Discovery of an Indirubin Derivative as a Novel c-Met Kinase Inhibitor with *In Vitro* Anti-Tumor Effects

Karyn Muzinga Ndolo¹, Su Jin An¹, Kyeong Ryang Park¹, Hyo Jeong Lee¹, Kyoung Bin Yoon¹, Yong-Chul Kim² and Sun-Young Han¹,*

¹College of Pharmacy and Research Institute of Pharmaceutical Sciences, Gyeongsang National University, Jinju 52828,
²School of Life Sciences, Gwangju Institute of Science & Technology, Gwangju 61186, Republic of Korea

**Abstract**

The c-Met protein is a receptor tyrosine kinase involved in cell growth, proliferation, survival, and angiogenesis of several human tumors. Overexpression of c-Met has been found in gastric cancers and correlated with a poor prognosis. Indirubin is the active component of Danggui Longhui Wan, which is a traditional Chinese antileukemic recipe. In the present study, we tested the anti-cancer effects of an indirubin derivative, LDD-1937, on human gastric cancer cells SNU-638. When we performed the *in vitro* kinase assay against the c-Met activity, LDD-1937 inhibited the activity of c-Met. This result was confirmed by immunoblot and immunofluorescence of phosphorylated c-Met. Immunoblot analysis showed that LDD-1937 decreased the expression of the Erk1/2, STAT3, STAT5, and Akt, downstream proteins of c-Met. In addition, LDD-1937 reduced the cell viability and suppressed colony formation and migration of SNU-638 cells. Furthermore, LDD-1937 induced G2/M phase arrest in the SNU-638 cells by decreasing the expression levels of cyclin B1 and CDC2. Cleaved-PARP, an apoptosis-related protein, was up-regulated in cells treated with LDD-1937. Overall, this study suggests that LDD-1937 may be a novel small-molecule with therapeutic potential for selectively inhibiting c-Met and c-Met downstream pathways in human gastric cancers overexpressing c-Met.

**Key Words:** Gastric cancer, Indirubin, c-Met, LDD-1937

**INTRODUCTION**

Gastric cancer has been pointed out to be one of the leading causes of cancer death in the world. Although the worldwide incidence of gastric cancer has declined over the recent decades, it remains one of the most important malignant diseases (Chan and Wong, 2015). It is known that the incidence of stomach cancer varies in different parts of the world (Ervik et al., 2016). In South Korea, gastric tumors were the first cause of cancer death in year 2015 (Annual report of cancer statistics in Korea in 2015, 2017). Unless diagnosed in the early stage, stomach cancers are difficult to cure. Notwithstanding, researchers are attempting to develop drugs with different mechanisms of action to enhance patients’ survival and to reduce mortality rates.

Indirubin is the major active component of the Chinese anti-chronic myelogenous leukemia (CML) traditional medicine recipe, Danggui Longhui Wan. Indirubin has been reported to inhibit DNA synthesis and cyclin-dependent kinases (Blazevic et al., 2015). Moreover, *in vitro* studies on indirubin derivatives revealed their inhibitory effects against protein kinases (Choi et al., 2010; Nam et al., 2012; Blazevic et al., 2015). Therefore, several indirubin derivatives were synthesized and tested as potential anti-neoplastic agents.

Currently, numerous anti-cancer agents have been developed and designed to target some specific molecular signaling pathways in cancer (Sawyers, 2004), and the receptor tyrosine kinases (RTKs) appear to be an important category of such therapeutic targets. Over 58 human RTKs have been discovered and described as having a key role in oncogenesis (Lemmon and Schlessinger, 2010). Among them, c-Met RTK has been an attractive cancer therapeutic target in recent years (You and McDonald, 2008; Zhu et al., 2014). The c-Met RTK and its natural ligand, hepatocyte growth factor (HGF), are involved in cell growth and proliferation, survival, angiogenesis, and metastatic progression. Upon stimulation
by ligand binding, c-Met dimerizes and phosphorylates itself. Subsequently, multiple downstream effector pathways such as phosphoinositide-3-kinase (PI3K)/Akt, mitogen-activated protein kinase (RAS/MAPK), signal transducer and activator of transcription (STAT) are activated (Christensen et al., 2005). Activation of these cascades leads to increased cell motility and invasion, angiogenesis, survival, growth and proliferation, and migration (Ma et al., 2003).

The c-Met RTK overexpression and/or amplification have been detected in a number of solid tumors including gastric tumors which are correlated with a poor prognosis, depth of tumor invasion, and metastasis in patients with gastric malignancies (Rodrigues and Park, 1994). This finding provides evidence for the key role of c-Met in gastric cancers. Thus, targeting the c-Met signaling pathway has come into the spotlight and has great potential in target-based cancer therapy. Among the various strategies to inhibit c-Met, inhibition of the auto-phosphorylation of c-Met and phosphorylation of its downstream signal pathways have emerged as intriguing targets for cancer therapy (Rodrigues and Park, 1994; Liu et al., 2010; Mughal et al., 2013). To date, numerous anti-cancer agents that block the c-Met pathway have been or are being evaluated in clinical trials, and some of them are entering late-stage trials for cancer interventions (Kang et al., 2014; Zhu et al., 2015a, 2015b). Although a number of these agents still require clinical validation, promising results have begun to emerge while the trials progress. The present study was carried out to identify and characterize an indirubin derivative, LDD-1937, as a c-Met kinase inhibitor.

**MATERIALS AND METHODS**

**Cell culture**

The human SNU-638 gastric cancer cell line was purchased from the Korean Cell Line Bank (Seoul, Korea) and grown as monolayers in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Hyclone, Logan, UT, USA) and maintained at 37°C in a humidified atmosphere at 5% CO₂.

**In vitro kinase assay**

The inhibitory activity of LDD-1937 against c-Met recombinant kinases was measured using the homogeneous time-resolved fluorescence (HTRF) assay. Concentrations of the enzyme, ATP, and substrate were optimized using an HTRF KinEASE kit following the manufacturer’s instruction (Cisbio, Codolet, France). The reaction was initiated by the addition of ATP to a mixture containing c-Met enzyme, peptide substrates, and serially diluted inhibitor in a kinase reaction buffer (250 mM HEPES (pH 7.0), 100 mM ATP, 0.5 mM orthovanadate, 5 mM MgCl₂, 1 mM DTT, 0.05% BSA, 0.1% NaN₃). Detection reagents were added, and then, the TR-FRET signal was measured with a Victor multi-label reader (Perkin Elmer, Waltham, MA, USA). The curve was fitted by nonlinear regression and the IC₅₀ was calculated using GraphPad Prism 5.01 (GraphPad, La Jolla, CA, USA).

**Immunofluorescence**

Cells in the exponential phase were dispensed onto a 8-well culture slide at a density of 5,000 cells per well and treated with LDD-1937. After a 5 h incubation, cells were fixed with 4% formaldehyde solution and permeabilized with 0.5% Triton X-100 solution. Slides were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (0.1 M phosphate buffer, pH 7.2) containing 0.2% Tween-20 (PBST) and then incubated overnight in primary antibodies at 4°C. The following day, cells were washed with PBST, incubated with Alexa Fluor 546 labeled secondary antibody (Life Technologies, Grand Island, NY, USA) at room temperature for an hour and then washed with 1% BSA in PBST. A coverslip was then mounted on the slides with Prolong Gold Antifade reagent with DAPI (Life Technologies), and the fluorescence images of the cells were acquired with a fluorescent microscope Axiosvert 200 (Carl Zeiss, Oberkochen, Germany).

**Immunoblot analysis**

Cells were lysed in SDS lysis buffer, and protein concentrations were measured using the SMART BCA Protein Assay kit (iNtRON Biotechnology, Seongnam, Korea). Samples were resolved on SDS-PAGE gels of 8, 10, or 15% and transferred to PVDF membranes (Millipore, Billerica, MA, USA) and then blocked with 5% nonfat milk and 5% BSA for 30 min at room temperature. Next, the membranes were incubated overnight with primary antibodies. The following day, the membranes were washed and then incubated with secondary antibodies for 30 min. at room temperature. Subsequently, the membranes were washed and immunoblots were detected using the ECL Select Western Blotting Detection Reagent (Amer sham ECL select, GE Healthcare, Chicago, IL, USA). Antibodies against phosphorylated c-Met (Tyr1313), c-Met, Akt, phosphorylated Erk1/2, Erk1/2, STAT3, and STAT5 were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phosphorylated Akt (Ser473), anti-phosphorylated STAT3 (Tyr705), anti-phosphorylated STAT5 (Tyr694), anti-cleaved PARP (Asp214) and anti-PARP were purchased from Cell Signaling Technology (Danver, MA, USA). An antibody against β-actin (Sigma-Aldrich) was used for the detection of β-actin as a loading control.

**Cytotoxicity assay**

Cell viability was assessed by a tetrazolium-based assay using the EZ-Cytox Cell Viability Assay kit (DaeilLab, Seoul, Korea). Cells were plated in 96-well plates (2,000 cells/well) and incubated with the LDD-1937 compound or dimethyl sulfoxide (DMSO) as a negative control. After 72 h of incubation, the viability of the SNU-638 cells was measured by the addition of 15 µL of EZ-Cytox reagent in each well. After incubation for 4 h, absorbance was measured using a Victor multi-label reader. The GI₅₀ was calculated by nonlinear regression using Prism version 5.01 (GraphPad).

**Anchorage-independent growth assay**

The anchorage-independent growth was assessed by soft agar clonogenic assay. Cells were dispensed into a 6-well plate (40,000 cells/well), suspended in 1.5 mL of medium containing 10% fetal bovine serum and 0.3% Noble Agar (BD biosciences, San Jose, CA, USA), in the presence or absence of LDD-1937, and layered over a base prepared of medium, 10% fetal bovine serum, and 0.6% agarose. The plates were incubated (37°C, 5% CO₂), and live colonies were stained with 0.005% crystal violet solution, visualized under a microscope and photographed using the Bio-Imaging System (DNR Bio-
Wound healing assay

Cells were grown and allowed to reach 90% confluency in 6 well-plates. Gaps 2 mm wide were introduced by scraping cells with a sterile 10 μL tip. The detached cells were removed, and cells were treated with varied concentrations of LDD-1937. After 24 h of incubation, the live cell images were obtained using a phase contrast microscope (Carl Zeiss).

Flow cytometric analysis

Cells were seeded in 6-well plates (20,000 cells/well) and then incubated overnight. After incubation, the cells were treated with different concentrations of LDD-1937 (0, 0.1, 1 or 10 μM) for 48 h. Then, the cells were detached, fixed, and treated with RNase A (50 μg/mL) and stained with propidium iodide (PI) (Sigma-Aldrich). Cell cycle distribution was analyzed by flow cytometry using the Accuri C6 (BD Biosciences).

RT-PCR

Total RNA was extracted using the ReliaPrep RNA cell mini-prep system (Promega, Madison, WI, USA) and reverse transcribed using the cDNA reverse-transcription kit (Life Technologies). The cDNA was amplified with cyclin B1, CDC2, and EF1α primers, using polymerase chain reaction (PCR): 94°C for 2 min. followed by 30 cycles at 95°C for 30 s, 57°C for 30 s, and 74°C for 90 s. The PCR products were electrophoresed on a 1% agarose gel and stained with Redsafe (iNtRON Biotechnology). Primer sequences used and the conditions of these reactions were as follows: cyclin B1: sense 5'-AAG AGC TTT AAA CTT TGG TCT GGG-3', antisense 5'-TTT CTT TGT AAG TCC TTG ATT TAC CAT G-3', size 319 bp. CDC2: sense 5'-GGT TCC TAG TGC AAT TCG-3', antisense 5'-TTT GCC AGA AAT TCG TTT GG-3', size 709 bp. EF1α: sense 5'-AGG TGA TTA TCC TGA ACC ATC C-3', antisense 5'-AAA GGT GGA TAG TCT GAG AAG C-3', size 234 bp.

RESULTS

LDD-1937 inhibits the kinase activity of c-Met in vitro

The indirubin derivative LDD-1937 compound (Fig. 1A) was reported as potent FLT3 inhibitor in vitro and in vivo, potentially useful for the treatment of acute myeloid leukemic patients (Lee et al., 2018). LDD-1937 was tested against the c-Met kinase activity using the purified recombinant c-Met protein as described in Materials and Methods. LDD-1937 potently inhibited the c-Met kinase activity in a concentration-dependent manner with an IC50 value of 0.239 μM (Fig. 1B).

LDD-1937 inhibits c-Met phosphorylation and downstream signaling pathways

The SNU-638 human gastric cancer cell line was used to assess the effect of LDD-1937 on c-Met phosphorylation and c-Met-dependent signaling pathways, including Erk1/2,
a dose-dependent manner, while the total expression level of a 1937 compound inhibited the auto-phosphorylation of c-Met in data showed that exposure of the SNU-638 cells to the LDD- with the immunofluorescence results, the immunoblot assay phosphorylated c-Met decreased dose-dependently. Consistent when the SNU-638 cells were treated with the LDD-1937 compound at the indicated concentration, the number of phosphorlated c-Met decreased dose-dependently. Consistent with the immunofluorescence results, the immunoblot assay data showed that exposure of the SNU-638 cells to the LDD-1937 compound inhibited the auto-phosphorylation of c-Met in a dose-dependent manner, while the total expression level of c-Met remained unchanged (Fig. 2B). To examine the down-stream signaling pathway by which LDD-1937 blocked the cell proliferation, the cellular phosphorylation of Erk1/2, STAT3, STAT5 and Akt was checked because the inhibition of the c-Met activity may cause the suppression of downstream signaling pathways, which are important for cell survival and proliferation (Christensen et al., 2005). Indeed, the data showed that phosphorylation of the signaling molecules was also inhibited by LDD-1937 in a dose-dependent manner (Fig. 2B).

**LDD-1937 inhibits the growth of the SNU-638 cells**

A cytotoxicity assay was undertaken to assess the effects of LDD-1937 on cell proliferation using SNU-638 cells. As shown in Fig. 3A, LDD-1937 exposure resulted in the inhibition of tumor cell growth with a GI50 value of 2.25 \( \mu \)M.

To examine the ability of LDD-1937 to cause a modulation in SNU-638 gastric cancer cell growth in soft agar, an anchor-age-independent growth assay was carried out. SNU-638 cells were grown in soft agar containing various concentra-tions of LDD-1937 for 4 weeks. The number of colonies of SNU-638 cells decreased after the treatment of LDD-1937 at 1 and 10 \( \mu \)M concentration (Fig. 3B). These results show the inhibition of the anchorage-independent growth of the SNU-638 cells by LDD-1937.

Cell migration was assessed by the wound healing assay under treatments of increasing concentrations of LDD-1937. A wound 2 mm wide was made in the monolayer, and the cells were treated with LDD-1937. Migration of the SNU-638 cells was checked after 24 h, revealing that the exposure to LDD-1937 interrupted the migration of the SNU-638 cells in a dose-dependent manner when compared with the untreated cells, in which the cells moved from the edge of the wound towards the center of the wound (Fig. 3C). Overall, these results correlated well with the inhibition of the c-Met activity.

**LDD-1937 inhibits cell proliferation through G2/M arrest**

The effect of LDD-1937 on the SNU-638 cell cycle progression was examined to understand its anticancer mechanism. As shown in Fig. 4A, exposure to LDD-1937 increased the cell population in the G2/M phase of the cell cycle, indicating cell cycle arrest in the G2/M phase. The G2/M population of the SNU-638 cells was increased from 22.7% in the control group to 45.6% in the 10 \( \mu \)M LDD-1937 treatment group (Fig. 4A).

The activation of the cyclin B1/CDC2 complex is essential for the G2/M transition and regulates the entry into mitosis during the cell cycle (Chen et al., 2008). Thus, the expression level of cyclin B1 and CDC2 mRNA was investigated to confirm the effect of LDD-1937 on the cell cycle through c-Met inhibition. The SNU-638 cells were treated with LDD-1937 for 24 h and subjected to RT-PCR. LDD-1937 decreased the mRNA levels of cyclin B1 and CDC2 dose-dependently (Fig. 4B). The results showed that the reduced c-Met activity by LDD-1937 results in decreased cyclin B1 and CDC2 expression, followed by cell cycle arrest at the G2/M phase. Inhibition of cell proliferation can also result from apoptotic cell death. The apoptotic cell death of the SNU-638 cells by LDD-1937 was confirmed by PARP cleavage. As shown in Fig. 4C, an increased level of cleaved PARP was observed after the LDD-1937 treatment, while the level of PARP remained unchanged.

**Fig. 3.** Effects of LDD-1937 on SNU-638 cell growth. (A) Cells at a density of 2,000 cells/well were seeded into 96-well plates and treated with the LDD-1937 compound. After incubation for 72 h, the viability of the SNU-638 cells was measured using the EZ-Cytox Cell Viability Assay kit. The percentage of cell growth was evaluated with 0.5% DMSO treatment as a negative control. (B) Cells were grown in soft agar in the presence or absence of LDD-1937. After 4 weeks of incubation, live colonies were stained with 0.005% crystal violet solution and photographed under a microscope. (C) SNU-638 cells were seeded into 6-well plates and allowed to reach 90% confluency. A wound was made on the monolayer, and cells were then washed and treated with varied concentrations of LDD-1937 for 24 h. The migration of cells was observed under a microscope at 24 h post-incubation.

---

Ndolo et al. LDD-1937 as c-Met Kinase Inhibitor

Ndolo et al. LDD-1937 as c-Met Kinase Inhibitor
DISCUSSION

Over the past decade, target-based cancer therapy has emerged as a promising approach for the management of cancer that may enhance the survival of cancer patients. There are approximately 40 FDA-approved small molecule inhibitors targeting kinases (Wilson et al., 2018). The c-Met RTK, as a target, appears to hold promise for future therapeutic applications.

To date, natural indirubin as well as its derivatives has widely been studied for molecular targeted therapy of cancer (Blazevic et al., 2015). Indirubin toxicity was reported to be low with mild side effects in the treatment of chronic myeloid leukemia, and researchers were stimulated by these facts to explore the use of indirubin and its derivatives also in other types of cancers.

In this study, we demonstrated that LDD-1937, an indirubin derivative, possessed a prominent inhibitory activity against c-Met kinase and its downstream signaling pathways, resulting in the inhibition of cell proliferation, migration, and induction of the G2/M phase arrest of the cell cycle in the human gastric cancer cell SNU-638. We first tested the inhibitory effect of LDD-1937 against c-Met kinase (Fig. 1B). Furthermore, the anti-cancer effect of this compound was characterized. To evaluate the ability of the inhibition of c-Met by LDD-1937, the auto-phosphorylation of c-Met and phosphorylation of its downstream signaling pathways in SNU-638 cancer cells were investigated. We found that LDD-1937 effectively inhibited c-Met phosphorylation in gastric cancer cells (Fig. 2). It is well established that activation and phosphorylation of c-Met involve numerous downstream targets (Rodrigues and Park, 1994). Based on our results, when we evaluated several potential targets of LDD-1937, we found that phosphorylation of Akt, Erk1/2, STAT3, and STAT5 is involved. Overall, through the inhibition of the c-Met kinase activity, LDD-1937 exhibited the ability to block effectively c-Met-related signaling cascades (Fig. 2B).

There have been several clinical trials of c-Met pathway modulators for gastric cancer patients. Rilotumumab, onartuzumab and ABT-700 are antibody-based therapeutics targeting HGF or c-Met receptor. Foretinib, tivantinib, and AMG-337 are small molecule c-Met inhibitors that have been underwent gastric cancer clinical studies. Clinical testing results for the c-Met modulators are disappointing in terms of efficacy (Bradley et al., 2017). However, subgroup of patients with c-Met amplification exhibited improvements in clinical trials of ABT-700 and AMG-337. From these results, patient selection with c-Met overexpression appears to be important in evaluation of clinical efficacy.

It has been reported that the deregulated HGF/c-Met signaling pathway has an important role in angiogenesis, tumor growth, proliferation, invasion, and metastasis (Ma et al., 2003). On the other hand, several studies have shown that downregulation of the c-Met activity leads to the inhibition of cell proliferation and invasion as well as the induction of apoptosis (Puri et al., 2007; Que and Chen, 2011). Similarly, when LDD-1937 was applied to the SNU-638 cells, it inhibited growth, proliferation and migration of the SNU-638 cells (Fig. 3).

Treatments with LDD-1937 appeared to induce cell cycle arrest at the G2/M phase as shown by the entrapment of cells in the G2/M phase, indicating a delay into the entry of mitosis which leads to the retardation of cell division (Fig. 4A). Moreover, LDD-1937 inhibited the expression of cyclin B1 and CDC2 mRNA levels (Fig. 4B). The cyclin B1/CDC2 complex is activated during the late G2 phase of the cell cycle, thereby triggering the initiation of mitosis (Smits and Medema, 2001). These results were consistent with the previously reported inhibition of CDKs and arrest of the G2/M phase of the cell cycle by indirubin and its derivatives (Hoessel et al., 1999; Moon et al., 2006; Blazevic et al., 2015).

Taken together, these observations have provided evidence that LDD-1937 possesses significant in vitro anti-cancer activity in gastric tumors. LDD-1937 inhibits cancer cell growth, proliferation, and cell cycle at G2/M phase and induces apoptosis. Although additional studies are yet needed,

---

**Fig. 4.** Effects of LDD-1937 on SNU-638 cell cycle and apoptosis. (A) SNU-638 cells were treated with the indicated concentration of LDD-1937 for 48 h. After staining with PI, the DNA content was measured by FACS analysis. (B) Cells were treated with LDD-1937 for 24 h at the indicated concentration, and total RNA extracted from the cells was subjected to RT-PCR. The cyclin B1 and CDC2 mRNA expression level was measured using specific primers. EF1α mRNA level was used as loading control. (C) SNU-638 cells were treated with various concentrations of LDD-1937 for 24 h and subjected to immunoblot analysis for measuring PARP and cleaved PARP levels.
LDD-1937 may be a potential anti-tumor agent targeting the c-Met pathway in gastric cancers expressing c-Met.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program (2015R1C1A2A01053928, 2018R1A2B6002081) through the National Research Foundation (NRF) funded by the Ministry of Science and ICT.

REFERENCES

Annual report of cancer statistics in Korea in 2015 (2017) Korea Central Cancer Registry. Available from: http://www.cancer.go.kr/
Blazevic, T., Heiss, E. H., Atanasov, A. G., Breuss, J. M., Dirsch, V. M. and Uhrin, P. (2015) Indirubin and indirubin derivatives for counteracting proliferative disease. Evid. Based Complement. Alternat. Med. 2015, 854089-854110.
Bradley, C. A., Salto-Tellez, M., Laurent-Puig, P., Bardelli, A., Rolfo, C., Taberner, J., Khawaja, H. A., Lawler, M., Johnston, P. G. and Van Schayckbroeck, S. (2017) Targeting c-MET in gastrointestinal tumours: rationale, opportunities and challenges. Nat. Rev. Clin. Oncol. 14, 562-576.
Chan, A. O. O. and Wong, B. (2015) Epidemiology of Gastric Cancer (M. F. Post, Ed.). Uptodate. Available from: http://www.uptodate.com/contents/epidemiology-of-gastric-cancer/.
Chen, H., Huang, Q., Dong, J., Wang, A. D. and Lan Q. (2008) Overexpression of CDC2/cyclin B1 in gliomas, and CDC2 depletion inhibits proliferation of human glioma cells in vitro. BMC Cancer 8, 29-40.
Choi, S. J., Moon, M. J., Lee, S. D., Choi, S. U., Han, S. Y. and Kim, Y. C. (2010) Indirubin derivatives as a potent FLT3 inhibitors with anti-proliferative activity of acute myeloid leukemic cells. Bioorg. Med. Chem. Lett. 20, 2033-2040.
Christensen, J. G., Burrows, J. and Salgia, R. (2005) c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. Cancer Lett. 225, 1-26.
Envik, M., Lam, F., Fertay, J., Mery, L., Soerjomataram, I. and Bray, F. (2016) Cancer Today. International Agency for Research on Cancer, Lyon, France. Available from: http://gco.iarc.fr/today/.
Hoesssel, R., Leclerc, S., Endicott, J. A., Nobel, M. E., Lawrie, A., Tunnah, P., Leost, M., Damiens, E., Marie, D., Marko, D., Niederberger, E., Tang, W., Eisenbrand, G. and Meijer, L. (1999) Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases. Nat. Cell Biol. 1, 60-67.
Kang, Y. K., Muro, K., Ryu, M. H., Yasui, H., Nishina, T., Ryu, B. Y., Kamiya, Y., Akinaga, S. and Boku, N. (2014) A phase II trial of a selective c-Met inhibitor tivantinib (ARQ 197) monotherapy as a second- or third-line therapy in the patients with metastatic gastric cancer. Invest. New Drug 32, 355-361.
Lee, D. H., Sung, E. S., Ahn, J. H., Huh, J. W. and You, W. K. (2015a) Development of antibody-based c-Met inhibitors for targeted cancer therapy. Immunotargets Ther. 4, 35-44.
Lee, H. J., Lee, J., Jeong, P., Choi, J., Baek, J., Ahn, S. J., Moon, Y., Heo, J. D., Choi, Y. H., Chin, Y. W., Kim, Y. C. and Han, S. Y. (2018) Discovery of a FLT3 inhibitor LDD1937 as an anti-leukemic agent for acute myeloid leukemia. Oncotarget 9, 924-936.
Lee, J. J. X., Chan, J. J. and Choo, S. P. (2015b) Clinical development of c-MET inhibition in hepatocellular carcinoma. Diseases 3, 306-324.
Lemmon, M. A. and Schlessinger, J. (2010) Cell signaling by receptor tyrosine kinases. Cell 141, 1117-1134.
Liu, X., Newton, R. C. and Scherle, P. A. (2010) Developing c-Met pathway inhibitors for cancer therapy: progress and challenges. Trends Mol. Med. 16, 37-45.
Ma, P. C., Maulik, G., Christensen, J. and Salgia, R. (2003) c-Met: structure, functions and potential for therapeutic inhibition. Cancer Metastasis Rev. 22, 309-325.
Moon, M. J., Lee, S. K., Lee, J. W., Song, W. K., Kim, S. W., Kim, J. I., Cho, C. H., Choi, S. J. and Kim, Y. C. (2006) Synthesis and structure-activity relationships of novel indirubin derivatives as potent anti-proliferative agents with CDK2 inhibitory activities. Bioorg. Med. Chem. 14, 237-246.
Mughal, A., Aslam, H. M., Sheikh, A. and Khan, A. M. H. (2013) c-Met inhibitors. Insect. Agent. Cancer 8, 13.
Nam, S. K., Scuto, A., Yang, F., Chen, W. Y., Park, S. M., Yoo, H. S., Koning, H., Bhatia, R., Cheng, X., Merz, K. H., Eisenbrand, G. and Jove, R. (2012) Indirubin derivatives induce apoptosis of chronic myelogenous leukemia cells involving inhibition of Stat5 signaling. Mol. Oncol. 6, 276-283.
Purr, N., Khramtsov, A., Ahmed, S., Nallasuva, V., Hetzel, J. T., Jagadeeswaran, R., Karczman, G. and Salgia, R. (2007) A selective small molecule inhibitor of c-Met, PHA665752, inhibits tumorigenicity and angiogenesis in mouse lung cancer xenografts. Cancer Res. 67, 3529-3534.
Que, W. and Chen, J. (2011) Knockdown of c-Met inhibits cell proliferation and invasion and increases chemosensitivity to doxorubicin in human multiple myeloma U266 cells in vitro. Mol. Med. Rep. 4, 343-349.
Roddries, G. A. and Park, M. (1994) Autophosphorylation modulates the kinase activity and oncogenic potential of the Met receptor tyrosine kinase. Oncogene 9, 2019-2027.
Sawyers, C. (2004) Targeted cancer therapy. Nature 432, 294-297.
Smits, V. A. and Medema, R. H. (2001) Checking out the G(2)/M transition. Biochim. Biophys. Acta 1519, 1-12.
You, W. K. and McDonald, D. M. (2008) The hepatocyte growth factor/c-Met signaling pathway as a therapeutic target to inhibit angiogenesis. BMJ Rep. 41, 833-839.
Wilson, L. J., Linley, A., Hammond, D. E., Hood, F. E., Coulson, J. M., MacEwan, D. J., Ross, S. J., Slupsky, J. R., Smith, P. D., Eyers, P. A. and Prior, I. A. (2018) New perspectives, opportunities, and challenges in exploring the human protein kinome. Cancer Res. 78, 15-29.
Zhu, K., Kong, X., Zhao, D., Liang, Z. and Luo, C. (2014) c-Met kinase inhibitors: a patent review (2011-2013). Expert Opin. Ther. Pat. 24, 217-230.