lung, liver, breast, and ovarian cancers [10,11]. In continuation to our earlier work on ludartin to look for more potent analogs effective against breast cancer cell lines, we designed a strategy to synthesize some more compounds using a diastereoselective Michael addition and epoxidation reactions.

Ludartin was subjected to epoxidation using *m*-CPBA to furnish 1,10β-epoxyludartin (**11**) (**Fig. 2**, **Table 1**), which furnished only a single diastereomer with a β-configuration in conformity with the literature reports [12]. To confirm that epoxidation at the 1,10-double bond is directed by the already existing C 3(4) epoxide, a ring opening of the 3(4) epoxide in ludartin was carried out as described previously [9]. It was observed that in an opened epoxide structure, epoxidation at the 1,10-double bond gives a diastereomeric ratio of β- and α-epoxides in a 85 : 15 ratio, validating that it is the 3(4) epoxide geometry that gives rise to the diastereoselective formation of only the β-epoxide in ludartin. After epoxidation of ludartin (**3**) to give 1,10β-epoxy ludartin (**11**), both **3** and **11** were subjected to diastereoselective Michael addition using a range of amines. Reductive amination was carried out by refluxing a solution of ludartin (**3**) or its epoxy analog (**11**) in acetonitrile and an appropriate amine for 8–24 h (**Fig. 2**). The reaction was completely clean, furnishing products

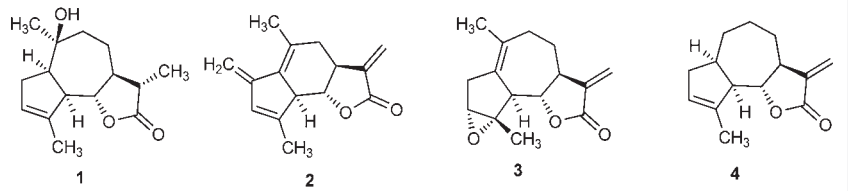


Fig. 1 Sesquiterpene lactones that show aromatase inhibitory activity.

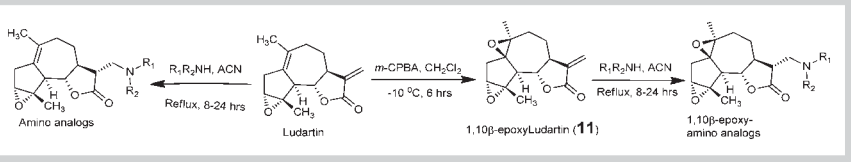
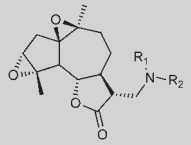
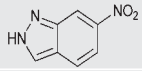
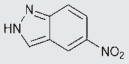


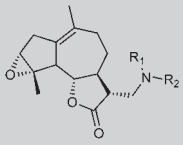
Fig. 2 Synthesis of amino analogs, epoxy ludartin and epoxy-amino analogs.

Table 1 Various 1,10-epoxy-11*R*,13-dihydroamino ludartin analogs.

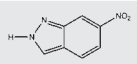
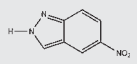


General structure of epoxy-amino analogs

S. No	Product	R_1R_2NH	Yield (%)[a]
1	**12**		**70**
2	**13**		**65**
3	**14**		**92**
4	**15**		**90**
5	**16**		**84**
6	**17**		**78**



General structure of amino analogs

S. No	Product	R_1R_2NH	Yield (%)[a]
1	**18**		**70**
2	**19**		**65**
3	**20**		**67**
4	**21**		**92**
5	**9**		**90**
6	**22**		**84**
7	**23**		**78**

[a] Represents the yield obtained after the purification of the compounds.

with satisfactory yields (60–96%) (**Table 1**). A library of 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 the α-methylene protons (13 H_2) of ludartin (**3**) as well as its epoxy analog (**11**). Since ludartin bears five contiguous chiral centers (C-3,4,5,6,7), Michael addition creates one more chiral center at the C-11 position, with *R* configuration [8]. Further epoxidation created two more chiral centers in the molecule, making the total number of chiral centers in any 1,10-epoxy-11*R*,13-aminoludartin analog equal to eight. Ludartin, along with its analogs, were studied in a colorimetric sulphorhodamine B cytotoxicity assay against a panel of three breast cancer cell lines, including T47D, MCF-7, and MDA MB-231. The analogs that depicted an appreciable amount of growth inhibition in a preliminary assay were again evaluated at different concentrations (10–100 µM) to obtain their IC_{50} values given in **Table 2**. The parent ludartin that exhibited activity against human lung, liver, prostate, leukemia, and neuroblastoma cell lines as reported earlier [5] was found to be profusely active against T47D, MCF-7, and MDA MB-231 cell lines with IC_{50} values of 2.8, 0.5, and 0.67 µM, respectively. Epoxidation of ludartin lead to the formation of a single diastereomer (**11**), with 1,10-epoxide having a β-configuration. This isomer (**11**) in turn represents the 3(4)-α-epoxy analog of a novel, noteworthy, and clinically significant antitumor arglabin (**5**), which was used to treat various types of cancers like lung, liver, and ovarian cancers in the former USSR [10, 11]. Some studies have identified a few potent analogs

Table 2 Cytotoxicity of the compounds against various breast cancer cell lines.

Compound	T47D		MCF-7		MDAMB-231	
	%GI	IC_{50}[a]	%GI	[a]IC_{50}	%GI	[a]IC_{50}
3	75	2.80	96	0.50	92	0.67
9	22	–	26	–	19	–
11	58	14	96	0.75	94	1.20
12	40	80	15	–	55	45
13	51	49	0	–	68	36
14	23	–	17	–	34	nd
15	35	–	96	–	68	–
16	16	–	0	–	23	nd
17	15	–	70	45	41	64
18	21	–	42	65	21	–
19	50	20.6	83	1.00	62	7.6
20	44	17.1	83	1.21	62	14.8
21	24	–	43	60	27	90
22	21	–	25	–	36	–
23	21	–	41	65	31	–
Doxorubicin	99	0.41	99	0.22	100	0.10

[a] IC_{50} values given are in µM. Ludartin (**3**) and doxorubicin served as positive controls.

of arglabin and ludartin (**Fig. 3**) [4, 10, 11]. Although, epoxidation of ludartin at the 1,10-double bond decreased the activity against the T47D cell line with an IC_{50} of 14 µM compared to the parent ludartin with an IC_{50} of 2.8 µM against the same cancer

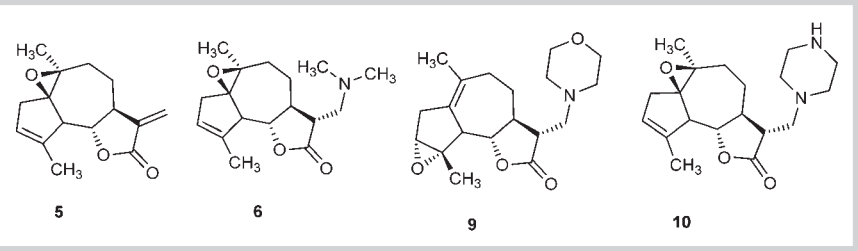


Fig. 3 Arglabin (**5**), dimethylamino arglabin (**6**), 13-morpholinoludartin (**9**) and 13-piperdino arglabin (**10**).

cell line. However, this modification almost led to the retention of a comparable cytotoxic effect against the MCF-7 and MDA MB-231 cell lines displaying IC_{50}s of 0.75 and 1.20 µM, slightly less than the standard ludartin with IC_{50}s of 0.5 and 0.67 µM against the same cancer cell lines, respectively. Among all of the compounds, the parent ludartin (**3**) and its 1,10-epoxy analog (**11**) were the most active because of the intact α-methylene-γ-lactone moiety and that the reductive amination at this moiety leads to analogs with a reduced cytotoxic effect, but enhances the cell line selectivity, which was evident from our previous studies as well [7,8]. Surprisingly, the dimethyl amino analogs (**12** and **18**) of 1,10-epoxy ludartin (**11**) and ludartin (**3**) were inactive against all of the cancer cell lines. Contrary to this, the ethyl methyl amino analog (**19**) of ludartin demonstrated a drastic change in activity with IC_{50}s of 1.00, 7.60, and 20.6 µM against MCF-7, MDA MB-231, and T47D cell lines, respectively. However, the introduction of one more ethyl group, instead of methyl furnishing a diethylamino ludartin (**20**), enhanced the selectivity of the parent ludartin against the MCF-7 cell line with an IC_{50} of 1.21 µM. This analog was previously [4] found to be active only against THP-1 (3.5 µM) and HCT-116 (3.7 µM). However, epoxidation of this analog led to a loss of bioactivity. The piperdino analog of ludartin, which depicted a selective cytotoxic effect against THP-1 cell lines (IC_{50}), was found to be completely inactive against the breast cancer cells. This study provides new insights into the synthesis of ludartin analogs, wherein the epoxy analogs of ludartin representing the epoxy analogs of an antitumor arglabin have been exploited for their new biological potential against breast cancer cell lines, prompting us to study these compounds further. In this regard, compound **11** is currently undergoing an investigation in our laboratory.

Material and Methods

^1H and ^{13}C NMR spectra were recorded on Bruker 100, 400, and 500 MHz Bruker spectrometers using TMS as an internal standard. Chemical shifts (δ) are expressed as ppm. Mass spectra were recorded on Shimadzo lab solutions. Chromatography was carried out using ordinary phase column chromatography silica gel 60–120 mesh (Merck), and precoated TLC plates with silica gel 60 F_{254} (Merck, 0.25 mm). Detection was done by using 1% $Ce(SO_4)_2$ − 10% aq. H_2SO_4, *para*-anisaldehyde, and ninhydrin reagent followed by heating, and spraying by dragendorff's reagent.

General procedure for the synthesis of 1,10β-epoxy analog of ludartin (11)

m-CPBA was added to a solution of ludartin (**3**) in CH_2Cl_2 at − 10 ° C and the reaction mixure was left for stirring. As measured by TLC profiling, the reaction was complete after 6 h. The reaction mixture was worked up in CH_2Cl_2 washed with Na_2SO_3, $NaHCO_3$, and saturated brine solution. The organic layers so obtained were dried under vaccuo to afford a single diastereomer **11** which was separated using silica gel column chromatography.

General procedure for the synthesis of 1,10-β-epoxy-11R,13-dihydroamino analogs of compound 11

A solution of compound **11** (30 mg, 0.121 mmol) in acetonitrile (2 mL) and amine (0.121 mmol) was heated under reflux for 8–15 h either in the presence of the base (Et_3 N/DBU) or, more often, without the base. After cooling, the reaction mixture was evaporated under vaccuo and the residue obtained was subjected to normal silica gel column chromatography using hexane-EtOAc as the eluent to furnish the pure product.

Supporting information

The spectral data of all of the compounds as well as a detailed description of the bioevaluation study are available as Supporting Information.

Acknowledgements

Shabir H. Lone is thankful to CSIR-India for providing a financial grant in the form of SRF (Budget head: P-81101). The financial assistance from DST under GAP-2100 is highly acknowledged.

Conflict of Interest

The authors declare no conflict of interest.

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received March 7, 2016
revised March 25, 2016
accepted April 10, 2016

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DOI http://dx.doi.org/10.1055/s-0042-106973
Planta Med Int Open 2016; 3: e51–e54

ISSN 2509-6656

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