Supporting Information

Diastereoselective Synthesis of 1,10\beta\text{-Epoxy-11}R,13-dihydroamino Analogs of Ludartin as Anti-breast Cancer Agents

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Fig. 1S $^1$H and $^{13}$C NMR of (11R)-13-(diethylamine)-11,13-dihydroludartin.
Fig. 2S $^1$H and $^{13}$C NMR of (11R)-13-(piperidine)-11,13-dihydroludartin.
Fig. 3S $^1$H and $^{13}$C NMR of (11R)-13-(morpholine)-11,13-dihydroludartin.
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Fig. 10S $^1$H and $^{13}$C NMR of (11R)-13-(dimethylamine)-11,13-dihydroludartin.
Fig. 11S $^1$H and $^{13}$C NMR of (1R)-13-(ethylmethylamine)-11,13-dihydroludartin.
Spectral Analysis of Compounds

1(10)-β-epoxy-ludartin (11): \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 6.14 (1H, s, \(J = 3\)Hz), 5.44 (1H, s, \(J = 3\)Hz), 3.75 (1H, t, \(J = 10.7\) Hz, 10.6 Hz), 3.41 (1H, s), 2.70 (1H, d, \(J = 10.7\) Hz), 2.30-2.40 (m, 3H), 2.0-1.9 (m, 1H), 1.68 (s, 3H), 1.3-1.45 (m, 2H), 1.26 (s, 3H) 1.25 (m, 1H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 169.28, 138.75, 119.32, 81.02, 69.17, 65.58, 61.81, 59.83, 54.27, 53.00, 37.36, 35.48, 23.86, 20.72, 18.76. ESI-MS at \(m/z\) = 263.12 (calculated for C\(_{15}H_{18}O_4\), 262.12).

1(10)-β-epoxy-(11R)-13-(dimethylamine)-11,13-dihydroludartin (12): \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 3.77 (1H, t, \(J = 10.6\) Hz, 10.8 Hz, ), 3.39 (1H, s), 3.32 (1H, d, \(J = 10.8\) Hz), 2.96 (s, 2H), 2.64 (1H, d, \(J = 17.3\) Hz), 2.51 (3H, s), 2.35 (1H, d, \(J = 17.3\) Hz), 2.05 (m, 2H), 2.0 (s, 6H), 1.59 (3H, s), 1.25 (3H, s), 1.23 (s,1H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 176.31, 80.07, 68.24, 65.34, 61.46, 60.12, 54.27, 51.43, 45.03, 37.22, 34.61, 28.31, 25.21, 23.21, 20.36, 19.12. ESI-MS at \(m/z\) = 308.18 (calculated for C\(_{17}H_{25}NO_4\), 307.17).

1(10)-β-epoxy-(11R)-13-(diethylamine)-11,13-dihydroludartin (13): \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 3.80 (t, \(J = 10.8\), 10.7, 1H), 3.41 (s, 1H), 2.91-2.79 (m, 1H), 2.71-2.36 (m, 5H), 2.37-2.23 (m, 4H), 2.23-2.08 (m, 1H), 2.00-1.77 (m, 3H), 1.68 (s, 3H), 1.64 (s, 3H), 1.58-1.29 (m, 2H), 1.25 (s, 3H), 0.97 (m, 3H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 177.79, 81.02, 68.30, 64.56, 61.21, 60.09, 54.34, 54.07, 52.68, 47.36, 45.21, 37.75, 35.48, 25.70, 21.98, 19.70, 12.21. ESI-MS at \(m/z\) = 336.21 (calculated for C\(_{19}H_{29}NO_4\), 335.20).

1(10)-β-epoxy-(11R)-13-(morpholine)-11,13-dihydroludartin (14): \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) 3.72 (m, 5H), 3.39 (s, 1H), 2.80 (d, \(J = 17.6\) Hz), 2.64 (s, 1H), 2.46 (s, 5H), 2.27 (m, 4H), 1.95 (d, \(J = 12.4\) Hz, 1H), 1.84 (d, \(J = 17.5\) Hz, 1H), 1.63 (s, 1H), 1.36 (m, 1H). \(^{13}\)C NMR (125 MHz,
$\text{CDCl}_3 \ \delta \ 176.37, \ 80.95, \ 68.92, \ 67.21, \ 65.61, \ 61.17, \ 60.00, \ 57.40, \ 54.12, \ 54.07, \ 54.95, \ 44.09, \ 37.73, \ 35.38, \ 25.45, \ 20.40, \ 18.75. \ ESI-MS \ at \ m/z = 349.18 \ (calculated \ for \ C_{19}H_{27}NO_{5}, \ 350.19)$. 

$\text{1(10)-\beta-epoxy-(11R)-13-(5-nitroindazole)-11,13-dihydroludartin (15)}$: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.72 (s, 1H), 8.28 (s, 1H), 8.19 (s, 1H), 7.68 (s, 1H), 4.78 (s, 2H), 3.79 (t, $J = 10.8, 10.8$ Hz, 1H), 3.38 (s, 1H), 2.83 (m, 1H), 2.38 (m, 2H), 2.05-1.65 (m, 2H), 1.56 (s, 3H), 1.38 (s, 3H), 1.25 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 175.05, 141.54, 137.12, 124.77, 123.54, 122.43, 118.86, 110.27, 80.92, 68.58, 65.28, 61.23, 59.72, 53.80, 51.81, 47.40, 46.18, 37.34, 35.34, 24.72, 20.29, 18.49. ESI-MS at $m/z = 425.15$ (calculated for C$_{22}$H$_{23}$N$_3$O$_6$, 426.16). 

$\text{1(10)-\beta-epoxy-(11R)-13-(6-nitroindazole)-11,13-dihydroludartin (16)}$: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.58 (s, 1H), 8.11 (s, 1H), 8.03 (d, $J = 10.6$ Hz, 1H), 7.82 (d, $J = 8.8$ Hz, 1H), 4.80 (m, 2H), 3.90-3.69 (m, 1H), 3.38 (s, 1H), 2.88 (dt, $J = 12.8, 4.7$ Hz, 1H), 2.32 (d, $J = 7.7$ Hz, 1H), 2.24-2.11 (m, 2H), 2.02 (s, 1H), 1.85 (t, $J = 11.9$ Hz, 2H), 1.56 (m, 2H), 1.32 (s, 3H), 1.26 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 174.66, 147.00, 139.23, 134.80, 127.05, 122.06, 115.97, 106.77, 80.44, 68.92, 65.84, 60.94, 59.74, 53.63, 51.98, 46.99, 46.09, 37.15, 35.03, 24.64, 20.42, 19.00. ESI-MS at $m/z = 425.15$ (calculated for C$_{22}$H$_{23}$N$_3$O$_6$, 426.16). 

$\text{1(10)-\beta-epoxy-(11R)-13-(1,2,4-triazole)-11,13-dihydroludartin (17)}$: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.1 (s, 1H), 7.94 (s, 1H), 4.50 (m, 2H), 3.76-3.68 (t, $J = 10.7, 10.6$ Hz, 1H), 3.34 (s, 1H), 2.91 (d, $J = 10.8$ Hz, 1H), 2.65 (m, 2H), 2.43 (d, $J = 17.1$ Hz, 1H), 2.24-1.84 (m, 4H), 1.69 (s, 3H), 1.26 (s, 3H), 1.23 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 173.79, 150.78, 144.22, 80.45, 68.74, 65.48, 61.23, 59.72, 53.40, 51.70, 47.03, 45.78, 33.97, 32.21, 26.06, 21.37, 19.07. ESI-MS at $m/z = 332.15$ (calculated for C$_{17}$H$_{21}$N$_3$O$_4$, 331.15).
(11R)-13-(dimethylamine)-11,13-dihydroludartin (18): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.76 (1H, t, $J = 10$ Hz, 10 Hz), 3.45 (1H, s), 3.35 (1H, d, $J = 10.8$ Hz), 2.96 (s, 2H), 2.67 (1H, d, $J = 17.5$ Hz), 2.56 (s, 3H), 2.41 (1H, d, $J = 17.5$ Hz), 2.05 (m, 2H), 1.99 (s, 6H), 1.6 (3H, s), 1.59 (3H, s), 1.23 (s, 1H). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 176.40, 135.85, 133.29, 80.96, 67.23, 64.05, 55.30, 51.80, 44.85, 34.39, 33.66, 29.89, 27.62, 22.82, 22.71, 19.30. ESI-MS at $m/z$ = 292.18 (calculated for C$_{17}$H$_{25}$NO$_3$, 291.18).

(11R)-13-(ethylmethylamine)-11,13-dihydroludartin (19): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.73 (1H, t, $J = 10$ Hz, 10 Hz), 3.38 (1H, s), 2.98 (1H, d, $J = 10.7$ Hz), 2.81-2.53 (m, 3H), 2.5-2.26 (m, 4H), 2.18 (m, 3H), 2.17-1.87 (m, 5H), 1.65 (s, 3H), 1.60 (s, 3H), 1.3-1.14 (m, 1H), 1.08-0.90 (m, 2H) $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 177.24, 135.66, 133.62, 80.96, 67.43, 64.08, 55.77, 55.45, 52.15, 52.09, 46.17, 44.43, 34.65, 34.47, 27.92, 23.86, 19.17, 12.28. ESI-MS at $m/z$ = 306.20 (calculated for C$_{18}$H$_{27}$NO$_3$, 305.19).

(11R)-13-(diethyl amine)-11,13-dihydroludartin (20): Colorless liquid, $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.63 (t, $J = 10.2,10.6$ Hz, 1H), 3.38 (s, 1H), 3.02 (d, $J = 10.7$ Hz, 1H), 2.87 (dd, $J = 13.6, 4.5$ Hz, 1H), 2.75-2.63 (m, 2H), 2.61-2.41 (m, 4H), 2.35-1.97 (m, 5H), 1.68 (s, 3H), 1.63 (s, 3H), 1.21 (m, 2H), 1.00 (t, $J = 7.1, 7.8$ Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 177.39, 135.55, 133.34, 80.23, 67.14, 63.89, 55.51, 52.29, 51.79, 47.10, 44.90, 34.25, 33.28, 28.06, 22.35, 19.15, 11.59. ESI-MS at $m/z$ = 320.21 (calculated for C$_{19}$H$_{29}$NO$_3$, 319.21).

(11R)-13-(piperidine)-11,13-dihydroludartin (21): Colorless semisolid paste, $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 3.64 (t, $J = 10.2$ Hz, 1H), 3.38 (s, 1H), 3.02 (d, $J = 10.6$ Hz, 1H), 2.81-2.66 (m, 2H), 2.56 (m, 1H), 2.51-2.32 (m, 6H), 2.28-1.95 (m, 4H), 1.68 (s, 3H), 1.63 (s, 3H), 1.54 (d, $J = 5.6$ Hz, 4H), 1.42 (d, $J = 5.1$ Hz, 2H), 1.22 (m, 1H). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 176.81,
ESI-MS at m/z 332.21 (calculated for C_{20}H_{29}NO_{3}, 321.21).

(11R)-13-(morpholine)-11,13-dihydroludartin (9): Colourless solid, {\textsuperscript{1}H} NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 3.75-3.57 (m, 5H), 3.39 (s, 1H), 3.03 (d, \(J = 10.6\) Hz, 1H), 2.80 (m, 1H), 2.76-2.58 (m, 2H), 2.41 (m, 6H), 2.12 (m, 4H), 1.68 (s, 3H), 1.63 (s, 3H), 1.31-1.15 (m, 1H). \({\textsuperscript{13}}\)C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 176.73, 135.40, 133.40, 80.28, 67.16, 66.89, 63.85, 57.22, 55.47, 54.08, 51.91, 44.17, 34.40, 33.47, 27.69, 22.52, 19.13. ESI-MS at m/z 334.19 (calculated for C_{19}H_{27}NO_{4}, 333.19).

(11R)-13-(5-nitroindazole)-11,13-dihydroludartin (22): Brownish solid, {\textsuperscript{1}H} NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.63 (s, 1H), 8.16 (s, 1H), 8.04 (d, \(J = 8.8\) Hz, 1H), 7.84 (d, \(J = 8.8\) Hz, 1H), 5.30 (s, 1H), 4.83 (t, \(J = 22.7\) Hz, 2H), 3.72 (t, \(J = 10.2\) Hz, 1H), 3.36 (s, 1H), 2.92 (d, \(J = 18.5\) Hz, 2H), 2.68 (d, \(J = 17.6\) Hz, 1H), 2.42 (d, \(J = 17.7\) Hz, 1H), 2.20-1.93 (m, 3H), 1.65 (s, 3H), 1.55 (s, 3H), 1.24 (d, \(J = 12.3\) Hz, 1H). \({\textsuperscript{13}}\)C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 174.86, 146.91, 139.04, 135.24, 134.32, 133.39, 126.98, 121.76, 115.76, 106.55, 80.49, 67.00, 63.62, 53.44, 51.79, 46.92, 46.14, 33.79, 33.19, 27.16, 22.51, 19.02. ESI-MS at m/z 410.16 (calculated for C_{22}H_{23}N_{3}O_{5}, 409.16).

(11R)-13-(6-nitroindazole)-11,13-dihydroludartin (23): Brownish solid, {\textsuperscript{1}H} NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.63 (s, 1H), 8.16 (s, 1H), 8.04 (d, \(J = 8.8\) Hz, 1H), 7.84 (d, \(J = 8.8\) Hz, 1H), 5.30 (s, 1H), 4.83 (t, \(J = 22.7\) Hz, 2H), 3.72 (t, \(J = 10.2\) Hz, 1H), 3.36 (s, 1H), 2.92 (d, \(J = 18.5\) Hz, 2H), 2.68 (d, \(J = 17.6\) Hz, 1H), 2.42 (d, \(J = 17.7\) Hz, 1H), 2.20-1.93 (m, 3H), 1.65 (s, 3H), 1.55 (s, 3H), 1.24 (d, \(J = 12.3\) Hz, 1H). \({\textsuperscript{13}}\)C NMR (101 MHz, CDCl\textsubscript{3}) \(\delta\) 174.86, 146.91, 139.04, 135.24, 134.32, 133.39, 126.98, 121.76, 115.76, 106.55, 80.49, 67.00, 63.62, 53.44, 51.79, 46.92, 46.14, 33.79, 33.19, 27.16, 22.51, 19.02. ESI-MS at m/z 410.16 (calculated for C_{22}H_{23}N_{3}O_{5}, 409.16).
Bioevaluation

For bioevaluation studies, RPMI-1640 medium, streptomycin, fetal bovine serum, sodium bicarbonate, phosphate buffer saline, sulphorhodamine, trypsin, doxorubicin (> 98% purity), and gentamycin sulphate were purchased from Sigma Chemicals Co. Glacial acetic acid was from Fischer Scientific and trichloroacetic acid (TCA) was from Merck Specialities Private limited. All of the human cancer cell lines (A-549, THP-1, PC-3, HCT-116) were obtained from ATCC Sigma. All of the cells used were grown in RPMI-1640 medium containing 10% FBS, 100 unit penicillin/100 µg streptomycin per ml medium. Cells were allowed to grow in a carbon dioxide incubator (Thermoscientific USA) at 37°C with 98% humidity and a 5% CO₂ gas environment.

In the present study, the cytotoxic effect of the different analogs was evaluated using the sulphorhodamine B (SRB) assay. The SRB dye binds to the basic protein of cells that have been fixed to tissue culture plates by TCA. As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell number. In the present case, all cells lines seeded in flat-bottomed 96-well plates were allowed to adhere overnight, and then media containing different analogs (varying concentrations) were added. The plates were assayed for 48 h. The cells were fixed by adding 50 µL of ice-cold 50% TCA to each well for 60 min. The plates were washed five times in running tap water and stained with 100 µL per well SRB reagent (0.4% w/v SRB) in 1% acetic acid for 30 min. The plates were washed five times in 1% acetic acid to remove unbound SRB and allowed to dry overnight. SRB was solubilized with 100 µL per well, 10 mM tris-buffer, shaken for 5 min, and then the optical density was measured at 570 nm.