Review

Protein kinases as therapeutic targets to develop anticancer drugs with natural alkaloids

Hanlin Ye¹, Linlin Wang¹, Li Ma², Maksim Ionov³, Gan Qiao¹, Jianlin Huang¹, Li Cheng¹, Yuxi Zhang¹, Xiaohui Yang¹, Shousong Cao¹,*, Xiukun Lin¹,*

¹Department of Pharmacology, School of Pharmacy, Southwest Medical University, 646000 Luzhou, Sichuan, China, ²Rizhao Hospital of Traditional Chinese Medicine, 276826 Rizhao, Shandong, China, ³Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Targeting MAPK pathway
4. Targeting PI3K/AKT/mTOR pathway
5. Targeting EGFR
6. Targeting JAK/STAT signaling pathway
7. Discussion
8. Author contributions
9. Ethics approval and consent to participate
10. Acknowledgment
11. Funding
12. Conflict of interest
13. References

1. Abstract

**Background:** Protein kinases play an important role in cell proliferation, differentiation, mobility and cell cycle arrest etc. These enzymes act as important targets in developing anticancer agents. Over the years, a large number of protein kinase inhibitors have been discovered and developed as anticancer agents for the treatment of human cancer. The drug-resistance and off-targeting limit their efficiency for the treatment of human cancer.

**Materials and methods:** Alkaloids are an important class of natural products with broad spectrum biological activities. In the past decades, numerous alkaloids with significant anticancer activity by inhibiting protein kinases were identified. In the present mini-review, we will present the key enzymes including mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) and janus-activated kinases/signal transducer and activator of transcription (JAK/STAT) targeted by alkaloids and highlight the special sites targeted by alkaloids on protein kinases and/or reversing drug resistance. Additionally, the challenge and prospect of developing alkaloids as new anticancer agents are also discussed.

**Conclusion:** Alkaloids suppressed tumor growth through targeting different signaling pathways mediated by protein kinases of cancer cells. It is conceivable that novel alkaloids anticancer agents with promising clinical value will be developed in the future.

2. Introduction

Protein kinases catalyze the transfer of a phosphate group to a specific amino acid in a protein molecule. Since the phosphate groups are charged negatively, the 3D structures of the specific protein will be changed when adding the phosphate group, leading to activation and inactivation of the specific proteins. There are several kinds of protein kinases with different selection towards substrates, and tyrosine protein kinases and serine threonine protein kinases are the main subfamilies [1]. It is well established that protein kinases function as a kind of important regulators in cellular events involving DNA damage-repair, cell proliferation, motility and apoptosis, etc. [2, 3]. Most protein kinases such as PI3K, Akt, EGFR and MAPK are usually highly expressed and/or activated in tumor cells, and these enzymes have been acted as major targets in developing novel anticancer agents [4]. Nowadays, almost a quarter...
of all newly developed anticancer drugs belongs to protein kinase inhibitors. Gefitinib (Iressa), a selective EGFR inhibitor, was approved in 2005 by the US Food and Drug Administration (FDA) to treat non-small cell lung cancer [5, 6]. In 2005, a multi-target tyrosine kinase inhibitor, Sorafenib (Nexavar; Onvx/Bayer), was approved by FDA to treat advanced primary liver cancer and primary kidney cancer (advanced renal cell carcinoma) [7–9]. However, the off-targets and drug resistance of protein kinase inhibitors limit their efficiency and application in the treatment of cancer patients [10, 11]. Therefore, it is urgent to develop novel agents overcoming the drawback of protein kinase inhibitors.

Alkaloids, a class of nitrogen-containing organic compounds usually with complex ring structures, are widely distributed in natural organisms. Alkaloids display diverse biological activity, including antiviral, antimicrobial, antiinflammatory, and anticancer etc. [12]. Over the past decades, a large number of alkaloids have been applied clinically for the treatment of different malignant tumors [13, 14]. Taxol and vincristine, a kind of microtubule inhibitors are used to treat human cancers [15, 16]. Irinotecan and topotecan developed from camptothecin are also commonly used in cancer treatment [17]. Over the years, numerous alkaloids have been found as protein kinase inhibitors, and some of them display potent anticancer activity [18]. We discussed and highlighted the therapeutic targets according to their pathways (Fig. 1) and the special sites targeted by alkaloids on protein kinases and/or reversing drug resistance. In addition, the challenge and prospect of developing alkaloids as new anticancer agents were also presented in this mini-review.

3. Targeting MAPK pathway

MAPK, a class of serine-threonine kinases, mediates intracellular signal transduction related to cell proliferation, differentiation, death, survival and transformation [19]. The classical MAPKs consist of extracellular signal-regulated kinase (ERK), p38 MAPK and c-Jun NH2-terminal kinases (JAK)/stress-activated protein kinases (SAPK). There are many subtypes of these kinases, including ERK1-8, p38-α, -β, -γ, -δ, and JNK1-3 [20, 21]. ERK1/2 are the ultimate components of the cell signal cascade of Ras/Raf/MEK/ERK signal [22]. ERK1/2 are able to phosphorylate more than 250 known substrates that regulate a variety of biological functions, including cell proliferation, gene transcription, cell cycle progression, migration, adhesion, survival and metabolism [23]. p38 MAPK is usually activated by the upstream MKK3 and MKK6 kinases, and sometimes by MKK4. Activated p38 MAPK plays an important role in cell growth, cell cycle arrest, apoptosis and differentiation [24]. Therefore, these molecules have become important targets for anticancer agents.

Over the years, numerous alkaloids affecting MAPK signaling have been identified. Dehydrocorydalin (DHC) (Fig. 2), an isoquinoline alkaloid isolated from Corydalis yanhusuo, Corydalis tuber or Corydalis bulbosa [25], displays diverse biological activities, including inhibition of antibody-mediated and cell-mediated allergy, inhibition of proinflammatory cytokine expression, and promotion of myoblast differentiation [26]. In recent years, the anticancer effects of DHC attract great attention; DHC was able to induce apoptosis [27], and inhibit tumor metastasis [28]. Recent study showed that DHC could inhibit the proliferation and metastasis of melanoma cells by down-regulating the MEK1/2/ERK1/2 cascade and the phosphorylation levels of MEK1/2 and ERK1/2 were down-regulated by DHC in a time- and dose-dependent manner [29]. DHC significantly inhibited the proliferation of melanoma cells with IC50 values of 39.73 µM. DHC was also capable of inhibiting the cell cycle-related proteins, including CDKs and cyclins, leading to cell arrest at G0/G1 phase in malignant melanoma.

Aconitum szechyanianum Gay, a traditional Chinese medical herb, is used to treat inflammation related diseases in China for more than the 500 years [30]. Its extract displays potent anticancer activity in several cancer cells including hepatoma SMMC-7721, gastric cancer SGC-7901 and esophageal cancer Eca-109 cells both in vitro and in vivo [31]. Recent studies showed that the extracts of Aconitum szechyanianum Gay were able to induce apoptosis via targeting p38 MAPK pathway in hepatoma HepG2 cells, cervical cancer Hela cells and lung cancer A549 cells; treatment with the extract down-regulated the level of p38 MAPK phosphorylation, and induced mitochondria-dependent apoptosis. Four alkaloids (Fig. 2) responsible for the anticancer effect were isolated from its dried roots including 3-acetylaconitine (C20H19NO12), songorine (C22H23NO12), aconitine (C34H47NO11) and deoxyaconitine (C33H47NO10) [32].

3α-acetylatesberosine (Fig. 2), an indole alkaloid and isolated from Melodinus suaveolens, showed potent anticancer effect against human promyelocytic leukemia HL-60, human liver cancer SMMC-7721, human lung cancer A549, human breast cancer MCF-7 and human colon cancer SW480 cells with IC50 values of 0.2–0.6 µM [33, 34]. Recent study showed that the compound is capable of inhibiting DNA damage-repair through MAPK pathway in glioblastoma U87 and T98G cells with the IC50 values of 1.7 µM and 4.3 µM, respectively [35]; treatment of glioblastoma U87 cells with the compound led to phosphorylation of JNK and ERK, resulting in cumulative DNA damage.

Several alkaloids including Isolensinine (Fig. 2), liensinine (Fig. 2), and neferine (Fig. 2), are isolated from the seed embryo of lotus (Nelumbo nucifera Gaertn), and display potent anticancer activities in several cancer cells [36–38]. Neferine inhibited the proliferation of hu-
Fig. 1. Schematic diagram of protein kinase pathways targeted by natural alkaloids. The protein kinase pathways including MAPK, PI3K/AKT/mTOR, JAK/STAT pathways play a significant role in the proliferation, survival and differentiation of cells. Natural alkaloids are able to interfere with some specific molecules related to protein kinase.

Man osteosarcoma cells by inducing G1 phase arrest [36]. Isoliensinine induced apoptosis in triple negative human breast cancer cells through targeting p38 MAPK and JNK pathways [38]. Treatment with isoliensinine (20 µM) resulted in increased expression of the phosphorylation of p38 and JNK in human breast cancer MDA-MB-231, MDA-MB-436, and MDA-MB-468 cells. The results suggested that isoliensinine may be developed as a novel anticancer agent for the treatment of breast cancer. Berberine (BBR) (Fig. 2), an isoquinoline alkaloid, is extracted from *Coptis chinensis*. BBR exhibits broad pharmacological effects, such as antiinflammatory, anti-diabetes, antibacterial, liver protection, neuroprotection and so on [39]. In recent years, it has been proved to be a promising anticancer agent for the treatment of breast cancer, colon cancer, ovarian cancer, melanoma, adhesive tape blisteroma and leukemia [40]. BBR inhibited the proliferation, invasion and metastasis of different cancer cells by inducing apoptosis, cell cycle arrest and autophagy in cancer cells. BBR was able to inhibit the growth of human non-small cell lung cancer A549 and PC-9 cells with the IC_{50} values of 58.33 µM and 49.00 µM, respectively. Recent study confirmed that berberine induced apoptosis through p38 MAPK and JNK pathways, treatment with BBR (80 µM) led to down-regulation of the expression of p-p38 MARK and p-JNK in non-small cell lung cancer [41].

4. Targeting PI3K/AKT/mTOR pathway

PI3K/AKT/mTOR pathway, activated by tyrosine kinase receptor or other cytokines, plays an essential role in regulating cell proliferation, growth, survival, exercise, metabolism and immune response [42, 43]. It is well documented that PI3K/AKT/mTOR pathway is over activated in up to 60% of cancers [44]. The activation of PI3K/AKT/mTOR pathway promotes uncontrolled proliferation, genomic instability and metabolic reprogramming of cancer cells [45]. Over the years, numerous anticancer agents are used for the treatment of human malignancies in clinic via inhibiting PI3K/mTOR signaling pathway.
Tetrandrine (TET) (Fig. 3), a bisbenzylisoquinoline alkaloid and calcium channel blocker isolated from *Stephania tetrandra* S.Moore, is used for the treatment of silicosis and rheumatoid arthritis as a traditional Chinese medicine [46]. Recent studies have shown that PI3K/AKT/mTOR Pathway plays a critical role in TET induced apoptosis in several cancer cells; treatment of human gastric cancer cells with TET resulted in inactivation of AKT/mTOR phosphorylation [47]. TET was also capable of inducing autophagy via targeting PI3K/AKT signaling in MCF-7 and MDA-MB-231 cell [48]. Clinical trial showed that the combination of TET and traditional cancer drugs including daunorubicin, etoposide and cytarabine (150 mg/m²) was effective in the treatment of multidrug resistant acute myeloid leukemia, pretreatment with TET (2 mg/kg) and daunorubicin, etoposide, and cytarabine resulted in around 42% complete remission and the p-glycoprotein was decreased significantly in the tumors of acute myeloid leukemia patients [49]. Cepharanthine (CEP) (Fig. 3), another kind of bisbenzylisoquinoline alkaloid, is isolated from *Stephania cepharantha* Hayata [50]. CEP (15 µM) induced apoptosis and inhibited proliferation significantly in human leukemic Jurkat T cells. Both MAPK and PI3K/AKT/mTOR pathways play a key role in anticancer effect of CEP, treatment with CEP (15 µM) inhibited the expression of p-Pi3K and p-mTOR and upregulated the expression of p-JNK and p-p38 in leukemia Jurkat T cells [51].
Piperlongumine (Fig. 3), a natural pyridine alkaloid, is extracted from *Piper longum* [52]. Studies have shown that piperlongumine displays broad biological activities including antianxiety, antiangiogenic, antidepressant, anticancer, antibacterial, and antidiabetic activities [53]. Piperlongumine exhibits strong cytotoxicity in various cancer cells, but it does little harm to normal human cells [54]. PI3K/AKT/mTOR play an important role in piperlongumine induced apoptosis; treatment with the compound led to down-regulation of p-Akt and p-mTOR in both A549 and U937 cancer cells [55, 56]. Studies also found that p38 was involved in piperlongumine-induced cancer cell growth inhibition; treatment with the compound (20 μM) up-regulated the expression of p-p38 [57]. The combination of piperlongumine with chemotherapeutic agents such as doxorubicin and cisplatin increased the sensitivity of cancer cells significantly [58]. Piperlongumine combined with TRAIL (TNF related apoptosis inducing ligand) or p53 activator APR-24 showed synergistic anticancer potential in several human cancer cells including human colon cancer HT-29, prostate cancer DU145, breast cancer MDA-MB-231, and head and neck squamous carcinoma (HNSC) cells [59].

Veratramine (Fig. 3), a natural steroidal alkaloid, is isolated from the plants of lily family [60, 61]. Veratramine is able to inhibit cell growth both in vitro and in vivo via targeting PI3K/Akt/mTOR; treatment with veratramine (19.81 μM) resulted in down-regulation of the phosphorylation levels of PI3K, AKT and mTOR in a concentration-dependent manner in liver cancer HepG2 cells [62]. *In vivo* study showed that the compound was capable of inhibiting the tumor growth significantly with low toxicity for the mice without obvious affection on blood cell count, body weight, and liver and kidney function [62].

Nitidine chloride (NC) (Fig. 3), a benzoanthraquinone alkaloid isolated from *Zanthoxylum nitidum* (Roxb) DC, shows significant anticancer activity against a variety of cancer cells including ovarian cancer, colorectal cancer, liver cancer, and renal carcinoma [63–66]. NC treatment led to down-regulation of AKT phosphorylation and suppressed the metastasis of renal cell carcinoma 786-O and A498 cells as well as glioblastoma U87 and U251 cells [63]. NC also inhibited the activation of STAT3 and ERK pathways and suppressed the expression of Bcl-2, Bax, cyclin D1, CDK4, VEGF-A and VEGFR2 in human hepatocellular carcinoma HepG2, HCCLM3, and Huh7 cells.
Fig. 4. Chemical structures of alkaloids that target epidermal growth factor receptor (EGFR).

[64] Magnoflorine (MAG) (Fig. 3), an apomorphine alkaloid, is isolated from Coptidis Rhizoma [67]. MAG exhibits wide spectrum activities including anti-inflammatory, anti-anxiety, anticancer etc. [68, 69]. MAG induced apoptosis and autophagy via targeting AKT/mTOR and p38 MAPK pathways [70]. Treatment with MAG led to decreased expression of p-PI3K, p-AKT and p-mTOR. In vivo study showed that MAG inhibited the growth of MCF-7 tumors bearing in nude mice and reduced the side effects of doxorubicin (DOX) in experimental animals. The tumor inhibitory rate has been increased from 30% (DOX alone) to 70% when the mice were treated with the combination of Mag/DOX (both at 3 mg/kg) [70].

5. Targeting EGFR

Epidermal growth factor receptor (EGFR), a 170 kDa transmembrane glycoprotein, is composed of a single polypeptide chain. The occurrence and development of various cancers are associated with its mutations, overexpression or signal regulation disorders [71]. Therefore, EGFR and its related signal factors have become the critical targets for the discovery and development of novel chemotherapeutic drugs MCF-7.

Cyclovirobuxine D (CVB-D) (Fig. 4) is the main active ingredient extracted from Buxus microphylla. CVB-D is widely used to treat cardiovascular diseases such as coronary heart disease, angina pectoris and arrhythmia in China [72, 73]. In recent years, there are numerous reports to show that CVB-D inhibited EGFR. CVB-D can inhibit the proliferation, colony formation, cell cycle process, and induced apoptosis through mitochondrial-mediated pathway in human gastric cancer MGC-803 and MKN28 and hepatocellular carcinoma HepG2 and HCC-LM3 cells [74]. Previous study revealed that CVB-D induced autophagic death through targeting AKT/mTOR signal pathway; treatment with CVB-D led to upregulation of p-AKT and p-mTOR in MCF-7 cells [75]. Recent study showed that both EGFR and PI3K/AKT were involved in CVB-D induced growth inhibition of cancer cells; treatment with CVB-D (10–40 µM) led to down-regulated expression of p-FAK, p-EGFR and EGFR proteins, inhibiting the proliferation, migration and invasion of human hepatoma HepG2 and HCCLM3 cells [76]. Oxymatrine (OM) (Fig. 4) belonged to a quinoline alkaloid is isolated from Sophora flavescens. OM displays diverse pharmacological effects, such as anti-inflammation, anti-virus, immune regulation and anti-apoptosis [77–80]. OM induced apoptosis in human pancreatic cancer PANC-1 cells by regulating the expression of Bcl-2, IAP families and the release of cytochrome C [81]. Both PI3K/AKT/mTOR and EGFR are involved in OM induced growth inhibition of cancer cells; treatment with OM down-regulated the phosphorylation levels of AKT and mTOR, inhibited the expression of EGFR, inhibited the invasion and metastasis of human glioblastoma U251MG cells [82]. OM can also inhibit angiogenesis of pancreatic cancer PANC-1 cells via targeting
NF-κB pathway [83]. OM (1 mg/mL) combined with erlotinib (2 µM) exhibited a stronger inhibitory effect than erlotinib (2 µM) alone in human glioma U251MG cells [82]. The inhibitory rate was increased from 30% to around 75% in the combination treatment compared with erlotinib alone.

Matrine (Fig. 4) and oxymatrine (Fig. 4) are the main active components of *Sophora flavescens* [84]. Matrine exhibits cytotoxicity in several cancer cells including hepatocellular carcinoma [85], gastric cancer [86], breast cancer [87] and lung cancer [88]. Matrine displays anticancer activity through targeting different cellular pathways in cancer cells; matrine could inhibit cancer cell proliferation, invasion, metastasis, and angiogenesis, induce differentiation and apoptosis, reverse multidrug resistance [89]. In order to increase the anticancer activity, a derivative of matrine, WM130 (C\textsubscript{30}N\textsubscript{4}H\textsubscript{40}SO\textsubscript{5}F) was developed [90]. WM130 inhibited the proliferation, migration and invasion of hepatocellular carcinoma Huh-7 cells more powerful than that of the parent compound matrine with IC\textsubscript{50} value around 40.61 µg/mL. EGFR plays a critical role for the effect of WM130 on the growth inhibition in cancer cells; WM130 down-regulated the level of EGFR phosphorylation in a dose-dependent. Further study showed that PI3K/Akt was also involved in WM130 induced growth inhibition in cancer cell; WM130 up-regulated the expression of PTEN resulting in the inactivation of AKT/ERK pathway [91]. In vivo study showed that treatment with WM130 (40 and 80 mg/kg) significantly inhibited (43.43% and 53.92%) the growth of Huh-7 tumors bearing in nude mice.

Noscapine (Fig. 4), a benzylisoquinoline alkaloid derived from the opium poppy *Papaver somniferum*, is widely used as a cough suppressant in Chinese folk medicine. Studies have shown that noscapine displays anticancer activity both in vitro and in vivo [92–95]. Noscapine induced growth inhibition of cancer cells is dependent on EGFR-related signal pathway; treatment with noscapine resulted in inactivation of EGFR and AKT, and the reduction of the expression of cyclin D1 and CDK4/6, leading to G1 arrest in osteosarcoma MG63 cells [96]. Treatment with noscapine also led to down-regulation of VEGF in hypoxic human glioma U87 cells [97, 98].

### 6. Targeting JAK/STAT signaling pathway

JAK/STAT signaling pathway plays an essential role in numerous cellular functions including cell proliferation, differentiation, apoptosis, immunomodulation and hematopoiesis [99]. There are four members of JAK family; JAK1, JAK2, JAK3 and TYK2. STAT families are composed of seven members; STAT1-4, STAT5A/5B and STAT6 [100]. The activation of JAK/STAT pathway depends on a variety of cytokines and growth factors including interleukin, interferon and EGF family members [101]. The binding of cytokines and other ligands to the receptor leads to the activation of janus kinase (JAK), and the activated JAK phosphorylates the tyrosine residues in the tail of the cytoplasm receptors [11]. In the process of tumorigenesis and development, over-activated JAK/STAT signals contribute to cancer cells proliferation, survival, invasion and neoangiogenesis [102]. Therefore, this pathway has attracted more and more attention as a new target for anticancer drugs.
Dentatin (Fig. 5), a carbazole alkaloid isolated from *Murraya koenigii*, has been found to exhibit a variety of anticancer activities [103, 104]. Dentatin can induce apoptosis in prostate cancer PC-3 and LNCaP cells [105], and breast cancer MCF-7 cells [106]. Recent study showed that JAK/STAT signaling plays a critical role in dentatin induced apoptosis and autophagy in cancer cells [107]. Dentatin was able to down-regulate the phosphorylation of JAK1, JAK2, STAT1 and STAT3, and the compound is also capable of increasing the expression of Beclin-1 and LC3II, leading to autophagic cell death in HT-29 cancer cells. Dentatin can also up-regulate the expression of cyclin D1, down-regulate the expression of cyclin A/B1, induce S-phase arrest in HT-29 cells. Lycorine (Fig. 5), a isoquinoline alkaloid extracted from *Amaryllidisaceae* displays broad spectrum of pharmacological activities such as anti-inflammatory, emetic, anti-malaria, anti-viral and so on [108, 109]. Lycorine can inhibit the growth of cancer cells through different mechanisms. Lycorine inhibited the growth and metastasis of prostate cancer [110]. The compound significantly inhibited the migration, proliferation and invasion of prostate cancer PC-3M, DU145, LNCaP and 22RV1 cells with the IC_{50} values around 5–10 μM. Treatment with lycorine (5 μM) led to down-regulation of p-EGFR, p-JAK1, p-JAK2 and p-STAT3. In vivo trial showed that lycorine inhibited the growth of tumor significantly; administration of lycorine (10 mg/kg) by i.p. inhibited the tumor growth with an inhibition rate of 78%. Additionally, lycorine can affect the JAK/STAT3 pathway via other regulators; lycorine was able to down-regulate the expression of p-JAK2 and p-STAT3 by inhibiting HDAC in human acute myeloid leukemia K562 cells and human multiple myeloma U266, RPMI8226 and KM3 cells [111]. Lycorine was also capable of inhibiting the activation of JAK/STAT3 pathway via up-regulating the expression of SOCS1 in multiple myeloma cells [112].

Camptothecin (CPT) (Fig. 5), a quinoline alkaloid, was isolated from *Camptotheca acuminata*. It’s analogs such as irinotecan and topotecan are used clinically for the treatment of ovarian, colorectal and other cancers by inhibiting topoisomerase-I (Top-I) [113]. Moreover, CPT is also able to inhibit the phosphorylation of JAK2 and STAT3; the level of JKA2 is closely associated with the sensitivity of CPT in colon cancer cells [114].

### 7. Discussion

Table 1 (Ref. [25–41, 46–70, 72–83, 90–98, 103–114]) summarizes the natural alkaloids and their main targets in protein kinase pathways. However, it should be kept in mind that some of the alkaloids are able to target multiple pathways related to protein kinases. EGFR plays critical roles in WM130 induced growth inhibition of cancer cells. However, PI3K/Akt is also involved in WM130 induced growth inhibition of cancer cells [91]. NC treatment lead to down-regulation of AKT phosphorylation and inhibition of metastasis in several cancer cells [63]. NC also inhibited the activation of STAT3 and ERK pathways in human hepatocellular carcinoma HepG2, HCCLM3, and Huh7 cells [64]. Both EGFR and PI3K/AKT were involved in CVB-D induced inhibitory effect on cancer cells [111].

Although the pathways of PI3K/Akt/mTOR, JAK/STAT, EGFR and p38 MAPK account for most of the protein kinase enzymes, there are still many other protein kinases responsible for the occurrence, development, proliferation, invasion, metastasis and mitosis of cancer cells. A ser/thr protein kinase, protein kinase C (PKC) plays very important roles in regulating a variety of cellular functions in cell development and proliferation. Several pathophysiological conditions including cancers are development by the dysregulation of PKC [115]. A benzophenanthridine alkaloid, chelerythrine isolated from Chelidonium majus (L), acting as a PKC inhibitor, was able to induce apoptosis significantly in Dalton’s lymphoma cells [116]. The combination of berberine (10 μM) and AS2O3 (5 μM) inhibited the growth, migration and invasion of human malignant glioma U87 cells and decreased the expression of PKC α and ε [117]. CDKs, an important class of protein kinases, control key transcriptional events and critical cell cycle checkpoints in response to intracellular and extracellular signals. CDK inhibitors also act as an important class of targets for developing novel anticancer agents [118]. A vitality of alkaloids targeting the growth of CDKs-mediated cancer cells have been identified as newly discovered anticancer agents. Rohitukine, a chromone alkaloid isolated from *Amoora rohituka* (Roxb.), exhibited inhibitory effect on the growth of leukemia HL-60 and Molt-4 cells through down-regulating the expression of CDK2/A and CDK9/T1 [119]. An oxaoporphine alkaloid, lycasine isolated from *Asimina triloba* inhibited the growth of hepatoma HepG2 and lung cancer NCI-H460 cells. Lycasine induced S-phase arrest via down-regulation of the expression of cyclin A2/B1/D1/E1 and CDK2/6 [120]. FLT3, a type III receptor tyrosine kinase acts as a proto-oncogene involved in crucial steps of haematopoesis such as proliferation, differentiation and survival. In recent years, FLT3 has been developed as an important marker in different haematological malignancies [121]. Homoharringtonine (HHT), an alkaloid isolated from Cephalotaxus species, was able to suppress the expression of FLT3/p-FLT3 and inhibit the growth of human AML MV4-11 and MOLM-13 cells in nanomolar level [122]. A benzyalmine alkaloid, 2-acetyl-benzylamin, isolated from *Adhatoda vasica*, displayed potent anticancer effect by down-regulation of JAK2 and FLT3. Over the past decades, the development of protein kinase inhibitors as novel anticancer drug has advanced dramatically, and many kinase inhibitors have been approved by the US FDA to treat various human disorders; among them more than forty protein kinase inhibitors are used for cancer treatment [4]. Even though significant ad-
Table 1. Summary of alkaloids as protein kinase inhibitors and their specific targets.

<table>
<thead>
<tr>
<th>Name of Alkaloids</th>
<th>Sources</th>
<th>Targets and cell lines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrocorydaline</td>
<td><em>Corydalis yanhusuo</em></td>
<td>MEK1/2 and ERK1/2, A375, MV3 cells</td>
<td>[25–29]</td>
</tr>
<tr>
<td>Aconitum szechyanum Gay</td>
<td><em>Aconitum pendulum Busch</em></td>
<td>SMCC-7721, SGC-7901, Eca-109, HepG2, Hela and A549 cells</td>
<td>[30–32]</td>
</tr>
<tr>
<td>3α-acetonyltabersonine</td>
<td><em>Melodinus suaveolens</em></td>
<td>p38 MAPK</td>
<td>[33–35]</td>
</tr>
<tr>
<td>Isoliensinine, liensinine and neferine</td>
<td><em>Nelumbo nucifera Gaertn</em></td>
<td>p38 MAPK</td>
<td>[36–38]</td>
</tr>
<tr>
<td>Berberine</td>
<td><em>Coptis chinensis</em></td>
<td>p38 MAPK</td>
<td>[39–41]</td>
</tr>
<tr>
<td>Tetrandrine</td>
<td><em>Stephania tetrandra S.Moore</em></td>
<td>P38 MAPK</td>
<td>[46–49]</td>
</tr>
<tr>
<td>Cepharanthine</td>
<td><em>Stephania cepharantha Hayata</em></td>
<td>p38 MAPK</td>
<td>[50, 51]</td>
</tr>
<tr>
<td>Piperlongumine</td>
<td><em>Piper longum</em></td>
<td>p38 MAPK</td>
<td>[52–59]</td>
</tr>
<tr>
<td>Veratramine</td>
<td>Plants of the lily family</td>
<td>p38 MAPK</td>
<td>[60–62]</td>
</tr>
<tr>
<td>Nitidine chloride</td>
<td><em>Zanthoxylum nitidum (Roxb) DC</em></td>
<td>U87, U251, 786-O and A498 cells</td>
<td>[63–66]</td>
</tr>
<tr>
<td>Magnoflorine</td>
<td><em>Coptidis Rhizoma</em></td>
<td>AKT/mTOR</td>
<td>[67–70]</td>
</tr>
<tr>
<td>Cyclovirobuxine D</td>
<td><em>Buxus microphylla</em></td>
<td>EGFR, MGC-803, MKN28, HepG2 and HCCLM3 cells</td>
<td>[72–76]</td>
</tr>
<tr>
<td>Oxymatrine</td>
<td><em>Sophora flavescens.</em></td>
<td>EGFR and VEGF, PAN-1 and U251MG cells</td>
<td>[77–83]</td>
</tr>
<tr>
<td>WM130</td>
<td><em>Sophora flavescens.</em></td>
<td>EGFR and ERK, Hah7 cells</td>
<td>[90, 91]</td>
</tr>
<tr>
<td>Noscapine</td>
<td><em>Papaver somniferum</em></td>
<td>EGFR, MGC-803, HepG2 and HCCLM3 cells</td>
<td>[92–98]</td>
</tr>
<tr>
<td>Dentatin</td>
<td><em>Murraya koenigii</em></td>
<td>JAK/STAT</td>
<td>[103–107]</td>
</tr>
<tr>
<td>Lycorine</td>
<td><em>Amaryllidaceae</em></td>
<td>PC-3M, DU145, 22RV1, U256, RPM18226 and KM3 cells</td>
<td>[108–112]</td>
</tr>
<tr>
<td>Camptothecin</td>
<td><em>Camptotheca acuminata</em></td>
<td>JAK/STAT</td>
<td>[113, 114]</td>
</tr>
</tbody>
</table>

Advancement has been made in the development of kinase inhibitors as novel anticancer agents, the drug resistance and off-targets hinder the application of these kinds of drugs. Several approaches have been developed to overcome the drug resistance or off-targets of protein kinase inhibitors; targeting alternative binding sites on the protein kinases or affecting other pathways that are required for the kinase transformation [123]. Nuciferine, an alkaloid isolated from *Nelumbo nucifera* and *Nymphaeaceaeulera*, was able to inhibit the tumor growth via inhibiting P3K/akt/mTOR pathway, and the compound can also reverse the drug resistance via inhibiting the P-gp expression in several cancer cells [124]. Bisindolylmaleimide alkaloid (BMA), isolated from *Streptomyces staurosporeus*, is considered as a PKC inhibitor, and a newly synthesized BMA analogue, BMA097, exhibits anticancer effects both in vitro and in vivo. BMA is able to bind directly to the SH2 domain of STAT3 and inhibit the phosphorylation of STAT3 [125]. These drugs provide an alternative approaches for overcoming the drug resistance associated with protein kinase inhibitors.

Isoquinoline represents one of the most important class of alkaloids in cancer therapy. The structure/activity relationship (SAR) of isoquinoline has been investigated and the result revealed that the substitutions of 11H-benzothieno [3, 2-b]quinoline-4-carboxamides and indololo[1,2-b]quinoline-6-carboxamides at the pseudo-peri position to the carboxamide side chain is crucial for its cytotoxicity against several cancer cells [126]. Moreover, in protoberberine alkaloids, substitution of methoxyl to hydroxy group at the C-9 position was very important for their anticancer efficacy [127]. DHC and BBR share similar
structure as that of isoquinoline and both of the compounds inhibited the cancer cell growth by inhibiting p38MAPK pathway significantly. CPT and its analogs as TOP-1 inhibitor display similar anticancer effect.

The anticancer alkaloids reviewed here are found from different kinds of natural organisms and display diverse mechanisms for their anticancer activities. Several anticancer agents developed from alkaloids exhibit unique anticancer mechanism. For example, 3α-acetonyltabersonine could inactivate the DNA damage-repair by inhibiting MAPK signaling pathway. In the past several decades, alkaloids from the nature resource have served as an important class of agents in the treatment of different human diseases. It should be predicated that in the future nature alkaloids will provides us more and more novel anticancer drugs with significant therapeutic efficacy and low side effects considering the wide distribution and broad activities of alkaloids.

8. Author contributions
XL and SC designed the work. HY, LW, LM, MI, GQ, JH, LC, YZ, XY, SC and XL collected and reviewed the references. HY wrote the first draft. XL and SC wrote and reviewed the final version of the manuscript. All authors discussed and contributed to the manuscript.

9. Ethics approval and consent to participate
Not applicable.

10. Acknowledgment
Not applicable.

11. Funding
The study was supported the International Collaborative Project of the MOST of China (No#: 2017YFE0195000). We are also grateful for the support by the Taishan Talents project of Shandong province and the Natural Sci. Foundation in Shandong Province of China (No#: ZR2020MH421, ZR2020MH420, ZR2020QH360).

12. Conflict of interest
The authors declare no conflict of interest.

13. References


[97] Pedersen MW, Tkach V, Pedersen N, Berezin V, Poulsen HS. Expression of a naturally occurring constitutively active variant of the epidermal growth factor receptor in mouse fibroblasts


**Keywords:** Alkaloids; Protein kinase inhibitors; Anti-cancer activity; MAPK; PI3K/AKT/mTOR; JAK/STAT

**Send correspondence to:** Shousong Cao, Department of Pharmacology, School of Pharmacy, Southwest Medical University, 646000 Luzhou, Sichuan, China, E-mail: shousongc@gmail.com