EurJOC
European Journal of Organic Chemistry

Minireviews
doi.org/10.1002/ejoc.202100204

Chemistry Europe
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Strigolactones: Phytohormones with Promising Biomedical Applications

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Dedicated to Professor Franco Cozzi on the occasion of his 70th birthday.

Strigolactones are a class of carotenoid-derived plant hormones, which impact many functions of plant metabolism in response to environmental stress. They also act as exogenous signals perceived by both mycorrhizal fungi and parasitic plants. The peculiar chemical skeleton of these molecules and their mode of action in plants inspired possible applications in biomedical fields. In this minireview, an overview of the most recent applications of Strigolactones and analog derivatives as anti-cancer, anti-inflammatory, and antiviral drugs is described.

1. Introduction

Strigolactones (SLs) are a class of carotenoid-derived molecules. Since their first isolation in 1966 as promoting agents for the germination of root parasitic Striga plants,[1] diverse endogenous plant functions have been described for SLs in the control of shoot and root branching, fruit ripening, secondary growth, senescence, stress response,[2] biotic and abiotic stress resistance.[3] These new bio properties give rise to recognition of SLs as novel phytohormones. In 2005 it was reported that the release of SLs by plant roots stimulates the hyphal branching in arbuscular mycorrhizal (AM) fungi and enhances the efficiency of symbiosis.[4] The multiple and versatile roles of SLs make them interesting molecules in basic science to deepen the knowledge of fundamental molecular mechanisms. Moreover, research into SLs may promote modern agriculture and sustainable agronomic practices.[5]

Considering the important role of phytohormones in the survival of plants and the molecular mechanism at the base of their action, researchers are keenly interested to screen the potential benefits for human beings. It is well established that phytohormones not only govern important physiological traits in plants but also have impacts on human physiological processes.[6] Phytohormones with therapeutic activity can be exploited for cancer treatment as well as for their anti-inflammatory and antiviral activity mainly because they are biocompatible and have well-characterized functions.[7]

Owing to their new bio properties and the high potential of applications of SLs in the biomedical field there is a strong stimulus in the discovery of structurally related either natural or synthetic compounds with SL- like activity. Currently, at least 25 naturally occurring SLs have been identified and characterized.[8]

2. Chemistry of Strigolactones

Natural SLs have complex structures with a core composed of a tricyclic ABC lactone ring system which is linked to a butenolide D-ring by an enol-ether bridge (Figure 1).[9] Stereochemistry plays a crucial role in the fine-tuning of the biological properties ascribed to SLs.[10] Depending on the different orientation of the B/C junction, the naturally occurring SLs can be divided into two families strigol- (3aR,8aS in strigol, Figure 1) and orobanchol-type SLs, (3aS,8aR) in orobanchol, Figure 1) while the D-ring is always R configured (Figure 1). In the biosynthetic pathways, the AB-rings can be modified by demethylation, hydroxylation, epoxidation, acetoxylation,[11] giving rise to the structural diversification present in natural SLs.[12]

SLs which exhibit the A-, B-, C- core structure have been also termed as "canonical strigolactones" to distinguish from SL-like compounds that do not have the canonical A-, B-, and/or C-part and are therefore termed 'non-canonical SLs' (Figure 1). However, in all cases, the butenolide (D-ring) is connected to the rest of the molecule through an enol ether bridge exhibiting *E* stereochemistry at the double bond.

Having highlighted the potential that lies in the application of SLs both in agriculture and in biomedicine (the latter will be discussed below) and that natural SLs are produced and extruded in micromolar concentration, an inevitable prerequisite for harnessing such potential is that synthetic SL products need to be developed. A selection of the huge number of synthetic SLs that have been produced so far are represented in

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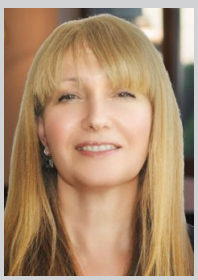
Figure 2 and include GR24,[13] Nijmegen-1,[14] Strigolactams,[15] indole derivatives EGO10, Th-EGO, and EDOT.[16] The core bioactivity of these synthetic SLs mainly depends on the presence of the D-ring, although the side-chain functional groups have been shown to affect SL function and activity.[17]

The design and synthesis of SL analogs are generally based on the assumption that the bioactiphore required for bioactivity in SLs is the D-ring. Although the contribution of the A-ring to activity is low, when present it should have the required stereochemistry (molecular freedom) to get reasonably active analogs.[18] We have demonstrated that structural modification of the D-ring into a γ-lactam functional group may give insight into the variations in SL binding interaction with its receptor.[19] Fluorescent SLs have also been developed, which were used to track SL trafficking and perception.[16,20] All these synthetic SLs have greatly contributed to improving our understanding of the biological role of SLs.

Among the synthetic SLs which have been mostly employed in biomedical applications, a special focus is reserved for GR24 (**13** and 14, Figure 2) and indolyl derivatives Th-EGO and EDOT

Prof. Cristina Prandi is Full Professor of Organic Chemistry at the Chemistry Department of the University of Torino where she is also Vice Rector for Research; her main interests are related to organometallic chemistry, gold catalysis, and target-oriented synthesis. More recently she focused her interest on the study of organometallic transformations in unconventional solvents. She has carried out research on the synthesis of bioactive phytohormones analogs focusing on SAR (structure-activity relationship) studies and the design of active derivatives. Furthermore, she has moved her interest in the field of bioimaging studies developing fluorescent-tagged molecules. She also carried out research on the use of plant metabolites analogs for their potential anticancer activity. Prof. Prandi is the leader of several national projects and chaired the Cost Action FA1206 dedicated to Strigolactones. She was invited to give lectures to several Scientific meetings. She is the author of 112 scientific publications, 7 patents, 2 book chapters, editor of 3 special issues and 2 books, and of 80 communications in national and international congresses.



Prof. Hinanit Koltai, Ph.D., is a Senior Research Scientist and the Director of Academic Affairs at the Agricultural Research Organization, Volcani Center, Israel. She is a leading Author of more than 100 peer-reviewed publications and more than 30 book chapters and invited reviews and she holds 15 international patents. She is the Editor of books and a member of Editorial boards in international scientific journals. She teaches plant development and medical cannabis courses in Bar Ilan University, Israel. Research in Koltai lab is focused on deciphering the "entourage effect" between cannabis compounds while specifying active pharmaceutical ingredients (API) from cannabis and their medical activity at the physiological and molecular levels in human cells, 3D printed models, and human tissues. The lab combines state-of-the-art chemistry and cellular and molecular biology tools, making discoveries in this field. Research results are IP protected; they are published in international scientific journals and form a scientific basis for the development of what might become the next generation of cannabis-based medical products and elite cannabis strains.

Prof. Yoram Kapulnik is the Executive Director of the U.S.-Israel Binational Agricultural Research and Development Fund (BARD) as of August 1, 2017. He is the former Director General of the Volcani Center in Israel (2011–2016) and before this position, he served as the head of numerous Institutes within the Volcani Center. Born and raised in Israel, Prof. Kapulnik received his doctorate in Soil Microbiology from the Hebrew University and as a Fulbright Fellow, completed his post-doctoral work at the University of California, Davis (1984–1986). He worked for more than 25 years as a Professor of Plant physiology and biotechnology at the Volcani Center, Israeli Ministry of Agriculture. His early work focused on the study of Plant-microorganism interactions, mainly in plant roots, and their impact on crop productivity. Later, he concentrated on the identification and utilization of natural products produced by numerous plant species and their health benefit to humans. Prof. Kapulnik holds 14 patents, trained more than 65 graduate and post-graduate students in his lab, and has published more than 170 articles in his field in international scientific journals and edited 3 books. During the years, Prof. Kapulnik served on numerous national and international research evaluation panels, chaired the board of the Israeli Gene Bank, the natural "food security" commission, and the national working group to develop curriculum frameworks for agricultural education in Israel.

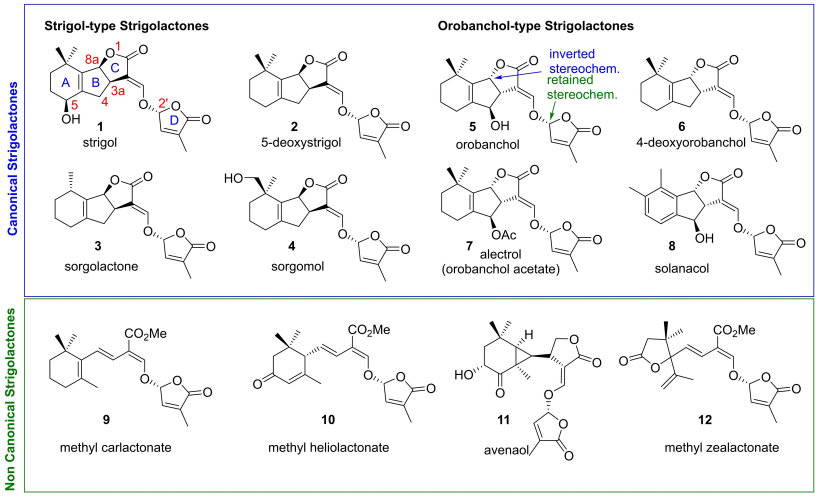


Figure 1. Natural SLs with ABC-ring system in canonical SLs, as illustrated for 1–8 structures. 5-deoxystrigol (2) and 4-deoxyorobanchol (6) illustrate the differences in orientation of the BC ring junction which defines the two families strigol-like and orobanchol-like. In non-canonical SLs the ABC core is no longer in place as shown in 9–12 while the butenolide D-ring is present with the same R configuration at the 2′ in the whole series of natural SLs.

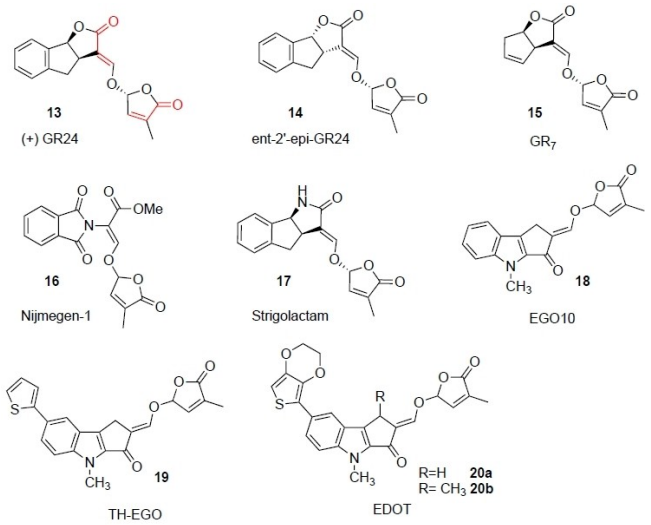
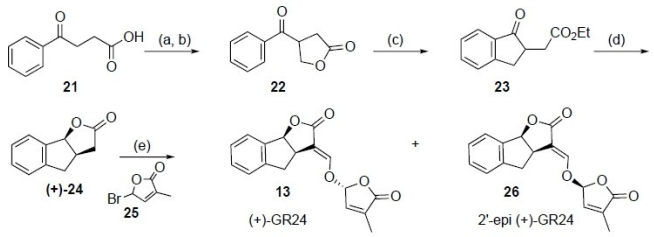


Figure 2. A selection of synthetic SLs. In compound 13 the Michael acceptor functions are highlighted in red.

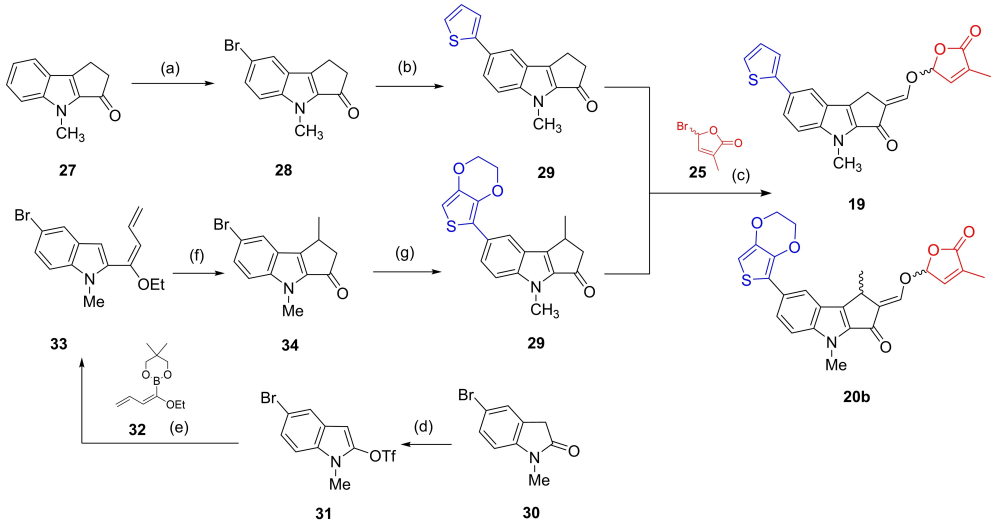


Scheme 1. Enantioselective McErlean synthesis of (+)-GR24 13. Reagents and conditions: (a) HCHO, NaOH (aq); (b)HCl; (c) H_2SO_4, 100 °C, AcCl, EtOH; (d) (S,S)-RuTsDPEN (4 mol%) HCO_2H, $^iPr_2NEt$, then PPTS, 90 %, 92 % ee to 99 % ee after crystallization; (e) HCO_2Me, KOtBu, then 25.

(19 and **20** respectively, Figure 2). Enantioselective synthesis of (+) GR24 **13** has been reported (Scheme 1).[21] The key steps are an intramolecular Ritter reaction to access indanone **23** and a lactonization catalyzed by the Noyori' s (S,S)-RuTsDPEN catalyst to enantiopure (+)-**24**. Remarkably the use of (R,R)-RuTsDPEN gave (−)-**24** and pave the way to obtain the other two stereoisomers of GR24.

ThEGO **19** (and EDOT **20 a**, both without substituents on the C-ring), and **20 b** (Figure 2) were synthesized according to the sequence shown in Scheme 2.[16]

4-Methyl-1,2-dihydrocyclopenta[b]indol-3(4H)-one **27**[16] has been reacted with NBS to selectively obtain 7-bromo analog **28** in 67 % yield and then functionalized by means of a Suzuki-Miyaura cross-coupling reaction in order to introduce a thienyl substituents specifically in position 7 of the A ring. The last step, the linkage of the D ring has been performed under basic conditions in the presence of ethyl formiate and bromo butenolide **25. 19** was obtained with a 50 % overall yield as a racemate. Purification by chiral HPLC furnished both the enantiomers for further biological investigations. A slightly different sequence has been applied to the synthesis of 20b. Commercially available 1-methylindolin-2-one has been selectively brominated with NBS in position 5 to afford 5-bromo-1-methylindolin-2-one **30** (Scheme 2) and converted into the corresponding triflate **31** with KHMDS as a base. Suzuki coupling of 31 with alkoxy dienyl boronate **32**[22] allows the installation of the chain needed for the construction of ring C (see Figure 1). This was realized according to a Nazarov electrocyclic process catalyzed by *o*-benezendisulfonimide (o-BDS).[23] Suzuki coupling with 2-(2,3-dihydrothieno[3,4-b][1,4]dioxin-7-yl)-5,5-dimethyl-1,3,2-dioxaborinane (not shown) and coupling with bromobutenolide **25**,[24] led to the final compound **20 b** as a mixture of diastereoisomers which were isolated for bioactivity evaluation and further applications



Scheme 2. Synthesis of TH-EGO **19** (same sequence was applied for the synthesis of **20a**). Reagents and conditions: (a) NBS, CAN, from 0 °C to 25 °C, 6 hs; (b) $Pd(OAc)_2$, K_3PO_4, SPhos, THF reflux 4 hs then thienyl boronate (or dimethyl (2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)boronate in case of **20b**; (c) *t*-OBuK, HCO_2Et, THF, 25 °C, 3 h then **25** in DME, 25 °C, 16 hs; (d) KHMDS 1.2 eq., $PhNTf_2$ 1 eq. THF,–78 °C, 2 h: (e) $Pd(OAc)_2$, K_3PO_4, SPhos, THF reflux 4 hs then **32**; (f) *o*-BDS, DCM, 80 °C; (g) $Pd(OAc)_2$, K_3PO_4, SPhos, THF reflux then ethoxythienyl boronate; (h) *t*-OBuK, HCO_2Et, THF, 25 °C, 3 h then **25** in DME, 25 °C, 16 hs.

3. Biomedical Applications

3.1. Anticancer activity

Various plant-derived bioactive compounds have been shown to inhibit cancer cell growth and survival.[6] The first report on the antiproliferative activity of SLs was published by our group in 2012.[25] Based on the observation that SLs inhibit shoot branching in plants by blocking cell replications, six synthetic SLs analogs have been tested on breast cancer cell-line growth and survival. We show that SLs analogs are able to inhibit proliferation and induce apoptosis of breast cancer cells and at the same time are much less effective on 'non- cancer' lines. SLs analogs were also tested for their ability to inhibit the growth of mammosphere cultures that are typically enriched with cancer stem-like cells. We show that SLs are potent inhibitors of self-renewal and survival of breast cancer cell lines grown as mammosphere and even a short exposure leads to irreversible effects on mammosphere dissociation and induce cell death. Immunoblot analysis revealed that SLs analogs induce activation of the stress response mediated by both P38 and JNK1/2 MAPK modules and inhibit PI3 K/AKT activation.

Soon after the same set of SLs analogs were demonstrated to inhibit the growth and survival of an array of cancer-derived cell lines representing solid and non-solid cancer cells including prostate, colon, lung, melanoma, osteosarcoma, and leukemic cell lines, while normal cells were minimally affected.[26] While the mechanisms of SLs growth inhibition only begin to unfold, the obtained results indicate that SLs induces G2 cell cycle arrest in all cells regardless of their underlying genetic alterations, e.g. p53, k-ras, or nuclear receptor status. We show that SLs are effective in targeting human primary prostate cancer cells while being significantly less toxic to normal prostate cells of the same patient, suggesting that SLs might be a treatment option in advanced prostate cancer.[26] The treatment of cancer cells with SLs analogs was marked by activation of the stress-related MAPKs: p38 and JNK and induction of stress-related genes; cell cycle arrest and apoptosis are marked by increased percentages of cells in the sub-G1 fraction and Annexin V staining. In addition, the response of patient-matched conditionally reprogrammed primary prostate normal and cancer cells was tested. The tumor cells exhibited significantly higher sensitivity to the two most potent SLs which were 19 and 20 analogs with increased apoptosis confirmed by PARP1 cleavage compared to their normal counterpart cells.[26]

To further examine the anti-cancer activity of SLs in vivo, we have examined their effects on the growth and viability of MDA-MB-231 tumor xenografts model either alone or in combination with paclitaxel.[27] Indeed, a challenge arises in identifying therapeutic combinations that will target both the hyperproliferative cells as well as the slow-growing cancer stem-like cells that are capable of self-renewal and survival after therapy. Moreover, productive combinations may create synergistic responses permitting the use of the lowest possible drug dosages to effectively target all cancer cells and reduce toxic side effects. As a matter of fact, further insight into the mechanism of SLs activity suggests that like paclitaxel, treatment with SLs leads to an effect on the integrity of the microtubule network and to inhibition of cell migration.[27] Furthermore, the concurrent administration of 19 and paclitaxel lead to an additive growth inhibition of MDA-MB-231 cultured cells. The additive effect was apparent only when the compounds were administrated at relatively low concentrations. At higher concentrations of 19 or paclitaxel, no additive effects were apparent between the two compounds in cultured cells.[27] The additive effect obtained with concurrent administration of 19 and paclitaxel suggest that the two compounds affect cell proliferation by a similar mechanism. Compounds **19** and **20**

were found to affect the integrity of the microtubules network. In few hours after treatment microtubule bundles were formed in the cytosol and around the cell nucleus. Paclitaxel was shown to successfully target class I and III b-tubulin, and lead to straightening of the protofilaments, inducing a more GTP-like configuration in the microtubule protofilaments, resulting in formation of microtubule bundles (Figure 3). However, it is yet to be determined whether SLs are microtubules targeting agents.[27]

A further investigation on the molecular mechanisms at the base of the SLs effects on the inhibition of tumor growth was reported.[28] SLs were shown to induce DNA damage in the form of DNA double-strand breaks (DSBs). There are two major pathways of DSBs repair: an error-prone non-homologous end joining (NHEJ) that often includes processing of the DNA broken ends before ligation, and the error-free homology-directed repair (HDR) that uses a homologous sequence (most often a sister chromatid) as a template for repair.[29] In addition to DSBs induction, SLs simultaneously impair DSBs repair, mostly homology-directed repair (HDR) and to a lesser extent non-homologous end joining (NHEJ).[28] In response to SLs, RAD51, the homologous DSB repair protein, is ubiquitinated and targeted for proteasomal degradation and it fails to co-localize with γH2AX foci. Interestingly, SLs synergize with DNA damaging agents-based therapeutics. The combination of PARP (poly ADP ribose polymerase) inhibitors and SLs showed an especially potent synergy, but only in BRCA1-proficient cells. No synergy was observed between SLs and PARP inhibitors in BRCA1-deficient cells, suggesting that by inducing a "BRCAness" phenotype, SLs can sensitize cells to chemotherapeutic drugs such as PARP inhibitors to enhance their efficacy and supporting a role for SLs in HDR impairment. The data suggest that SLs increase genome instability and cell death by a unique mechanism of inducing DNA damage and inhibiting DNA repair.[28]

One of the challenging requirements for synthetic SLs is their stability in solvent media in which they are formulated, as this contributes to efficacy. Environmental conditions, such as temperature and pH,[13] might induce degradation of the molecules for example SLs are well known to be prone to hydrolysis at basic pH. Nanocarriers facilitate selective drug delivery and sustained release at target sites, thereby enhancing drug efficacy and reducing toxicity. Furthermore, glutathione (GSH)/pH-responsive nanosponges (GSH/pH–NS) allow the controlled release of various drugs in response to the intracellular GSH concentration and pH.[30] NS are pH- and GSH-responsive owing to the presence of disulfide bridges and carboxyl groups in the GSH/pH–NS polymer matrix. Thus, a high intracellular GSH concentration, as is the case in tumors compared to normal tissue (0.5–10 mM vs. 2–20 μM),[31] can promote drug release from nanoparticles containing redox-sensitive chemical groups.[32] To increase SLs efficacy and selectivity, compounds **19** and **20a** were loaded into glutathione/pH-responsive nanosponges (GSH/pH–NS) to selectively deliver SLs to prostate cancer cells and thus enhance their therapeutic efficacy (Figure 4).[33] SLs were then readily incorporated into the GSH/pH–NS, kinetic analysis revealed that release of **19** and **20a** from the GSH/pH–NS was accelerated at acidic pH and in the presence of a high GSH concentration. Evaluation of the effects of **19**- and **20a**-loaded GSH/pH–NS on the growth of DU145 (high GSH) and PC-3 (low GSH) prostate cancer cells revealed that the GSH/pH–NS inhibited the proliferation of DU145 cells to a greater extent than free SLs over a range of concentrations. These findings indicate GSH/pH–NS are efficient tools for controlled delivery of SLs to prostate cancer cells and may enhance the therapeutic efficacy of these compounds.

Simplified synthetic SLs analogs were tested on HCC (Hepatocellular Carcinoma) cell line- HepG2 and evaluated for their capability to induce cell proliferation inhibition and

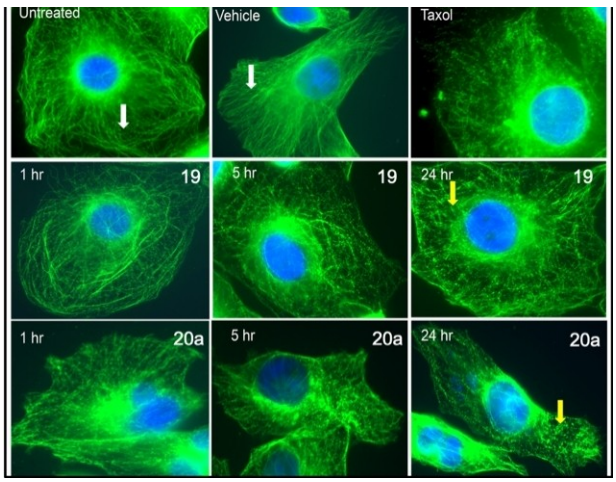


Figure 3. Fluorescent images of MDA-MB-231 cancer cells immunostained for α-tubulin (Green staining- Alexa Fluor 488) following 19 (15 μM), paclitaxel (25 nM), 20a (15 μM), vehicle control treatments or untreated control for 1, 5 or 24 hr. Blue nuclei staining- DAPI. Images were taken using Zeiss Axiovert 200 M Fluorescence inverted microscope at ×63 magnification. White arrow denotes microtubule filaments; yellow arrow denotes microtubule aggregates.

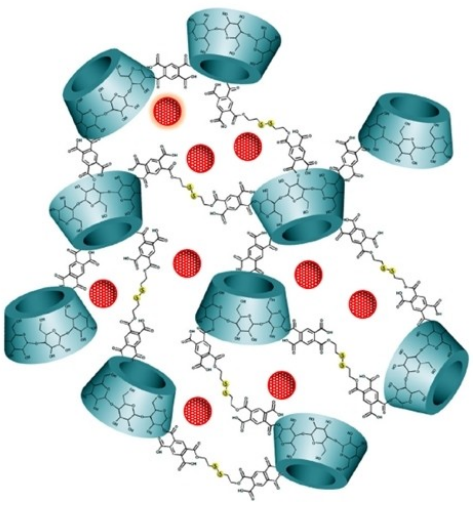


Figure 4. Active compounds (red balls) included into Glutathione responsive nanosponges.

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apoptosis (Figure 5).[34] Compared to natural SLs (see Figure 1), compounds shown in Figure 5 lack the ABC ring system, while they retain the D-butenolide ring. These synthetic simplified SLs are also known as mimics.[35] Primary WST-1 assays, followed by annexin-V/7AAD staining, demonstrated the anti-proliferative effects.[33] The SLs analogs **38** and **39** were found to significantly reduce HepG2 cell viability in a dose- and time-dependent manner and induce apoptosis. Interestingly, though **38** and **39** strongly affected cancer cell proliferation, both compounds showed a moderate anti-proliferative effect on normal cells.[33] Further studies are needed to investigate if compounds **38** and **39** target microtubules dynamics directly or the expression of its tubulin structure.

GR24 (Figure 1) has been demonstrated to have a potent anti-angiogenic activity both *in vivo* and *in vitro*.[36] The *in vitro* results show that GR24 inhibits the growth of endothelial cells and different cancer cell lines with a micromolar range of half inhibitory concentration (IC50) values.[36] In addition, GR24 inhibits key steps of the angiogenic process *in vitro*, such as tubulogenesis, invasion, extracellular matrix remodeling capacity, migration, and adhesion of endothelial cells at non-cytotoxic concentrations which interferes with several key steps of the angiogenic process, such as proliferation, differentiation, migration and ECM degradation by endothelial cells. In the *in vivo* tests, GR24 shows a great inhibitory effect on vasculature formation in the chicken chorioallantoic membrane and in two different zebrafish models.[36] GR24 is proposed to promote changes in the cytoskeleton organization of endothelial cells, and the proposed mechanism of action for this compound involves the inhibition of VEGFR2 activation in response to VEGF, leading to a decrease in focal adhesion kinase (FAK) signaling.[36] Unfortunately, the authors did not specify if the GR24 they used in the experiments is a pure enantiomer or a mixture of stereoisomers.

3.2. Anti-inflammatory activity

Besides cancer-related studies, more recently natural SLs and analogs have been claimed to have potential activity in other biomedical fields.

Two plant-derived compounds, the SL analog GR24, and pinosylvin have been investigated for their effect on glucose metabolism. The aim of the study was to establish the role of SLs in adipogenesis and inflammation of murine adipocytes.[37] 3T3-L1 preadipocytes were differentiated into adipocytes and were treated with GR24 and pinosylvin. Resveratrol was used as a reference treatment. The effects of the target compounds on adipogenesis and inflammation were explored by different methods such as cytotoxicity assays, lipid staining, western blotting, and ELISA. GR24 upregulated SIRT1 and enhanced the production of NADþ, an essential SIRT1 substrate. GR24, pinosylvin, and resveratrol attenuated adipogenesis *via* inhibition of the expression of PPARg and C/EBPa and protected against inflammation by inhibiting TNF-α-stimulated IL-6 secretion. GR24 also inhibited NF-kB activation.[37]

In a recent study, racemic GR24 and derivatives of GR24 synthesized by reduction of the D ring were evaluated for their inhibitory activities on the release of some pro-inflammatory mediators (NO, TNF-α, and IL-6) as well as on migration of neutrophils and macrophages in fluorescence-labeled zebrafish larva models. Results showed that two optical isomers were active for studied parameters.[38]

The SAR (structure-activity relationship) study realized by the authors revealed that, among all the stereoisomers of both GR24 and reduced GR24 (Figure 6), the most promising compounds for the treatment of inflammatory diseases were the unreduced (+)-GR24 **13** (Figure 2) and its enantiomer (−)-GR24 (not shown). The in vitro anti-inflammatory experiments revealed a similar or even better inhibitory effect on the release of inflammatory mediators and cytokines (NO, TNF-α, and IL-6) as compared to dexamethasone. Moreover, the migration of neutrophils and primitive macrophages was also measured in an in vivo zebrafish model; the results also revealed strong inhibition by the same compounds. It was demonstrated that the anti-inflammatory activity of **13** and its enantiomer was due to, at least in part, their ability to suppress the activation of NF-κB and MAPK cascades, resulting in decreased NO, TNF-α, and IL-6 levels. These results further confirm that compounds with the preserved D-ring as in natural SLs generally exhibit improved anti-inflammatory activity, which suggests that structures with the reduced D-ring would be an effective strategy to develop anti-inflammatory agents.[38]

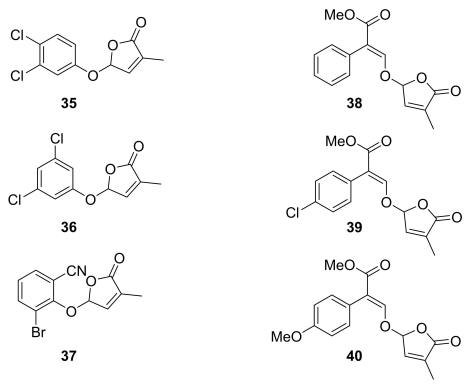

Figure 5. Simplified SLs analogs tested on HCC cell line HepG2.

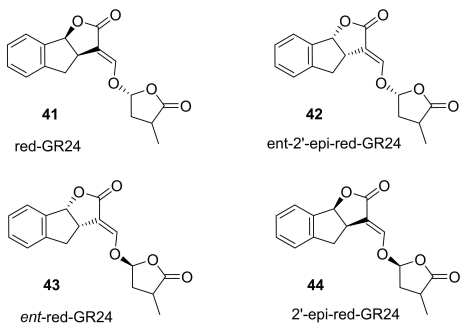

Figure 6. Stereoisomers of GR24 reduced at the D-ring.

Racemic GR24 was also studied in its interactions with several signaling molecules and enzymes involved in inflammation, glucose metabolism, and cytoprotection by using both in vitro and in silico analysis.[39] Additionally, molecular docking and ADMET (chemical Absorption, Distribution, Metabolism, Excretion, and Toxicity) analyses of this compound were performed to elucidate its potential as a therapeutic candidate for further in vivo and clinical studies. Interestingly, in view of further application of GR24 as a drug, in their computational work, the authors show that GR24 is in line with the Lipinski and Veber "rules of five"[40] which is the most widely used set of criteria for evaluating drug-likeness. In addition, its molecular weight is less than 500 g/mol, and it has the recommended polar surface area and lipophilicity for cellular permeability as well as a medium level BBB partition coefficient indicating that it may pass through BBB and reach CNS cells.

Neuropathological changes in Alzheimer's disease (AD) are directly linked to the early inflammatory microenvironment in the brain. In a recent study, SLs analog GR24 either as a racemic mixture or as pure enantiomers (compound **13** and its enantiomer, Figure 2) was used in phenotypic screening tool on SIM-A9 microglial cell line, showing a remarkable potency in the suppression of neuroinflammatory and neurotoxic mediators.[41] More in detail, GR24 demonstrated a marked potency in the suppression of lipopolysaccharide (LPS)-induced neuroinflammatory/neuro-toxic mediators by regulating NF-κB, Nrf2, and PPARγ signaling. The results shown by the authors demonstrated that GR24 markedly downregulated proinflammatory and upregulated cytoprotective genes/proteins and signaling molecules in both microglial and BBB endothelial cells. Overall, in this study, findings suggest that, in the treatment and prevention of neuro-inflammatory/neurodegenerative disorders, GR24 may provide a novel multipotent therapeutically active structure on which in silico optimized SL-like pharmacophores can be developed and synthesized for further preclinical and clinical investigations.

3.3. Antiviral

The antiviral activity of a panel of synthetic SLS was tested against HMCV (Human Citomegalovirus) with the aim of identifying new druggable targets for anti-HCMV therapy to be used in place of nucleoside therapy whose side effects are reported as very serious.[42] Compound **19** and **20a** (Figure 2) markedly inhibit the replication of different HCMV strains in vitro. Moreover, SLs do not affect the first steps of HCMV infection, i.e., attachment and entry, rather, they exert their role in the late phases of the viral cycle. In particular, the authors show that an SL-dependent trigger for apoptosis of the infected cell may be a novel strategy against HCMV infection. Finally, in silico molecular docking simulations have been used to predict the interactions between the SL analogs and the modeled structure of the putative target IE1, which is known to inhibit apoptosis.[42] Antiviral therapy that is based on molecules exerting their effects by targeting cellular proteins instead of specific viral proteins is a promising solution, as they are not associated with drug resistance. The potent SL in vitro antiviral activity warrants further in vivo studies that can validate the potential use of SLs in the prevention and/or control of HCMV infections.

4. Conclusions

SLs represent a class of phytohormones with unique structural features. The double Michael acceptors functional group (highlighted in red in compound **13**, Figure 2) makes them potential bioactive molecules for biomedical applications. As outlined in this minireview, an increasing number of publications encompass the role of SLs as therapeutically active compounds in the diversified field from cancer to anti-inflammatory to antiviral applications. Due to their complex structure, isolation and/or synthesis of these compounds on a sufficient multi-gram scale to study their biological effects is a challenging procedure. Also, stereochemistry plays a crucial role in natural SLs. Unfortunately, only a few of the studies herein selected use enantiopure compounds. Further investigations are needed to finely tune the role of each enantiomer. Due to the sometimes complex structure of natural SLs, model analogs with simpler structures retaining biological activity have been designed and synthesized. It can be envisaged that structure-activity relationship studies as well as *in silico* simulation might lead to target-specific structural analogs of SLs and lead candidates to be developed into new efficient drugs.

Acknowledgements

We would link to acknowledge Italian Minister for Research (MUR), Huvepharma Italia srl, CRT (Cassa di Risparmio di Torino), Regione Piemonte.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Strigolactones · Synthesis · Anticancer · anti-inflammatory · Plant hormones

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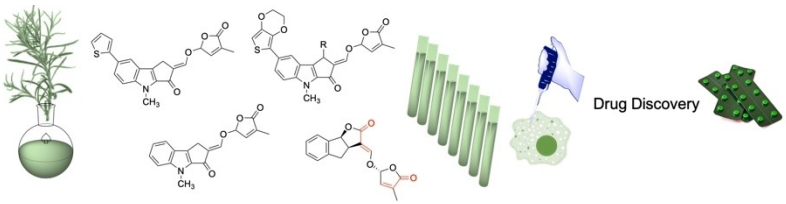
Manuscript received: February 18, 2021
Revised manuscript received: April 2, 2021
Accepted manuscript online: April 9, 2021

MINIREVIEWS



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Strigolactones (SLs) are carotenoid-derived plant hormones. Their effects on plant metabolism and the elucidation of their mode of action on plant cells inspired applications in biomedical fields. In this Minireview, the state of the art of using SLs as anticancer, anti-inflammatory, or antiviral bioactive compounds is reported.