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# Synthesis of imidazo[1,2-*f*]phenanthridine derivatives under a metal- and base-free condition and their anticancer activity

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ABSTRACT

A series of imidazo[1,2-*f*]phenanthridine derivatives were synthesized from phenanthridin-6-amine and aldehydes through sulfur endorsed oxidative cyclization reaction under a metal- and base-free condition with atom economy strategy and evaluated their anticancer activity. Among all the synthesized derivatives, the compound **3d** and **3f** exhibited $IC_{50}$ values of 2.18 ± 0.08 and 2.24 μM ± 0.71 μM, respectively. Compound **3d** had the strongest inhibitory activity against HT-29 cells with an $IC_{50}$ value being 1.25 μM ± 0.22 μM, which was even stronger than that of paclitaxel.



## Introduction

The phenanthridine core moiety is an important structural unit present in a variety of natural products with a wide spectrum of physiological activities and pharmacological properties [1–4]. Investigations in heterocyclic chemistry are intriguing because of the potential for the discovery of novel scaffolds with diverse chemical and biological properties [5]. The imidazole framework is a particularly important subset, widely implicated in many biochemical processes [6–8]. The imidazole moiety plays a pivotal role in the activity of many natural metabolites in addition to pharmacological compounds and has a rich chemistry which makes it an interesting constituent in drug discovery [9]. Fused nitrogen-containing heterocyclic compounds are extremely significant organic molecules, which are frequently found as cardinal units in natural alkaloids, agrochemicals, and pharmaceutical drugs [10–13]. HYPERLINK "SPS:refid::bib10_bib11_bib12_bib13"

Among nitrogen-containing heterocyclic compounds, imidazo[1,2-*f*]phenanthridine has prominent and particular biological properties.

Zephycandidine A (Fig. 1)(A) was identified as the first naturally occurring imidazo[1,2-*f*]phenanthridine alkaloid. It was originally isolated from *Zephyranthes candida* and exhibited potent cytotoxic activity against tumor cells [14]. It is noteworthy that dihydro-imidazo-phenanthridinium derivatives have affinity to bind DNA and show cytotoxicity toward cancerous cells (Fig. 1)(B, C) [15].

Due to the prodigious value in pharmaceuticals and biological chemistry, the development of efficient methods for the synthesis of imidazo[1,2-*f*]phenanthridine and their derivatives have attracted considerable attention. One of the typical synthetic routes to imidazo[1,2-*f*]phenanthridine was developed by Zhang, et al., by reacting phenanthridin-6-amine and chloroacetaldehyde in the presence of $Na_2CO_3$ and isopropanol at 80 °C overnight (Scheme 1, Eq. (**a**)) [16]. Zhao, et al., synthesized benzimidazole-fused phenanthridines from 2-arylbenzimidazoles and aryl halides in the presence of Pd catalyst, Xphos ligand, $K_2CO_3$ base in DMF, 160 °C, for 72 h (Scheme 1, Eq. (**b**)) [17].

Among the published methods, metals and bases are utilized for the synthesis of the compounds. It is, however, challenging to remove a trace amount of transition metals from the biheteroaryl products due to the intimate interaction between the metal and heteroatoms. From the viewpoint of sustainable chemistry, therefore, it is imperative to establish a simple and facile procedure for the synthesis of imidazo[1,2-*f*]phenanthridine and its derivatives under a metal- and base-free condition.

Elemental sulfur widely exists in nature, which is nontoxic and stable under normal conditions. Moreover, sulfur serves as an effective oxidant in organic synthesis to allow oxidative coupling

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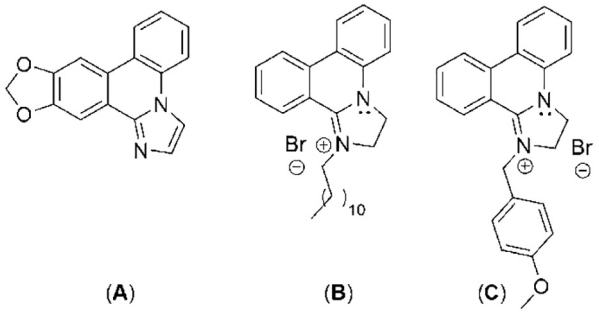
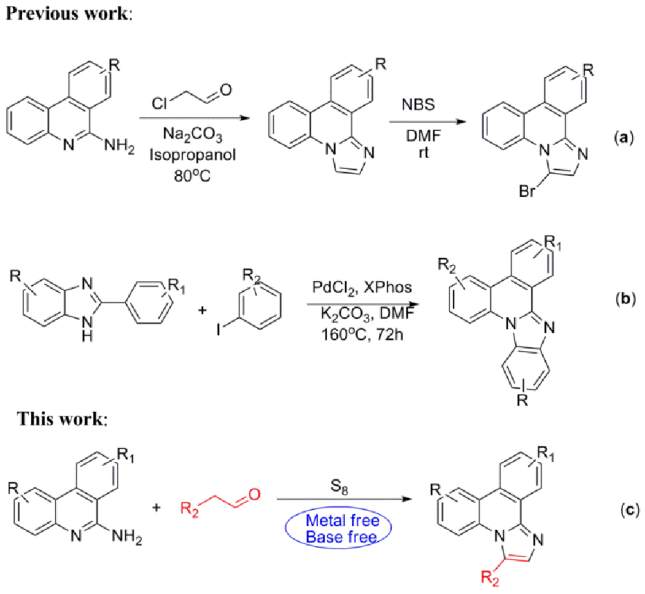



**Fig. 1.** Bioactive imidazo[1,2-*f*]phenanthridine derivatives.

reactions to take place. Here, we reported a novel strategy for the oxidative annulation of phenanthridin-6-amine and readily available aldehydes to form imidazo[1,2-*f*]phenanthridine derivatives under a metal- and base-free condition (Scheme 1, Eqn (**c**)), and evaluated their anticancer activity.

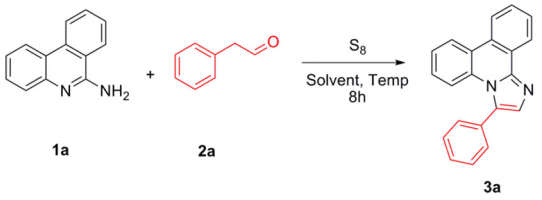
## Results and discussion

In this work, we described the synthesis of imidazo[1,2-*f*]phenanthridine derivatives by means of sulfur initiated oxidative cyclization under a metal- and base-free condition. We set out our investigation using a coupling reaction between readily available phenanthridine-6-amine (**1a**) and phenylacetaldehyde (**2a**). A high yield of annulation reaction to form 3-phenylimidazo[1,2-*f*]phenanthridine (**3a**) was, however, not achieved initially (Table 1, entry 1). When the reaction was conducted with NMP, toluene, and cyclohexane, we observed the desired product formation, but only in low yield. There is no desired product formation in chlorobenzene as a solvent. A trace amount of the product was detected using a mixture of 1,4-dioxane and toluene as a co-solvent. We then attempted to improve the yield by screening the solvent system and found that polar media were superior to low polar solvents (entries 1–7). The further solvent screening revealed that a mixture of cyclohexane/DMSO as a co-solvent could enhance the




**Scheme 1.** Schematic representation for the synthesis of imidazo[1,2-*f*]phenanthridine derivatives.

**Table 1**
Optimization of reaction conditions.




| Entry | Additive | Solvent | Temp(°C) | Time | Yield (%) |
|---|---|---|---|---|---|
| 1 | $S_8$ | DMF | 120 | 8 h | 33 |
| 2 | $S_8$ | DMSO | 120 | 8 h | 72 |
| 3 | $S_8$ | Toluene | 120 | 8 h | Trace |
| 4 | $S_8$ | 1,4-dioxane | 120 | 8 h | Trace |
| 5 | $S_8$ | Cyclohexane | 120 | 8 h | 26 |
| 6 | $S_8$ | NMP | 120 | 8 h | 18 |
| 7 | $S_8$ | Chlorobenzene | 120 | 8 h | nd |
| 8 | $S_8$ | Cyclohexane/DMSO(5:1) | 120 | 8 h | 73 |
| 9 | $S_8$ | Cyclohexane/DMSO(4:2) | 120 | 8 h | 74 |
| 10 | $S_8$ | Cyclohexane/DMSO(3:3) | 120 | 8 h | 77 |
| **11** | **$S_8$** | **Cyclohexane/DMSO(2:4)** | **120** | **8 h** | **86** |
| 12 | $S_8$ | Cyclohexane/DMSO(1:5) | 120 | 8 h | 75 |
| 13 | $S_8$ | Cyclohexane/DMSO(2:4) | rt | 8 h | nd |
| 14 | – | Cyclohexane/DMSO(2:4) | 120 | 8 h | nd |
| 15[a] | $S_8$ | Cyclohexane/DMSO(2:4) | 120 | 8 h | 66 |
| 16[b] | $S_8$ | Cyclohexane/DMSO(2:4) | 120 | 8 h | 79 |
| 17[c] | $S_8$ | Cyclohexane/DMSO(2:4) | 120 | 8 h | 73 |
| 18 | $S_8$ | Cyclohexane/DMSO(2:4) | 60 | 8 h | 47 |

Reaction conditions: 1a (0.2 mmol), 2a (0.4 mmol), $S_8$ (0.4 mmol), solvent (0.6 mL).
[a] 1 eq of $S_8$.
[b] Under $N_2$ condition.
[c] 1 eq of 2a, nd means not detected.

**Table 2**
Synthesis of 3-aryl or alkylimidazo[1,2-*f*]phenanthridines from **1a** and various aldehydes.

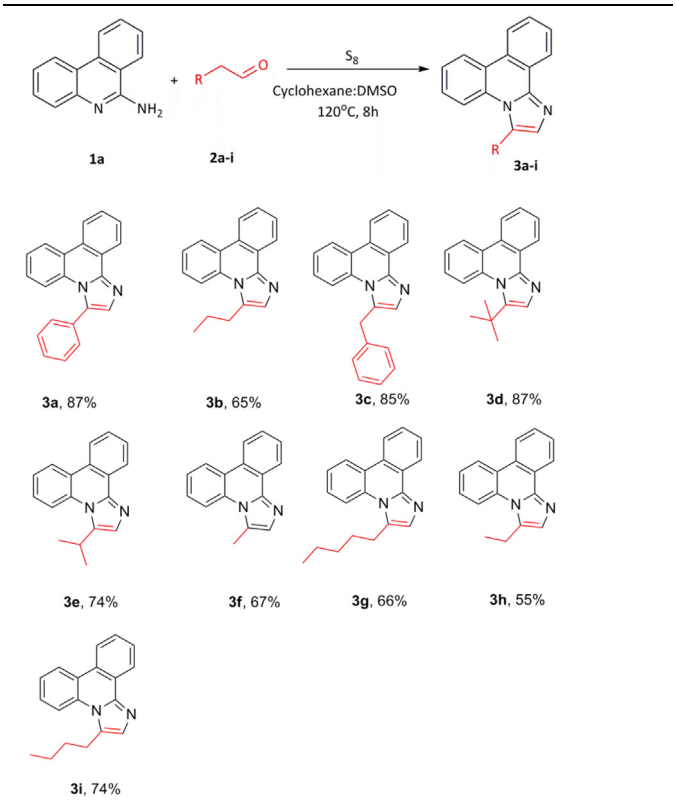
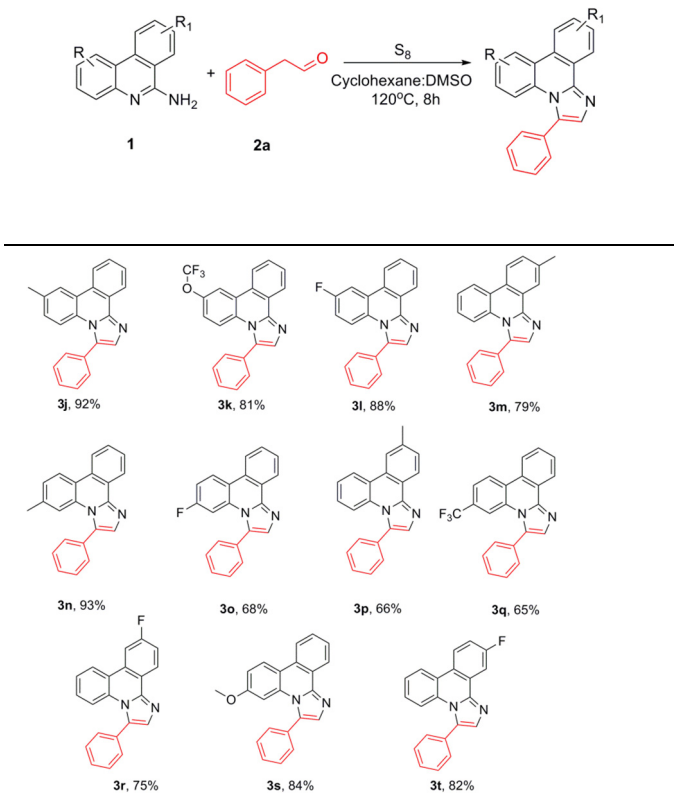



**Table 3**
Synthesis of 3-arylimidazo[1,2-*f*]phenanthridines from **2a** and various phenanthridine-6-amines.




oxidative annulation (entries 8–12). We found that the use of a co-solvent system of cyclohexane:DMSO at a ratio of 1:2 resulted in marked improvement of the yield. It is most likely that DMSO served not only as a co-solvent but also as a co-oxidant [18]. In addition, we envisaged that the reaction was not initiated in the absence of sulfur. In fact, a reasonable amount of the target product was detected in the presence of 1 eq of sulfur (entry 15). The yield of the product was reduced under an inert condition and with 1 eq of 2a (entry 16 and 17). Finally, the best yield was observed in the reaction condition with cyclohexane/DMSO (1:2) in the presence of 2 eq sulfur at 120 °C for 8 h in an air atmosphere (entry11).

Using the optimized reaction condition, we synthesized a series of imidazo[1,2-*f*]phenanthridines from structurally diverse aldehydes and phenanthridine-6-amines, as shown in Tables 2 and 3. First, various aldehydes were treated with phenanthridine-6-amine (**1a**) to produce target products (Table 2). Phenylacetaldehyde and phenylpropionaldehyde were successfully reacted with phenanthridine-6-amine to produce 3-phenyl- and 3-benzyl-imidazo[1,2-*f*]phenanthridine with good yields (**3a**, **3c**). Aliphatic aldehydes were reacted with phenanthridine-6-amine of 3-alkylimidazo[1,2-*f*]phenanthridine to give a good yield, too. It is noteworthy that branched chain aldehydes (**3d**, **3e**) produced 3-alkylimidazo[1,2-*f*]phenanthridine more efficiently than linear chain aldehydes.

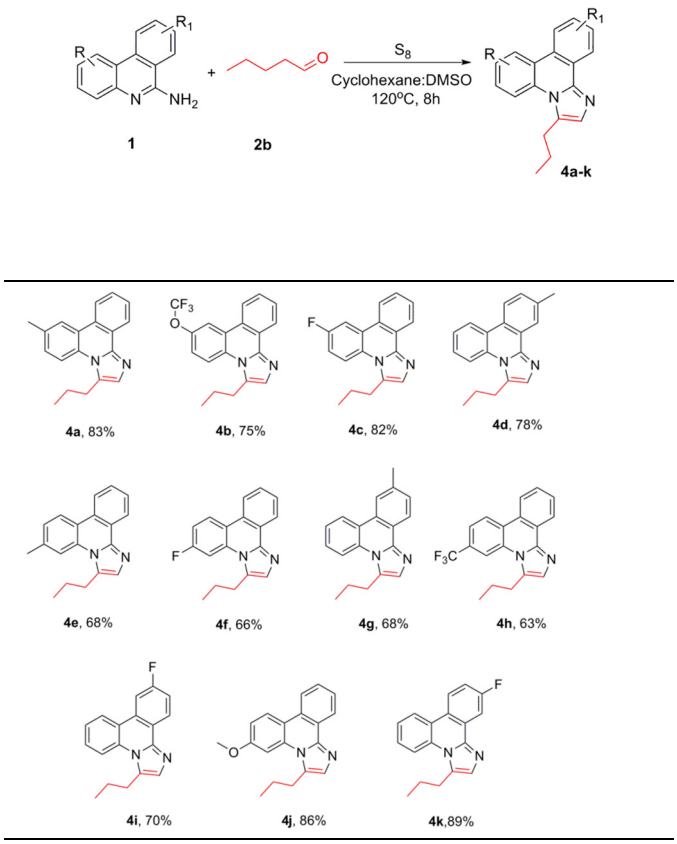
Subsequently, we explored the substrate scope of differentially substituted phenanthridine-6-amines with phenylacetaldehyde (Table 3). Methyl group isomers of phenanthridine-6-amine at positions of 6, 7, 10, and 11 reacted with phenylacetaldehyde (**2a**) to give annulated products with excellent yield (**3j**, **3m**, **3n**, **3p**). Phenanthridine-6-amine isomers with an electron-withdrawing fluoro group at positions of 6, 7, 10, and 11 reacted with phenylacetaldehyde (**2a**) to give desired products with moderate to good yield. Electron donating methoxy group-containing phenanthridine-6-amine reacted with **2a** to give a good yield of the desired product (**3s**). Trifluoromethoxy group-containing phenanthridine-6-amine reacted with **2a** to give a product with a good yield (**3k**).

We next examined the substrate scope of differentially substituted phenanthridine-6-amines with pentanal (**2b**) (Table 4). Methyl group isomers of phenanthridine-6-amine at positions of 6, 7, 10, and 11 reacted with pentanal (**2b**) to give desired products with moderate to good yield (**4e**, **4a**, **4g**, **4d**). Phenanthridine-6-amines with an electron-withdrawing fluoro group at positions of 6, 7, 10, and 11 reacted with pentanal (**2b**) to give target products with good yield. Electron donating methoxy group-containing phenanthridine-6-amine reacted with **2b** to give a good yield of the desired product (**4j**). Trifluoromethoxy group-containing phenanthridine-6-amine reacted with **2b** to give the product with good yield (**4b**).

Based on experimental results and earlier reports [18], we proposed a plausible reaction mechanism in Scheme 2.

We next examined the anticancer activity of the 31 newly synthesized compounds **3a**–**t**, **4a**–**k** and paclitaxel as positive control by using a cell counting kit-8 (CCK-8) method against 11 cancer cell lines: SK-OV-3 cells, A549 cells, HepG2 cells, AGS cells, MDA-MB-231 cells, HL-60 cells, HT-29 cells, HCC827 cells, K562 cells, PC-3 cells, H1299 cells, with a normal human fetal lung fibroblast cell line (MRC-5 cells) being used as a reference [19]. At first, the growth inhibition of 11 different cancer cells was observed by the treatment with **3a**–**t** and **4a**–**k** compounds at the concentration of 20 μM for 48 h. As shown in Fig. S1A–K, all the synthesized compounds had antiproliferation activity to different degrees against all tested cancer cells. It was worthy of note that compounds **3d** and **3f** exhibited marked inhibi-

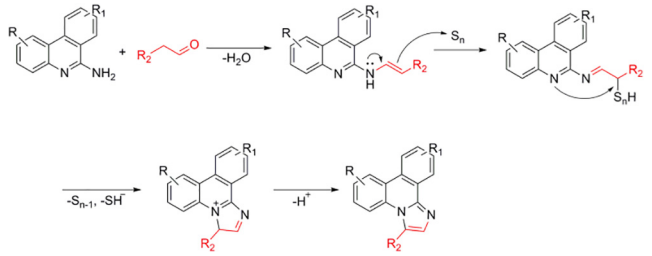
**Table 4**
Synthesis of 3-alkylimidazo[1,2-*f*]phenanthridines from **2b** and various phenanthridine-6-amines.




tory activity at 20 μM compared to other compounds, in particular, on A549, HepG2, HT-29, and K562 cells. Furthermore, the dose-dependency in the antiproliferative activity of **3d** and **3f** was examined using four cancer cell lines at compound concentrations of 0, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 μM (Fig. 2).

Results showed that: (1) the compounds **3d** and **3f** both exhibited strong antiproliferative activity against four cancer cells with $IC_{50}$ values of less than 10 μM, (2) **3d** and **3f** exhibited a low $IC_{50}$ value (2.18 ± 0.08 and 2.24 ± 0.71 μM, respectively) and a high selectivity-Index (SI, 16.0 and 10.1, respectively) against A549 cells, and (3) **3d** showed strongest inhibition activity to HT-29 cells with a smallest $IC_{50}$ value of 1.25 ± 0.22 μM and the highest SI of 27.9 among four cancer cell lines, in which the antitumor activity of **3d** was more potent than that of paclitaxel, suggesting that HT-29 cells are susceptible to **3d** (Table 5, Fig. 2C).

In addition, the cytotoxic effect of **3d**, **3f**, and paclitaxel (as a positive control) on MRC-5 cells was examined as shown in Fig. 2E and Table 5. Compounds **3d** and **3f** showed moderate cytotoxicity against




**Scheme 2.** Plausible reaction mechanism.

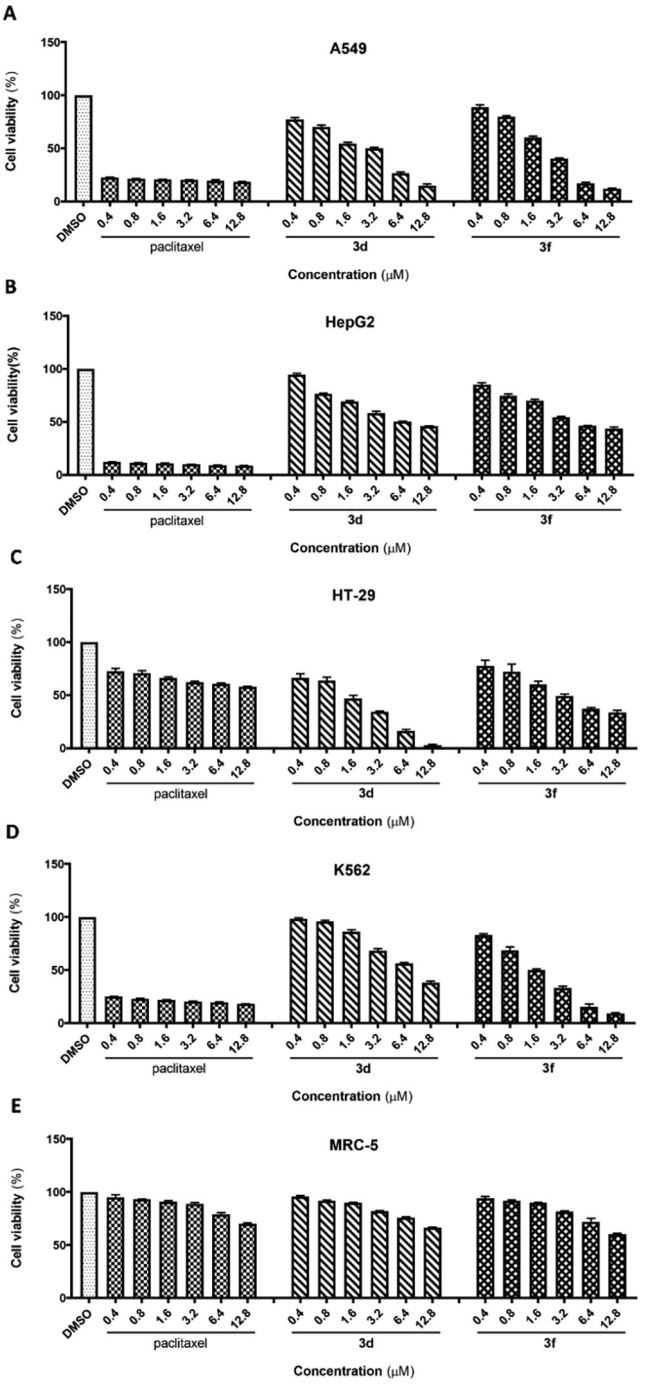



**Fig. 2.** Effect of **3d** and **3f** on A549, HepG2, HT-29, K562, and MRC-5 cells. Viability of the tumor cell lines was determined after treatment with compounds **3d** and **3f** at concentrations of 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 μM for 48 h using a Cell Counting Kit-8 assay. Paclitaxel. a positive control; 1% DMSO:1% DMSO solution in corresponding culture medium as a reference.

**Table 5**
$IC_{50}$ values (μM) for A549, HepG2, HT-29, K562, and MRC-5 cell lines treated with **3d**,**3f** and a positive control drug (paclitaxel). The values are expressed as mean ± SD (in triplicates).

| Cancer cells | Paclitaxel | **3d** | **3f** |
|---|---|---|---|
| **A549** | 0.275 ± 0.120 (212)[a] | 2.18 ± 0.08 (16.0) | 2.24 ± 0.71 (10.1) |
| **HepG2** | 0.0850 ± 0.0401 (686) | 5.96 ± 0.52 (5.85) | 7.17 ± 0.89 (3.15) |
| **HT-29** | 48.1 ± 0.90 (1.21) | 1.25 ± 0.22 (27.9) | 3.28 ± 0.52 (6.89) |
| **K562** | 0.235 ± 0.130 (248) | 7.83 ± 0.71 (4.46) | 1.62 ± 0.79 (13.9) |
| [b]**MRC-5** | 58.3 ± 0.9 | 34. 9 ± 0.5 | 22.6 ± 0.8 |

[a] Selectivity-Index (SI).
[b] MRC-5: Normal human fetal lung fibroblast cell line.

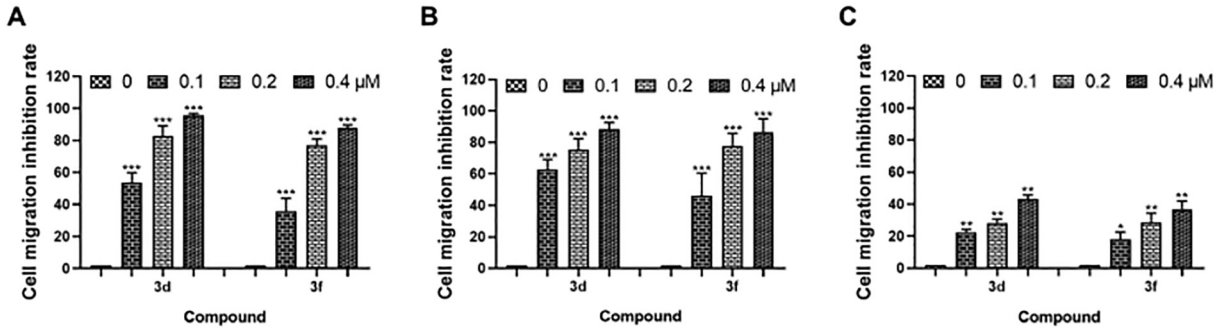



**Fig. 3.** Effect of compounds **3d** and **3f** on the mobility of A549 (A), HepG2 (B), and HT-29 (C) cell lines. The effect of **3d** and **3f** on the mobility of tumor cells was determined at the concentration of 0, 0.1, 0.2, and 0.4 μM for 48 h, respectively. Cell inhibitory rate (%) for suppression of the migration in cancer cells as a function of concentrations of compounds **3d** and **3f**. The % cell inhibitory rate was calculated by the equation: cell inhibitory rate (%) = $(1 - D_{drug}/D_{control}) \times 100\%$, where $D_{drug}$ is the mean distance of cell migration in drug group, $D_{control}$ is the mean distance of cell migration in control group; Values are the means ± S.D (*P < 0.05, **P < 0.01, ***P < 0.001, significantly different compared with the control group).

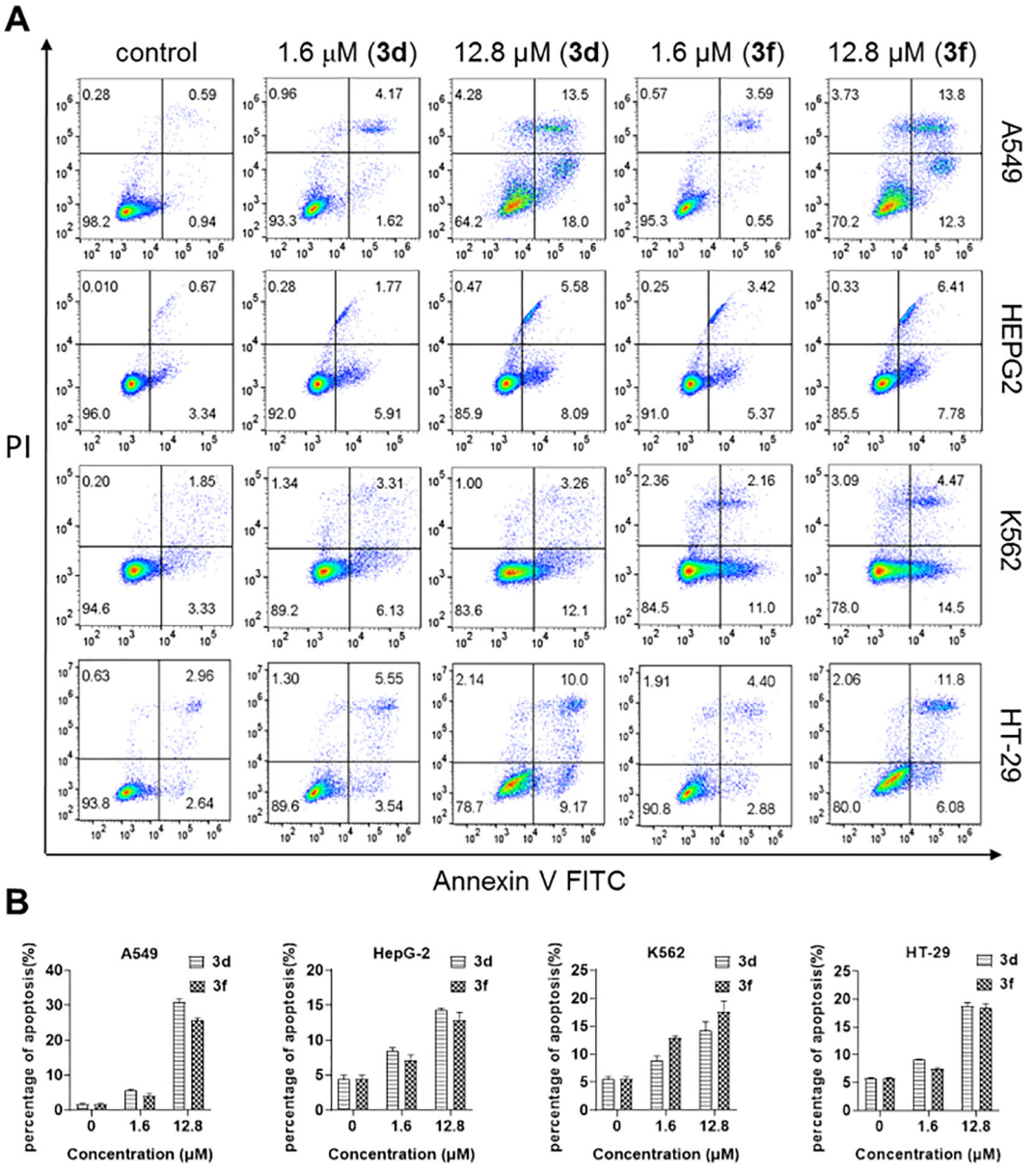



**Fig. 4.** (A) Representative cytograms on flow cytometric analysis of apoptotic cells induced by **3d** and **3f** using Annexin V-FITC/PI double staining method. A549, HepG2, K562, HT-29 cells were treated with Annexin V-FITC/PI at **3d** and **3f** concentrations of 1.6 and 12.8 μM for 48 h. B) Quantitative analysis of **3d** and **3f** induced apoptosis. Mean and SD values were from two independent experiments.

normal MRC-5 cells with $IC_{50}$ values of 34.9 ± 0.5 μM and 22.6 ± 0.8 μM, respectively, which are lower than that of paclitaxel.

Taken together, both compounds **3d** and **3f** possessed strong cytotoxic activity against four examined cancer cell lines, especially, **3d** against HT-29 cells. This implies that **3d** may further be optimized as a leading compound in the development of therapeutics for colorectal cancer.

The death of cancer patients is attributed to the metastasis and invasion of tumor cells. It is reported that telomerase activity is closely related to the metastasis and invasion of tumor cells [20,21]. Through TRAP ASSAY, however, we found that compounds **3d** and **3f** failed to inhibit the metastasis and invasion of tumor cells through inhibition of telomerase activity [19,21]. We, therefore, further examined the effect of **3d** and **3f** on the migration of A549, HepG2 and HT-29 cells by using a wound healing assay. Since K562 is non-adherent, the cell line was not included in this assay. As shown in Figs. 3 and S2, the compounds **3d** and **3f** could significantly inhibited the migration of tumor cells in a concentration-dependent manner and showed the strongest inhibitory effect on the migration of A549 cells with the concentration gradients of 0.1 μM, 0.2 μM, and 0.4 μM in all three cancer cells. It is likely that **3d** had the stronger inhibitory activity on the migration of the tumor cell lines than **3f**, consisting with the experimental results obtained by CCK-8 (Table 5). These results suggest that the new compounds **3d** and **3f** can effectively inhibit the mobility of the three cancer cell lines at low concentrations.

The induction of cell death by enhancing apoptosis, a type of programmed cell death, is a common strategy for treating tumors with small molecular compounds. Upon cell membrane eversion during apoptosis, Annexin-V binds to phosphatidylserine (PS). As the process of apoptosis proceeds, the permeability of cell membranes increases, which allows propidium iodide (PI) to enter the cells and bind to DNA. By using a suitable fluorescent dye, therefore, the process of apoptosis can be detected by flow cytometric analysis. As shown in Fig. 4, the proportion of apoptotic cells increased with increasing the concentration of **3d** and **3f**, when the four tumor cell lines, A549, HT-29, HepG2, and K562 cells, were treated with the compounds. The compound **3d** exhibited more potent apoptotic ability with the proportions of apoptotic cells ranging from 13.67% ± 1.06% to 31.5% ± 1.89% than **3f** at the concentration of 12.8 μM, except for K562. Noticeably, compounds **3d** and **3f** exhibited the most potent apoptotic effect on A549 cells, in particular **3d** with the highest apoptotic percentage being 31.5% ± 1.89%, mostly consisting with the experimental results obtained in other assay systems.

## Conclusions

In summary, we have established a novel synthetic route of imidazo[1,2-*f*]phenanthridines from phenanthridin-6-amine and aldehydes in the presence of elemental sulfur. This oxidative cyclization under a metal- and base-free condition generally proceeds with high yield. The strategy thus represents a synthetic route of imidazo[1,2-*f*] phenanthridines with atom economy. When exposed to A549 cell lines, the compound **3d** and **3f** exhibited $IC_{50}$ values of 2.18 ± 0.08 and 2.24 μM ± 0.71 μM, respectively. Compound **3d** had the strongest inhibitory activity against HT-29 cells with an $IC_{50}$ value being 1.25 μM ± 0.22 μM, which was even stronger than that of paclitaxel, suggesting that colorectal cancer is a preferable target of this compound. Our findings in this study demonstrate that imidazo[1,2-*f*] phenanthridine derivatives could be potential candidates for developing anti-colorectal cancer drugs.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.152908.

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