

# Therapeutic value of steroidal alkaloids in cancer: Current trends and future perspectives

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Discovery and development of new potentially selective anticancer agents are necessary to prevent a global cancer health crisis. Currently, alternative medicinal agents derived from plants have been extensively investigated to develop anticancer drugs with fewer adverse effects. Among them, steroidal alkaloids are conventional secondary metabolites that comprise an important class of natural products found in plants, marine organisms and invertebrates, and constitute a judicious choice as potential anti-cancer leads. Traditional medicine and modern science have shown that representatives from this compound group possess potential antimicrobial, analgesic, anticancer and anti-inflammatory effects. Therefore, systematic and

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**Abbreviations:**  $\Delta\psi_m$ : mitochondrial membrane potential; AChE: acetylcholinesterase; APC4: anaphase-promoting complex 4; ASA: anticancer steroidal alkaloid; ATG5: autophagy-related protein 5; BChE: butyrylcholinesterase; bFGF: basic fibroblast growth factor; Cap-NMR: capillary nuclear magnetic resonance spectroscopy; CCND1: cyclin D1; CDKN2B: cyclin-dependent kinase inhibitor 2B; CHOP/GADD153: C/EBP homologous protein/growth arrest- and DNA damage-inducible gene 153; CNS: central nervous system; CRPC: castration-resistant prostate cancer; EC<sub>50</sub>: half maximal effective concentration; ED<sub>50</sub>: effective dose, for 50% of individuals getting the drug; EIF-2: eukaryotic initiation factor-2; EP4: E-prostanoid receptor 4; ER: endoplasmic reticulum; ERK: extracellular signal regulating kinase; ERK1/2: extracellular signal-regulated kinase 1 and 2; FAK: focal adhesion kinase; FDA: Food and Drug Administration; GADD45 $\alpha$ : growth arrest and DNA damage-inducible protein GADD45  $\alpha$ ; GIT: gastrointestinal tract; HER2: human epidermal growth factor receptor; HPLC: high-performance liquid chromatography; HPLC-ESIMS: high-performance liquid chromatography-electrospray ionization mass spectrometry;  $\kappa B\alpha$ : inhibitor of  $\kappa B\alpha$ ; LC-MS: liquid chromatography-mass spectrometry; LC-NMR-MS: liquid chromatography-nuclear magnetic resonance spectroscopy-mass spectrometry; LC-SPE-NMR: liquid chromatography-solid-phase extraction-nuclear magnetic resonance spectroscopy; LPS: lipopolysaccharide; MAP-2: microtubule-associated protein 2; Mcl-1s: Mcl-1 short form; MS: mass spectrometry; MUC1: Mucin 1; NF- $\kappa B$ : nuclear factor kappa B; NMR: nuclear magnetic resonance spectroscopy; NSCLC: non-small cell lung cancer; p38 MAPK: p38mitogen-activated protein kinase; PARP-1: Poli (ADP-Ribose) Polymerase-1; PKC $\alpha$ : protein kinase C- $\alpha$ ; RECK: reversion-inducing cysteine-rich protein with Kazal motifs; SAs: steroidal alkaloids; SCUBA: self-contained underwater breathing apparatus; SKP2: S-phase kinase-associ. protein 2; SMAC/DIABLO: second mitochondrion-derived activator of caspases/direct inhibitor of apoptosis-binding protein with a low isoelectric point; SMO: smoothed receptor; STAT3: signal transducer and activator of transcription 3; TAR: transactivation-responsive; TIMP-1: tissue inhibitor of metalloproteinase-1; TKIs: tyrosine kinase inhibitors; TNF $\alpha$ : tumor necrosis factor  $\alpha$ ; TopoII $\alpha$ : topoisomerase II $\alpha$ ; TXNRD1: thioredoxin reductase 1; U-PA: urokinase-type plasminogen activator; VEGF: vascular endothelial growth factor

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recapitulated information about the bioactivity of these compounds, with special emphasis on the molecular or cellular mechanisms, is of high interest. In this review, we methodically discuss the *in vitro* and *in vivo* potential of the anticancer activity of natural steroidal alkaloids and their synthetic and semi-synthetic derivatives. This review focuses on cumulative and comprehensive molecular mechanisms, which will help researchers understand the molecular pathways involving steroidal alkaloids to generate a selective and safe new lead compound with improved therapeutic applications for cancer prevention and therapy. *In vitro* and *in vivo* studies provide evidence about the promising therapeutic potential of steroidal alkaloids in various cancer cell lines, but advanced pharmacokinetic and clinical experiments are required to develop more selective and safe drugs for cancer treatment.

## Introduction

Small molecules containing a steroidal structure possess a wide range of pharmacological activities (e.g., antioxidant, neuroprotective and anti-hypercholesterolemic), irrespective of their highly conserved chemical structures and hormonal action (e.g., glucocorticoids, mineralocorticoids and gonadal steroids).<sup>1,2</sup> Various natural products have traditionally been used to treat or cure different diseases.<sup>3–9</sup> Recently, extensive studies have used natural and synthetically derived compounds to develop novel preventive or therapeutic agents for clinical applications for cancer treatment.<sup>10–16</sup> Over 60% of the available anticancer drugs are derived from natural sources, and this underlies the importance of these studies.<sup>17</sup> Understanding the structure–activity relationships and traditional applications of natural product-derived compounds can facilitate the design and synthesis of a vast array of novel therapeutics.

Prospective natural sources for drug discovery can be terrestrial or marine; those of terrestrial origin (plants, microorganisms, vertebrates and invertebrates) are easily accessible and offer a promising foundation for potentially new chemical entities (NCE). According to the World Health Organization (WHO), approximately 80% of the global population (representing approximately four billion people) living in developing countries depend on plant-based traditional medicines for their primary health care.<sup>18,19</sup> Ethnomedicinal properties of traditional medicines, along with their diverse medical applications in various diseases, have been well-characterized in several publications.<sup>20–26</sup> Goat bitter apple or soda apple (*Solanum aculeastrum*), thorn apple (*Solanum incanum* L.), tomato (*Lycopersicon esculentum* M.), false daisy (*Eclipta alba* L.) and Christmas box (*Sarcococca saligna*) are well-known plants possessing anticancer activity. Solamargine, tomatidine, solasonine,  $\alpha$ -solanine and  $\alpha$ -chaconine are natural products isolated from terrestrial plants and exhibit potent anticancer activity (Supporting Information S1, Tables 1–4). In addition to anticancer activity, steroidal alkaloids (SAs) also possess analgesic,<sup>27,28</sup> anti-inflammatory,<sup>27,29–31</sup> antimicrobial,<sup>32–40</sup> antithrombotic,<sup>41</sup> antiandrogenic<sup>42</sup> and antiarrhythmic<sup>43–45</sup> properties, which deem them a subject of high research interest.

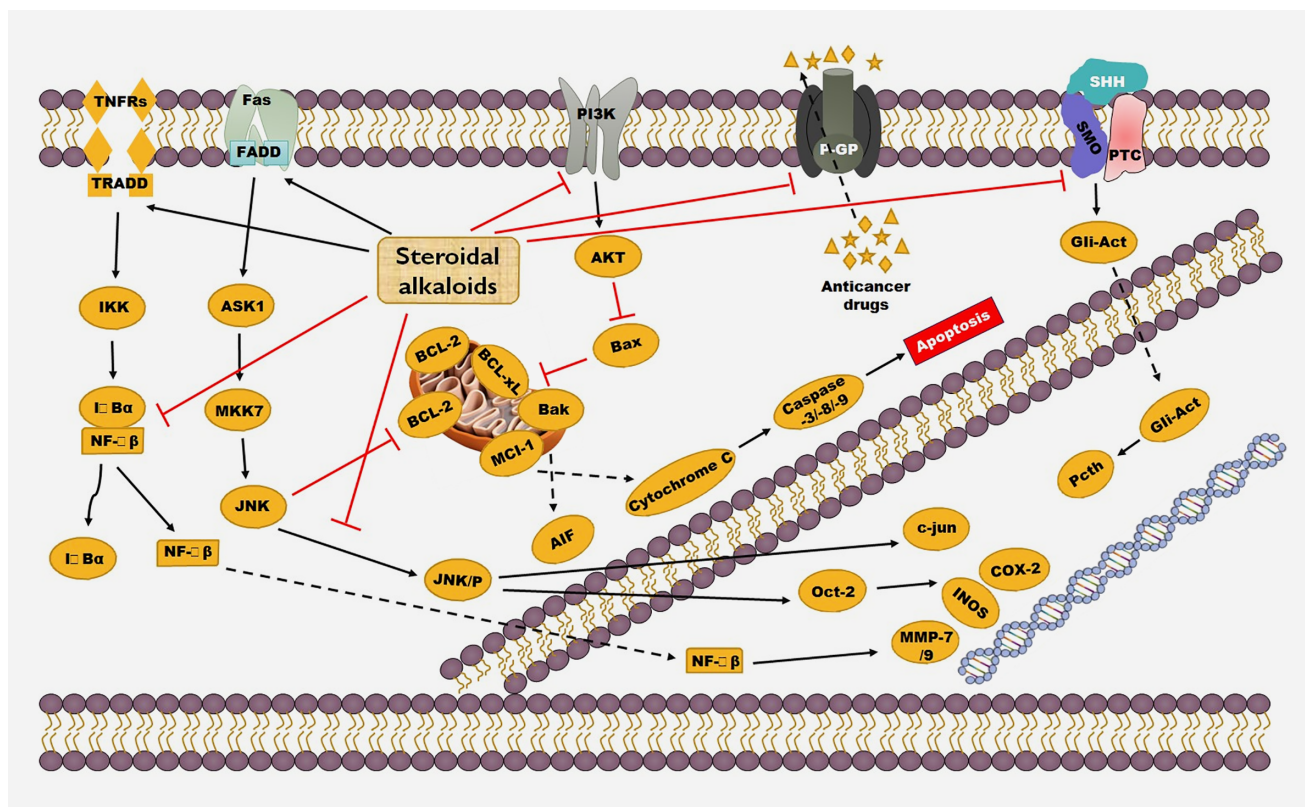
Conversely, marine organisms comprise ~50% of the total biodiversity of the Earth, and the marine ecosystem is an exceptional and vast reservoir of bioactive natural products with

unique chemical features.<sup>46</sup> Although few marine-derived drug candidates are clinically available, significant numbers of anticancer SAs (ASAs) have been discovered from marine sources.<sup>47</sup>

Over 10,000 bioactive compounds have been discovered from the marine ecosystem, and hundreds of novel compounds with innumerable pharmacological properties are identified annually.<sup>48</sup> Notably, sponges and worms are significant sources of ASAs. Although few ASAs have been obtained from the marine environment to date, among them, cephalostatin 1 is one of the most potent anticancer small molecules ever examined by the U.S. National Cancer Institute (NCI). Cephalostatin I is active at nanomolar concentrations and is noticeably more effective *in vitro* than paclitaxel in dispelling the mitochondrial membrane potential to induce apoptosis.<sup>49–51</sup>

Marine sources show great promise for the discovery of anticancer drugs, and the involvement of interdisciplinary fields, including pharmaceutical chemistry, pharmacology, analytical and organic chemistry, ecology, biology and biochemistry, has established marine anticancer drug discovery as a new field. Approximately seven marine-derived pharmaceutical elements have been approved by the FDA (Food and Drug Administration) for clinical use as drugs for various diseases. Brentuximab vedotin was approved for the treatment of Hodgkin's lymphoma and anaplastic large T-cell malignant lymphoma, cytarabine for leukemia, trabectedin for ovarian cancer and soft tissue sarcoma, eribulin mesylate for metastatic breast cancer, Ziconotide for pain, omega-3-acid ethyl esters for hypertriglyceridemia, vidarabine for recurrent epithelial keratitis caused by HSV, superficial keratitis and acute kerato-conjunctivitis; many other compounds are undergoing clinical trials.<sup>47,52–56</sup> Clinical trials involving cephalostatins 1 and 7 have been hindered because of the complications in harvesting the materials (0.1 g and <60 mg of cephalostatins 1 and 7, respectively, from 450 kg of the marine tube worm (*Cephalodiscus gilchristi*) from 60 to 80 m deep in sea waters near East Africa.<sup>57</sup> Synthesis of cephalostatin will render the bis-steroidal alkaloid more available and allow clinical trials. To expedite the design and development of cephalostatin, the identification and validation of the biological targets of cephalostatin should be coupled with quantitative structure–activity relationship (QSAR) studies.

To date, various articles have been published regarding the isolation, synthesis and anticancer effects of SAs; however, to



**Figure 1.** Anticancer mechanism of steroidal alkaloids. Reproduced with permission from the publisher of Ref. 54. Copyright © 2015, John Wiley and Sons.

the best of our knowledge, a review focusing on the mechanisms of action of SAs and their synthetic/semi-synthetic derivatives in various cancer cell lines, including their sources and inhibitory concentrations, has not yet been published. Secondary metabolites from natural products exhibit more “drug-likeness” (a qualitative concept for how “druglike” a compound is with respect to various parameters such as molecular weight, hydrogen bond and bioavailability, used in the field of drug design) properties than wholly synthetic molecules.<sup>58</sup> Therefore, we summarized the available information regarding these ASAs to assist future efforts toward anticarcinogenic drug discovery.

In this review, we systematically summarize the *in vitro* (Supporting Information S1, Tables 1–3) and *in vivo* (Supporting Information S1, Table 4) anticancer effects of natural, synthetic and semi-synthetic SAs, with emphasis on their mechanisms of action. Hereafter, we also highlight the molecular mechanisms involved in their anticancer effects (Supporting Information S1, Tables 1–4; Fig. 1), along with various *in silico* approaches involved in the development of anticancer leads from SAs.

## Steroidal Alkaloids

### Pharmacological properties

Steroidal alkaloids are secondary metabolites, which can be defined as organic molecules involved in the defense mechanisms of plants, with no role in growth, development or

reproduction. In addition to SAs, terpenes, phenolic compounds, flavonoids and coumarins are known as secondary metabolites.<sup>59–61</sup> They constitute an essential class of alkaloids generally isolated in the glycoalkaloid form, which characteristically occurs in higher plants from the Solanaceae, Liliaceae, Apocynaceae and Buxaceae families, and in amphibians and marine invertebrates (Fig. 2).<sup>62–64</sup> In addition to their different bio-potentialities, including analgesic,<sup>28</sup> antimicrobial,<sup>39</sup> and anti-inflammatory<sup>29</sup> activities, their anticancer potentials are being explored.<sup>65–67</sup> Numerous synthetic routes have also been developed for synthesizing SA analogs to achieve desired biological effects. Although there are multiple reports regarding the widespread pharmacological activities of SAs, few systematic reviews regarding the anticancer potentials and mechanisms of action of SAs are currently available.

### Structural insights and classification

SAs are characterized by a cyclopentanephenanthrene framework (steroidal scaffold), with a nitrogen atom fused as a fundamental fragment of the molecule, or in the ring or side chain.<sup>62</sup> Generally, SAs possess a 21-(pregnane), a 24-(cyclopregnane), a 27-carbon heterocyclic ring or combination of these. The C27 heterocyclic ring containing SAs are of two types: cholestane and C-nor-D-homosteroidal alkaloids, mostly occurring in the Solanaceae and Liliaceae families. Table 1 lists the different types of natural SAs.<sup>68</sup>

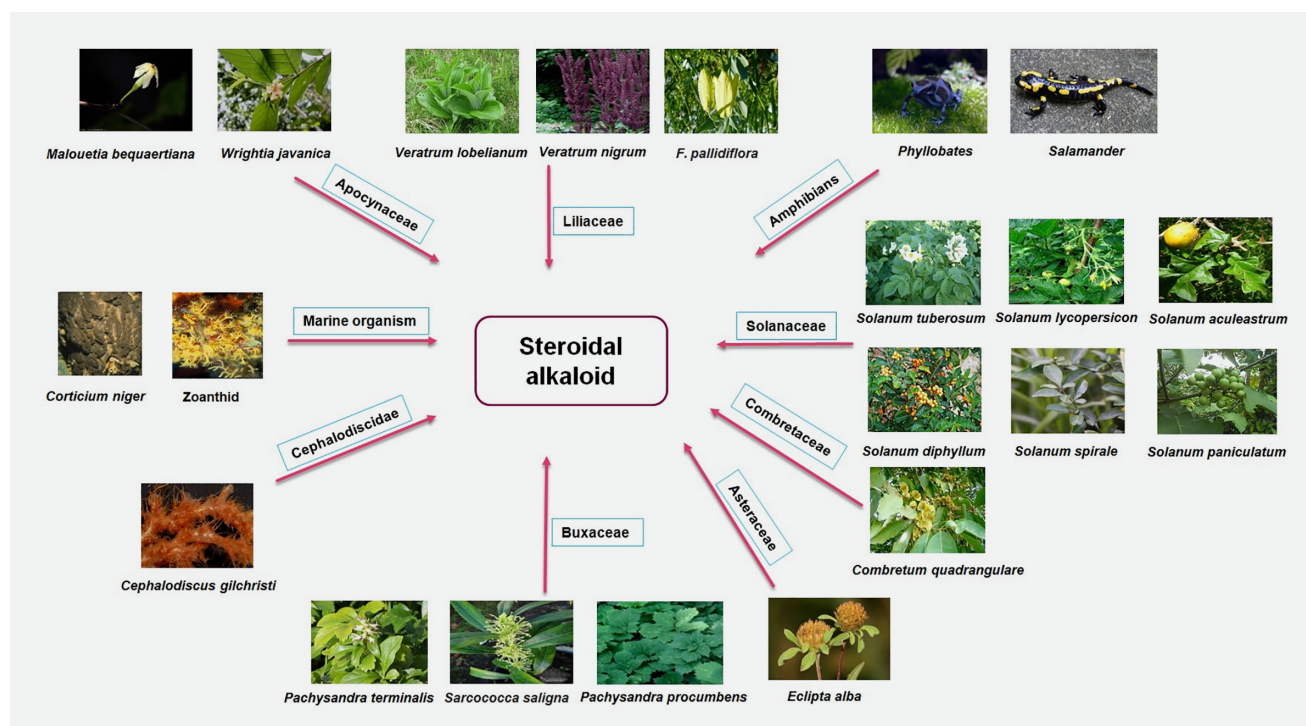


Figure 2. Vital sources of steroidal alkaloids. Reproduced with permission from the publisher of Ref. 54. Copyright © 2015, John Wiley and Sons.

### Mode of action of the anticancer activity exerted by SAs

**Induction of apoptosis.** Induction of apoptosis is one of the essential anticancer mechanisms of SAs. Apoptosis, or programmed cell death, is a highly synchronized and conserved cellular phenomenon maintained by a highly organized network of intrinsic cellular suicide machinery. When the homeostasis between cell proliferation and death is disturbed, apoptosis-inducing pathways are altered, which results in oncogenesis.<sup>69</sup> Receptor-controlled extrinsic pathways or mitochondrion-controlled intrinsic pathways can trigger apoptosis.<sup>70</sup> Additionally, other cellular organelles, including the endoplasmic reticulum (ER), cytoskeleton, lysosomes and nucleus, might participate in apoptotic communication by detecting impairments or by incorporating pro-apoptotic signals.<sup>71</sup> Various SAs activate apoptosis in specific cancer cell lines, including brioflin [increases Bax protein expression, suppresses Bcl-2, caspase-3 initiation and segmentation of poly (ADP-ribose) polymerase-1 (PARP-1) in HeLa cells], the 3-*O*-( $\beta$ -D-glucopyranosyl) etioline-induced receptor-mediated extrinsic apoptotic pathway, solamargine (upregulates TNFR-1), cephalostatin 1 (uses Smac/DIABLO for inducing apoptosis) (Supporting Information S1, Tables 1–4).<sup>50,51,57</sup>

**Cell cycle arrest.** Disruption of cell cycle progression plays a role in the anti-oncogenic effect of SA (Supporting Information S1, Tables 1 and 2). Mitogenic signals permit cells to enter controlled pathways, allowing traversing of the cell cycle.<sup>72</sup> CDKs are master regulators in the cell cycle.<sup>73,74</sup> Dysregulation or modification of

CDKs results in neoplasia.<sup>72</sup> Hyperactivation of CDKs results from mutations in CDK-regulated genes; CDK-inhibitor genes are linked to several cancer types. Thus, the inhibitors or modulators offer great promise for use in novel anticarcinogenic therapies.<sup>75,76</sup> SAs such as solamargine, ritterazine b and solanidine derivatives arrest the G<sub>0</sub>/G<sub>1</sub> and G<sub>2</sub>/M checkpoints in cancer cells (Supporting Information S1, Tables 1 and 2).<sup>30,50,77–81</sup>

**Anti-proliferative and anti-metastatic effects.** The molecular mechanism underlying the antiproliferative effects of SAs might involve the inhibition of various cell signaling pathways and proteins (such as MMP-2/9 and AKT) that allow the growth of cancer cells.<sup>82–88</sup> Inhibition of MMP-2 and MMP-9 by solanine is associated with the suppression of A2058 human melanoma cell migration and invasion, which contributes to the anti-metastatic activity of SAs.<sup>89</sup> Akt (protein kinase B) phosphorylation, which regulates various proteins involved in metastasis and cancer cell proliferation, is inhibited by  $\alpha$ -tomatine.<sup>90–93</sup> The anti-proliferative activity of  $\alpha$ -tomatine also involves an increase in p21WAF1/CIP1 levels and checkpoint kinase-2 activation (Supporting Information S1, Tables 1, 3 and 4). Additionally, SAs such as solasodine and cephalostatin 1 can induce DNA fragmentation and inhibit protein synthesis (Supporting Information S1, Tables 1, 2 and 4).<sup>50,51,57,80,94–100</sup>

### Anticancer effects of major SAs

**Tomatidine.** Goat bitter apple or soda apple (*S. aculeastrum*) and tomato (*S. lycopersicon* L.) are rich sources of tomatidine

and its glycosides such as  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tomatine.<sup>101</sup> Tomatidine exerts cytotoxic activity against HBL-100 cells.<sup>102</sup> MCF-7, HT-29 and HeLa cells were blocked at the G<sub>0</sub>/G<sub>1</sub> phase after treatment with tomatidine.<sup>103</sup> Tomatidine also obstructs the invasion and migration of A549 cells by upregulating TIMP-1 and RECK and downregulating MMP-2/9 expression.<sup>66</sup> By inhibiting the function of ABC transporters, tomatidine efficiently sensitizes carcinoma cells.<sup>104</sup>

Tomatidine glycosides are more cytotoxic against MDA-MB-231, KATO III and PC-3 cells, compared to tomatidine,<sup>105</sup> proving that the carbohydrate moiety plays a pivotal role in its cytotoxicity.  $\alpha$ -Tomatine exerts significant cytotoxicity against MCF-7, HL60, NCI-H460, AGS, K562 and HT-29 cells.<sup>106–108</sup> Lee *et al.*, 2011 reported that  $\alpha$ -tomatine treatment for 1 h kills PC-3 cells, compared to the normal prostate and liver cells.<sup>92</sup> The half-maximal effective concentration (EC<sub>50</sub>) of  $\alpha$ -tomatine for PC-3 cancer cells was  $1.67 \pm 0.3 \mu\text{M}$ , whereas those for the normal liver cells WRL-68 and the normal prostate cells RWPE-1 was higher ( $>5.0$  and  $3.85 \pm 0.1 \mu\text{M}$ , respectively) for 24-h-long treatment. The anti-metastatic activity of  $\alpha$ -tomatine has been well characterized through the inhibition of the signaling of the PI3K/Akt pathway, inhibition of FAK phosphorylation and modulation of I $\kappa$ B $\alpha$  protein expression.<sup>108</sup>  $\alpha$ -Tomatine can activate cellular apoptosis *via* caspase-3/–8/–9, Mcl-1 and Bak activation.<sup>92</sup>

**Solamargine and solasonine.** Solamargine and solasonine are two important spiroalane SAs. Solamargine exhibits selective and significant cytotoxic effects against HepG2, HCT116, MCF-7, HeLa, K562 and A549 cells, with an IC<sub>50</sub> value of 2.5, 3.8, 2.1, 6.0, 5.2 and 8.0  $\mu\text{M}$ , respectively, which are 2–3-fold lower than that for HL7702 human normal hepatocytes and H9C2 rat normal cardiomyoblasts (IC<sub>50</sub>, 13.5 and  $>20 \mu\text{M}$ , respectively).<sup>77</sup> Shiu *et al.*, 2007 reported that solamargine exhibited more cytotoxicity on breast cancer cells, compared to cyclophosphamide, methotrexate, epirubicin, cisplatin and 5-fluorouracil.<sup>78</sup> Furthermore, intravenous injection of solamargine (2.4 mg/kg) inhibited the progression of hepatocellular H22 cancer cells and Ehrlich ascites tumors in mice by 57.37% and 67.55%, respectively.<sup>79</sup>

**Solasodine.** Similarly, solasodine and its derivatives exert effective anticancer action on various human cancer cells, including colon, hepatocellular, lung, cervical, gastric, glioblastoma and breast cancer.<sup>80,94–97</sup> Solasodine hydrochloride monotherapy (intraperitoneal injection; 30 and 50 mg·kg<sup>–1</sup>·day<sup>–1</sup> of total 14 days) can reduce multidrug-resistant sarcoma S180 tumor growth by 67.4% and 80.1%, respectively, by boosting LAK (lymphokine-activated killer cell) and NK (Natural Killer) cell activity, stimulating lymphocyte proliferation and improving IL-2 production.<sup>98</sup> In mice, solasodine hydrochloride (15 mg/ml) combined with cyclophosphamide (20 mg/ml) can significantly reduce multidrug-resistant sarcoma S180 tumor growth, by inducing apoptosis,

followed by the downregulation of topoisomerase II and P-gp expression.<sup>99</sup>

Apoptosis induction exerts anticancer effects.<sup>109</sup> Similarly, the treatment of human osteosarcoma 1,547 cells with solasodine can induce apoptosis.<sup>100</sup> Solamargine can also induce apoptosis by modulating tumor necrosis factor receptor (TNFR) expression,<sup>80</sup> upregulating caspase-3/–9 and downregulating Bcl-2 and Bcl-xL expression<sup>78,80,81</sup> in lung cancer cells.

**$\alpha$ -Solanine and  $\alpha$ -chaconine.**  $\alpha$ -Solanine and  $\alpha$ -chaconine are important cholestane SAs. They possess almost similar structures, except side chains, where  $\alpha$ -solanine comprises glucose, galactose and rhamnose molecules, and  $\alpha$ -chaconine is constituted of glucose and two rhamnose molecules.<sup>110</sup> Tumor growth and metastasis was inhibited by  $\alpha$ -solanine *via* the downregulation of miR21 expression.<sup>111</sup> By inhibiting the phosphorylation of ERK1/2 and activating caspase-3,  $\alpha$ -chaconine can also induce apoptosis in the HT-29 cancer cell line.<sup>112</sup> PI3K/Akt and MAPK pathways regulate the expression of MMP-2/–9, and  $\alpha$ -solanine and  $\alpha$ -chaconine inhibit cancer cell invasion and migration by inhibiting Akt and ERK phosphorylation<sup>113</sup> and MMP-2/–9 activity.<sup>89</sup> The synergistic cytotoxic activities of  $\alpha$ -solanine and  $\alpha$ -chaconine are well established in HepG2 and AGS cells.<sup>114</sup>

**Pregnane alkaloids.** Various pregnane alkaloids display potent cytotoxic activity against several kinds of cancer cells. The role of sarcovagine D in PANC-1, SMMC-7721, HL60 and A549 cells is well established, with an IC<sub>50</sub> value of 0.96–16.69  $\mu\text{M}$ .<sup>115</sup> The migration of EGF-induced human breast cancer MB-MDA-231 cells was inhibited by the terminamines A–E and H isolated from Japanese pachysandra (*Pachysandra terminalis*).<sup>65</sup> Sarcovagine D and sarcocurinine A1 also exert potent anticancer activity against K562, PANC-1 and SK-BR-3 cells, with an IC<sub>50</sub> value of 2.25–5.00  $\mu\text{M}$ . *Wrightia javanica* contains another pregnane type SA, wrightiamine A, which acts as a cytotoxic agent against vincristine-resistant P388 cells.<sup>116</sup> SMMC-7721, MCF-7, HL60, SW480 and A549 cells become susceptible after treatment with veralkamine 3-(b-D-glucopyranoside), 6,7-epoxyverdine and 3-O-acetylveralkamine, isolated from *Veratrum taliense*.<sup>117</sup> Sarsaligenines A and sarsaligenines B also exert cell growth inhibitory activity in human leukemia HL60 cells with an IC<sub>50</sub> value of 2.87 and 3.61  $\mu\text{M}$ , respectively.<sup>115</sup>

**Cyclopamine.** Many studies have explored the cytotoxic potential of cyclopamine and its derivative in various cancer cell line (*in vitro* and *in vivo*) models. The cyclopamine C-nor-D-homosteroidal alkaloid has been characterized as an antagonist of the Hh signaling pathway and is teratogenic in animals.<sup>118</sup> In SMMC-7721, PLC/PRF/5 and Huh7 cells, cyclopamine is responsible for the induction of apoptosis *via* inhibition of the Shh signaling pathway and downregulation of Bcl-2 expression.<sup>119</sup> The molecular mechanism of apoptosis

Table 1. Examples of major SAs

Major types of SAs	Origin	Instances	Brief Chemistry of the representatives
Salamandra	Amphibians	Cycloneosamandione	In this type of steroidal alkaloid, the ring-A is altered, commonly into aza-homosteroids, except for Cycloneosamandione.
Jerveratrum Spirosolane Solanidine	Solanaceae, mainly found in food crops like potatoes, tomatoes	Jervine Soladulcidine Rubijervine	Here the side-chain of a C27 steroid has been transformed into a piperidine ring.  In this type, an extra ring is formed by C-16 to N bond formation.
Cerveratrum	Monocot families such as the Orchidaceae	Cevane	In this type of steroidal alkaloid, an abeo shift occurs from a procevine-type precursor, where the D ring has been expanded at the expense of the C ring. A widespread stereochemical difference has been observed within the basic cevane skeleton.
Conanine	Apocynaceae	Kurcholessine	Here almost all of the bases comprise an amino or an oxygen function at C-3 position. Some differences are observed in their skeletons such as methylation at the C-4 position.
Buxus		Cortistatin	This type of steroidal alkaloid is one of the largest groups, where the maximum structures are pentacyclic 4, 4, 14-trimethyl-9, 19-cyclopregnanes. Some of this group possess a tetracyclic system where the 9, 10 bond has been fused to give a seven-membered ring B. In some representatives, one or both carbon atoms attached to C-4 have been lost. Presence of a nitrogen function at C-3 and/or C-20 has been observed, which may be partially methylated, fully methylated or unmethylated. Slight structural differences remain to be isolated from various <i>Buxus</i> spp. In this group, the suffix letters used in the nomenclature designate the degree of methylation of the nitrogen atom(s).
Pregnane	Apocynaceae, Buxaceae and Didymelaceae		In this type, major representatives contain an amino group or related functional groups at C-3 or C-20, and regularly at other positions.
Cephalostatins/ ritterazines	marine worm Cephalodiscus gilchristi (Cephalostatin 1) and tunicate Ritterella tokioka (Ritterazine A)	Cephalostatin 1, Ritterazine A	The cephalostatin 1 structure is categorized by an asymmetric blending of the central pyrazine ring with two extremely oxygenated steroidal spiroketal subunits.  In ritterazine A, the left half is similar to that of cephalostatin 7, excluding an additional C-7' hydroxyl group. In the right half, the steroidal nucleus is totally rearranged.
Assorted steroidal alkaloids, including non-nitrogenous steroids connected by an ester or acetal bond to a nitrogen-containing unit	Marine sponges, toads,	Plakinamines, bufotoxin	In this group, the ester or acetal bond act as a linker between non-nitrogenous steroids and the nitrogen containing unit.

Abbreviation: SA, steroidal alkaloid.

induction by cyclopamine includes modulation of the AKT and ERK pathways.<sup>120</sup>

In prostate cancer, veratramine and jervine (structurally related to cyclopamine) exert anti-proliferative effects.<sup>121</sup> Veratramine inhibits cell proliferation and exerts cytotoxicity against PANC-1, NCI-H249, SW1990 and A549 cells, with an IC<sub>50</sub> value of 14.5, 8.5, 26.1 and 8.9  $\mu$ M respectively.<sup>67</sup> KAAD-cyclopamine selectively inhibits the Hh signaling

pathway by oncogenic mutations,<sup>122</sup> and exo-cyclopamine and its analogs also block the Hh signaling corridor in a Gli-dependent reporter assay.<sup>123</sup> Cyclopamine and KAAD-cyclopamine act as *in vitro* and *in vivo* anti-proliferative agents by targeting the smoothened (SMO) receptor.<sup>124</sup> Cyclopamine selectively targets and reduces Gli1 expression, resulting in the arrest of cell growth and induction of apoptosis in various leukemia and lymphoma cells.<sup>125</sup> Moreover,

cyclopamine displays noticeable anticancer effects in breast,<sup>126</sup> lung,<sup>127</sup> GIT/gastric, pancreatic, esophageal and biliary tract,<sup>128,129</sup> and prostate cancer;<sup>130</sup> leukemia;<sup>125</sup> and oral squamous cell carcinoma.<sup>131</sup>

**Cyclopregnane alkaloids.** Cortistatin A-D,<sup>117</sup> E-H<sup>132</sup> and J-L<sup>133</sup> exert antiproliferative activity against HUVECs, KB3-1, Neuro2A, K562 and NHDF cells; HUVECs; and HUVECs, respectively, with an IC<sub>50</sub> value of 2.3–14  $\mu$ M. Angiogenesis is the process of formation of new blood vessels from former blood vessels. Angiogenesis regulates tumor development and metastasis. Angiogenesis antagonists can be considered potential candidates as antitumor agents.<sup>133</sup> Cortistatin, a cyclopregnane alkaloid isolated from the Indonesian marine sponge *Corticium simplex* exerts cytostatic anti-proliferative action against HUVECs *via* inhibition of angiogenesis.<sup>133</sup> Among all cortistatins, cortistatin A exhibits discriminating anti-proliferative action against HUVECs, by inhibiting the migration and tubular development of HUVECs promoted by VEGF or bFGF.<sup>117</sup>

### Other Multifarious Pharmacological Activities of SAs

Apart from anticancer activity, different SAs possess various other pharmacological actions. For example, solasodine,  $\alpha$ -tomatine and neoverataline A and B possess antifungal activity;  $\alpha$ -chaconine, solanidine,  $\alpha$ -solanine and tomatidine exert anti-inflammatory activity; saliginnamide, N(a)-methyl epipachysamine-D and tomatidine show antibacterial activity; hookerianamide H and hookerianamide, N<sub>a</sub>-methylepipachysamine D and sarcovagine C exhibited antiplasmodial activity; solasodine showed anti-nociceptive and anti-estrogenic activity; (+)-(20S)-3-(benzoylamino)-20-(dimethylamino)-5 $\alpha$ -pregn-2-en-4 $\beta$ -ol and [(+)-(20S)-20-(dimethylamino)-3 $\alpha$ -(methylseneciylamino)-5 $\alpha$ -

pregn-12 $\beta$ -ol possess antiestrogenic activity; and cycloviobuxine showed cytoprotective effects.<sup>43–45,134,135</sup>

### Toxicity of SAs

The steroidal jerveratrum alkaloid cyclopamine (Fig. 3) and veratrum alkaloid jervine (Fig. 3) are potent teratogens that cause synophthalmia in sheep, rabbits, mice and hamsters.<sup>116,136–138</sup> Lipinski *et al.*, 2010 reported that cyclopamine and its semi-synthetic analog AZ75 can cause palate defects, lateral cleft lip and semilobar and alobar holoprosencephaly in mice, respectively.<sup>139</sup> Exposure to  $\alpha$ -chaconine might cause significant hepatotoxicity in normal human Chang liver cells.<sup>140</sup> In frog embryos, exposure to  $\alpha$ -chaconine and  $\alpha$ -solanine can induce neurological noxiousness (*viz.* spina bifida and other deformities).<sup>141</sup> According to Chaube *et al.*, 1976,<sup>141</sup> Smith *et al.*, 1996,<sup>142</sup> and Langkilde *et al.*, 2008,<sup>143</sup> the inhibition of cholinesterase enzyme activity in the human central nervous system (CNS) and cell membrane disruption in the gastrointestinal tract (GIT) may be attributed to the toxic effects of  $\alpha$ -chaconine and  $\alpha$ -solanine.<sup>144</sup> In hamsters, the teratogenic effects of solasodine may be attributed to the induction of spina bifida, exencephaly and cranial blebbing.<sup>145</sup> Structure–activity relationship analyses by Friedman *et al.*, 1991,<sup>141</sup> showed that the teratogenic effects of jervanes, solanidanes and spirosolanes were caused by C-5 and C-6 unsaturation in the steroidal skeleton and the olefinic linkage at C-5 and C-6 renders them more toxic. In pregnant and non-pregnant mice, three steroidal glycoalkaloids (solasodine, solanidine and tomatidine) induced hepatomegaly after being fed a diet containing these aglycones for 2 weeks.<sup>146</sup> Solanum potato glycoalkaloids, namely,  $\alpha$ -chaconine and  $\alpha$ -solanine, the aglycones solanidine and solasodine and the veratrum alkaloid jervine exert toxic effects in rainbow trout (*Oncorhynchus mykiss*) and the Japanese rice fish medaka (*Oryzias latipes*).<sup>147</sup>

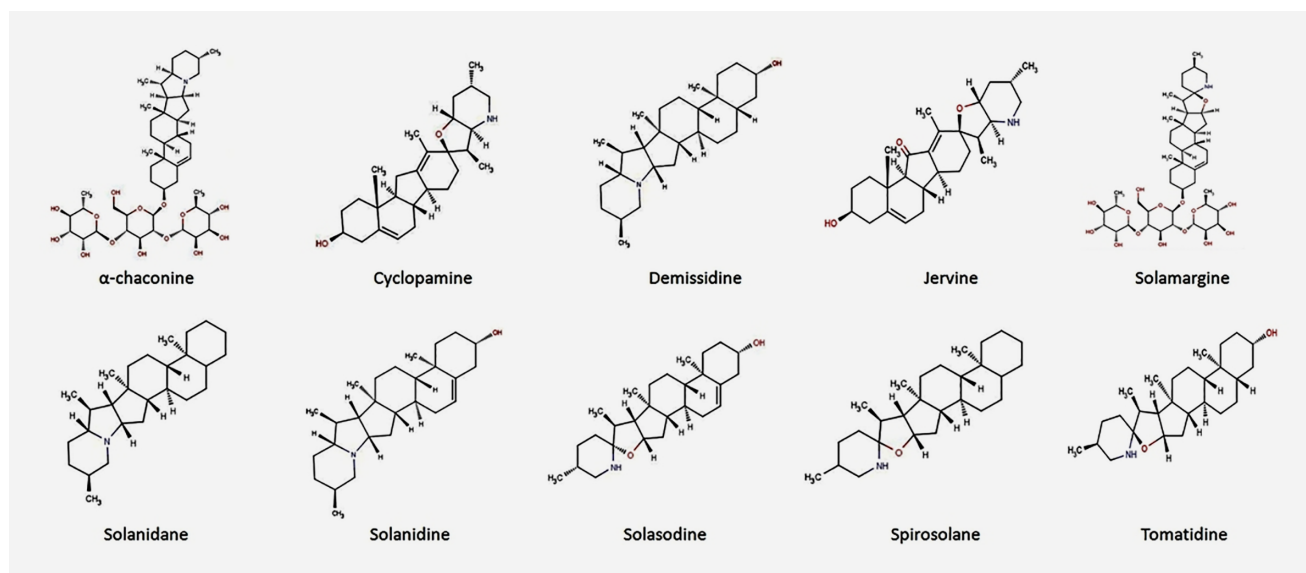


Figure 3. Chemical structure of typical steroidal alkaloids. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

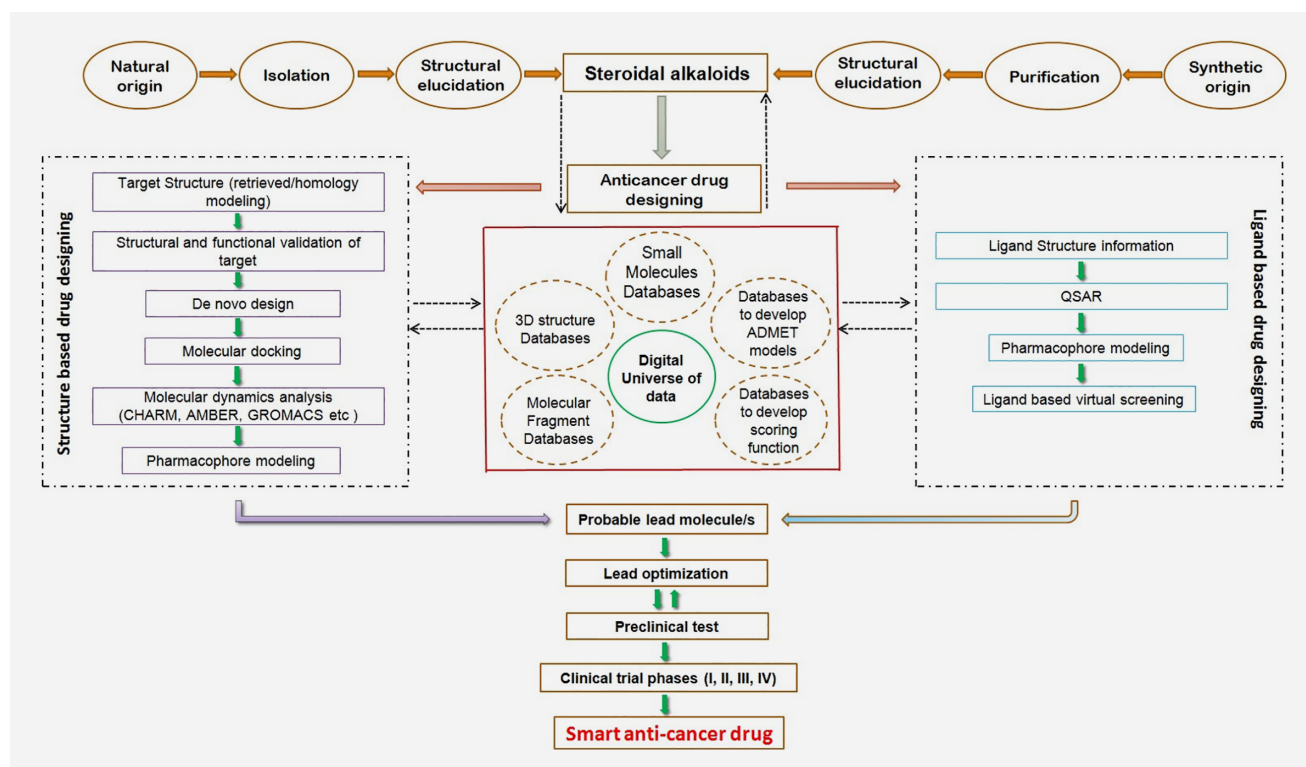


Figure 4. *In silico* approaches for smart anticancer drug development from steroidal alkaloids. [Color figure can be viewed at wileyonlinelibrary.com]

The SAs from potato peels (solanidine, demissidine,  $\alpha$ -chaconine and  $\alpha$ -solanine) are highly toxic to humans at concentrations of  $>1$  mg/g (dry weight).<sup>148,149</sup>

### ***In silico* approaches for exploiting anticancer leads from SAs**

Computer-aided drug design approaches are a promising area for the development of anticancer drug-like compounds using SA scaffolds. To avoid the huge costs and labor intensiveness incurred with random screening, different types of *in silico* approaches, including virtual screening, molecular modeling, molecular docking, QSAR, pharmacophore modeling, high-throughput screening, and cloud computing, are efficient and effective for anticancer drug discovery campaigns using SAs.<sup>150–152</sup> This technology has changed the scenario of rational design in the anticancer drug discovery process. Various web-based programs for QSAR [JRC QSAR Model Database, DemQSAR, OCHEM (Online Chemical Modeling Environment), and MC-3DQSAR] are available for the generation of potential leads.<sup>153–156</sup> For hit identification to lead optimization, structure and ligand-based virtual screening, other web-accessible servers and software (commercial/free versions; Supporting Information S2, Tables 1–3) are available. These computational methodologies provide a powerful toolbox for target identification, hit and lead generation and lead optimization. Synergism between dry and wet labs can facilitate the

discovery of cost-effective and reliable personalized medicines to improve anti-cancer drug discovery. Figure 4 is a flowchart illustrating the generation of smart anti-cancer drugs from SAs of natural and synthetic origins.

### **Recent trends and future directions**

Natural or synthetic secondary metabolites possess potent pharmacological activities. Among them, some have cleared the clinical trial parameters and are clinically available.<sup>157</sup> For instance, the FDA has approved vincristine, which is isolated from the leaves of the Madagascar periwinkle *Catharanthus roseus* (L.) G. Don (formerly *Vinca rosea* L.), for cancer treatment.<sup>158</sup> Dactinomycin, produced by *Streptomyces parvulus*, is administered as an antineoplastic antibiotic for the treatment of Kaposi's sarcoma, infantile fibrosarcoma and testicular cancer.<sup>159–161</sup> In 2005, paclitaxel (isolated from the bark of the Pacific yew (*Taxus brevifolia* Nutt.)) was approved by the FDA for use in the United States for the treatment of pancreatic, breast and non-small cell lung cancers.<sup>162</sup> In recent times, pharmaceutical companies have been focusing on drug discovery from natural and synthetic origins through combinatorial chemistry, which includes the generation of libraries containing millions of compounds,<sup>163</sup> *in silico* molecular docking, structure- and target-based virtual screening, molecular modeling, pharmacophore designing and receptor-based QSAR studies. Many



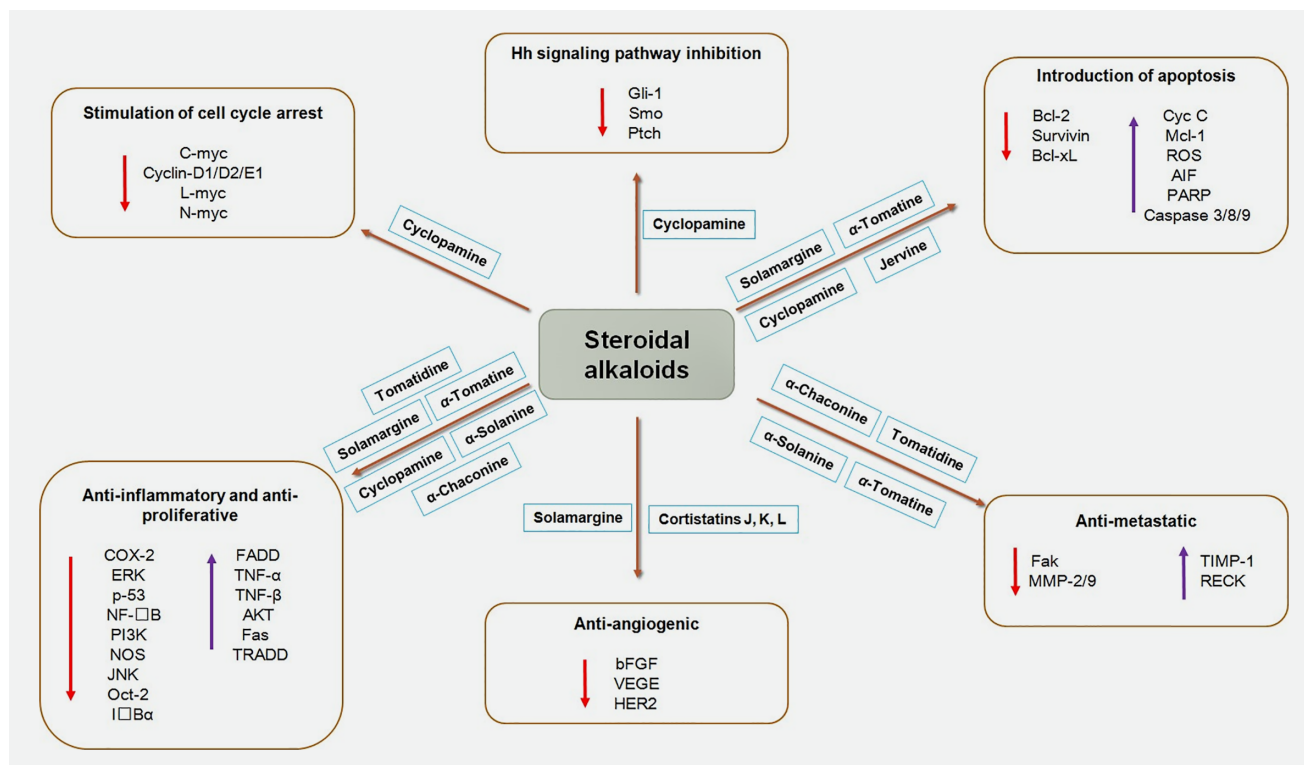


Figure 5. Steroidal alkaloids and major cellular signaling molecules. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

biotechnological companies also concentrate on lead identification and validation of natural products (or synthetic derivatives), and the development of these leads into drugs.<sup>163</sup> Numerous drugs of plant origin are currently undergoing clinical trials.<sup>164,165</sup>

Previously, drug discovery using natural sources was a time-consuming process, in particular, because of the recognition and structural elucidation of the bioactive compounds, which could take months or years. The rapidity of bioassay-guided fractionation, identification and structural elucidation of compounds can be significantly enhanced by using instruments and methods such as HPLC, MS or LC-MS, higher magnetic field-strength NMR, cap-NMR, LC-NMR-MS, LC-solid phase extraction (SPE)-NMR, LC-SPE-NMR in combination with HPLC-ESIMS, and robotics, to systematize high-throughput bioassays.<sup>166–169</sup>

Prompt screening of plant extracts for their biological activity is no longer a rate-limiting step because of the improvement of computerized high-throughput performances. Compounds with features such as autofluorescence or UV absorption often create problems in the screening process because of interference with the output, which can be overcome by pre-fractionation of the extracts.<sup>170</sup> Additionally, computational filtering methods have been applied with most high-throughput screening methods to minimize false-positive results.<sup>171</sup> In the future, dereplication can be implicated in the routine use of NMR-hyphenated techniques.<sup>168</sup> Furthermore, simple synthetic

methodologies can be implicated for bioassays, and novel analogs of the parent molecules can be designed and developed using upgraded combinatorial chemistry.

To meet the continuous demand, a constant and sufficient supply of anticancer drugs is essential. Plant cell culture is an alternative method for compounds with low synthetic yields, as altering the environmental conditions and medium can result in larger yield.<sup>172,173</sup> Paclitaxel and *Dioscorea* are successfully produced using cell culture techniques.<sup>174–177</sup>

### Summary and concluding remarks

The increasing incidence of cancer is a global concern. To avoid the toxicity associated with chemotherapy and irradiation, SAs are being investigated as alternative therapeutic measures. Most SAs are from different sources, and their mechanisms of action also vary. They can exert anticancer effects at micromolar or nanomolar concentrations (Supporting Information Files S1 and S2). This review discussed the potential of SAs as anticancer agents, with a focus on their natural and synthetic sources, *in vitro* or *in vivo* models for study, inhibitory concentrations and mechanisms of action.

The key molecular targets of SAs are summarized in Figure 5. They exert their anticancer effects by inducing apoptosis or autophagy by upregulating or downregulating apoptotic (Bax, Bcl2, Bcl-xL, Caspase-3/8/9, PARP, TNFR

I/II, Fas or HER2) and autophagic (LC3, AKT or mTOR) proteins. The inhibition of cell cycle progression at either G0/G1 or G2/M phase by interaction with CDKs is another mechanism underlying their anticancer activity. The antimetastatic action of SAs can be attributed to their interactions with, and the changes in the expression of ERK, RECK, TIMP and MMP-2/9 proteins. SAs such as solasodine block P-gp and inhibit multidrug-resistant cancers. They also disrupt the integrity of the cell membrane by changing cell morphology and DNA content, thereby exerting anticancer activity. SAs such as cyclopamine inhibits xenograft tumor growth *via* the Hh pathway;  $\alpha$ -tomatine reduces xenograft tumor growth by inducing apoptosis *via* interactions with survivin, p50 and p65. Apart from the potent anticancer and other pharmacological effects, some SAs exhibit toxic effects such as hepatotoxicity in normal human liver cells (Chang Liver cell line) and teratogenicity, palate defects and lateral cleft lips in sheep, rabbits, mice and hamsters. SAs such as  $\alpha$ -chaconine and  $\alpha$ -solanine are highly toxic to humans at concentrations of >1 mg/g (dry weight).

Although many plant-derived and synthetic SAs possess anticancer effects, the underlying molecular mechanisms remain to be established, predominantly with respect to the pharmacokinetics around the active site of the target protein. Such data is essential for clearance of standard clinical trial parameters and confirmation that these drugs are effective and safe. Understanding the crosstalk between SAs and associated signaling molecules will assist improved understanding of their molecular mechanisms of action and the development of anticancer drugs. Various biochemical and biophysical approaches, including co-crystallization and 3D-structure determination, can help understand the mechanisms underlying these interactions and develop more selective and less toxic anticancer drugs.

Combining existing chemotherapeutic drugs such as solamargine with cisplatin, MTX or 5-Fu can elevate the combined anticancer activity, thereby proving that combination therapy can exert synergistic or enhanced anticancer activity. *In silico* tactics, including molecular docking and QSAR studies, can be introduced to elucidate the molecular mechanisms, which can be validated by *in vitro* and *in vivo* experiments to aid significant development toward cancer prevention and treatment.

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### Cell line details

A2780 human ovarian cancer cell line  
 A431 skin epidermoid carcinoma  
 A549 human lung adenocarcinoma cell  
 AGS human Caucasian gastric adenocarcinoma

AGS human gastric cancer cell line  
 B16F10 murine melanoma  
 B16F10 murine melanoma  
 B16F2F mouse melanoma cell line  
 BGC-823 human stomach adenocarcinoma cell line  
 BPH-1 benign prostate hyperplasia epithelial cell line  
 C4-2B osteotropic prostate cancer cell line  
 DU145 human prostate adenocarcinoma cell line  
 GM07492A human lung fibroblasts  
 H1650 human lung cancer cells  
 H441 human adenocarcinoma  
 H460/multidrug-resistant counterpart of human NSCLC H460 cells  
 H520 squamous cell lung carcinoma  
 H661 large cell lung cancer  
 H69 small cell lung cancer  
 HBE human bronchial epithelial cell line SV40-transformed  
 HBL-100 human breast transformed epithelial cell line  
 HCT-116 colon cancer cell line  
 HeLa cervical carcinoma  
 HEP2 larynx cancer cell line  
 Hep3B hepatocellular carcinoma  
 HEPG2 liver cancer cell line  
 HL-60 human promyelocytic leukemia cell line  
 HL7702 normal hepatocyte  
 HT-29 colonic adenocarcinoma  
 HUVECs human umbilical vein endothelial cells  
 J16 human leukemia Jurkat T cells  
 K562/A02 multidrug-resistant counterpart of K562 cell line  
 K562 human myelogenous leukemia cell line  
 KB/VCR multidrug-resistant counterpart of KB cell line  
 KB squamous cell carcinoma cell line  
 KB3-1 KB epidermoid carcinoma cells  
 LLC Lewis lung carcinoma cells  
 M-109 Madison lung tumor  
 MCF-7 breast adenocarcinoma  
 MDA-MB-231 breast cancer cell line  
 MEL-28 human melanoma.  
 MG-63 human osteosarcoma  
 MGC-803 human gastric cancer cell line  
 MKN28 gastric cancer cell lines  
 MO59J human glioblastoma  
 MOLT-4 human leukemic T-lymphocytes cell line  
 NCI-H460 human lung large cell carcinoma  
 Neuro2A murine neuroblastoma cells.  
 NHDF normal human dermal fibroblast.  
 NIH3T3 mouse embryonic fibroblast cells  
 NSCLC-N6 human bronchopulmonary nonsmall-cell lung carcinoma cells  
 P388/ADM parental and the Adriamycin (doxorubicin)-resistant subline of mouse leukemia cells

P388/VCR	vincristine-resistant murine leukemia.	U2OS	human osteosarcoma
P-388	mouse lymphoid neoplasm.	U343	human glioblastoma
PC3	human prostate cancer cell line	U87	human primary glioblastoma cell line
RPE-1	human retinal pigment epithelial-1 cell line	V79	Chinese hamster lung fibroblasts.
RWPE-1	human normal prostate cell line	VERO	African green monkey kidney cell line
Saos-2	human osteosarcoma	WI-38	human lung epithelial fibroblast
SC115	Shionogi carcinoma	WM115	human melanoma cells line
SGC7901	gastric carcinoma	WM239	human melanoma cells line
SK-BR-3	breast carcinoma	WRL-68	human normal liver cell line
SMMC-7721	human hepatocarcinoma cell line	ZR-75-1	human breast cancer cell line
U251	human glioblastoma		

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