Selectively apoptosis-targeting compounds in gastro-intestinal cancers attract broad interest. Here, we investigated a synthetic sulfonamide, 4-bromo-N-(5-ethyl-5H-pyrido[4,3-b]indol-8-yl)benzenesulfonamide (L34). It showed high activity against gastric cancer cells SGC-7901, causing apoptosis, which was associated with downregulation of caspase-3 and XIAP, upregulation of cleaved caspase-3, and cleavage of PARP. Hence, L34 might be a potent chemotherapeutic agent against human gastric cancer.

Key words: anticancer compound; L34; apoptosis; gastric cancer

Gastric cancer represents the second most common cause of cancer-related deaths in the world, and the incidence is higher in Asia than in other geographical areas. As a systemic approach to control cancer, more and more studies of chemotherapy for gastric cancer are being done, and the existing evidence indicates that chemotherapy, especially when the disease is in an advanced stage, is commonly used and effective. Many novel chemotherapeutic agents, such as Taxanes, Fru-totecan, and S-1, have demonstrated activity in gastric cancer and offer hope of improving patient outcomes when administered with platinum-based regimens. However, chemoresistance is a common phenomenon of treatment failure. It can be intrinsic or acquired during treatment. Thus to develop more effective agents remains a big challenge. Recently, selective apoptosis-targeting compounds for gastrointestinal cancers attract broad interests.

Apoptosis, or programmed cell death, is a highly regulated process that involves the activation of a series of molecular events leading to cell death. It can be induced by various stimuli, including growth factor withdrawal, irradiation, cytotoxic drugs, and death receptor ligands. There are two major signalling routes in mammalian cells leading to apoptosis, the extrinsic pathway (triggered by death receptors) and the intrinsic pathway (mediated by mitochondria). In both the intrinsic and the extrinsic pathway, activation of caspases is a central event in the execution of apoptosis. One of the key discoveries in cancer research has been the recognition that anticancer chemotherapy kills cancer cells by activating the intrinsic or the extrinsic apoptosis pathway or both. Within the past few years, anticancer therapies inhibiting antiapoptotic signalling or actively inducing apoptosis in cancer cells, have entered clinical trial. Therapeutic agents targeting apoptosis pathways have burst onto the scene. Until now, agents targeting apoptosis pathways have primarily been tested alone or in combination with chemotherapy.

Small molecules with sulfonamide functionality, e.g., N-pyridinyl sulfonamide (ABT-751), chloroindolyl sulfonamide (Indisulam), and styrylpyridine N-oxide sulfonamide (HMN-214), have been reported to be potent anticancer agents, and are currently undergoing clinical trials in connection with a variety of cancers. This stimulated us to design and synthesize a series of sulfonamide derivatives, some of which have been screened for antitumor activity. Among them, a novel compound, L34 (4-bromo-N-(5-ethyl-5H-pyrido[4,3-b]indol-8-yl)benzenesulfonamide) (Fig. 1), showed potent antiproliferative activity against human cancer cells. The purpose of the present study was to investigate the anticancer activity and the molecular mechanism underlying L34 antitumor action in human gastric cancer cells. Data were presented as mean ± SD for three separate experiments. Comparisons between groups were performed by one-way ANOVA, followed by Bonferroni post hoc tests for multiple comparisons, with \( p < 0.05 \) considered significant considered significant versus the control group.

The novel compound L34 showed potent anti-tumor effects in many cancer cells. L34 exhibited excellent anti-proliferation activity against the tested human cancer cell lines of non-small-cell lung cancer A549 and colonic epithelial cell line HCT-116, Breast Cancer MCF-7, with IC50 values of 1.88 \( \mu \)M, 11.29 \( \mu \)M, 3.79 \( \mu \)M respectively (Table 1). Especially, it showed excellent anti-proliferative characteristics as for gastric cancer cells, with an IC50 of 1.83 \( \mu \)M. This suggests that L34 possesses significant anti-proliferation activities against human cancer cells especially gastric cancer cells. Moreover, we tested the effect of L34 on normal chang liver cells. The IC50 (34.40 \( \mu \)M) was much higher than cancer cells. This suggests that L34 was a less toxicity compound and can be used in clinical latter.

To determine whether apoptosis contributed to gastric cancer cell growth inhibition by the compound L34, PI staining and DAPI staining were used to examine its apoptosis-induction ability. It is recognized that apoptosis is responsive to agent-mediated antitumor activities, a distinct, intrinsic cell death program that occurs in various physiological and pathological situations.
characterized by typical morphological and biochemical hallmarks, including DNA fragmentation, chromatin condensation, cell shrinkage, and membrane blebbing.\(^{12}\) As shown in Fig. 1C and D, the percentage of apoptotic cells in the control group was 5.85%. Upon exposure to 0.25, 0.5, and 1\(\mu\)M L34 for 72 h, the percentages of apoptotic cells increased to 23.84%, 32.47% \((p < 0.05)\) and 55.14\% \((p < 0.05)\), respectively. In addition, L34 treatment influenced nuclear morphology. It can cause nuclear condensation and the fragmentation of the cell into apoptotic bodies. By DAPI staining and fluorescence microscopy, representative images of cells showing apoptotic bodies were noticeable (shown in Fig. 1B).

Mitochondrial depolarization is perhaps an early event during apoptotic cell death.\(^{13}\) Since L34 significantly increased apoptosis, we sought to measure the mitochondrial membrane potential by JC-1 staining in SGC-7901 cells. JC-1 selectively enters the mitochondria, where it forms monomers and emits green fluorescence (FL-1) when \(\Delta\psi_{\text{m}}\) is relatively low. At high \(\Delta\psi_{\text{m}}\), JC-1 aggregates and shows red fluorescence (FL-2). Thus the red and green fluorescence of JC-1 reflect the change in \(\Delta\psi_{\text{m}}\) of the mitochondrial membrane.\(^{14}\) As shown in Fig. 2A and B, the ratios of these specific apoptotic cells (exhibiting green fluorescence) increased to 19.72%, 40.4% \((p < 0.05)\), and 47.8% \((p < 0.05)\), after treatment for 72 h with various concentrations of L34 0.25, 0.5, and 1\(\mu\)M respectively. This indicates that the mechanism involved in apoptosis works partly through mitochondrial pathway.

The mechanisms of apoptosis are highly complex, involving an energy-dependent cascade of molecular events.\(^{15}\) Poly(ADP-ribose)polymerase (PARP) is an abundant nuclear enzyme that is responsible for poly(ADP-ribose)\(n\) response to DNA damage caused by numerous agents and during DNA base excision repair.\(^{10}\) It might contribute to cell death by depleting the cell of NAD and ATP, cleaving into 89- and 24-kDa fragments during drug-induced apoptosis in a variety of cells. Caspase-3 is primarily responsible for the cleavage of PARP during cell death, and it plays a key role in the execution of the apoptotic program. Activation of executor caspases 3 results in cleavage and inactivation of proteins involved in DNA repair mechanisms.\(^{17}\) These data reveal the high apoptosis-induction capability of L34 in SGC-7901 cells. Hence we investigated further some of the apoptosis-associated events in this process. PARP was investigated. L34 treatment (0.5 and 1.0\(\mu\)M, 72 h) efficiently induced cleavage of caspase-3 together with a cleavage of 85kDa of the inactive intermediate band of PARP in a dose-dependent manner (Fig. 2C). These results confirm that L34-induced

### Table 1. \(\text{IC}_{50}\) Values in Cell Lines Measured by MTT Assay

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell type</th>
<th>(\text{IC}_{50}) ((\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS49</td>
<td>Human non-small cell lung cancer</td>
<td>1.88</td>
</tr>
<tr>
<td>SGC-7901</td>
<td>Human gastric cancer cell</td>
<td>1.83</td>
</tr>
<tr>
<td>HCT-116</td>
<td>Human colon cancer cell</td>
<td>11.29</td>
</tr>
<tr>
<td>MCF-7</td>
<td>Human breast cancer cell</td>
<td>3.79</td>
</tr>
<tr>
<td>Chang Liver</td>
<td>Human hepatocyte-derived cell line</td>
<td>34.40</td>
</tr>
</tbody>
</table>

In brief, SGC-7901 cells were treated with L34 (0.5, 1.0, 2.0\(\mu\)M) for 48 h, then washed with PBS twice gently, and incubated with 2\(\mu\)L DAPI (5\(\mu\)g/mL) (Sigma, St. Louis, MO) for 5 min. Cells were examined by fluorescence microscopy. Apoptotic nuclei were identified by condensed chromatin as well as nuclear fragmentation (arrow). C and D, L34 induced apoptosis in SGC-7901 cells. PI staining and flow cytometry assay were used to characterize apoptosis. Briefly, SGC-7901 cells were treated with L34 (0.25, 0.5, 1.0\(\mu\)M) for 72 h, harvested, washed twice with PBS, and fixed with 70% ethanol overnight at 4 °C. Cells were washed once with PBS and digested in 400\(\mu\)L of PBS containing 50.0 mg/mL RNase (Amersco, USA) at 37 °C for 0.5 h. After incubation, they were stained with 200.0 mg/mL PI (Biosciences Pharmingen, San Diego, CA) at 4 °C for 30 min. Flow cytometry was performed on FACScan (BD Biosciences, Boston, MA), and collection and analysis of data were done using CellQuest software (BD Biosciences).
apoptosis was mediated by PARP cleavage and caspase-3 activation. In addition, XIAP is the most important member of the inhibitor of apoptosis protein (IAP) gene family in terms of ability to inhibit caspases and suppress apoptosis.\(^{18,19}\) Downregulation of XIAP expression can induce apoptosis and enhance chemotherapeutic sensitivity in human gastric cancer cells, playing a vital role in preventing cell death induced by various factors.\(^ {20}\) In the present study, a decreased expression level of XIAP was found, indicating that suppression of apoptosis was impaired. These findings indicate that L34 induces apoptosis by activating caspase cascade and blocking XIAP.

This study indicated that the novel compound L34 induced mitochondrial-mediated and caspase-dependent apoptosis, and possesses excellent anti-cancer capability \emph{in vitro}. L34 developed as an alternative chemical therapy for gastric cancer.

**References**