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Communication

Design, synthesis and biological evaluation of novel phthalazinone acridine derivatives as dual PARP and Topo inhibitors for potential anticancer agents

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ABSTRACT

In this study, we designed and synthesized a series of phthalazinone acridine derivatives as dual PARP and Topo inhibitors. MTT assays indicated that most of the compounds significantly inhibited multiple cancer cells proliferation. In addition, all the compounds displayed Topo II inhibition activity at 10 mol/L, and also possessed good PARP-1 inhibitory activities. Subsequent mechanistic studies showed that compound **9a** induced remarkable apoptosis and caused prominent S cell cycle arrest in HCT116 cells. Our study suggested that **9a** inhibiting Topo and PARP concurrently can be a potential lead compound for cancer therapy.

The structural integrity and stability of DNA are important for cell survival and normal physiological functions [1–3]. However, some endogenous or exogenous factors could directly or indirectly induce DNA damages or even mutation [4–6], which, if unrepaired, would lead to the development of various diseases, including cancer. Targeting pathways of DNA damage and repair are feasible strategy for treatment of cancer [7–9].

Topoisomerases (Topos) are necessary for the genome DNA correctly squeezed in a small cell nuclear. According to their structure and catalytic mechanism, Topos are classified into two major classes, during which Topo I cleaves one single-stranded DNA during each catalytic cycle, while Topo II cleaves double stranded DNA to resolve the DNA damage problems [10,11]. Overexpression of Topos may cause the instability of genome DNA and have been observed in many tumor cells. Targeting Topos have been demonstrated to be an effective way for cancer therapy [12–14]. Many acridine analogues, such as *m*-AMSA (Fig. 1), have entered clinical or preclinical trials as potent Topo inhibitors. Our group has also devoted much efforts in developing acridine-based Topo inhibitors for cancer therapy [15–19].

At the same time, poly(ADP-ribose) polymerases (PARPs) are well-known sensors of DNA damage and could repair the single-strand breaks (SSBs) in DNA *via* the base-excision repair (BER) pathway [20–22]. PARP-1 is a well-established target for developing anti-tumor drugs. PARP inhibitors could block PARP-mediated DNA damage repair and make tumor cells more sensitivity to cytotoxic agents. During the last decades, many PARP inhibitors (PARPi) have been reported [23–25]. Olaparib (Fig. 1) is the first PARP inhibitor approved by the FDA in 2014 for the treatment of advanced ovarian cancer. Different combinations of PARP inhibitors and genotoxic drugs are proceeded in clinical trials. These combinations including alkylating agents (temozolomide), cross-linking agents (cisplatin), Topo I inhibitors (topotecan and irinotecan) and Topo II inhibitors (etoposide) [26].

In this study, we intended to develop dual PARP and Topo inhibitors based on rational drug design strategy and our own studies reported previously [15,27–31]. We have developed 4-amidobenzimidazole acridine derivatives as first-in-class dual PARP and Topo inhibitors for cancer therapy [17]. As reported,

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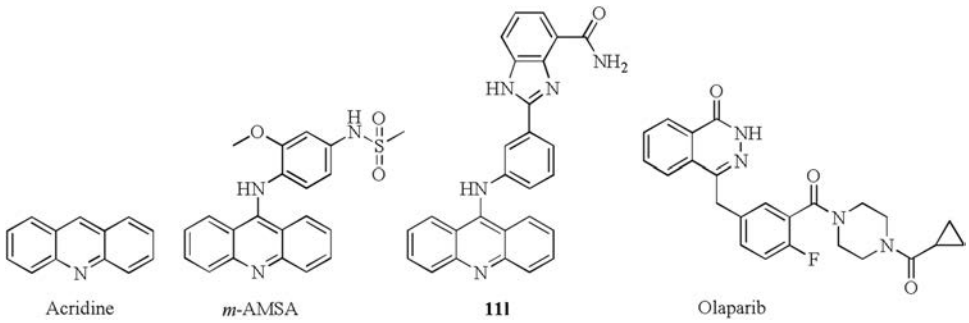


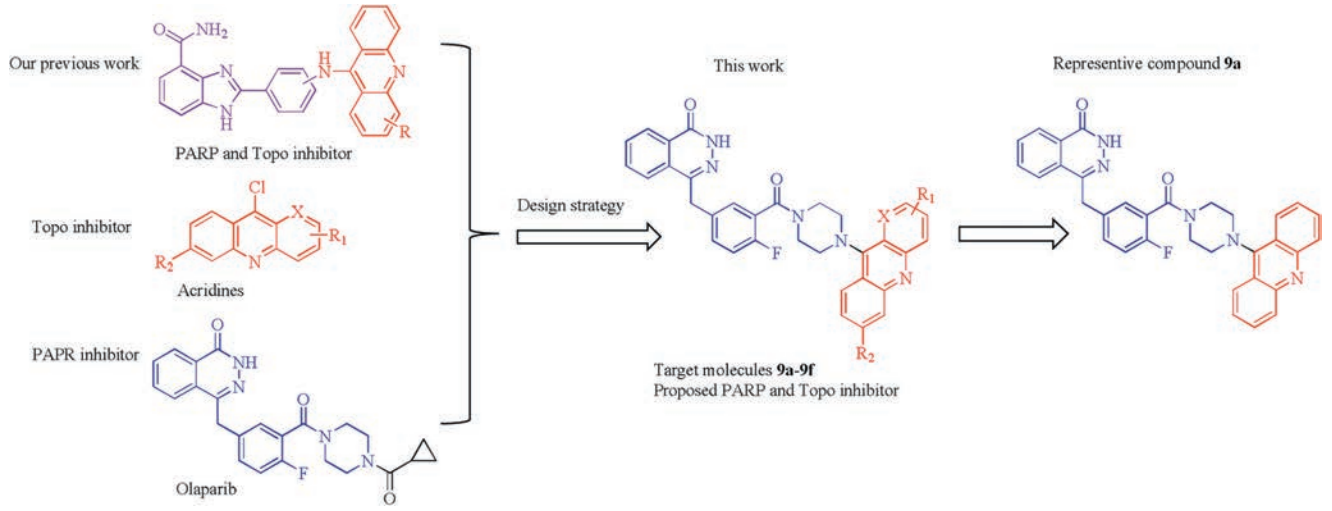
Fig. 1. Acridines and phthalazinone derivatives.

compound **11 l** (Fig. 1) showed good *in vitro* activities targeting Topo and PARP-1 and could efficiently suppress tumor growth in mice. Considering the relationship between PARP and Topo and the significant results, we hold the opinion that simultaneously targeting Topo and PARP-1 is a promising strategy for combating cancer. Phthalazinones has been recognized as an effective scaffold to develop PARP inhibitors especially since the approval of olaparib [32–34]. The phthalazinone structure of olaparib is a vital functional group to interact with PARP and the piperazine moiety is mainly used to improve the activity or adjust the physical and chemical properties. Therefore, the piperazine moiety can be modified or optimized without loss of PARP inhibitory activity based on the retention of the phthalazinone functional group (Scheme 1). Herein we developed a series of phthalazinone acridine derivatives by changing the substituent pattern of the acridine group and further evaluated their anti-tumor activities.

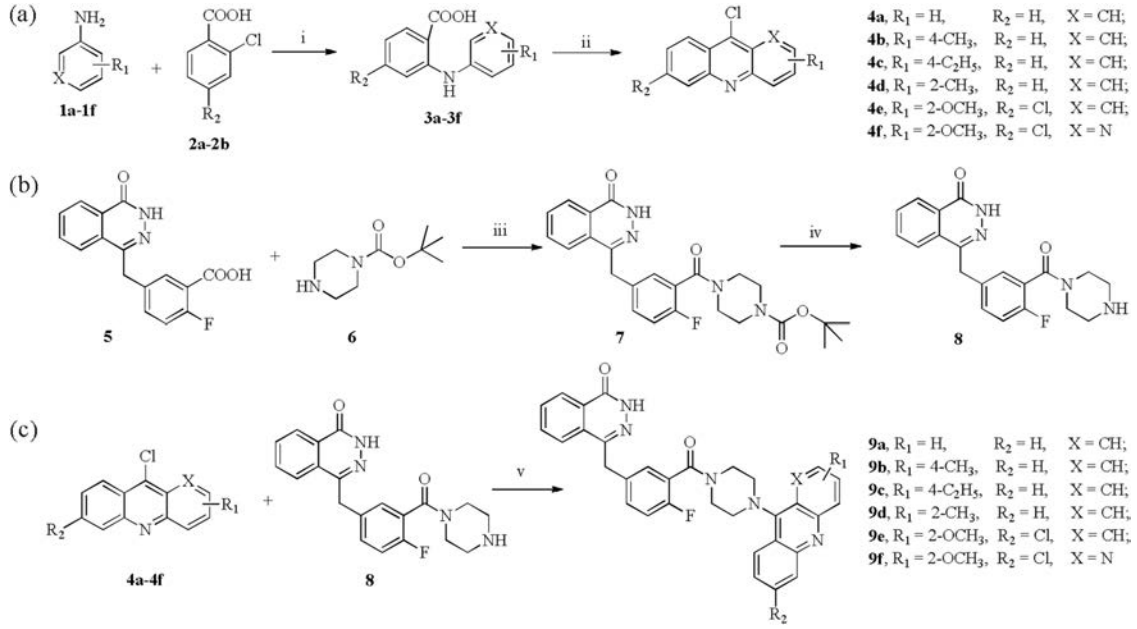
The synthesis of target molecules **9a**–**9f** is shown in Scheme 2. Firstly, compounds **3a**–**3f** were obtained by using 2-chlorobenzoic acid **2a**–**2b** reacted with phenylamine derivatives **1a**–**1f** *via* the Ullmann reactions. Then Friedel-Crafts acylation reactions were conducted in phosphorus oxychloride at 105 °C for 4 h to yield the corresponding acridine intermediates **4a**–**4f** [35]. Secondly, Compound **8** were synthesized according to the reported procedures [36]. At last, the desired compounds **9a**–**9f** were produced by the nucleophilic substitution reactions between the phthalazinone derivative **8** and corresponding 9-chloroacridines **4a**–**4f**. Target compounds were characterized with ^{1}H NMR, ^{13}C NMR, melting point and high resolution mass spectrum (Supporting information).

According to our previous studies, the size, length and the electron-negativity of the substituents on the acridine ring and the linker between two kinds of inhibitors had great effect on the antitumor activity [28]. Besides, methyl and methoxyl substitution at 2-position and 4-position of acridine could be helpful to its bioactivity as dual inhibitors of PARP and Topo [17]. In this report, PARP inhibitor of phthalazinone conjugated with Topo inhibitor acridine was used as a new scaffold compound for the development of PARP and Topo dual inhibitors. Compounds that were substituted by methyl, methoxyl or chloride substituted on the acridine ring were investigated. Using *m*-AMSA and olaparib as positive control, the cellular activities of the compounds were evaluated by the MTT assays. Different kinds of cancer cells including MCF-7 (human breast adenocarcinaoma cell line), K562 (human chronic myelogenous leukemia cells), HepG2 (human hepatoma cells), HeLa (human cervical cancer cells), HCT116 (human colon cancer cell line) and HCC1937 (human breast cancer cell line) were tested. Among the compounds we synthesized (Table 1), **9a** and **9c** displayed outstanding antiproliferative activities in all the tumor cells mentioned above. In particular, **9a** and **9c** showed similar activities to olaparib in MCF-7 and HCC1937 cells and greatly improved activities in the others. Further, compared with **9c**, **9a** with non-substitution of acridine showed better activities. While, compound **9f** with the N substituted at 1-position of acridine exhibited significantly reduced activity. In terms of cellular activity, **9a** is the most potent one among the compounds.

We then tested the activities of the synthesized compounds targeting PARP1 and Topo I/II enzymes using olaparib,



Scheme 1. Design strategy of dual Topo-PARP inhibitors.



Scheme 2. Synthesis of compounds **9a**–**9f**. Reagent and conditions: (a) (i) Cu, K_2CO_3, DMF, 130 °C, 4 h; (ii) $POCl_3$, 105 °C, 2 h; (b) (iii) HATU, trithylamine, DMF, 2 h, room temperature; (iv) HCl, H2O, EtOH, 3 h, room temperature; $NH_3 \cdot H_2O$, CH_2Cl_2; (v) Phenol, Ar, 120 °C, 1 h.

Table 1
In vitro antiproliferative potency and PARP-1 inhibitory activity of compounds **9a**–**9f**.

Compd.	R_1	R_2	X	MTT assays (IC_{50}[a], mol/L)						PARP-1 inhibitory activity (IC_{50}[a], mol/L)
				K562	MCF-7	HepG2	HeLa	HCT116	HCC1937	
9a	H	H	CH	3.94±0.38	13.80±0.58	11.7±0.40	13.11±1.02	12.88±0.54	7.96±0.61	11.85±0.65
9b	4-CH_3	H	CH	40.99±2.51	41.65±2.0	24.57±0.57	>50	46.10±2.51	48.86±1.97	87.89±0.20
9c	4-C_2H_5	H	CH	12.89±0.95	15.09±1.0	12.94±0.47	24.23±0.95	36.93±0.79	9.42±2.38	26.69±0.10
9d	2-CH_3	H	CH	>50	>50	34.56±1.86	>50	>50	>50	2.63±0.03
9e	2-OCH_3	Cl	CH	22.69±0.05	>50	25.53±0.33	43.59±2.06	25.95±0.49	8.65±2.48	3.12±0.08
9f	2-OCH_3	Cl	N	>50	21.51±1.5	>50	>50	>50	>50	130.7±9.80
m-AMSA				1.65±0.10	12.97±0.35	6.56±0.25	5.04±0.66	12.73±0.08	15.23±1.10	
Olaparib				>50	32.06±0.62	>50	>50	>50	9.39±0.51	0.98±0.009

[a] Data are expressed as the mean ± SD of at least duplicate determinations.

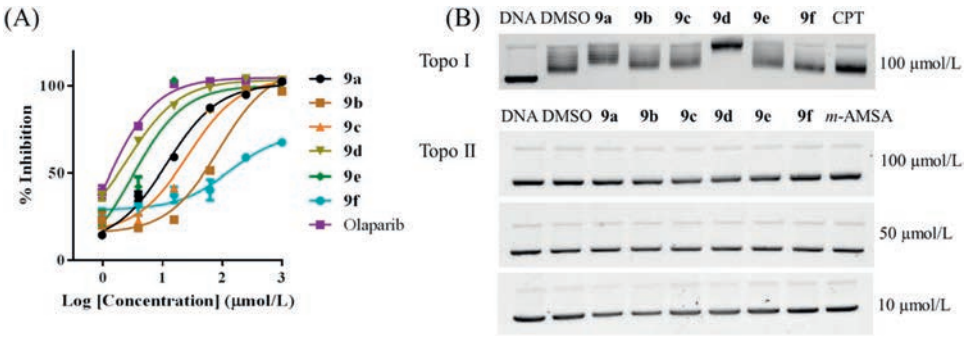


Fig. 2. PARP-1, Topo I and Topo II inhibitory activities. (A) PARP-1 inhibition activities of compounds **9a**–**9f** and olaparib at different concentrations. The IC_{50} values were determined by fitting the experiment data to log(inhibitor) *vs.* response-variable slope (three parameters) model in Prism. (B) Topo I: lane DNA: pBR322 DNA; lane DMSO: Topo I + pBR322 DNA; lane CPT: camptothecin + Topo I + pBR322 DNA; the others: compounds (100 μmol/L) + Topo I + pBR322 DNA. Topo II: lane DNA: pBR322 DNA; lane DMSO: Topo II + pBR322 DNA; lane *m*-AMSM: *m*-AMSM + Topo II + pBR322 DNA; the others: tested compounds at defined concentrations + Topo II + pBR322 DNA.

camptothecin (CPT) and *m*-AMSA as positive controls. As shown in Fig. 2A and Table 1, compounds **9a** and **9c** showed slightly weak PARP-1 inhibitory activities compared to olaparib. Compounds **9d** and **9e** showed similar PARP-1 inhibitory activities compared to olaparib. Compounds **9b** and **9f** showed poor PARP-1 inhibitory activities compared to olaparib. Compound **9d** showed the most potent PARP-1 inhibitory activity among the synthesized compounds, while its antiproliferative activities against cancer cells were weak (Table 1), which may attribute to the compound's physicochemical properties (*e.g.*, solubility, permeability,

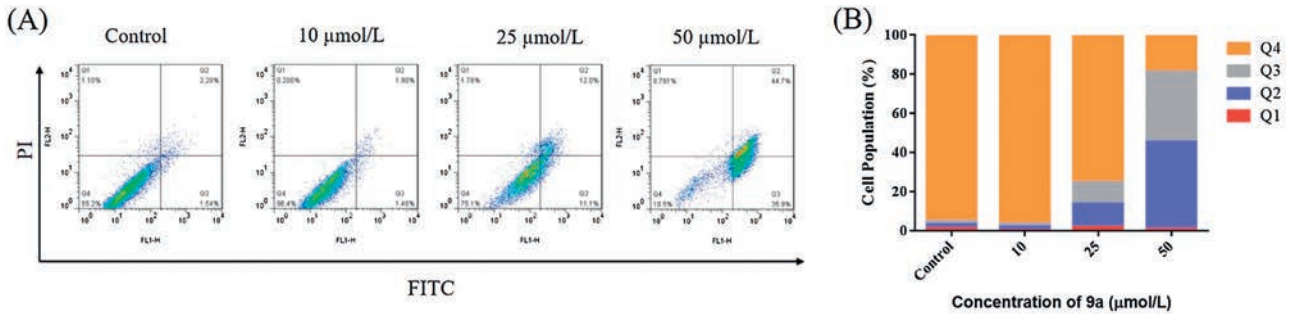


Fig. 3. Compound **9a** induced apoptosis. (A) HCT116 cells were incubated with different concentrations of compounds **9a** for 24 h followed by annexin V and PI staining and analyzed by flow cytometry. (B) Apoptosis analyzed by cell population distributions.

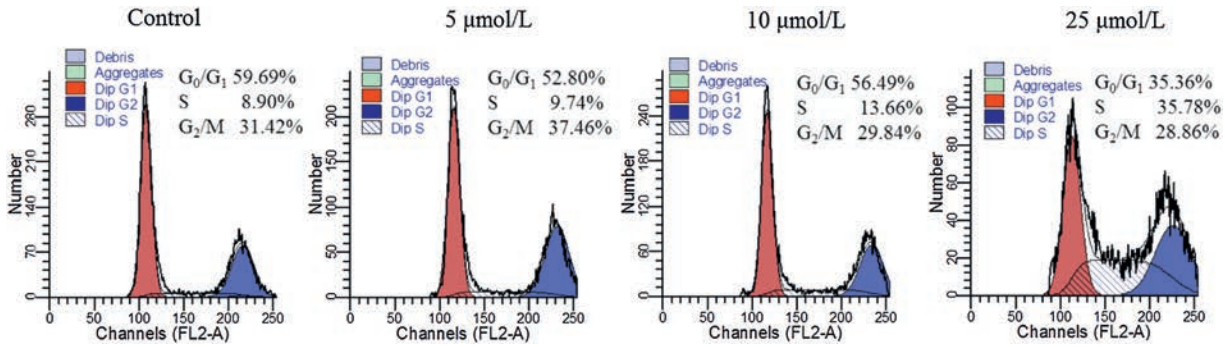


Fig. 4. Compound **9a** induced S cell cycle arrest.

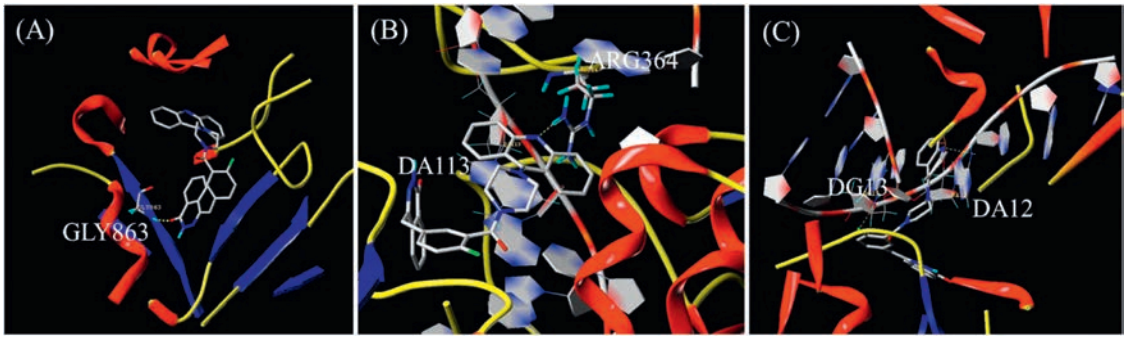


Fig. 5. Molecular models of Compound **9a** docked into (A) PARP-1, (B) Topo I & DNA complex and (C) Topo II & DNA complex.

lipophilicity, and stability) or the cellular environments (*e.g.*, cell membrane, pH, and metabolism) which could attenuate the amounts of compounds reaching the therapeutic target. The weak activity of **9f** against tumor cells and PARP1 might result from unfavorable solubility (Fig. S1 and Table S1 in Supporting information). Moreover, all the compounds displayed comparable Topo II inhibitory activities to *m*-AMSA at 10 μmol/L (Fig. 2B). These data suggest that all the compounds could potently inhibit Topo II, warranting them as dual PARP and Topo inhibitors.

DNA damage and its repair have been reported to have effect on the apoptosis pathway [37]. We choose **9a** for further study as it showed excellent cellular activities and desirable enzyme activities. To evaluate whether compound **9a** could induce apoptosis, an annexin-V/PI binding assay was conducted in HCT116 cells as shown in Fig. 3. Compared with DMSO controls, HCT116 cells treated with **9a** at different concentrations of 10, 25, and 50 μmol/L resulted in 0.5%, 22.9%, and 81.5% cells apoptosis, respectively, suggesting that compound **9a** could induce apoptosis in a dose dependent manner.

DNA damage caused by antitumor agents may activate DNA damage checkpoints, which arrest cell cycle progression, initiate DNA damage repair, or lead to cell death [37]. To evaluate whether compound **9a** could induce tumor cell cycle arrest, we then performed a flow cytometry assay to assess the effect of compound **9a** on the distribution of cell cycle. The cell cycle profiles of HCT116 after treating with compound **9a** in different concentrations for 48 h were illustrated in Fig. 4. The S-phase fraction was gradually increased from 8.90% in the untreated cells to 9.74%, 13.66%, and 35.78% in cells treated with compound **9a** at 5, 10, and 25 μmol/L, respectively, indicating that compound **9a** caused S cell cycle arrest in a dose-dependent manner.

To further understand the mechanism of compound **9a** interacting with the respective enzymes, **9a** was docked into the active sites of PARP1 (PDB code: 4UND), Topo I (PDB code: 1 K4T) and Topo II (PDB code: 4 G0U) using the SYBYL-X 1.3 protocol. As shown in Fig. 5A, the phthalazinone group of **9a** occupied the nicotinamideribose binding domain of PARP1, forming a hydrogen bond with Gly863. Moreover, the nitrogen atom of the acridine

group formed one more hydrogen bond with Arg364 of Topo I and the π-π stacking interaction between acridine with DA113 was observed (Fig. 5B). The docking results suggested that **9a** could form cleavage-complex with Topo I and DNA. Besides, the phthalazinone group of **9a** was parallel to DA12 and formed two hydrogen bonds with DA12 and DG13, leading to the formation of cleavage-complex with Topo II and double strands DNA (Fig. 5C). The above docking results may further confirm that **9a** could act as dual PARP and Topo I/II inhibitors.

In conclusion, Topos and PARPs are important for genome stability. Inhibitors targeting both of the targets may have synergistic antitumor effects. In this study, we designed and synthesized a new series of phthalazinone acridine derivatives targeting both PARP and Topo. Some of the compounds displayed potent antiproliferative activities against kinds of cancer cells and showed potential inhibitory potency against both PARP-1 and Topo I/II *in vitro*. Compound **9a** exhibited the most potent antiproliferative activity in its class. Further evaluation indicated that **9a** induced remarkable apoptosis, and caused prominent S cell cycle arrest. Taken together, compound **9a** is a promising dual-targeted inhibitor and is worth for further optimization for cancer therapy.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.06.019.

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