Advances and challenges in developing andrographolide and its analogues as cancer therapeutic agents

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Andrographolide (AGP), a naturally occurring bioactive compound, has been investigated as a lead compound in cancer drug development. Its multidimensional therapeutic effects have raised interest among medicinal chemists, which has led to extensive structural modification of the compound, resulting in analogues with improved pharmacological and pharmaceutical properties. Nevertheless, the analogues with the improved properties need to be rigorously studied to identify drug-like lead compounds. We scrutinised articles published from 2012 to 2018, to objectively provide opinions on the mechanisms of action of AGP and its analogues, as well as their potential as viable anticancer drugs. Preclinical and clinical data, along with the extensive medicinal chemistry efforts, indicate the compounds are potential anticancer agents with specific value in treating recalcitrant cancers such as pancreatic and lung cancers.

Introduction

Andrographolide (AGP) is the major bioactive compound present in *Andrographis paniculata*, a medicinal plant that has been widely used in complementary medicine, especially in Southeast Asia, China and India [1,2]. The compound possesses multidimensional therapeutic potentials such as anti-inflammatory and anticancer properties [3]. AGP (Fig. 1) is a labdane diterpene with an α,β-unsaturated γ-butyrolactone ring and two olefin bonds: $\Delta^{8(17)}$ and $\Delta^{12(13)}$. In addition, it has three hydroxyl (OH) groups at C-3 (secondary), C-14 (allylic) and C-19 (primary). As a bipolar compound, with the tendency to form H-bonds, AGP elicits biological responses by binding to substrates and/or receptors via its hydroxyl group terminus [4]. Poor selectivity alongside low solubility of AGP has been a major challenge affecting further development of the compound. Several attempts have been made to modify the structure of AGP to improve its potency and selectivity. To reduce random interaction with nontargeting sites or intramolecular (3- and 19-OHs) interactions, most studies have focused on modifying all or selected OH groups, for example esterification of C-3/14/19 OH groups using short- or long-chain aromatic or heterocyclic carboxylic acids and dicarboxylic acids [4].

Several attempts have been made to enhance the bioavailability of AGP through structural modification. Additionally, AGP presents a good modifiable pharmacophore for numerous pharmacological activities. Some analogues have exhibited improved bioavailability and enhanced therapeutic efficacy. Structure–activity relationship (SAR) studies on AGP revealed that an intact γ-butyrolactone ring, C12 = C13 and C8 = C17 double bonds, as well as the OH group at C-14 are crucial for sustenance of its cytotoxic activity [5]. To improve the potency and selectivity of anticancer activity of AGP, the compound has been subjected to systematic structural modifications in its side chains such as acylation of hydroxyls and introduction of a benzylidene moiety at C-3 and C-19 [2,6–10].

The early stages of cancer are usually asymptomatic. Upon initial diagnosis, most cancer patients are reported to be at an advanced stage [11]. Beside the surgical removal of tumours, chemotherapy is one common alternative for treatment of

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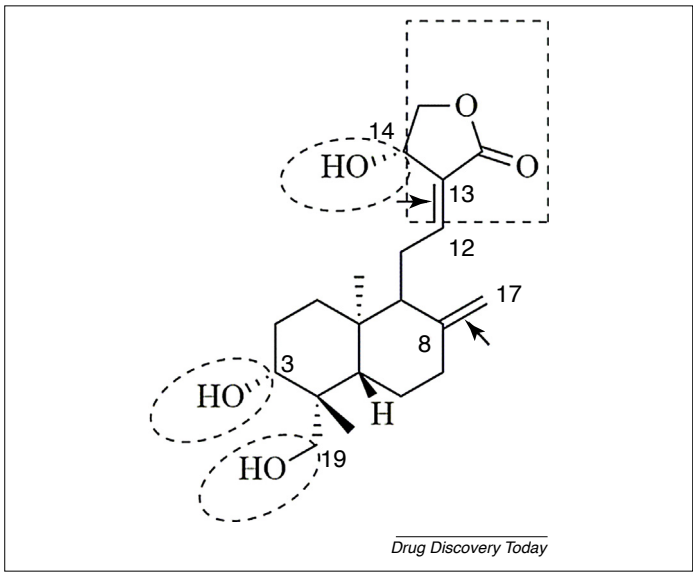

FIGURE 1

Chemical structure of andrographolide (AGP). To increase bioavailability and functionality, chemical modifications are often performed on the α,β-unsaturated γ-butyrolactone moiety (dashed box), the two double bonds C-8/17 and C-12/13 (black arrows) and three hydroxyls (dashed circles) at C-3, C-14 and C-19. Modifications at these positions have shown improvement of anticancer activity.

advanced cancer. Various studies have identified AGP and several of its analogues as potential chemotherapeutic agents by suppressing the growth of human prostate, breast, hepatoma and other tumour cells via different mechanisms of action. AGP was also tested in a clinical trial as a chemosensitiser against colorectal cancer (CRC) [12]. The following sections provide an in-depth review on the anticancer potential of AGP and its analogues against various cancer types (literature searches were limited to years 2012–2018).

Solid tumours

Gastric cancer

Gastric cancer (GC), commonly known as stomach cancer, is a leading cause of cancer-related deaths globally, with the highest incidence occurring in Asia, Latin America and the Caribbean. AGP is reportedly an effective cytotoxic agent for GC, owing to its potential to prevent GC cell proliferation. A study with human GC cell line SGC-7901 demonstrated a 36.6% decrease in G1 phase cells and significant increase in cells (>260%) at G_2/M phase after 48 h of treatment with a high dose of 40 μg/ml AGP (equivalent to ~114 μM) [13]. Similarly, the authors showed that AGP suppressed cell proliferation by modulating the levels of cell-cycle- and apoptosis-related proteins. Increasing concentrations of AGP (10, 20 and 40 μg/ml) led to upregulation of cell-cycle-inhibitory proteins (cyclin B1 and CDC2) and proapoptotic protein (Bax), as well as downregulation of antiapoptotic protein (Bcl-2). Similarly, AGP inhibited GC cell invasion by suppressing matrix metalloproteinase (MMP)-2 and MMP-9 activities through induction of tissue inhibitors of metalloproteinases (TIMP) expression (protein inhibitors of MMPs). Thus, inhibition of cancer cell proliferation by AGP could be achieved by blocking cell-cycle progression, promoting intrinsic apoptosis and/or repressing invasive activity [13].

Conversely, AGP modulates the extrinsic apoptotic pathway by acting as a sensitiser for tumour necrosis factor (TNF) – related apoptosis – inducing ligand (TRAIL) expression (Fig. 2) [14]. TRAIL, a member of the TNF family of ligands, is capable of selectively inducing apoptosis in cancer cells through interaction with the membrane death receptors: TRAIL-R1 (also known as DR4) and TRAIL-R2 (also known as DR5) [15,16]. Lim and colleagues [14] also reported TRAIL-induced apoptosis in human GC cell lines (either TRAIL-sensitive SNU601 and SNU638 cells or TRAIL-resistant AGS cells) treated with AGP. They observed an induced expression of DR5 (AGP concentration at ~20 μM) and DR4 (AGP concentration at ~30 μM), which subsequently activated the downstream caspase-8/caspase-3 pathway [14].

The cytotoxicity of an AGP analogue, 19-triisopropylsilyl-andrographolide (Fig. 3a), was determined using AGS and MKN-45 GC cells. The analogue induced cytotoxic activity by inhibiting topoisomerase IIα and increasing DNA damage marker γ-H2A.X (Fig. 2). This marker subjected the cancer cells to apoptotic cell death via activation of the caspase-3-dependent pathway [17]. In addition, this AGP analogue has a log*P* value (octanol–water partition coefficient) that is 3.8-times higher than AGP, which confers better penetration into cells. However, the binding mechanism to topoisomerase IIα is not well understood.

Prostate cancer

AGP was found to attenuate tumour growth in prostate cancer (PCa) cells by modulating certain cell cycle regulators (e.g., cyclin-dependent kinases), proinflammatory cytokines [e.g., interleukin (IL)-6] and chemokines (e.g., CXCL11, CXCR3 and CXCR7) [18–20]. IL-6 has a dual role in prostate cancer cell growth and differentiation, by acting as a paracrine growth factor in androgen-dependent prostate cancer cells (such as LNCaP) and as an autocrine growth factor in androgen-independent PCa cells (such as PC-3 and DU145) [21]. Chun and colleagues [19] demonstrated selectivity of AGP towards inhibition of IL-6 in three PCa cell lines: DU145, PC-3 and LNCaP cell lines. DU145 and PC-3 express the constitutive IL-6 autocrine loop, whereas LNCaP cells lack IL-6 yet express its receptor. In the latter, AGP managed to suppress paracrine IL-6-stimulated signalling pathways including the Janus kinase/signal transducer and activators of transcription (JAK/STAT) (Stat3 phosphorylation), the mitogen-activated protein kinase (MAPK)/Erk phosphorylation and the phosphoinositide 3-kinase (PI3K)/AKT (Akt phosphorylation) pathways (Fig. 2). The sensitivity of these PCa cells (LNCaP, DU145 and PC-3) towards AGP might be attributed to their androgen dependency, prostate-specific antigen (PSA) expression and p53 status [20]. Indeed, AGP displayed greater cytotoxic selectivity towards PC-3 cells (GI_{50} = 1.5 μM), which is androgen-independent and lacking p53 yet expressing PSA.

Administration of AGP to DU145-xenografted mice successfully delayed tumour growth with no obvious toxic effects. The compound (10 μM) significantly inhibited PCa cell growth by initiating apoptosis [19], through the intrinsic pathway, involving poly (ADP-ribose) polymerase (PARP), caspases and Bcl-2 family members [22]. Almost 86% of PC-3 cells were found to be at the stages of early and late apoptosis when treated with 10 μM AGP [23]. Similarly, Wong and colleagues [20] revealed that AGP induced apoptotic cell death by activating the caspase cascade and increasing the

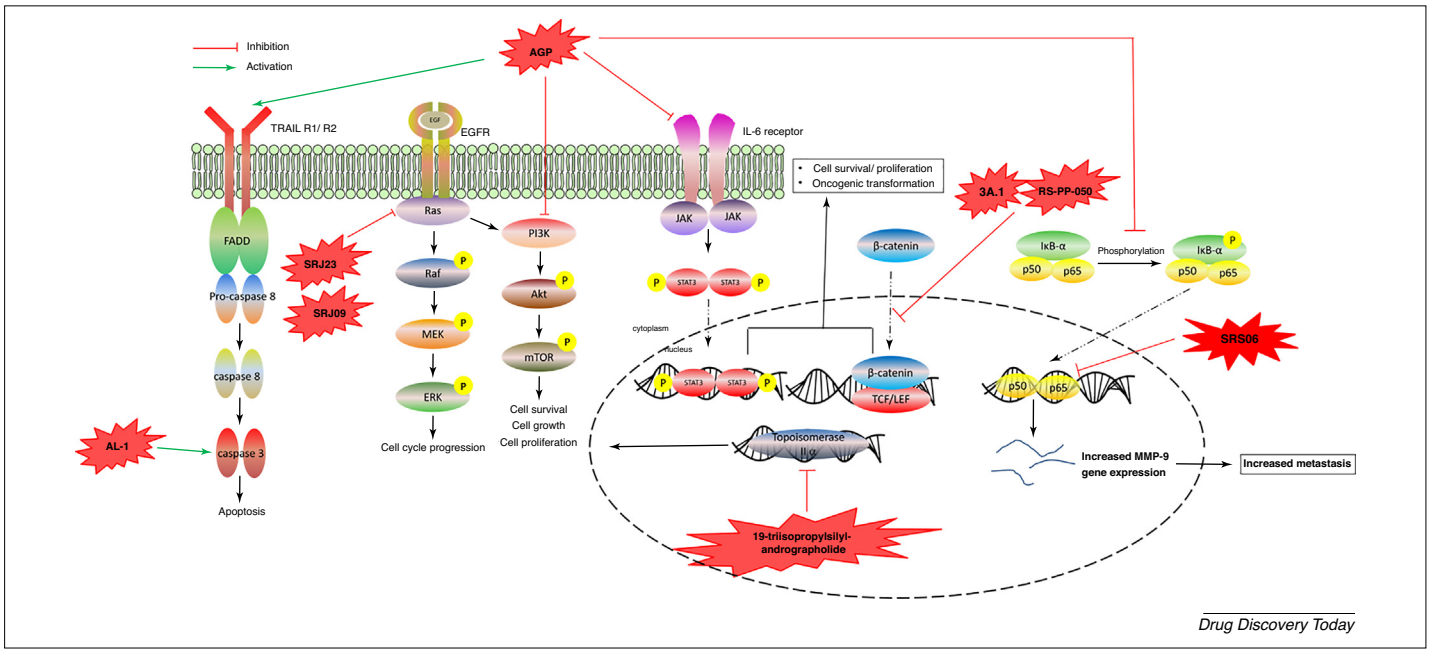

FIGURE 2

Molecular pathways for anti-cancer activity of AGP and selected analogues. AGP modulates extrinsic apoptotic pathway by acting as a sensitiser to induce the expression of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and the subsequent activation of the downstream caspase-8/caspase-3 pathway. AGP suppresses paracrine interleukin (IL)-6-stimulated signalling pathway. AGP suppresses paracrine IL-6-stimulated signalling pathways including the Janus kinase/signal transducers and activators of transcription (JAK-STAT) (Stat3 phosphorylation), which promotes aberrant cell growth and oncogenic transformation. AGP suppresses PI3K/Akt/mTOR pathway and causes the arrest of cell growth, proliferation, and survival. AGP inhibits phosphorylation of inhibitor of kappa B-alpha (IκB-α) whereas SRS06 inhibits p65 DNA binding activity, which both in turn prevent NF-κB nuclear translocation and decreases MMP-9 gene expression. AL-1 promotes apoptotic pathway through activation of caspase-3 and caspase-9 in a ROS-dependent mechanism. SRJ23 and SRJ09 attenuate oncogenic K-Ras by directly binding to an allosteric pocket on the protein and eventually inhibits the downstream MAPK signalling pathway, which is crucial for cell growth, proliferation, and survival. 19-Triisopropylsilyl-andrographolide acts as topoisomerase IIα poison and induces DNA breakage leading to apoptosis. RS-PP-050 and 3A.1 inhibit nuclear translocation of β-catenin, thereby halting Wnt/β-catenin signalling pathway, in a GSK-3β-dependent and GSK-3β-independent manner respectively.

expression of proapoptotic Bcl-2 family members, such as Bax and Bid. Furthermore, AGP arrested the cell cycle at G_2/M phase by downregulating the expression of cyclin-dependent kinase (CDK) 1, without modulating CDK4 and cyclin D1 expression. Additionally, AGP also induced p53- and reactive oxygen species (ROS)-dependent TRAIL-mediated extrinsic apoptotic cell death by elevating the expression of DR4 and DR5 receptors and stimulating the caspase-8/caspase-3 cascade in PC-3 cells (Fig. 2) [23]. A different study revealed the inhibition of cell growth and survival in AGP-treated PCa cell line C4-2b [18]. AGP was shown to modulate and block cell cycle progression by differential alteration of cell cycle regulators, including cyclin B1, cyclin A2 and cyclin E2, as well as chemokine receptors (CXCR3 or CXCR7). In addition, the chemokine (C-X-C motif) ligand C-X-C chemokine receptor (CXCL11-CXCR3/7) axes in PCa cells was attenuated, thus diminished cell viability and hindered cell migration.

To improve the antitumour activity of AGP, an analogue known as 3,19-(3-chloro-4-fluorobenzylidene)andrographolide (SRJ23, Fig. 3b) was synthesised and proven to selectively inhibit the growth of PCa cells in the National Cancer Institute (NCI) *in vitro* screen [24]. SRJ23 (GI_{50} = 0.4 μM) displayed a 50-fold improved inhibitory profile, compared with the parent compound AGP (GI_{50} = 19.95 μM), and induced G_2/M-phase-specific cell cycle arrest with a concomitant decrease in CDK1 expression, as well as apoptotic cell death of PC-3 via activation of the mitochondrial caspase-cascade signalling system [25].

Lung cancer

There are two categories of lung cancer, namely small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). SCLC is a highly malignant cancer which originated from fast-growing oat-like cells exhibiting neuroendocrine features and accounts for 15% of lung cancer cases, whereas NSCLCs make up the remaining 85% of lung cancers. NSCLC is further classified into three pathologic subtypes known as adenocarcinoma (the most prevalent type), squamous cell carcinoma and large cell carcinoma [26]. NSCLC is usually associated with a relatively poor prognosis and high risk of tumour relapse. The relapse that occurs following first-line platinum-based chemotherapy, such as cisplatin treatment, is attributed to acquired resistance [27]. The mechanism underlying cisplatin resistance is inconclusive, yet enhanced autophagy was reported to play a major part [28]. It is factual that many clinically relevant anticancer therapeutic agents induce autophagy [29]. In cancer cells, autophagy could promote cellular survival through inhibition of the negative autophagy regulator: the mammalian target of rapamycin complex 1 (mTORC1) [30]. Similarly, Mi *et al.* [27] illustrated the inhibitory effect of AGP on pro-survival autophagic processes via activation of phosphatase and tensin homologue (PTEN)-dependent Akt/mTOR cascade in the cisplatin-resistant NSCLC cells (*in vitro* model: A549/DDP). Suppression of autophagy re-sensitised the NSCLC cells to cisplatin-mediated apoptosis. Meanwhile, the anticancer effect of the compound was further enhanced upon *in vivo* administration of

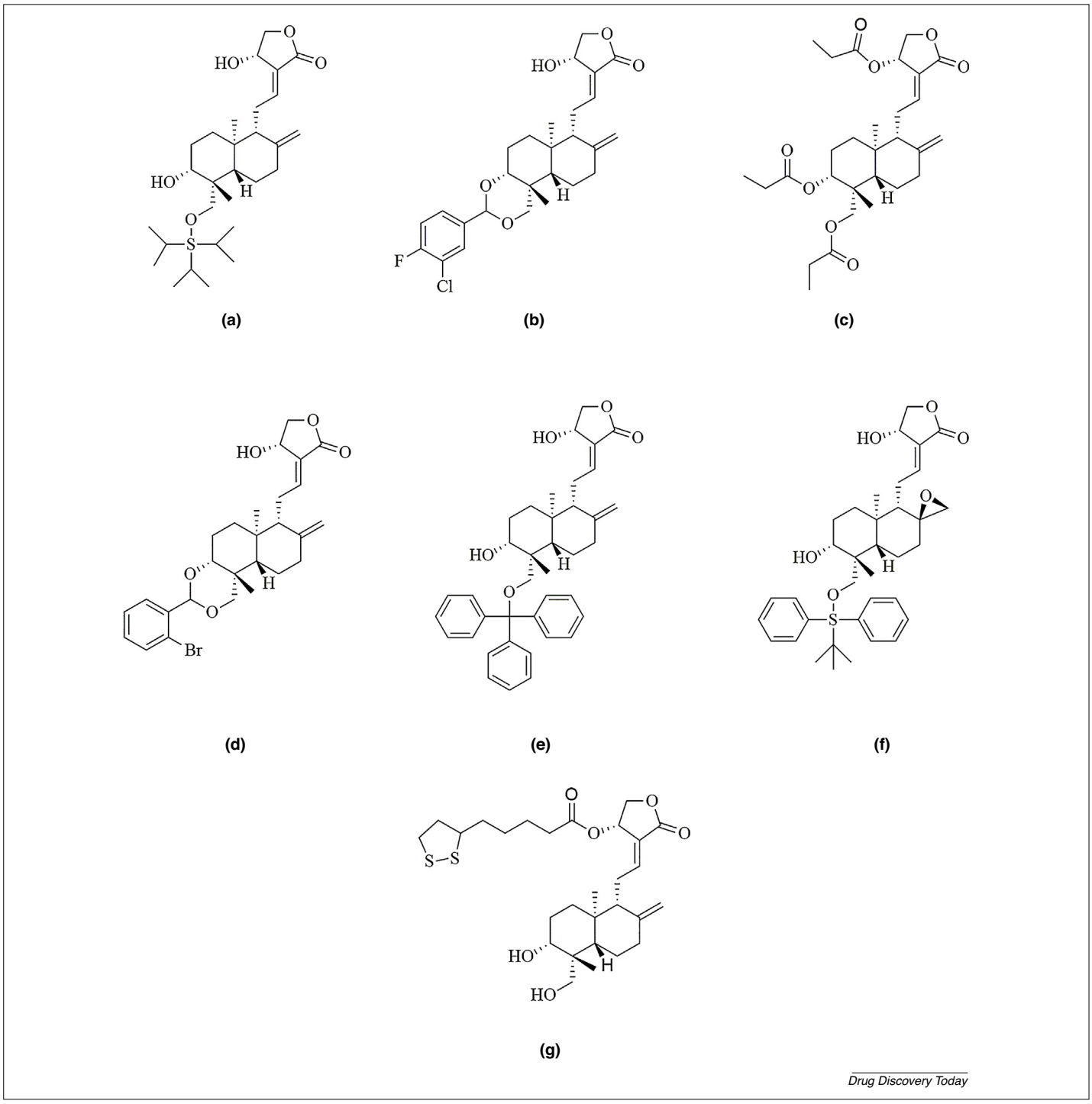

FIGURE 3

Chemical structures of andrographolide (AGP) analogues. (a) 19-triisopropylsilyl-andrographolide causes DNA-damage-induced apoptosis in gastric cancer cells. (b) SRJ23 was reported to inhibit growth of prostate cancer cells. (c) SRS06 was revealed to induce apoptotic cell death in non-small-cell lung cancer (NSCLC) cells. (d) SRJ09 was reported to inhibit the growth of breast cancer cells. (e) RS-PP-050 and (f) 3A.1 suppress colon cancer proliferation and induce apoptosis. (g) AL-1 exerts cytotoxicity against chronic myelogenous leukaemia.

AGP–cisplatin combination (5 mg/kg and 0.75 mg/kg, respectively). Synergistic antitumour activity was also observed in an *in vivo* study involving administration of the combination 100 mg/kg AGP plus 20 mg/kg paclitaxel to an A549 NSCLC xenograft [31]. The underlying molecular mechanism of this anticancer synergy is not clearly understood but AGP was proposed to potentiate paclitaxel-mediated antiproliferative and apoptotic cell death of NSCLC cells by increasing the intracellular ROS accumulation.

AGP attenuation of pulmonary tumour formation in vascular endothelial growth factor (VEGF)-induced transgenic mice could occur via the suppression of angiogenic VEGF expression and blockage of the cell cycle at G_2/M phase, with a concomitant

downregulation of cyclin A and cyclin B [32]. The antiangiogenic potential of AGP is attributed to its ability to downregulate the expression of hypoxia-inducible factor-1 (HIF-1), which is a master regulator of cellular response to hypoxia that participates in tumour growth and angiogenesis, through sustained suppression of its upstream PI3K/Akt signalling pathway [33]. Apart from the suppression of tumour angiogenesis, AGP (<5 μM) was shown to suppress migration and invasion of A549 lung cancer cells in wound-healing and *in vitro* trans-well assays [34].

Using the A549 NSCLC cell line, Lim *et al.* [35] described a distinct inhibitory activity exhibited by an AGP analogue: 3,14,19-tripropionylandrographolide (SRS06) (Fig. 3c). It was found to promote apoptotic cell death by downregulating the levels of nuclear factor-kappa B (NF-κB) protein, a major regulator of DNA transcription, and inhibiting p65 DNA binding activity (Fig. 2) at a relatively low concentration (5 μM). Indeed, SRS06 showed higher potency relative to the parent compound. Another NSCLC cell line, H3255 harbouring an epidermal growth factor (EGFR) mutation, showed increased DNA fragmentation, decreased Na^+-K^+-ATPase and protein kinase C activity as well as decreased transforming growth factor (TGF)-β1 and VEGF levels upon treatment with AGP at concentrations below 5 μM [36].

Breast cancer

AGP has also shown activity towards breast cancer [37–40]. This malignancy can be categorised into two groups: invasive (infiltrating), which accounts for ~80% of breast cancer patients, and noninvasive (*in situ*) [41]. AGP inhibited the survival of MDA-MB-231, a highly aggressive triple-negative breast cancer cell line. The compound had an IC_{50} of 30 μM and it induced this inhibition via the induction of ROS accumulation, caspase-dependent mitochondrial-mediated intrinsic apoptosis and cell cycle arrest at the G_2/M phase. Interestingly, at a similar concentration, AGP did not induce cytotoxicity in normal human breast epithelial cells (MCF-10A) [37]. Zhai and colleagues [42] revealed that AGP (10 μM) inhibition of MDA-MB-231 cellular proliferation occurred via suppression of inhibitor of kappa B-alpha (IκB-α) phosphorylation, prevention of NF-κB nuclear translocation and consequential abrogation of MMP-9 gene expression (Fig. 2). The action of AGP in MDA-MB-231 cells was also reported to involve HIF-1 inactivation [40]. Under hypoxic conditions (1% O_2), AGP significantly suppressed tumour growth and angiogenesis by reducing HIF-1α activity and hypoxia-mediated VEGF expression, through inhibition of the upstream PI3k/Akt/mTOR signalling cascade at relatively low concentrations.

In contrast to the findings by Banerjee *et al.* [37], AGP reportedly induced non-phase-specific cell cycle arrest in MCF-7 cells, a breast cancer model expressing oestrogen receptors [43]. AGP predominantly arrested the cell cycle at G_1- and G_2/M phases at the early time point (24 h treatment period) but induced an S block at later time points (48 h and 96 h treatment periods). Interestingly, two AGP analogues, namely SRJ23 (Fig. 3b) and 3,19-(2-bromobenzylidene) andrographolide (SRJ09) (Fig. 3d), were reported to induce G_1 phase-specific cell cycle perturbation in MCF-7 cells, with a concomitant increase in p21 expression and decrease in CDK4 expression [24]. This suggests that a substituent of benzylidene pharmacophore at 3-19-positions of the AGP structure improves the potency of the compound and cell cycle phase specificity (Fig. 3). Further study on SRJ09 revealed that this analogue prompted MCF-7 breast cancer cell death through an extrinsic apoptotic pathway independent of Bcl-2 and p53 [44].

Liver cancer

Hepatocellular carcinoma (HCC) is a chronic type of liver cancer. AGP has been reported in many studies associated with HCC and identified its effectiveness in impeding carcinogenesis through activation of several pathways including autophagic cell death, apoptosis and inhibition of tumour angiogenesis [45–50]. AGP induced apoptosis by activating the antioxidant systems in *in vitro* (in hepatoma Hep3B cells) and *in vivo* (liver tissues obtained from rats with diethylnitrosamine-induced HCC) models. This included the upregulation of intracellular reduced glutathione (GSH), superoxide dismutase (SOD), glutathione-S-transferase (GST) levels and the downregulation of malondialdehyde (MDA) and nitric oxide (NO) levels [45]. However, the augmentation of GSH level was reportedly temporary, owing to the potential of AGP to bind and deplete its cellular level, thereby causing an increased glutathione peroxidase activity, ROS production and eventual apoptotic cell death [46]. Furthermore, AGP-induced cell death could be autophagy-mediated, via the activation of liver cancer cells (Huh-7, QGY-7703 and BEL-7402) cyclophilin-D-induced mitochondrial dysfunction and elevation of intracellular ROS levels, independently of caspase-associated apoptosis [47].

The antiangiogenetic potential of AGP in hepatoma Hep3B cells and tumour-bearing Hep3B-xenografted nude mice occurred by blocking the expression of VEGFD and VEGFA, as well as phosphorylation of VEGF receptor 2 (VEGFR2) and its downstream targets such as the MAPK proteins crucial for cell growth, proliferation and survival [48,49]. Yang and colleagues also revealed a more pronounced antiangiogenic activity of ADN-9, a 15-benzylidene-substituted analogue of AGP (exact structure was not disclosed), on human umbilical vein endothelial cells (HUVECs) [50]. It produced a stronger inhibitory effect on the VEGF-induced capillary-like tube formation associated with attenuation of VEGF/VEGFR2/AKT signalling and VEGF-induced nuclear translocation of NF-κB at non-toxic concentrations (1.25–5 μM). This analogue also exerted a higher antimetastatic effect against murine hepatoma H22 in orthotopic and subcutaneous xenograft models compared with AGP [50].

Aberrant expression of microRNAs (miRNAs), which constitute a class of small noncoding RNAs participating in regulation of the expression of oncogenes or tumour suppressor genes, has been implicated in the initiation, progression and metastasis of HCC [51]. Lu *et al.* [52] demonstrated the AGP-induced alteration of a miRNA expression profile in Hep3B and SMCC7721 liver cancer cells; whereas few crucial miRNAs were identified to possibly contribute to inhibition of hepatoma tumour growth. These include miR-222-3p, miR-23a-3p, miR-106b-5p and miR-30b-5p. The expression of these miRNAs was elevated *in vivo* and *in vitro*, with concomitant reduction in the expression of their downstream target genes involved in hepatoma tumour growth and development [52].

Oral cancer

Oral cancer is a type of head and neck cancer, wherein ~95% of the cases are oral squamous cell carcinomas (OSCCs). OSCC is

characterised by poor prognosis and low survival rate owing to its high invasive and metastatic properties [53]. In addition, resistance to chemo- and radio-therapy complicates the effectiveness of treatments in advanced OSCC patients. Metastasis and resistance to chemo- and radio-therapy were believed to arise from the presence of cancer stem cells (CSCs) [54]. A previous study investigated the ability of AGP to reduce the cancer stemness and invasiveness in oral cancer stem cells (OCSCs) [55]. They discovered that the weakening effect was mediated through an overexpression of the tumour suppressor miRNA-218 – a miRNA that regulates self-renewal ability, epithelial–mesenchymal transition (EMT) and EMT-associated traits such as cell invasion, migration and chemoresistance [55]. The downregulation of this miRNA has been implicated in different tumours including glioma, thyroid cancer and NSCLC [56–58]. Furthermore, in this study, AGP was shown to exert a moderate effect on suppressing tumour-initiating activity, yet it played a sensitiser role in combination with radiation, evidenced by almost 80% synergistic reduction in invasion ability and clonogenicity which subsequently suppressed tumorigenesis in OCSCs [55]. Another study reported the *in vivo* antioncogenic activity of AGP on OSCC by abolishing NF-κB activation and averting tumorigenesis [59]. It is noteworthy that overexpression of miR-218 is associated with downregulation of its direct functional downstream target: EGFR-coamplified and overexpressed protein (ECOP), which governs the transcriptional activity of NF-κB and associated apoptotic response [60].

Colorectal cancer

CRC is one of the most commonly diagnosed solid tumours and is usually associated with a high recurrence rate caused by development of acquired resistance to chemotherapies such as 5-fluorouracil (5-FU) and cisplatin [61,62]. The anticancer potential of AGP has been widely evaluated in CRC cells and the results obtained were promising. In a study conducted by Wang and colleagues [61], AGP was shown to directly bind and stabilise Bax, without altering its mRNA level. Using an in-house developed 5-FU-resistant HCT116 cancer cell line (HCT116/5-FUR), treatment with AGP (10 μM) significantly increased Bax expression and synergistically enhanced 5-FU-induced apoptosis. The synergistic effect could also be mediated through other mechanisms, including an elevated ratio of proapoptotic Bax:antiapoptotic-Bcl-2 protein expression, activation of caspases and increased association of death ligand to receptors that augment release of cytochrome c [62]. These proved that AGP could reverse chemotherapy resistance and act as a sensitiser in CRC cells towards chemotherapy-induced apoptosis, via either intrinsic (mitochondrial) or extrinsic (death receptor) pathways.

Tumour invasion and metastasis are major concerns of CRC tumour relapse. MMP-2 is an example of proteins implicated in the control of tumour cell invasion and metastasis. At subcytotoxic concentrations (<3 μM), AGP suppressed MMP-2 activity without affecting its expression [63]. Inhibition of the MAPK signalling cascade, especially attenuation of Erk activation, could principally contribute to the anti-invasive activity of AGP in CRC [63]. Treatment of HCT-116 colon cancer cells with SRJ09 showed improved antiproliferative and apoptogenic effects. In contrast to the ability of AGP to arrest the cell cycle at G_1/S and G_2/M phases in colon cancer cells, SRJ09 significantly induced G_1-phase-specific cell cycle arrest accompanied by an increase in p21 and decrease in CDK4 protein levels [24,64]. The non-phase specificity of AGP in blocking cell cycle progression could be due to AGP-induced ROS generation and accumulated endoplasmic reticulum stress (ER stress), which entirely affect cell survival [64]. Additionally, *in vitro* assessments have demonstrated the potential of SRJ09 to penetrate through the DLD-1 colon cancer multicell layer (MCL) by diffusion and induce greater cytotoxicity when compared with the parent compound (IC_{50} = 41 μM, which is fourfold lower than that of AGP) [65].

The anti-colorectal-cancer activities of two AGP analogues: 19-*O*-triphenylmethyl andrographolide (RS-PP-050) (Fig. 3e) and 19-tert-butyldiphenylsilyl-8,17-epoxy andrographolide (3A.1) (Fig. 3f), were previously investigated. Both compounds were revealed to have acted via the Wnt/β-catenin signalling pathway in HT-29 colon cancer cells [66,67]. Mechanistically, RS-PP-050 inhibits β-catenin phosphorylation in a GSK-3β-independent pathway, whereas 3A.1 functions through suppression of total β-catenin protein expression in a GSK-3β-dependent pathway (Fig. 2). The clinical relevance of AGP in combination with capecitabine is being studied (Table 1); the trial started in 2014 and ~52 colorectal cancer patients have joined the study.

Non-solid tumours: T cell acute lymphoblastic leukaemia (T-ALL), multiple myeloma (MM), lymphoma

Haematological malignancies are of three major types: leukaemia, lymphoma and myeloma. Although there is a paucity of data on the activity of AGP on these non-solid tumours, several aspects of antitumour potential in these tumours are somewhat similar to what is obtainable in solid tumours. The anticancer potential of AGP in malignancies such as T-ALL, chronic myelogenous leukaemia (CML), B cell lymphoma and multiple myeloma were previously studied [68–71].

Yang *et al.* [68] investigated the *in vitro* and *in vivo* cytotoxic effect of AGP on human T-ALL Jurkat cells. Besides significant Jurkat tumour shrinkage in xenografted nude mice (AGP dose = 100 mg/kg), they also reported that AGP induced p53- and p38MAPK-dependent apoptotic cell death by inhibiting the PI3K/AKT pathway. Conversely, the analogue of AGP, andrographolide-lipoic acid conjugate (AL-1) (Fig. 3g) was found to moderately affect solid tumours and be able to induce greater cytotoxicity on human CML K562 cells than AGP, via ROS-dependent DNA oxidative damage and a mitochondrial-mediated apoptosis mechanism (Fig. 2) [72]. The involvement of the downregulated Toll-like receptor 4 (TLR4)/NF-κB signalling pathway in multiple myeloma (MM) OPM1 cells was reported by Gao and Wang [69]. Additionally, AGP induced caspase-dependent apoptosis in ordinary MM cells and MM cancer stem cells [69,70]. In a study by Yang *et al.* [71], a series of lymphoma cell lines [including Ramos, Burkitt p53-mutated lymphoma; Granta, mantle cell lymphoma (MCL); HF-1, follicular lymphoma (FL); and SUDHL4, diffuse large B cell lymphoma (DLBCL)] were studied and it was depicted that the cytotoxic effects of AGP occurred via accumulation of ROS and ROS-mediated activation of caspases, which led to mitochondrial apoptotic cell death.

Future perspectives

This review addresses the latest advances in the development of AGP and its analogues in cancer therapy by targeting

TABLE 1

Summary of clinical trials involving AGP and/or *Andrographis paniculata* interventions

Registry	Disease condition	Refs Identification number	Study design	Intervention, dosage, route, regime	Recruitment Sstatus (Phase)	Sponsor
ClinicalTrials.gov	Colorectal cancer	[12] NCT01993472 [79]	Randomised, open-label	Two cycles of combined andrographolide (500 mg, i.v.d. o.d., d1–14, q3w) and capecitabine (1250 mg/m^2, p.o. b.i.d., d1–14, q3w)	Ongoing (Phase II)	Gu Yanhong
Australian New Zealand Clinical Trial Registry (ANZCTR)	Chronic hepatitis C (risk factor for liver cancer)	U1111-1160-2405 ACTRN12614000966695 [80]	Randomised, double-blind, placebo-controlled	Sylimarin + other antioxidants including *Andrographis paniculata* extract (standardised to contain 34.8 mg AGP), b.i.d., p.o., for 6 months	Completed (Phase 2)	John Hunter Hospital Charitable Trust, Australia

tumorigenesis, angiogenesis and metastasis with varied mechanisms of action. Beneficial pharmaceutical chemistry and pharmacological properties of these versatile compounds discussed in this review strongly suggest that they are promising therapeutic leads useful for the treatment of breast, lung, liver, colon and pancreatic cancers. It is anticipated that the recent clinical trial involving AGP in combination with the antimetabolite capecitabine would show positive results. AGP analogues, by contrast, have not advanced into any clinical trials.

Despite the potential benefits of AGP in various clinical conditions including cancer, the compound has shown poor bioavailability owing to its rapid biotransformation into 14-deoxy-12-sulfo-andrographolide, efflux by P-glycoprotein (P-gp) and low aqueous solubility (~74 μg/ml in water at 25 °C) [73,74]. Structural modification is one of the common ways through which bioavailability could be improved. With the application of an *in silico* approach, absorption, distribution, metabolism, and excretion (ADME) properties of AGP and its analogues could be predicted at the early stages of development. One instance is QikProp software, established by Schrödinger, USA, which calculates and predicts several ADME properties including oral bioavailability and permeability; and successfully evaluated a series of 3,19-*O*-acetal analogues of AGP with calculated descriptors that lie within the standard range of values exhibited by 95% of all known oral drugs [7]. The increased calculated log*P* values imply an improvement of the passive molecular transport across the plasma membrane. Previous studies have shown convergence to an interesting point where the addition of halogen substituents on the AGP structure (halogenated AGP analogues) helped to improve potency against cancer cells in terms of antiproliferation, ROS generation, apoptosis and cell cycle arrest [75,76]. Remarkably, AGP and its benzylidene analogues (SRJ09 and SRJ23) are capable of binding the oncogenic Ras, commonly known as an undruggable molecular target owing to lack of a well-defined binding pocket to accommodate small bioactive molecules (Fig. 2) [77]. Yet, the analogues were revealed to impair Ras activation (Fig. 2) and inhibit the downstream MAPK signalling cascade more potently relative to AGP [76]. Indeed, our unpublished data identified halogen-based structural modification to possess better values of the predicted octanol–water partition coefficient for AGP analogues (data not shown), thus improving its permeability across the plasma membrane that subsequently might enhance its interaction with oncogenic Ras. Besides, it is noteworthy that replacing hydrogen with halogen could protect the compound from being easily metabolised by the cytochrome P450 system [78].

Concluding remarks

In view of the favourable *in vitro* and *in vivo* results discussed above, AGP analogues could be developed for the treatment of breast, lung, liver, colon and pancreatic cancers. More specifically, SRJ09 and SRJ23 with novel mechanisms of action targeting the oncogenic K-Ras are potential candidates that could have clinical benefits in the treatment of cancers that are addicted to Ras signalling, such as pancreatic, lung and colon cancers.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements: author contributions

Hon Liong Soo: overall data collection and manuscript drafting; Shun Ying Quah: illustration of cancer pathways, anticancer mechanisms and graphical drawing; Ibrahim Sulaiman: clinical trial data collection and overall language checking; Sreenivasa Rao Sagineedu: writing of chemistry component (e.g., SAR); Jonathan Chee Woei Lim: generating ideas and checking scientific content; Johnson Stanslas: coordinating members' roles, supervising outline of the manuscript, editing and the corresponding author.

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