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Triterpenoid Dimerization as a Bioactivity-Modulating Strategy: From Natural Pristimerin Dimers to Semi-Synthetic Oleanolic Acid Derivatives †

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Abstract

Dimerization is a potentially useful strategy for modulating triterpenoid bioactivity, although its effects are strongly structure-dependent. Natural pristimerin–pristimerin dimers provide a useful reference showing that naturally occurring dimerization does not necessarily increase biological potency. The main focus of this proceeding is therefore placed on a controlled semi-synthetic series of oleanolic acid (OA) dimers (OADs, **2a**–**2n**) and their C-3-acetylated derivatives (AcOADs, **3a**–**3n**). In previous studies, the OAD and AcOAD series were characterized and evaluated using MTT cytotoxicity assays, selectivity index determination, and antioxidant assays, while the OAD series was additionally examined by molecular docking and ADMETox prediction. In the four-cell MTT panel, OA showed IC50 values of 18.15–19.62 μM, whereas thirteen of the fourteen OADs displayed markedly increased cytotoxic activity, with values generally in the low-micromolar range; compound **2d** gave IC50 values of 1.12–1.64 μM. Post-dimerization acetylation further lowered the mean IC50 in ten of fourteen matched OAD/AcOAD pairs, although the effect remained strongly linker-dependent. Selected derivatives also showed enhanced antioxidant activity. Molecular docking and ADMETox predictions provided additional support for further evaluation of the OAD series. Overall, the results indicate that controlled dimerization can provide a practical and potentially economical strategy for increasing the biological activity of oleanolic acid, provided that linker architecture and subsequent functionalization are appropriately optimized.

Keywords: triterpenes; oleanolic acid; pristimerin; triterpene dimers; oleanolic acid dimers; cytostatic activity; antioxidant activity; ADMETox

1. Introduction

Dimerization provides a conceptually simple means of modifying a natural-product scaffold by covalently linking two molecular units. In triterpenoid chemistry, such a transformation can substantially alter molecular size, shape, conformational freedom, lipophilicity, and the spatial presentation of functional groups. These changes may increase or decrease biological activity depending on the structure of the monomer and the mode of connection [1,2]. Dimerization is therefore particularly interesting from a medicinal-chemistry perspective when the coupling reaction can be performed in a controlled and synthetically practical manner.

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Natural pristimerin–pristimerin dimers provide a useful reference for considering the consequences of triterpenoid dimerization. Cangorosins, xuxuarines, scutionins, and related Celastraceae metabolites display considerable structural and stereochemical diversity, arising from different modes of connection between two quinone-methide triterpenoid units [3–7].

However, their structural complexity is not accompanied by a consistent increase in biological potency. Available biological studies indicate generally weak or variable antibacterial and cytotoxic effects compared with related monomeric quinone-methide triterpenoids [1,7]. Similarly, in an antigiardial study containing pristimerin-derived metabolites, the monomeric compounds pristimerin and tingenone were among the most active constituents [8]. These observations demonstrate that dimerization itself should not be regarded as an intrinsic potency-enhancing event.

Against this natural background, oleanolic acid (OA) provides a deliberately controlled semi-synthetic model of triterpenoid dimerization. Earlier studies demonstrated that C-28-linked OA dimers can show stronger antiproliferative activity than the parent triterpene [9,10]. Subsequently, a systematic series of fourteen OA dimers (OADs, **2a**–**2n**) was obtained in which the length of the linker was varied from one to twelve carbon atoms and two four-carbon unsaturated analogues with cis and trans geometry were included [11]. These compounds were further investigated for cytostatic activity, antioxidant properties, molecular docking, and predicted ADMETox parameters [12]. Finally, acetylation of the free C-3 hydroxyl groups afforded the corresponding AcOADs **3a**–**3n** [13]. This series therefore provides a controlled platform for examining not only whether dimerization can enhance OA bioactivity, but also how linker architecture and a simple post-dimerization modification affect the magnitude of that enhancement.

The aim of this proceeding is to examine triterpenoid dimerization as a strategy for modulating biological activity, with particular emphasis on the previously reported semi-synthetic oleanolic acid dimers. Natural pristimerin dimers are used as a biological and structural reference illustrating that naturally occurring molecular pairing does not necessarily increase potency. The principal analysis focuses on the OAD and AcOAD series and evaluates whether controlled dimerization can provide a simple and potentially economical route to derivatives with enhanced cytotoxic and antioxidant activity. Particular attention is given to linker architecture, selectivity, and the additional effect of C-3 acetylation. No new wet-laboratory experiments are reported here; the quantitative analysis is based on the previously published OA datasets [11–13].

2. Methods

2.1. Natural Pristimerin Dimers as a Contextual Reference

A focused literature component was retained to provide a natural reference for triterpenoid dimerization. The recent review of pristimerin–pristimerin dimers [7], together with representative primary studies [3–6,8], was used to summarize the structural diversity and reported biological consequences of naturally occurring dimer formation. This component was not intended as a new systematic review or meta-analysis. Its purpose was limited to establishing a context for the subsequent analysis of the semi-synthetic OA series. All quantitative structure–activity comparisons presented below are based on the OAD and AcOAD datasets [11–13].

2.2. Semi-Synthetic Oleanolic Acid Dimer Series

The preparation and characterization of the OAD and AcOAD series have been reported previously [11,13]. Oleanolic acid was converted into symmetrical dimers by linking the C-28 carboxyl functions, which are attached at C-17 of the oleanane skeleton, using α,ω-

dihalogenated linker reagents under K_2CO_3/DMF conditions at approximately 80 °C. The resulting series comprised saturated linkers containing one to twelve carbon atoms and two unsaturated four-carbon linkers with cis or trans geometry. The corresponding AcOADs **3a**–**3n** were subsequently obtained by acetylation of the free C-3 hydroxyl groups with acetic anhydride [13]. Detailed synthetic, purification, and spectroscopic procedures are available in the original reports.

2.3. Biological and Computational Data

Cytotoxicity data used for the paired comparison were obtained by the MTT assay in SKBR-3 breast adenocarcinoma, SKOV-3 human ovarian cystadenocarcinoma, PC-3 prostate carcinoma and U-87 glioblastoma cells. Human dermal fibroblasts (HDF) served as the non-malignant reference for calculating the Selectivity Index (SI = IC50,HDF/IC50,cancer) [11,13]. Antioxidant activity was evaluated with DPPH and, in the extended study, CUPRAC assays [11–13]. The OAD series was also examined by QSAR/PASS analysis [11], blind docking with CB-Dock2 against focal adhesion kinase (FAK; PDB 1MP8), and ADMETox prediction with ADMETlab 2.0 [12].

For Table 1, the four cancer-cell IC50 values reported for each OAD and matched AcOAD were converted to a simple arithmetic mean. This descriptive mean is used only to visualize the direction and magnitude of the acetylation effect across the matched series; it is not a pooled pharmacological endpoint and no inferential statistics were applied to the cross-cell-line average. No quantitative cross-study comparison was performed for the natural pristimerin dimers because of the heterogeneity and limited extent of the available biological data.

Table 1. Mean MTT IC50 values for matched non-acetylated and acetylated OA dimers. Values are arithmetic means across SKBR-3, SKOV-3, PC-3 and U-87 cells calculated from the published datasets [11,13].

Linker	OAD	Mean IC_{50} (μM)	AcOAD	Mean IC_{50} (μM)	AcOAD/OAD
1 C	**2a**	3.64	**3a**	2.16	0.59
2 C, sat.	**2b**	6.55	**3b**	3.06	0.47
3 C, sat.	**2c**	7.68	**3c**	3.26	0.42
4 C, sat.	**2d**	1.38	**3d**	3.26	2.36
4 C, cis	**2e**	2.43	**3e**	3.21	1.32
4 C, trans	**2f**	4.98	**3f**	6.87	1.38
5 C, sat.	**2g**	5.37	**3g**	4.27	0.79
6 C, sat.	**2h**	5.66	**3h**	4.94	0.87
7 C, sat.	**2i**	3.34	**3i**	1.95	0.58
8 C, sat.	**2j**	10.19	**3j**	2.77	0.27
9 C, sat.	**2k**	7.02	**3k**	2.46	0.35
10 C, sat.	**2l**	18.54	**3l**	5.82	0.31
11 C, sat.	**2m**	4.17	**3m**	1.92	0.46
12 C, sat.	**2n**	6.92	**3n**	9.89	1.43

A ratio below 1.00 denotes a lower mean IC_{50} after acetylation. The mean is a descriptive comparison across different cancer cell lines and should not be interpreted as a single pharmacological endpoint.

3. Results and Discussion

3.1. Natural Pristimerin Dimers as a Reference Point for Controlled Dimerization

Natural pristimerin–pristimerin dimers illustrate the structural diversity that can arise when two triterpenoid units become covalently connected. Different ether-bridge connectivities, oxidation states, and stereochemical arrangements generate several families of natural dimers, including cangorosins, xuxuarines, and scutionins [3–7].

Their structures demonstrate that dimerization can profoundly remodel the three-dimensional architecture of a triterpenoid scaffold. Their biological behavior, however, does not indicate a general potency advantage. Available data suggest that many pristimerin–pristimerin dimers possess weak or variable antibacterial and cytotoxic activity compared with related monomeric quinone-methide triterpenoids [1,7]. Natural pristimerin dimers are therefore used here primarily as a reference showing that molecular pairing alone is insufficient to guarantee enhanced activity. This provides the rationale for examining whether a deliberately controlled mode of dimerization can produce a more predictable biological gain in the oleanolic acid series.

3.2. Controlled Dimerization of Oleanolic Acid: Synthetic Simplicity and Linker Design

The OA series differs fundamentally from the natural pristimerin dimers because the connection between the two triterpenoid units was introduced deliberately and varied systematically. OADs **2a**–**2n** were obtained from OA using defined dihalogenated linkers under K_2CO_3/DMF conditions at 80 °C (Figure 1). An important practical feature of the developed method is its simplicity: only one dimeric product was obtained in each reaction, and the crude products could be isolated by crystallization or precipitation without silica-gel column chromatography [11]. The method therefore provides a comparatively simple and economical route for examining whether dimerization can convert OA into derivatives with substantially increased biological activity.

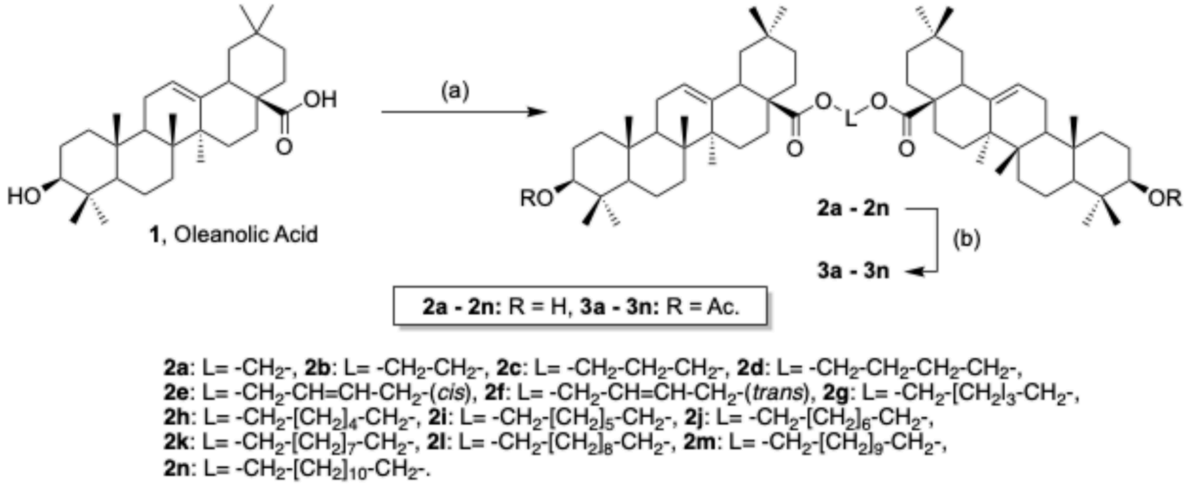


Figure 1. General synthesis of oleanolic acid dimers (OADs, **2a**–**2n**) and their acetylated derivatives (AcOADs, **3a**–**3n**). Reagents and conditions: (**a**) K_2CO_3, dry DMF, 80 °C; appropriate dihalogenoalkane or dihalogenoalkene; (**b**) $(CH_3CO)_2O$, reflux.

3.3. Dimerization Markedly Enhances the Cytotoxic Activity of Oleanolic Acid

The experimental MTT data demonstrate a clear overall gain in cytotoxic activity after OA dimerization. The parent OA showed IC50 values of 18.15–19.62 μM across SKBR-3, SKOV-3, PC-3, and U-87 cells, corresponding to a four-line mean of 18.80 μM [11]. Thirteen of the fourteen OADs were markedly more active than OA, with IC50 values generally in the low-micromolar range. The strongest effect was observed for the saturated four-carbon dimer **2d**, which gave IC50 values of 1.12–1.64 μM and a mean value of 1.38 μM. In contrast, the ten-carbon dimer **2l** showed a mean IC50 of 18.54 μM, close to the parent compound. Dimerization therefore provided a substantial activity gain for most derivatives, but the magnitude of that gain was strongly dependent on linker architecture rather than increasing monotonically with linker length [14].

Potency and selectivity also did not move in parallel. Compound **2h**, carrying a saturated six-carbon bridge, was less potent than **2d** but gave SI values of 2.87–3.24 across the four cancer cell lines, whereas the trans-unsaturated four-carbon analogue **2f** had SI values below 0.6 [11]. These differences are large enough to change how the same cytotoxicity result should be interpreted. For a dimeric scaffold, selecting only the lowest tumor-cell IC50 can therefore favor compounds that also damage the non-malignant reference cells.

3.4. Post-Dimerization Acetylation as a Second Activity-Modulating Step

To determine whether the biological properties of the dimeric scaffold could be further tuned by a simple peripheral modification, the free C-3 hydroxyl groups of OADs **2a**–**2n** were acetylated to give AcOADs **3a**–**3n** [13]. The matched series allows the effect of acetylation to be considered independently for each linker architecture (Table 1).

Acetylation lowered the mean IC50 in ten of the fourteen matched linker classes (Table 1), showing that the biological properties of the dimeric OA scaffold could often be further improved by a simple post-dimerization modification. The effect was particularly pronounced for the eight-, nine-, and ten-carbon saturated linkers. Mean IC50 values decreased from 10.19 to 2.77 μM for **2j**/**3j**, from 7.02 to 2.46 μM for **2k**/**3k**, and from 18.54 to 5.82 μM for **2l**/**3l**. The eleven-carbon pair also improved from 4.17 μM for **2m** to 1.92 μM for **3m** [11,13].

The same table also shows why the acetylated series should not be described as uniformly superior. Acetylation made the highly active four-carbon saturated dimer **2d** less active (1.38 μM for **2d** versus 3.26 μM for **3d**). The cis- and trans-unsaturated four-carbon compounds **2e** and **2f** also lost activity after acetylation, and the twelve-carbon derivative shifted from 6.92 μM for **2n** to 9.89 μM for **3n**. In other words, the effect of masking the C-3 hydroxyl group is linker-dependent and may reflect changes in molecular conformation and physicochemical behavior. It does not impose one directional effect on the entire series.

The individual AcOAD data are still strong enough to explain the conclusion of the original study: several acetylated derivatives were active in the low-micromolar range. Compound **3m** gave 1.76–2.31 μM across the four cancer lines, and **3i** gave 1.90–2.00 μM [13]. However, selectivity again varied. Some AcOADs had SI values below 1, while **3m** gave values of about 2.0–2.6. A medicinal-chemistry decision based on these compounds therefore needs both potency and a broader assessment of normal-cell toxicity.

3.5. Antioxidant Activity and Computational Assessment

Antioxidant testing added a second, non-identical SAR. OADs **2a**, **2b** and **2f** were stronger than OA in the DPPH assay [11], while several OADs gave CUPRAC Trolox-equivalent values around 0.04 mg/mL [12]. The two assays need not rank compounds identically because they probe different redox chemistry. Their combined result is nonetheless useful: dimerization altered both cytotoxic and antioxidant behavior, but the structural optimum was assay-dependent. The acetylated series also retained substantial antioxidant activity, with selected AcOADs reaching DPPH Trolox-equivalent values above 0.04 mg/mL [13].

The computational studies add hypotheses rather than a confirmed mechanism. Blind docking of the OADs to FAK (PDB 1MP8) identified favorable poses, with the unsaturated linker compounds among the stronger predicted binders [12]. In the reported CB-Dock2 analysis, compound **2f** reached a Vina score of −11.6 kcal/mol in the largest identified cavity. This result is compatible with target engagement but does not demonstrate that FAK is responsible for the MTT phenotype. Direct biochemical binding or inhibition measurements would be needed to make that connection.

ADMETlab 2.0 predictions were favorable for several absorption, distribution, metabolism, and toxicity parameters, although very low predicted aqueous solubility and non-optimal logS, logP, and logD values remained important limitations [12]. The result is encouraging as an early filter, but the molecular size and lipophilicity of triterpenoid dimers make experimental pharmacokinetics particularly important. None of the current in vitro datasets establish oral exposure, tissue distribution, metabolic stability or antitumor efficacy in vivo. The same restraint applies to the antioxidant measurements: chemical radical-scavenging or copper-reduction assays do not by themselves predict cellular antioxidant effects.

4. Conclusions

Natural pristimerin dimers provide an important reference showing that triterpenoid dimerization can generate considerable structural diversity without necessarily increasing biological potency. The semi-synthetic OA series demonstrates that the outcome can be markedly different when dimerization is introduced in a controlled manner. Using a comparatively simple synthetic procedure, two OA units were connected through defined linkers to generate a series in which thirteen of fourteen OADs showed substantially stronger cytotoxic activity than the parent compound. The magnitude of this improvement depended strongly on linker length and geometry, demonstrating that dimerization is not a universal potency multiplier but can become an effective strategy when the mode of molecular connection is rationally controlled.

Post-dimerization acetylation provided an additional level of optimization, lowering the mean IC50 in ten of fourteen matched OAD/AcOAD pairs while reducing activity in four others. Together with the observed changes in antioxidant properties, these results show that controlled dimerization can provide a practical and potentially economical route from moderately active oleanolic acid to substantially more active derivatives. Further development should focus on compounds combining high potency with favorable selectivity, while direct target validation, solubility, pharmacokinetics, and in vivo efficacy remain necessary before their therapeutic potential can be assessed.

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Abbreviations

The following abbreviations are used in this manuscript:

OA	Oleanolic acid
OAD	Oleanolic acid dimer
AcOAD	Acetylated oleanolic acid dimer
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
SI	Selectivity Index

DPPH	2,2-diphenyl-1-picrylhydrazyl
CUPRAC	Cupric Reducing Antioxidant Capacity
FAK	Focal Adhesion Kinase
ADMETox	absorption, distribution, metabolism, excretion and toxicity

References

1. Happi, G.M.; Ntabo, V.K.; Tcho, A.T.; Wansi, J.D. Naturally Occurring Dimeric Triterpenoids: Occurrence, Chemistry and Bioactivities. *Phytochemistry* **2022**, *200*, 113242. [CrossRef] [PubMed]
2. Paquin, A.; Reyes-Moreno, C.; Bérubé, G. Recent Advances in the Use of the Dimerization Strategy as a Means to Increase the Biological Potential of Natural or Synthetic Molecules. *Molecules* **2021**, *26*, 2340. [CrossRef] [PubMed]
3. Lara-Nah, D.; Cab-Torres, G.D.; Graniel-Sabido, M.J.; Marrero-Carballo, R.; Quijano-Quiñones, R.F.; Mirón-López, G.; Mena-Rejón, G.; Cáceres-Castillo, D. Comparative Study of Pristimerin Oxidation in the Biomimetic Synthesis of Celastraceae Triterpene Dimers. *J. Mol. Struct.* **2025**, *1339*, 142358. [CrossRef]
4. Jacobsen, N.E.; Wijeratne, E.M.K.; Corsino, J.; Furlan, M.; Bolzani, V.D.S.; Gunatilaka, A.A.L. Biomimetic Synthesis of Xuxuarines Eα and Eβ: Structure Revision of Rzedowskia Bistriterpenoids. *Bioorg. Med. Chem.* **2008**, *16*, 1884–1889. [CrossRef] [PubMed]
5. González, A.G.; Rodríguez, F.M.; Bazzocchi, I.L.; Ravelo, A.G. New Terpenoids from *Maytenus blepharodes*. *J. Nat. Prod.* **2000**, *63*, 48–51. [CrossRef] [PubMed]
6. Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. Revised Structures of Cangorosins, Triterpene Dimers from *Maytenus ilicifolia*. *J. Nat. Prod.* **1997**, *60*, 111–115. [CrossRef]
7. Günther, A.; Bednarczyk-Cwynar, B. Bridging Two Worlds: Structural and Pharmacological Aspects of Natural Triterpenoid Dimers: Pristimerin-Pristimerin-Type Dimers. *Molecules* **2026**, *31*, 1386. [CrossRef] [PubMed]
8. Mena-Rejón, G.J.; Pérez-Espadas, A.R.; Moo-Puc, R.E.; Cedillo-Rivera, R.; Bazzocchi, I.L.; Jiménez-Diaz, I.A.; Quijano, L. Antigiardial Activity of Triterpenoids from Root Bark of *Hippocratea excelsa*. *J. Nat. Prod.* **2007**, *70*, 863–865. [CrossRef] [PubMed]
9. Cheng, K.-G.; Su, C.-H.; Yang, L.-D.; Liu, J.; Chen, Z.-F. Synthesis of Oleanolic Acid Dimers Linked at C-28 and Evaluation of Anti-Tumor Activity. *Eur. J. Med. Chem.* **2015**, *89*, 480–489. [CrossRef] [PubMed]
10. Cheng, K.; Liu, J.; Sun, H.; Xie, J. Synthesis of Oleanolic Acid Dimers as Inhibitors of Glycogen Phosphorylase. *Chem. Biodivers.* **2010**, *7*, 690–697. [CrossRef] [PubMed]
11. Günther, A.; Zalewski, P.; Sip, S.; Ruszkowski, P.; Bednarczyk-Cwynar, B. Oleanolic Acid Dimers with Potential Application in Medicine—Design, Synthesis, Physico-Chemical Characteristics, Cytotoxic and Antioxidant Activity. *Int. J. Mol. Sci.* **2024**, *25*, 6989. [CrossRef] [PubMed]
12. Günther, A.; Zalewski, P.; Sip, S.; Bednarczyk-Cwynar, B. Exploring the Potential of Oleanolic Acid Dimers–Cytostatic and Antioxidant Activities, Molecular Docking, and ADMETox Profile. *Molecules* **2024**, *29*, 3623. [CrossRef] [PubMed]
13. Günther, A.; Zalewski, P.; Sip, S.; Ruszkowski, P.; Bednarczyk-Cwynar, B. Acetylation of Oleanolic Acid Dimers as a Method of Synthesis of Powerful Cytotoxic Agents. *Molecules* **2024**, *29*, 4291. [CrossRef] [PubMed]
14. Hoenke, S.; Wiengarn, I.; Serbian, I.; Al-Harrasi, A.; Csuk, R. Synthesis of amide-spacered dimers of ursolic and oleanolic acid. *Mediterr. J. Chem.* **2019**, *9*, 24–36. [CrossRef]

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