Review

Gypsogenin Fighting for A Position in The Pentacyclic Triter-penes Game of Thrones on Anti-Cancer Therapy: A Critical Review

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Gypsogenin Fighting for a Position in the Pentacyclic Triter-Penes Game of Thrones on Anti-Cancer Therapy: A Critical Review

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Abstract: In the last decade, gypsogenin has widely grabbed the attention of medicinal chemists by virtue of its prominent anti-cancer potential. Despite its late identification, gypsogenin proved itself as a new anti-proliferative player battling for a front position among other classic pentacyclic triterpenes such as oleanolic acid, glycyrrhetinic acid, ursolic acid, betulinic acid, and celastrol. Herein, we present the most important reactions of gypsogenin by modification of its four functional groups. Furthermore, we demonstrate insights into the anti-cancer activity of gypsogenin and its semisynthetic derivatives and go further by introducing our perspective to judiciously guide prospect rational design. The present article opens a new venue for a better exploitation of gypsogenin chemical entity as a lead compound in cancer chemotherapy. To the best of our knowledge, this is the first review article exploring the anti-cancer activity of gypsogenin derivatives.

Keywords: pentacyclic triterpenes; gypsogenin; anti-cancer

1. Introduction

Cancer is the second major worldwide cause of mortality preceded with cardiovascular diseases [1–4]. Medicinal chemists are continuously urged to innovate new chemical entities to overcome resistance, reduce side effects, and enhance the efficacy of commercial drugs in the hard-fought battle against cancer [5–10]. Many natural products have provided skeletons and structural references for the invention of modern drugs [11–14]. Found in higher plants, pentacyclic triterpenes (PTs) are bio-nutrient phytochemicals endowed with diverse range of bioactivities such as hepatoprotective [15–17], anti-inflammatory [18–20], anti-hypertensive [17,21–23], anti-atherosclerotic [21,22], anti-viral [24–26], anti-fibrosis [27–29], and anti-ulcer effect [18,30,31]. In particular, PTs have ubiquitous applications in terms of anti-cancer drug discovery [32–37].

The literature is loaded with a plenty of success stories linking PTs derivatives with a prominent role in the prevention of cancer initiation, promotion, angiogenesis, and progression through disrupting different intermittent mechanisms and pathways. The number of scientific publications and citations linking PTs and cancer has been soaring over the past twenty years according to Web

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of Science database (Figure 1). PTs are generally non cytotoxic albeit minor derivatizations can lead to dramatic changes in activity.

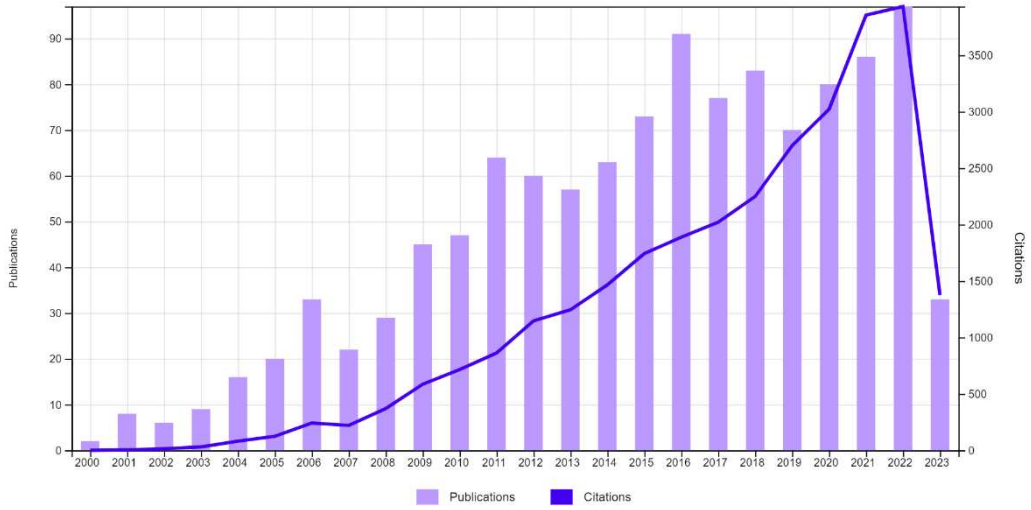

Figure 1. Number of citations and scientific publications containing research linking triterpenes with anti-cancer activity over the period 2000 – 2023. Data was obtained from the Web of Science database by searching the keywords triterpene cancer.

PTs comprise four main chemical skeletons namely oleanane, ursane, lupane, and friedelane. Oleanolic acid, from the oleanane type, is one of the most extensively studied PTs in terms of medicinal chemistry. Oleanolic acid suppresses proliferation of hepatocellular carcinoma [38,39], human bladder cancer [40], breast cancer [41,42], lung carcer [43], and colon cancer [44,45]. To have such diverse activities, oleanolic acid modulates multiple cell signaling pathways [46]. Two oleanolic acid derivatives, CDDO and CDDO-Me, have already entered clinical trials for treatment of solid tumors and lymphoma allowing oleanolic acid to top the throne of pentacyclic triterpenes in terms of chemotherapy [47,48]. Glycyrrhetinic acid is another representative of oleanane-type triterpenoids with ubiquitous anti-cancer activities [49–54]. Ursolic acid [44,55–58], betulinic acid [59–63], and celastrol [64–67], representing ursane, lupane and friedelane type triterpenoids, respectively, were reported to possess multifaceted anti-cancer properties.

Gypsogenin (3-hydroxy-23-oxoolean-12-en-28-oic acid), a less-explored PT, extracted from *Gypsophila oldhamiana* in a saponin form linked with sugar moieties. It is generated as a pure sapogenin by acid hydrolysis [68]. It has an oleanane type skeleton and possesses four active sites, C-3 hydroxyl, ring C double bond, C-23 aldehyde group and C-28 carboxylic acid, which are amenable for a wide range of chemical transformation (Figure 2). The hydroxyl, alkene, and carboxyl groups exist in most PTs. Nevertheless, the aldehyde group is unique as other classic triterpenes lack such group which represents a structural alert for most medicinal chemists due to its high reactivity [69].

Previously, aldehydes used to have an unfavorable reputation due to their toxicity and metabolic instability. Nonetheless, in modern chemical biology, they increasingly have been applied as covalent probes to target lysine residues in proteins through forming a covalent imine-adduct. In this regard, roblitinib development as a an exquisitely selective inhibition of FGFR4 signaling was based on presence of aldehyde group. The latter is responsible for making a reversible-covalent bond with the target while avoiding safety concerns of irreversible covalent inhibitors [70]. Taken together, the aldehyde group will play an important role in drug discovery in the 21st century to find ligands for traditionally undruggable targets [69,71]. This may give an advantage to gypsogenin over other PTs.

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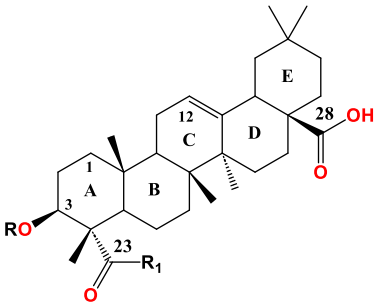
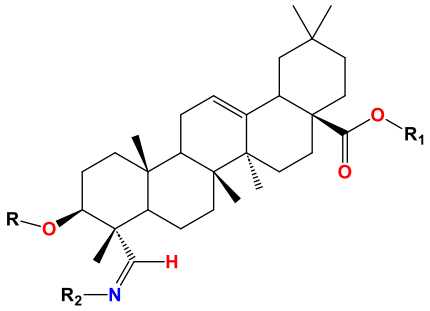
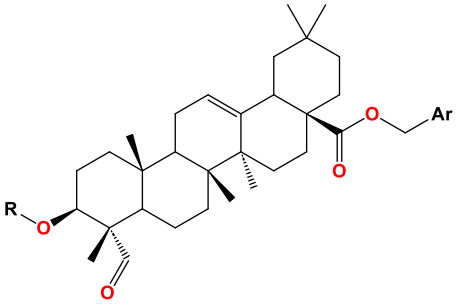


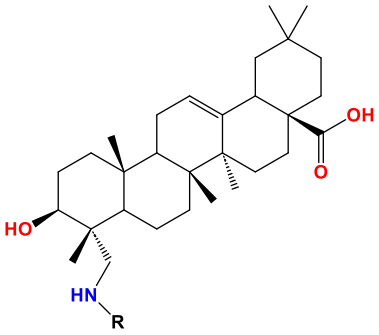
Gypsogenin, R = H, R_1=H
Gypsogenic acid, R = H, R_1=OH
1b, R = Ac, R_1= H



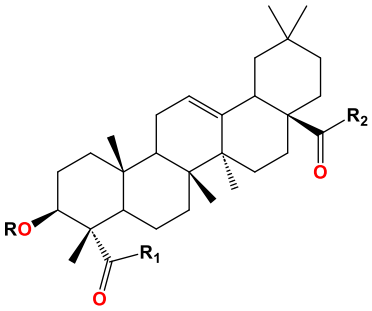
1a, R = H, R_1 = H, R_2 = OH
1d, R = OAc, R_1 = H, R_2 = OH
1f, R = H, R_1 = benzyl, R_2 = OH
4, R = H, R_1 = H, R_2 = -(2,4 dinitrophenyl)hydrazono



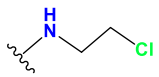
1c, Ar = phenyl, R=H
1h, Ar = phenyl, R=Ac
GP2, Ar = 3,5-bis(trifluoromethyl)phenyl, R = H
GP5, Ar = 4-cyanophenyl, R = H



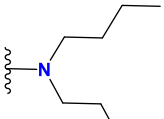
6c, R = piperazinyl phenyl
6l, R = 4-tolyl
6j, R = 4-isopropyl phenyl
6i, R = 4-anisyl,
9a, R = methyl
10, R =



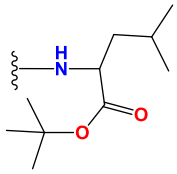
5, R = Ac, R_1 = H, R_2 =



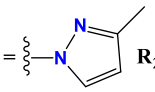
7g, R = Ac, R_1 = H, R_2 =



8, R = Ac, R_1 = H, R_2 =

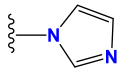


8f, R = Ac, R_1 =



R_2 =

9, R = Ac, R_1 = H, R_2 =



14c, R = Ac, R_1 = OH, R_2 =

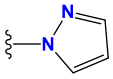


Figure 2. Structure of gypsogenin, gypsogenic acid, and their bioactive derivatives through reaction with 3-OH, C-23 -CHO or -COOH, and C28 -COOH.

Recently, gypsogenin proved itself as an outstanding entity that can enter the competition between PTs for a front position as a lead anticancer agent. Most of previous reports linked

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gypsogenin to anti-cancer effect. It is unlikely to find other bioactivity for gypsogenin and its derivatives; one example is the observed strong inhibition of acetylcholinesterase by which makes them potential treatment for Alzheimer's disease [72]. Stunningly, the first carboxamide series of gypsogenin came out in 2018 which points out the shortage of enough structure-activity relationship (SAR) studies on this precious PT [68]. Moreover, it was not there gypsogenin derivatives with modified ring C before 2023.

Several PTs exhibit limited water solubility and low bioavailability, which can be addressed by derivatization [73]. Derivatization not only optimizes triterpenes pharmacokinetics but also pharmacodynamics. Herein, we summarized chemical modifications of gypsogenin four functional groups and focused on the anti-cancer effect of gypsogenin and its semi-derivatives. We generated SAR for gypsogenin and its derivatives against leukemia, breast cancer and lung cancer. We present our recommendations for prospective work and the missing information that should be addressed. Our study represents a cornerstone reference for any future research linking gypsogenin and cancer. We believe that future extensive SAR studies of gypsogenin will advance it to a front position in the pentacyclic triterpenes game of thrones on anti-cancer therapy.

2. Gypsogenin extraction and chemical transformation:

The difficulty of isolation of gypsogenin from plants and the high price of commercially available gypsogenin limited its extensive SAR studies. One extraction example showed that starting with 20 kg of air-dried roots of *Gypsophila oldhamiana* yields as low as 1.3 g of pure gypsogenin. The procedures were initiated by water extraction of the water soluble saponins before drying under vacuum. The mixture is subjected to acid hydrolysis using 10% HCl for 72 h before neutralization with NaOH and extraction with ethyl acetate. After evaporation, the mixture is applied to column chromatography using 10 : 1 hexane–ethyl acetate eluent to give rise gypsogenin as a white solid [68].

As we mentioned above, gypsogenin has four functional groups that can be feasibly modified to enhance its pharmacodynamic and pharmacokinetic profile. The 3-OH group was acetylated using the conventional method used for other PTs—reflux with acetic anhydride in dry pyridine—as described by Emirdag *et al*. [74]. Addition of dimethyl amino pyridine (DMAP) as a catalyst is used elsewhere to improve yield [72,75]. The 3-OH group was recently oxidized forming the 3-keto analogue. This was achieved by mixing gypsogenin with Dess-Martin periodinane in dichloromethane at 0 °C for 15 minutes [75]. The authors also reported 3-OH etherification using different alkyl bromides in presence of potassium iodide and potassium carbonate in dimethyl formamide (DMF) at 60 °C [75]. Dehydration of gypsogenin by thionyl chloride in (DMF) eliminates the 3-OH group and produces its unsaturated 2,3 dehydro- analogue [75].

Gypsogenic acid (**Figure 2**), the dicarboxylic acid analogue of gypsogenin, can be isolated from *Gypsophila oldhamiana* roots, especially if a portion of gypsogenin is transformed into gypsogenic acid during the hydrolysis step. In addition, its 3-acetyl analogue was synthesized through oxidation of 3-acetyl gypsogenin by sodium hypochlorite and hydrogen peroxide in presence of sodium dihydrogen phosphate at room temperature [76]. A similar oxidation process could be achieved by vigorous stirring with potassium permanganate in ethanol water mixture at room temperature [76].

The 4-aldehyde group of gypsogenin is versatile and has been reacted in different ways. Its oximation using hydroxylamine hydrochloride in pyridine at 105 °C afforded compound **1a** in a good yield (**Figure 2**) [68,74]. It was also reacted with thiosemicarbazide in a 1:1 MeOH: water mixture under reflux forming a thiosemicarbazone analogue [74]. Another amination of gypsogenin's 4-aldehyde was performed in acetic acid using phenyl hydrazine or 2,4-dinitrophenylhydrazine solvent at room temperature; the later resulted in the formation of Schiff base **4** [68].

We have performed reductive amination of gypsogenin's 4-aldehyde group using different amines and sodium triactoxyborohydride, for *in situ* reduction of the formed Schiff base, in dichloroethane solvent at room temperature (compound **6c**,**6l**, **6j**, **6i** and **10**) [77,78]. The yield of this reaction was generally poor due to low solubility of gypsogenin in dichloroethane. That is why another group has performed this reaction in methanol while using sodium borohydride for as a reducing agent to afford compound **9a** [75].

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Oxidation of ring C was recently conducted using different conditions leading to different products. Stirring of gypsogenin with hydrogen peroxide and formic acid in dichloromethane at room temperature afforded the epoxide congener (**2**). On the other hand, oxidation of gypsogenin using selenium dioxide in acetic acid under reflux gave rise to the 11-keto derivative (**3**) (**Figure 3**). The produced enone system of ring C imitate that is naturally found in glycyrrhetinic acid.

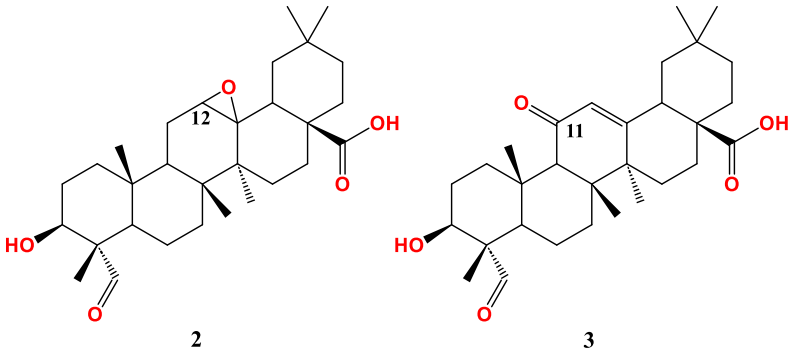

Figure 3. Gypsogenin derivatives with modified ring C.

The fourth functional group of gypsogenin is the 28-COOH which is widely found in PTs. A feasible esterification process involves activation by potassium carbonate in DMF at room temperature followed by addition of appropriate alkyl bromide. This was applied for synthesis of **1c** [**78**], **GP2**, and **GP5** [79] with good yields. Different amides of 3-acetyl gypsogenin were produced by activation of the carboxyl group with oxalyl chloride followed by addition of the appropriate amine in presence of triethyl amine as a catalyst in dichloromethane [68,75]. This general method was applied for the synthesis of amides shown in **Figure 2** such as compounds **5**, **8** [75] and **9** [76] in good yields. Bisamidation was performed for 3-acetyl gypsogenic acid adopting the same procedures to afford derivatives such as **8f** through reaction with two different amines for each carboxyl group [76]. Some reported bisamides were synthesized by reacting 3-acetyl gypsogenic acid dichloride with the two molar equivalents of the same appropriate amine [72,76].

3. Anti-cancer effect of gypsogenin, gypsogenic acid, and their semisynthetic derivatives

3.1. Anti-leukemic activity

In 2007, gypsogenic acid, the 4-carboxyl analogue of gypsogenin **(Figure 2)**, didn't show observable activity against chronic myeloid leukemia (K562) and acute myeloid leukemia (HL-60) where its IC_{50} exceeded 100 μM for both cells [80]. Another study was in accordance with this where gypsogenic acid IC_{50} against K562 was 227.6 μM, however HL-60 was more sensitive (IC_{50} 61.1 μM) [81]. The latter value is in discrepancy with the previous report by Lee group [80]. Gypsogenic acid demonstrated low activity against other lymphoid leukemias SKW-3 and BV-173 (IC_{50} 79.1 and 41.4 μM, respectively) [81].

Later on, we found that gypsogenin highly outperforms gypsogenic acid with IC_{50} 12.7 μM against K562 highlighting the crucial role of 4-aldehyde group [79]. Simultaneously, Emirdag *et al.*, revealed that gypsogenin has anti-proliferative effect on HL-60 (IC_{50} 10.4 μM) by inducing apoptosis [74,82]**.** Acetylation of its 3-OH group by acetic anhydride in presence of pyridine afforded **1b** with no significant impact on activity against HL-60 (**1b** IC_{50} 10.77 μM). Gypsogenin activity is increased by oximation of its aldehyde group (compound **1a** IC_{50} 3.9 μM). Mutually, the 3-acetylated oxime analogue **1d** surpassed the activity of **1b** (IC_{50} 5.9 μM) [74]. Gypsogenin benzyl ester **1c** has IC_{50} 8.1 μM, however **1c** acetylation product **1h** has IC_{50} 6.7 μM [74] **(Figure 3)**.

By virtue of its notable apoptotic effect, **1c** was further benchmarked for its effect on K562 cell line where it showed moderate activity (IC_{50} 9.3 μM) [83]. However, this study represented a turning point for a better understanding of gypsogenin's molecular target. **1c** inhibited ABL1 tyrosine kinase

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with IC_{50} 8.71 μM. This is assumed to be the main target for its cytotoxic effect on K562. It is needless to say that the presence of other off targets cannot be excluded. Concomitantly, **1c** inhibited other kinases such as C-terminal Src kinase (CSK) and Lyn kinase isoform B; LYN B (IC_{50} 1.5 μM and 2.9 μM, respectively) [83]. It is clear that oximation of **1c** is detrimental for its activity on both K562 and HL-60 as the respective IC_{50} value of **1f** is 21.3 μM and 10.6 μM [83].

Ciftci *et al*. moved forward with a structure activity relationship study of 1c and succeeded in enhancing its activity [79]. As mentioned above, the free aldehyde group is crucial for activity against leukemia. Therefore, Ciftci *et al*. came up with phenyl substituted esters of 1c keeping a free 4-aldehyde group [79]. Compounds GP2 and GP5 have IC_{50} 4.7 and 3.1 μM, respectively, against K562 cells. Additionally, IC_{50} of GP2 and GP5 for ABL1 tyrosine kinase was 7.1 μM and 6.1 μM, respectively. Both compounds have induced an explicit apoptosis effect, especially GP2 whose apoptosis induction was clearer than imatinib; a gold standard ABL1 kinase inhibitor for CML therapy. Concomitantly, GP2 suppressed the downstream signaling of extracellular signal-regulated kinase (ERK) phosphorylation [79]. In a similar vein, both compounds exhibited moderate activity on MT-2 and Jurkat cells. Of interest, the IC_{50} of GP5 for MT-2 and Jurkat was 7.2 μM and 4.8 μM, respectively. The authors evaluated both compounds for their effect on peripheral blood mononuclear cells (PBMC) and calculated the selectivity index as the ratio of the IC_{50} between PBMC and K562 cells. The higher selectivity index of GP2, 11.0, than GP5, 8.0, reflects a more satisfactory safety profile of GP5.

A recent report by Ulusoy *et al*. showed that reductive amination of the 4-aldehyde group with different aromatic and alicyclic amines has either reduction or complete abrogation of anti-K562 activity [78]. The hit compound in this study, **6l**, has IC_{50} 11.3 μM which is even less active than the parent compound, gypsogenin [78]. Furthermore, **6l** inhibited ABL1 kinase in a moderate fashion (IC_{50} value of 13.0 μM). This is another evidence of the crucial role of the 4-aldehyde group for anti-K562 activity (**Figure 4**). In addition, **6l** has less effect on MT-2 and Jurkat than those of **GP-2** and **GP-5**. Compound **6l** has moderate effect on a panel of kinases at 30 μM of drug concentration specially BRK, BTK, LYN B, and SRC. Compound **6j** with the more hydrophobic 4-isopropyl substitution exhibited less activity (IC_{50} 23.8 μM), whereas presence of a bulky *N*-piperazinyl benzyl moiety abolished activity as for **6c** (IC_{50} > 100 μM). The activity is also abolished in presence of electron donating substitution as for **6i** (IC_{50} > 100 μM).

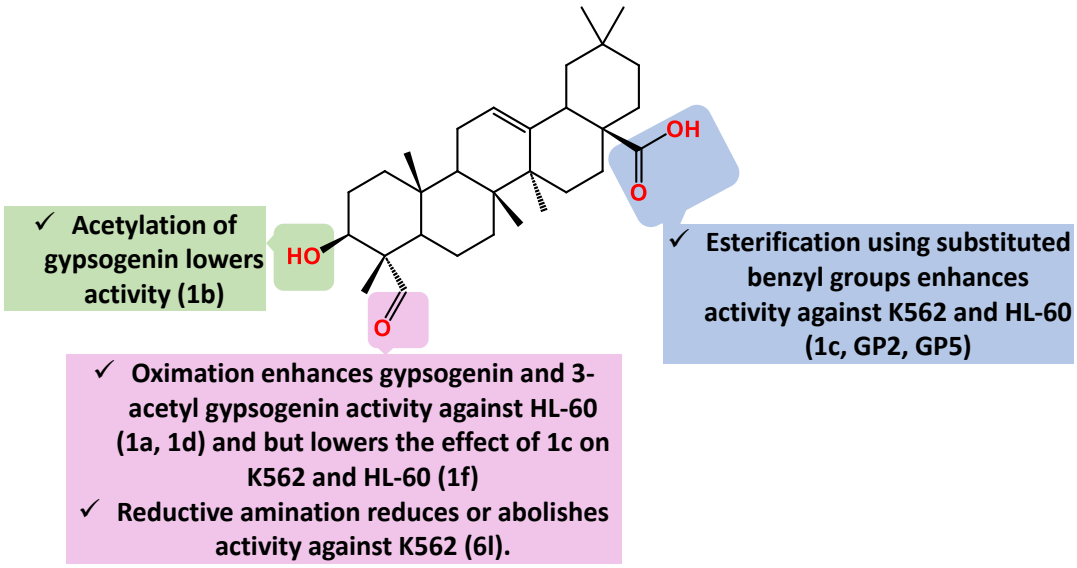

Figure 4. Summary of gypsogenin derivatives SAR pertaining to cytotoxicity against K562 and HL-60 cells.

Surprisingly, there is no report linking between gypsogenin or gypsogenic acid carboxamides and leukemia till now. This is the same case for gypsogenin derivatives with modified ring C (**Figure**

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3). In a word, gypsogenin benzyl esters are the most active derivatives against K562 and HL-60 leukemias till now. The SAR pertaining to activity against K562 and HL-60 is afforded in **Figure 4**.

3.2. Anti-breast cancer activity

Gypsogenin has moderate cytotoxic activity on MCF-7 (IC_{50} 9.0 μM) however its benzyl ester derivative 1c has IC_{50} 5.1 μM [74]. Surprisingly, substituted benzyl esters such as GP2 and GP2 showed less activity than gypsogenin with respective IC_{50} 51.58 μM and 15.3 μM. Notably, the 3-acetyl analogues of gypsogenin and 1c, namely 1b and 1h, possess less activity (IC_{50} 20.5 μM and 65.1 μM, respectively). However, oximation of gypsogenin and 1c slightly improves their cytotoxic effect as seen for 1a and 1f. The exact mechanism of action is not elucidated [74].

Wu *et al.* found that gypsogenic acid has weak antiproliferative effect on MCF-7 (IC_{50} 26.8 μM) which also highlight the role of 4-aldehyde group. The authors highly enhanced gypsogenin and gypsogenic acid activity through mono-and bisamidation [76]. Gypsogenin carboxamide with imidazole, compound **9**, has IC_{50} 3.7 μM which is similar to gypsogenic acid mono-amide of C28 with pyrazole, compound **14c,** with IC_{50} 3.8 μM. Gypsogenic acid bisamide of both C23 and C28, compound, **8f** demonstrated a pronounced activity (IC_{50} 4.1 μM). The favorable safety profile of those carboxamides is shown through measuring their activity on human umbilical vein endothelial cells (HUVEC cells). **8f** possesses the highest selectivity index (24.0) among the mentioned active compounds.

Another evidence of the efficiency of gypsogenin amides was disclosed this year by Sun *et al* [75]. Two strong amides namely, 5 and 8 possess IC_{50} 5.7 μM and 13.8 μM, respectively, towards MCF-7. They also synthesized compound 9a which is afforded by reductive amination using methylamine; its IC_{50} is 11.3 μM which is more than gypsogenin (IC_{50} 9.0 μM). The selectivity index of 5, 8, and 9a exceeds 30 when related to their effect on HUVEC.

Ring C oxidized gypsogenin derivatives have been recently developed (**Figure 3)** [75]. The epoxide derivative (**2**) has IC_{50} 26.6 μM on MCF-7. In parallel with this, the 11-keto derivative (**3**) has a similar activity (IC_{50} 25.3 μM) implying that modification of this ring by oxidation reduces MCF-7 sensitivity. Conclusively, gypsogenin carboxamides are the best cytotoxic entities against MCF-7 when compared to other derivatives **(Figure 5)**.

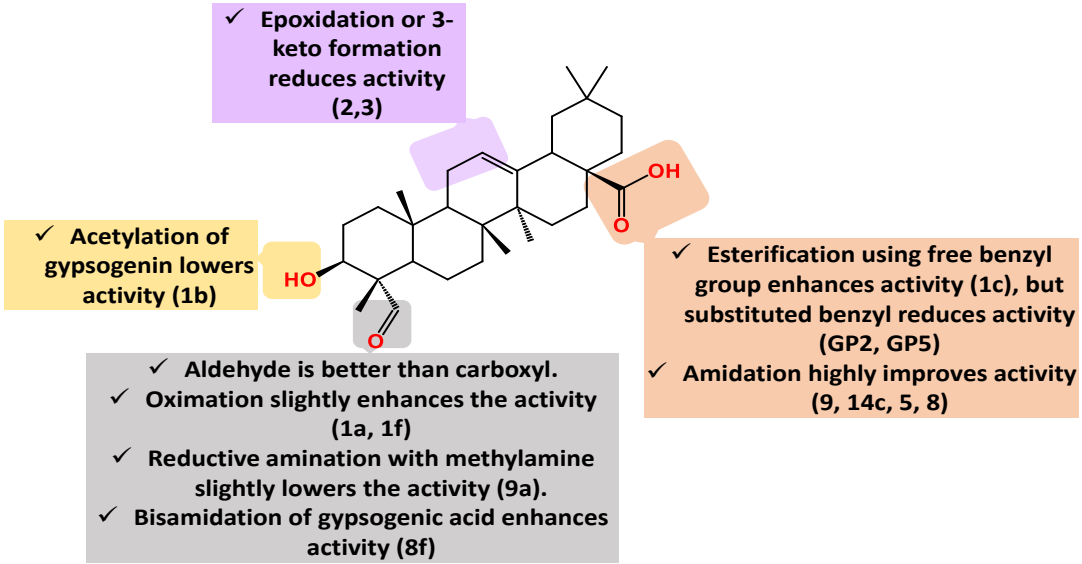

Figure 5. Summary of gypsogenin derivatives SAR pertaining to cytotoxicity against breast cancer cells.

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3.3. Anti-lung cancer activity

Gypsogenin can inhibit the growth and metastasis of Lewis lung cancer through inhibition of tumor angiogenesis and induction of apoptosis [84]. Different molecular targets were implicated in this mechanism. Gypsogenin downregulated mutant P53 and vascular endothelial growth factor (VEGF). It reduces the expression of Bcl-2 protein and raises Bax expression, promoting tumor apoptosis. The anti-proliferative effect of gypsogenin, 3-acetyl gypsogenin, (**1b**), and 3-acetyl gypsogenic acid against A549 lung cancer cells is moderate (IC_{50} 19.6, 30.8, and 23.7 μM) [68,76]. Oximation of gypsogenin and **1b** keeps the activity without significant change [68]. 2,4-dinitrophenyl)hydrazono derivative of gypsogenin (**4**) demonstrated strong cytotoxic effect on A549 cells (IC_{50} 3.1 μM) [68]. In accordance, the amino product (**9a**) exhibited stronger cytotoxic effect (IC_{50} 1.5 μM) [75].

The two carboxamides **9** and **14c** showed a bit higher activity than compound **4** (IC_{50} 2.5 and 2.8 μM, respectively) [76]. Both compounds destroyed the cell membranes and increase their permeability, which led to the outflow of intracellular nucleic acid, but they weakly induce apoptosis and arrest the cell cycle [76]. Another anti-lung cancer hit derivative is gypsogenic acid bisamidation product of (**8f**) whose IC_{50} is 2.0 μM. However, it is noteworthy that mono-amidation products **9** and **14c** surpass (**8f**) activity but with lower selectivity index for HUVEC.

Concomitantly, compounds **5** and **8** showed sub-micromolar effect on A549 (IC_{50} 0.5 μM and 0.9 μM, respectively), induced both apoptosis through damaging the cell membrane and cell cycle arrest. Combining *in silico* and *in vitro* tools defined VEGF1 as gypsogenin target. Remarkably, compound **5** showed higher binding affinity to VEGF1 than the parent compound which is in accordance with the cytotoxicity results. Gypsogenin esters showed disappointing results as for **GP2** whose IC_{50} exceeds 100 μM and **GP5** which is less active than the parent compound (IC_{50} 24.5 μM).

The epoxide analogue **(2)** has almost same activity as the parent compound (IC_{50}.18.7 μM) whereas the 11-keto derivative **(3)** has a slightly better activity (IC_{50}.13.5 μM)). In conclusion, gypsogenin carboxamides are the most active anti-proliferative entities against A549 **(Figure 6)**.

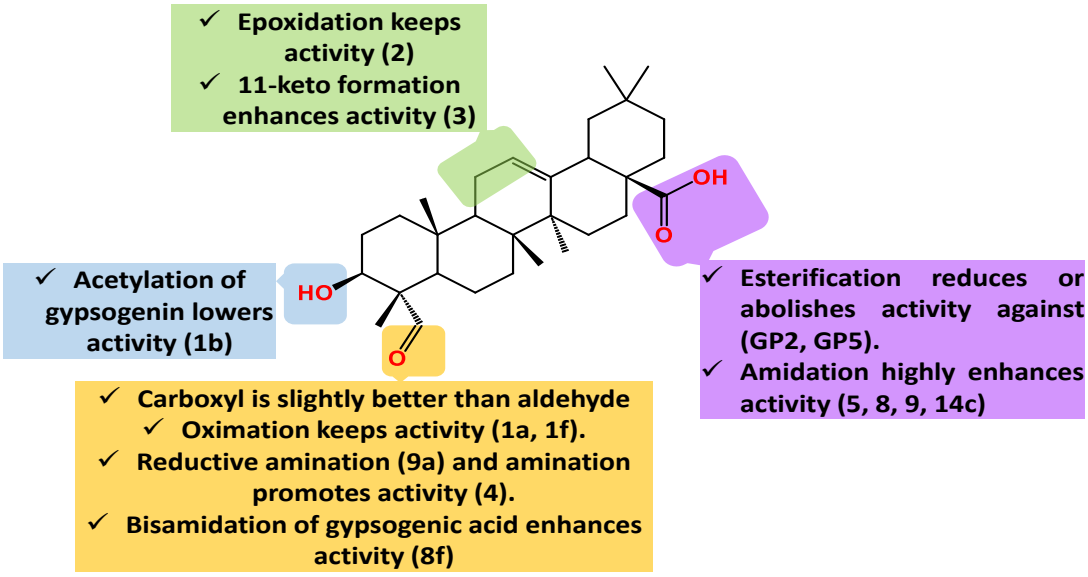

Figure 6. Summary of gypsogenin derivatives SAR pertaining to cytotoxicity against lung cancer cells.

3.4. Other anti-cancer activities

A batch of gypsogenin derivatives demonstrated other notable anti-cancer effects. In this regard, we will focus only on compounds with at least single digit micromolar IC_{50}. Compound **1a** has a remarkable anti-proliferative activity against SaoS-2 cells (osteosarcoma) and HeLa cells (cervical cancer). Its 3-acetylated derivative (**1d**) also has a similar effect on SaoS-2 but not on HeLa. It is

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noteworthy that gypsogenin has IC_{50} 7.8 against SaoS-2 which is better than **1a** and **1d,** and 3-acetyl gypsogenin **(1b)**. On the other hand, **1d** is distinguished by its high activity against HT-29 cells (colorectal adenocarcinoma**)** [74] **(Table 1)**.

Table 1. Gypsogenin derivatives with different cytotoxic activities.

Compound	**Cell line name and IC_{50} μM**		
	HT-29 [74]	**Saos-2 [82]**	**HeLa [74]**
Gypsogenin	**10.4**	**7.8**	**22.4**
1a	**10.8**	**7.9**	**8.7**
1b	**11.1**	**8.2**	**35.0**
1d	**6.7**	**8.9**	**>100**
		LOVO [75]	
2		**> 30**	
3		**17.8**	
5		**7.2**	
8		**0.8**	
9a		**5.8**	
	LOVO [68]	**HePG2** [68]	**SKOV3** [68]
4	**2.9**	**10.0**	**9.7**
7g	**3.5**	**12.5**	**13.1**
	HepG2 [76]	**TE-1** [76]	**MC3-8** [76]
8f	**3.6**	**5.4**	**4.8**
9	**4.0**	**4.7**	**2.9**
14c	**2.2**	**4.2**	**2.6**
		HeLa [79]	
GP2		**35.2**	
GP5		**5.6**	
	U251	**T98G**	**U87**
10 [77]	**5.8**	**8.1**	**17.0**

Other study showed that gypsogenin suppressed gastric cancer cells NCI-N87 proliferation via targeting VEGF and MM-9 and promoting the expression of caspase-3 and Bax proteins [84]. Compounds **4** and **7g** were reported mainly for targeting colon cancer cells (LOVO) through strong induction of apoptosis and dose-dependent S-phase arrest in cells pre-treated with either of them. Both compounds exhibited moderate effect on SKOV3 (ovarian cancer) and HepG2 cells (Hepatocellular carcinoma) [68]. The amino compound **9a** also exhibited notable activity against LOVO. Compounds 2 and 3 showed no or moderate activity towards LOVO [75]. The most active compound against LOVO cells is compound **8** with submicromolar cytotoxicity implying that gypsogenin carboxamides are usually on the top among other derivatives [75] (**Table 1**).

Three amides were reported by Wu *et al*., **9**, **14c**, and **8f** with outstanding activities against HepG2, TE-1 (esophageal cancer), and MC3-8 (colon cancer) cells [76]. Gypsogenin 28-COOH ester **GP5** showed better activity on HeLa cells than **GP2** [79]. Ciftci *et al*., revealed new derivatives that suppress glioma proliferation through EGFR inhibition. The amino derivative compound **10** has the strongest effect against EGFR and glioma cells U251, T98G, and U87, consequently (**Table 1**). The titled compound clearly induced apoptosis in U251 in a comparable fashion to cisplatin. The study revealed that gypsogenin benzyl esters were less effective than **10** on glioma cells [77] **(Table 1)**. Furthermore, at 30 μM concentration, compound **10** showed moderate inhibition for a panel of other kinases including ABL1 tyrosine kinase.

4. Conclusion and Future Directions

Befitting with its anticancer promise, we presented a critical review of gypsogenin and its derivatives. Gypsogenin possesses a versatile and unique aldehyde group that can be utilized for making covalent interactions with undruggable targets. We dissected how was gypsogenin utilized for semi-synthesis by reacting its four functional groups then we demonstrated bioactivity of the most notable derivatives in literature. So far, gypsogenin carboxamides demonstrated high cytotoxic activity against breast and lung cancer. The bisamides of gypsogenic acid possess prominent activity as well, however their anti-leukemic activity is yet to be explored. Gypsogenin benzyl esters showed pronounced activity against CML. Ring C modified gypsogenin derivatives are weak antiproliferative agents against lung and breast cancer, but they haven't been tested for their anti-leukemic effect. Gypsogenin and its derivatives were reported to target kinases such as ABL1 and VEGF. The selectivity index of some active compounds is high reflecting their potential high safety. Further medicinal chemistry studies on gypsogenin are urgently needed to afford more active hits and elucidate their other plausible targets.

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