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Click chemistry inspired facile synthesis and bioevaluation of novel triazolyl analogs of Ludartin ☆

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ABSTRACT

A convenient and modular synthesis involving diastereoselective Michael addition followed by regioselective Huisgen 1,3-dipolar cycloaddition reaction was carried out to furnish 1,4-disubstituted-1,2,3-triazoles of Ludartin. This reaction scheme involving Michael addition followed by regioselective Huisgen 1,3-dipolar cycloaddition reaction leading to the formation of triazolyl analogs is being reported for the first time. All the triazolyl products were characterised using spectral data analysis. Sulphorhodamine B cytotoxicity screening of the resulting products against a panel of five human cancerous cell-lines revealed that few of the analogs display promising broad spectrum cytotoxic effect. Among all the synthesized compounds, only **3q** displayed the best cytotoxic effect with IC_{50} values of 12, 11, 38, 39 and 8.5 μM but less than the standard Ludartin (**1**) with IC_{50} values of 6.3, 7.4, 7.5, 6.9 and 0.5 μM against human neuroblastoma (T98G), lung (A-549), prostate (PC-3), colon (HCT-116) and breast (MCF-7) cancer cell lines, respectively. The present synthesis was designed based on the previous literature reports of Ludartin as an aromatase inhibitor. Our work provides an initial study on structure–activity relationship of triazolyl analogs of sesquiterpene lactones in general and Ludartin (**1**) in particular.

Sesquiterpene lactones (SLs) form one of the largest biogenetically homogenous group of secondary metabolites known. SLs have been a subject of vast number of phytochemical/biological studies in the past four decades, mainly due to the fact that many of them display various conspicious biological activities.[1–3] These biological activities are attributed to the presence of electrophilic structure elements in SLs which undergo covalent reaction with functional biological molecules resulting in their deactivation.[1–3] The alkylation of free sulphur moieties of enzymes and other functional proteins by SLs is responsible for SL bioactivity.[1–3] This is in harmony with the pearsons hard-soft acid base principle (HSAB) that soft nucleophiles and electrophiles react more readily with each other than with reactants classified as hard electrophiles and nucleophiles, for example, non-conjugated carbonyl groups and amino or hydroxyl groups, respectively.[4,5]

Among the sesquiterpene lactones only a few have reached clinical trials which include artemisinin from *Artemisia annua* L, thapsigargin from *Thapsia garganica* and parthenolide from *Tanacetum parthenum*.[6] However owing to the relatively non selective mechanism of action, most of the SLs are not suitable for drug development. To address this non specificity issue amino prodrug approach has been developed to improve their pharmacokinetic potential.[7] Following the amino prodrug approach effective structure–activity relationships have been developed for various guaianolides (Fig. 1A) including, Ludartin[8] (**1**), arglabin[9] and other non-guaianolide sesquiterpene lactones (Fig. 1B) like alantolactone,[10] isoalantolactone,[10] costunolide,[11] parthenolide,[12–15] α-santonin,[16] helenalin[17] and ambrosin.[18] As a result few synthetic derivatives which include (11*R*)-13-(dimethyl-amine)-11,13-dihydroarglabin and (11*R*)-13-(dimethyl amine)-11,13-dihydroparthenolide have reached to clinical trials.[6,9]

Arglabin along with its dimethylamine analog, (11*R*)-13-(dimethylamine)-11,13-dihydroarglabin is an approved anticancer agent in several countries for treatment of lung, liver, breast and ovarian cancers. [9] (11*R*)-13-(dimethylamine)-11,13-dihydro-arglabin has less side effects than other chemotherapeutic agents.[9]

Ludartin (**1**), position isomer of Arglabin, shows gastric cytoprotective effect[19] and also inhibits aromatase enzyme which is involved in hormone-dependent breast cancer.[20,21] Earlier, we reported the structure–activity relationship of amino analogs of Ludartin (**1**) at its highly reactive α-methylene-γ-lactone moiety.[8] Some of the amino derivatives of Ludartin (**1**) were found to be potent and selective cytotoxic agents and the results were in fine tune with those reported for Arglabin by *R. Csuk* and coworkers that the Michael addition at the exocyclic double bond leads to derivatives with reduced/or equal cytotoxic effect but cell line

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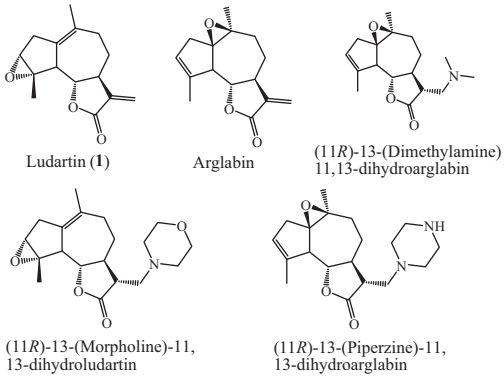


Figure 1A. Some bioactive guaianolides that have been studied for SAR and their active analogs.

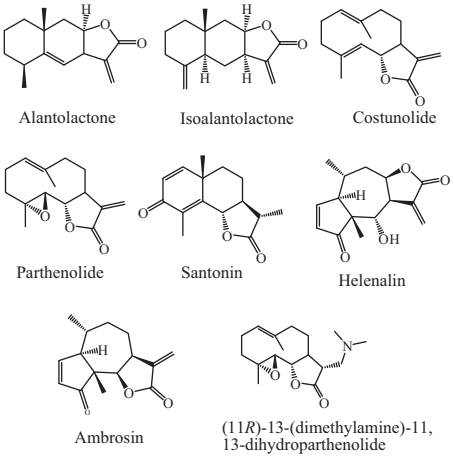


Figure 1B. Some bioactive Sesquiterpene lactones apart from guaianolides.

dependent selectivity.[9] Keeping in view the fact that Ludartin inhibits aromatase enzyme involved in hormone-dependent breast cancer and that the triazole based aromatase inhibitors in general represent the third generation frontline therapy for early and even advanced cases of breast cancer in postmenopausal women[21], we designed a reaction strategy, to develop effective triazole based analogs to rationalise lead properties of Ludartin (**1**) especially against breast cancers, involving a combination of Michael addition and Huisgen 1,3-dipolar cycloaddition reaction.

In view of the broad spectrum cytotoxic potential of triazoles in general, the triazole analogs of Ludartin were screened against other cancer cell-lines available, apart from breast cancer cells (MCF-7). An exhaustive literature survey revealed that such triazolyl formation has not been reported on any sesquiterpene lactone so far using click chemistry approach and represents the first of its kind on any sesquiterpene lactone and thus constitutes an initial structure–activity relationship of triazolyl derivatives of sesquiterpene lactone class of natural products in general and Ludartin (**1**) in particular.

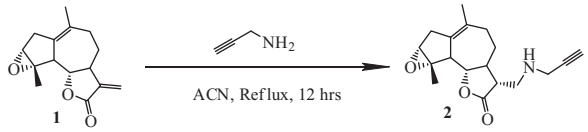
Ludartin (**1**) was subjected to Michael addition using propargyl amine in acetonitrile under reflux at its highly reactive α-methylene-γ-lactone motif described previously (Scheme 1A).

This reaction serves two important purposes: one it gives adducts that react in a *retro*-michael's fashion at the target site as is hypothesized for amino analogs of sesquiterpene lactones[8] and simultaneously acts as a template (terminal triple bond) for the second step of the reaction. Formation of propargylated product (**2**) in the first step 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**).

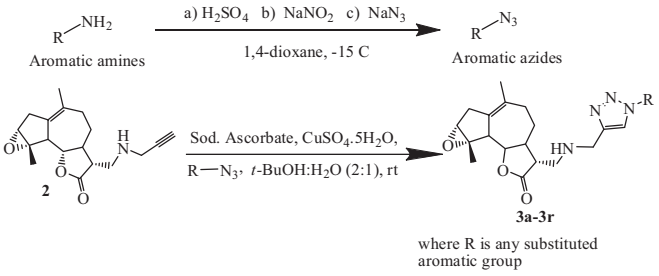
Such reaction creates one more chiral center at C-11 position whose configuration was determined as *R* on the basis of correlations deduced from NOESY described earlier.[8] On the other hand aromatic azides were prepared from their corresponding aromatic amines by diazotisation with sodium nitrite in acidic conditions followed by displacement with sodium azide. These aromatic azides were allowed to undergo 1, 3-dipolar cycloaddition reaction typically called Huisgen cycloaddition with the terminal acetylene bearing Michael adduct (**2**) under sharpless click chemistry conditions ($CuSO_4{\cdot}5H_2O$ and sodium ascorbate in *t*-BuOH/H_2O (2:1)) to afford regioselectively 1,4-disubstituted-1,2,3-triazoles in good to excellent yields[22] (Scheme 1B, Table 1). Under these conditions a series of such analogs was synthesized to look for structure–activity relationship studies. All the triazolyl products were characterised using spectral data analysis. Formation of products could easily be confirmed by a very down field *H*-5 proton signal (almost around 8.0 Hz) and other proton resonances in the aromatic region. Further characterisation of the products was done using ^{13}C, DEPT-NMR and HRMS as well as ESI-MS.

Ludartin (**1**) and its triazolyl analogs were then studied in a colorimetric Sulphorhodamine B (SRB) cytotoxicity assay[23] against a panel of five human cancer cell lines viz. neuroblastoma (T98G), lung (A-549), prostate (PC-3), colon (HCT-116) and breast (MCF-7). Preliminary cytotoxicity screening of the analogs was carried out at 50 μM concentration and cell death was determined. Ludartin (**1**) served as a positive control in this assay. The analogs which exhibited significant cytotoxic effect, greater than 50% growth inhibition at the preliminary screening concentration were further assayed at different concentrations (5–50 μM) to generate the IC_{50} values given in Table 2. The values are the average of the triplicate analysis.

From the cytotoxicity profile it is clear that the parent molecule, Ludartin (**1**) demonstrated to be cytotoxic not only against human leukaemia (THP -1), lung (A-549), colon (HCT-116), and prostate (PC-3) as described earlier[8] but proved to be effectively cytotoxic against breast (MCF-7) and neuroblastoma (T98G) cancer cells with IC_{50} values of 0.5 and 6.3 μM respectively. Among the synthesized triazolyl analogs, only five compounds **3a**, **3d**, **3e**, **3q** and **3r** exerted broad spectrum cytotoxic effects against the tested cancer cell-lines at their preliminary screening concentration (50 μM) and hence IC_{50} values of these analogs were evaluated. Compound **3q**

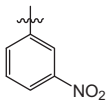
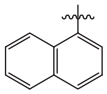
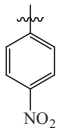
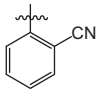
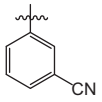
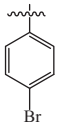
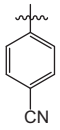
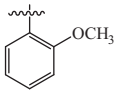
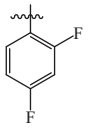
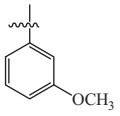
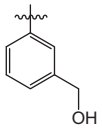
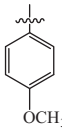
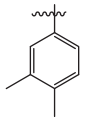
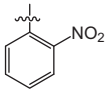
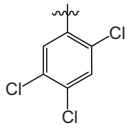


Scheme 1A. Preparation of amino propargyl of Ludartin.[8]



Scheme 1B. Preparation of aromatic azides and the triazoles of **2**.

Table 1
Preparation of different 1,4-disubstituted-1,2,3-triazole analogs of Ludartin (**1**)

S.No.	R	Product	Yield[a] (%)	S.No.	R	Product	Yield[a] (%)
1		**3a**	72	10		**3j**	74
2		**3b**	76	11		**3k**	90
3		**3c**	68	12		**3l**	76
4		**3d**	60	13		**3m**	71
5		**3e**	92	14		**3n**	85
6		**3f**	90	15		**3o**	82
7		**3g**	68	16		**3p**	77
8		**3h**	60	17		**3q**	86
9		**3i**	68	18		**3r**	74

[a] Refers to isolated yields, 'R' refers to any substituted aromatic group.

was the most effective analog depicting IC_{50} values of 8.5, 11 and 12 μM against breast (MCF-7), lung (A-549) and neuroblostoma (T98G) cancer cell lines respectively. Compound **3d**, showed reduced but a bit selective cytotoxic effect against human lung (A-549) and colon (HCT-116 cell-lines) (IC_{50} values of 24 and 26 μM respectively), with no such cytotoxic effect against neuroblastoma (T98G), prostate (PC-3) and breast cancer cells (MCF-7). Contrary to **3d**, **3e** exhibited decreased cytotoxicity with IC_{50} values ranging between 32-52 μM against the tested cancer cell lines. Compound **3a** was little bit potent against neuroblastoma (T98G) and breast (MCF-7) (IC_{50} of 21 μM against both) as compared to that of lung (A-549), prostate (PC-3) and colon (HCT-116) cancer cells displaying IC_{50} values of 24, 54 and 37 μM respectively. However 2,4,5-trichloro-analog (**3r**) showed better cytotoxicity against breast (MCF-7) (IC_{50} of 12 μM) followed by neuroblastoma (T98G), colon (HCT-116), lung (A-549) and prostate (PC-3) with IC_{50} of 17, 18, 21 and 33 μM respectively.

However among all the tested compounds, none of them exhibited a selective cytotoxic effect against a particular cell line as was true of the amino analogs described earlier.[8]

Persual of the literature revealed a good agreement between our results of Ludartin (**1**) against breast cancer cells (MCF-7, IC_{50} of 0.5 μM) with those of earlier reports of this molecule, that it inhibits the aromatase[20] enzyme involved in breast cancer leading us to propose that the most potent triazolyl analogs of Ludartin (**1**) that is, compounds **3q** and **3r** (IC_{50} values of 8.5 and 12 μM) respectively against this particular cell line (MCF-7) may also be acting in the same fashion. However, in vivo studies are warranted to investigate the molecular mechanisms of action responsible for the antiproliferative activity of the most active compounds. The work has already been initiated in this direction.

Comparing the results of our earlier study[8] with that of the current work made us to conclude that the amino analogs (previously reported) were better than the triazolyl analogs both in terms of activity as well as selectivity towards particular cell-line. Among the amino analogs (Table 3) morpholino (**11**), 6-nitro- (**17**) and 5-nitroindazole (**18**) derivatives exhibited potent and selective cytotoxic effect against leukemia (THP-1), prostate (PC-3) and lung (A-549) cancer cell lines respectively. Similarly the diethyl (**2**) and piperidine (**10**) analogs exerted the cytotoxic effect against both

Table 2
Cytotoxicity profile of Ludartin and its triazolyl analogs against five human cancer cell lines

Compound	T98G		A-549		PC-3		HCT-116		MCF-7	
	% GI[a]	IC_{50}	% GI[a]	IC_{50}	% GI[a]	IC_{50}	% GI[a]	IC_{50}	% GI[a]	IC_{50}
3a	73	21	77	24	56	54	58	37	77	21
3b	32	nd	21	nd	08	nd	48	nd	00	nd
3c	37	nd	32	nd	28	nd	06	nd	12	nd
3d	17	nd	68	24	00	nd	79	26	22	nd
3e	58	52	74	39	28	nd	72	35	79	32
3f	34	nd	21	nd	40	nd	38	nd	00	nd
3g	00	nd	08	nd	00	nd	00	nd	06	nd
3h	00	nd	20	nd	00	nd	00	nd	00	nd
3i	00	nd	17	nd	00	nd	00	nd	00	nd
3j	00	nd	00	nd	00	nd	00	nd	00	nd
3k	00	nd	26	nd	00	nd	00	nd	00	nd
3l	42	nd	46	nd	22	nd	20	nd	12	nd
3m	00	nd	10	nd	00	nd	00	nd	00	nd
3n	00	nd	00	nd	00	nd	00	nd	00	nd
3o	12	nd	19	nd	21	nd	00	nd	29	nd
3p	00	nd	47	nd	00	nd	11	nd	13	nd
3q	99	12	73	11	46	38	49	39	84	8.5
3r	77	17	91	21	73	33	74	18	81	12
1	97	6.3	97	7.4	97	7.5	97	6.9	100	0.5

IC_{50} values are expressed in μM concentration and represent the average of the triplicate analysis.
'nd' means not determined in the given concentration range.
T98G, A-549, PC-3, HCT-116 and MCF-7 are the human neuroblastoma, lung, prostate, colon and breast cancers cells, respectively.
[a] % GI refers to growth inhibition measured at 50 μM concentration.

Table 3
Comparision between the potent amino and triazolyl analogs of Ludartin

Compound	Amino analogs of Ludartin				Compound	Triazolyl analogs of Ludartin				
	IC_{50}					IC_{50}				
	A549	HCT-116	PC-3	THP-1		A549	HCT-116	PC-3	MCF-7	T98G
1	7.4	6.9	7.5	3.1	**1**	7.4	6.9	7.5	0.5	6.3
6	–	3.7	–	3.5	**3a**	24	–	–	21	–
10	–	4.0	–	3.9	**3q**	11	–	–	8.5	12
11	–	–	–	2.8	**3r**	21	–	18	12	17
17	–	–	2.2	–						
18	2.6	–	–	–						

Compound **1** refers to the parent molecule Ludartin, **6**, **10**, **11**, **17** and **18** refers to diethyl, piperidine, morpholine, 6-nitro and 5-nitroindazole analogs respectively described in earlier study[8] while as **3a**, **3q** and **3r** refer to variously substituted triazolyl analogs of Ludartin.

colon (HCT-116) and leukemia (THP-1) cell-lines. However among the triazolyl analogs only one compound (**3q**) depicted a good cytotoxic potential against the breast cancer cell line (MCF-7) with IC_{50} of 8.5 μM but that too less than the parent Ludartin (**1**) showing IC_{50} of 0.5 μM. Despite low bioactivity of the triazolyl analogs synthesized this study provides new insight with regard to the bioactivity of parent Ludartin (**1**) against breast (MCF-7) and neuroblastoma (T98G) cell-lines making us to conclude that Ludartin is highly active against breast cancer cells and that the modification at the highly active α-methylene-γ-lactone moiety of Ludartin (**1**) leads to analogs with reduced cytotoxic effect compared to Ludartin (**1**) itself, thereby making the presence of α-methylene-γ-lactone moiety essential for the desired biological effect.

In summary, a range of triazolyl analogs were synthesized using a diastereoselective Michael addition followed by regioselective Huisgen's cycloaddition reaction and studied for their behaviour against a panel of five human cancerous cell lines. Compound **3q** proved to be the best analog with IC_{50} of 8.5 μM against MCF-7 cell line after standard Ludartin (**1**) with IC_{50} of 0.5 μM against the same cell-line. The results are an indicative of the fact that the analog **3q** may be acting by inhibition of the aromatase enzyme involved in hormone-dependent breast cancer, as is true of the parent Ludartin (**1**). However further studies especially the in vivo studies need to be carried out for revealing the exact mechanism of action. This study provides an initial structure–activity data, based on triazolyl synthesis of sesquiterpene lactones in general and Ludartin (**1**) in particular.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.01.018.

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22. Isolation of Ludartin (**1**): Ludartin was isolated from *Artemisia amygdalina* Decne as described previously.[24] *Synthesis of (11R)-13-(propargyl amine)-11, 13-dihydroludartin (**2**):* A solution of **1** (1000 mg, 4.65 mmol) in acetonitrile (2 ml) and propargyl amine (0.121 mmol) was heated under reflux for 12 h in presence of base DBU. After cooling the reaction mixture was evaporated under vaccuo on a rotary evaporator and the residue obtained was subjected to normal silica-gel column chromatography using Hexane-EtOAc as eluent to furnish the pure product. *Spectral data of compound (**2**).* Yield 80%; R_f = 0.5 (5% MeOH-DCM); IR (KBr cm^{-1}) 3017, 2944, 1755; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (t, J = 10.8, 10,8 Hz, 1H), 3.46 (s, 1H), 3.38 (s, 1H), 3.06–2.97 (m, 2H), 2.89 (dd, J = 12.1, 6.7 Hz, 1H), 2.71 (d, J = 17.7 Hz, 1H), 2.51–2.33 (m, 2H), 2.21 (m, 3H), 2.10–1.97 (m, 1H), 1.89 (d, J = 4.4 Hz, 2H), 1.68 (s, 3H), 1.62 (s, 3H), 1.30–1.18 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.84, 135.09, 133.33, 81.44, 80.50, 71.80, 66.90, 63.86, 52.80, 51.78, 46.87, 45.38, 38.66, 34.20, 33.52, 27.39, 22.55, 19.19. ESI-MS (m/z): 302 $(M+H)^+$. *Synthesis of triazole analogs of compound **2** (**3a–3r**):* To a solution of compound **2** (25 mg, 0.083 mmol) in t-BuOH:H_2O (2:1, 3 ml), sodium ascorbate (2.0 mg, 0.012 mmol) and $CuSO_4{\cdot}5H_2O$ (2 mg, 0.0075 mmol) were added at room temperature. To this mixture, aryl azide (0.12 mmol) was added and the reaction mixture was sonicated till its completion. The crude mixture was extracted with ethyl acetate (3 × 20 ml) and the combined organic layer was dried over sodium sulfate and purified through column chromatography to give pure **3a**–**3r** in 60–92% yield. *Spectral data of compound **3e**:* Yield 92%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.65 (s, 4H), 5.30 (s, 1H), 4.06 (s, 2H), 3.70 (t, J = 10.8, 10.7 Hz, 1H), 3.38 (s, 1H), 3.11–2.83 (m, 3H), 2.76–2.62 (m, 1H), 2.42 (m, 2H), 2.21–1.83 (m, 4H), 1.68 (s, 3H), 1.63 (s, 3H), 1.25 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.20, 146.94, 135.38, 133.36, 132.96, 132.61, 122.32, 121.23, 121.83, 119.92, 111.36, 80.76, 66.82, 63.82, 52.98, 53.82, 46.54, 45.77, 44.54, 34.16, 33.50, 27.32, 22.57, 19.11. HR-MS (m/z): 501.1310 for $[M + 1]^+$. Similarly other compounds were isolated and characterised using spectral analysis.
23. Sulphorhodamine B assay for % growth inhibition: The SRB assay was used to screen the semisynthetic analogs of Ludartin for cell cytotoxicity. Various human cancer cell lines, human breast cancer cell line (MCF-7, at a density of 7×10^3 cells per mL per 100 μL per well), human lung carcinoma cell line (A-549, at a density of 8×10^3 cells per mL per 100 μL per well), human prostate cancer cell line (PC-3, at a density of of 8×10^3 cells per mL per 100 μL per well), human colon cancer cell line (HCT-116, at a density of 1×10^4 cells per mL per 100 μL per well) and human neuroblastoma cell line (T98G, at a density of 1×10^4 cells per mL per 100 μL per well) used in this study were purchased from European collection of cell culture (ECACC) USA) and seeded in flat-bottomed 96-well plates. The cells were incubated at 37 °C for four hours in a humidified atmosphere containing 5% CO2 and then media containing samples at different concentrations were added. The plates were incubated for the next 48 h. The cells were fixed by adding 50 μL per well of ice cold 50% TCA to each well for 60 min maintained at 4 °C. 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 and allowed to dry overnight. SRB was solubilised with 100 μL per well 10 mM Tris-base, shaken for 5 min and the OD was measured at 570 nm with reference wavelength of 620 nm.[25] Further the IC50 values on the cancer cells of different tissue origin used for screening were determined by non-linear regression analysis using graph pad software.[26]
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