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Synthesis and anti-tumor activities of *N*′-benzylidene-2-(4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetohydrazone derivatives

Jiangping Lou [a,†], Zhen Liu [b,†], Yan Li [b], Mi Zhou [b], Zhengxi Zhang [b], Shu Zheng [a], Renxiao Wang [b,*], Jian Li [a,*]

[a] School of Pharmacy, East China University of Science and Technology, 130 Mei Long Road, Shanghai 200237, People's Republic of China
[b] State Key Laboratory of Bioorganic Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, People's Republic of China

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ABSTRACT

A compound with a cyclic thienopyrimidine moiety and an aceto-hydrazone moiety in its chemical structure was discovered in a cell-based screening to have noticeable cytotoxicity on several tumor cell lines. A total of 38 derivatives of this compound were synthesized at five steps with high yields. These compounds were tested in standard MTT assays, and several compounds exhibited improved cytotoxic activities. The most potent compounds have IC_{50} values of 10–20 μM on A549, HeLa, and MBA-MD-231 tumor cells. Flow cytometry analysis of several active compounds and subsequent examination of caspase activation indicate that they induce caspase-dependent apoptosis in tumor cells. In addition, these compounds do not have obvious effect on a normal cell line HEK-293T, demonstrating the desired selectivity against tumor cells. Results from a fluorescence polarization-based in vitro binding assay indicate that this class of compounds does not significantly interrupt the interactions between Mcl-1 and Bid. Their cytotoxicity is achieved presumably through other mechanisms.

The discovery and development of effective anti-cancer therapies has been accelerated in recent years by various molecular targeted techniques and strategies.[1] Nevertheless, in vitro screening with tumor cell lines is still an essential approach to the identification of compounds with desired potency.[2] One famous example is the NCI-60 platform, which is a panel of 60 human tumor cell lines made available by the US National Cancer Institute.[3] Millions of compounds have been screened on the NCI-60 platform so far, and such efforts have led to the discovery of many compounds with clear pharmaceutical implications.

As part of our efforts on discovering new potential anti-cancer compounds, we screen the compounds in our inventory, which are either purchased from a commercial source or synthesized by ourselves, whenever enough samples are available. In our cell-based screening, each compound is tested at a single concentration of 20 μM in a standard MTT assay on three selected tumor cell lines, including A549 (human alveolar epithelial cell), HeLa (human cervical tumor cell), and MBA-MD-231(human breast tumor cell). Recently, a compound, that is, **1** in Figure 1, was discovered in our screening, which exhibited notable cytotoxicity at the tested concentration. This compound was originally purchased from the Specs catalog (Specs ID = AF-399/40713992). The IC_{50} values of this compound in the MTT assay were 40 and 16 μM on HeLa and MDA-MB-231 cells, respectively, but its effect on A549 cells was rather low (Fig. 1). Considering the biological potency and synthetic accessibility of compound **1**, it may serve as a reasonable lead compound for further developments.

From the viewpoint of a medicinal chemist, two distinctive moieties are included in the chemical structure of this compound: a cyclic thienopyrimidine moiety and an acetohydrazone moiety (**2** in Fig. 2). Our literature survey indicates that some known compounds containing such moieties are indeed associated with anti-cancer activities. A number of tetrahydrobenzothieno-pyrimidine (**3** in Fig. 2) and arylthienopyrimidine (**4** in Fig. 2) derivatives were reported as anti-cancer, anti-bacterial and anti-microbial agents.[4–6] The molecular targets of these compounds are often unclear. At present, two possible molecular targets of these compounds have been reported, that is, 5-HT_{1A} receptor[7] and 17-β-hydroxysteroid dehydrogenase.[8,9] Both of them are related to cancers. The thienopyrimidine moiety is therefore a valuable scaffold for developing new anti-cancer compounds. Our literature survey also found several acetohydrazone derivatives that exhibit broad-spectrum anti-tumor and anti-microbial activities. The oxoquinoxaline derivatives (**5** in Fig. 2) are reported to show anti-tubercular, anti-bacterial and anti-fungal activities.[10] Such compounds with an aryl group instead of oxoquinoxaline will cause distinct cytotoxic activities.[11] In particular, an arylpiperazinyl aceto-hydrazone derivative (PAC-1, **6** in Fig. 2) was demonstrated to induce apoptosis in cancer cells by activating procaspase-3 to caspase-3.[12] Given these facts, it is reasonable to

* Corresponding authors.
E-mail addresses: wangrx@mail.sioc.ac.cn (R. Wang), jianli@ecust.edu.cn (J. Li).
† These authors made equal contributions to this work.

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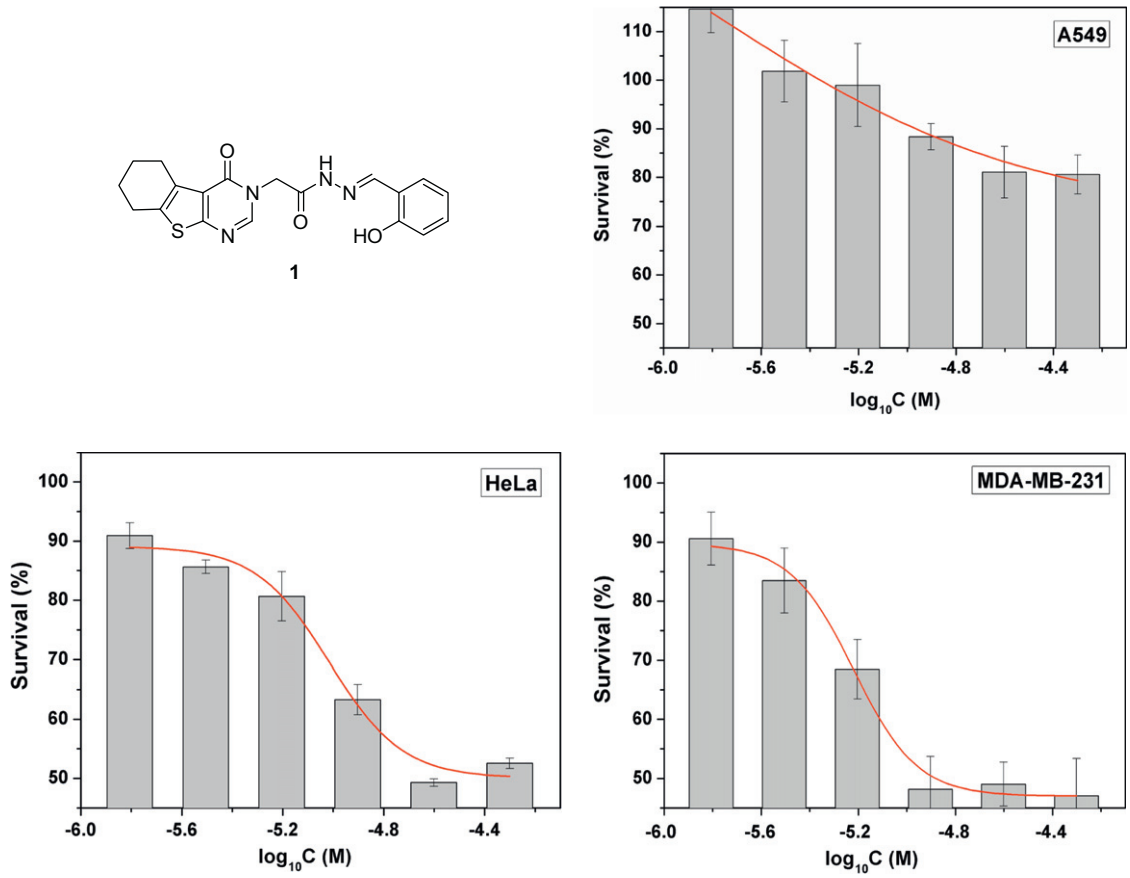

Figure 1. The lead compound (**1**) identified in our screening and its dose-dependent cytotoxicity on three selected tumor cell lines.

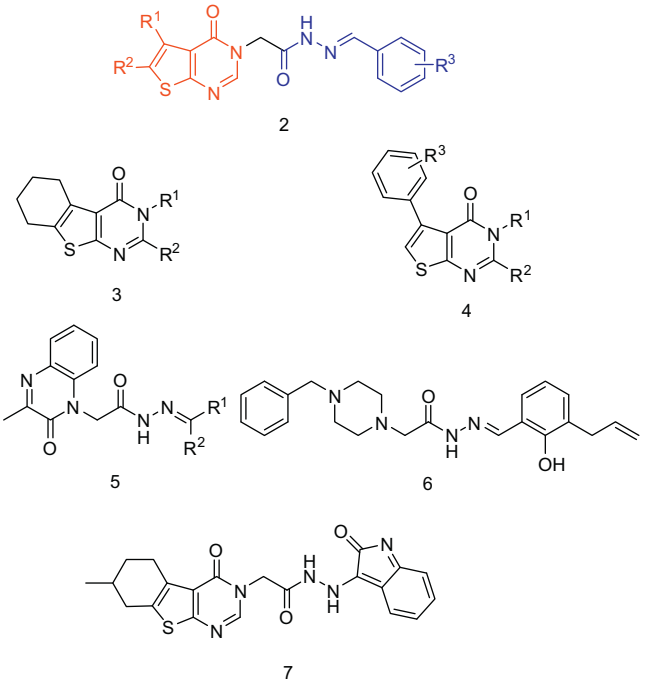

Figure 2. The structural scaffold of the compounds synthesized in this study (**2**) and some structurally similar compounds reported in literature (**3**–**7**).

expect that a compound combining these two moieties may have anti-tumor activities. In fact, one known compound (**7** in Fig. 2) is close to compound **1**. According to the record in the PubChem BioAssay database (CID: 5711212),[13] this compound inhibits the interactions between Mcl-1 and Bid proteins and thus may induce apoptosis in tumor cells as other effective Mcl-1 inhibitors.

The above analysis provides additional supports that the active compound identified in our screening, that is, compound **1**, is worth further developments. In this study, we have synthesized a total of 38 derivatives (**12–49**) of compound **1**. Chemical synthesis of this class of compounds can be completed relatively efficiently in high yields (10–30% total yield over five steps). The synthetic methods for the preparation of compounds **1** and **12–49** is illustrated in Scheme 1. Using our method described previously,[14] ketones, cyanoacetates, and sulfur were mixed and subjected to microwave irradiation for several minutes to produce 2-amino-thiophene (**8**). In this reaction, basic aluminum oxide was used as solid support and morpholine as base catalyst. Compound **8** was cyclized to thieno[2,3-*d*]pyrimidine (**9**) by condensation with formamide.[15] Compound **10** was the obtained by reacting ethyl bromoacetate and **9**, which then was converted to the hydrazide **11**, the key intermediate, by refluxing with 85% hydrazine monohydrate in ethanol. Various aromatic aldehydes were reacted with compound **11** to produce the target compounds **1** and **12–49**.

All of the synthesized compounds (Table 1) were then evaluated at a single concentration of 20 μM on A549, HeLa, and MDA-MB-231 cells. Inhibitions of cell growth were determined using a standard MTT assay after 48-hour treatment. Among them, compounds **24**, **33**, **35**, **37** and **45** exhibited inhibition ratio over 50% on at least one tumor cell line (Table 1). These compounds were then tested at multiple concentrations to determine their cytotoxicity quantitatively. In this experiment, cell line HEK-293T, that is, human embryonic kidney cells transformed with adenovirus 5 DNA and